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JAMES L. ABBRUZZESE, EDITORS

PANCREATIC CANCER



M.D. ANDERSON SOLID TUMOR ONCOLOGY SERIES

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Raphael E. Pollock, M.D., Ph.D.

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MERRICK I. ROSS, M.D.

Douglas B. Evans, M.D.

*Department of Surgical Oncology
The University of Texas
M. D. Anderson Cancer Center
Houston, Texas*

Peter W. T. Pisters, M.D., F.A.C.S.

*Department of Surgical Oncology
The University of Texas
M. D. Anderson Cancer Center
Houston, Texas*

James L. Abbruzzese, M.D.

*Department of Gastrointestinal
Medical Oncology
The University of Texas
M. D. Anderson Cancer Center
Houston, Texas*

Editors

Pancreatic Cancer

With 109 Illustrations



Springer

Douglas B. Evans, M.D.
Professor of Surgery
Department of Surgical Oncology
The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030, USA

Peter W. T. Pisters, M.D., F.A.C.S.
Associate Professor of Surgery
Department of Surgical Oncology
The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030, USA

James L. Abbruzzese, M.D.
Professor of Medical Oncology
Chairman, Department of
Gastrointestinal Medical Oncology
The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030, USA

Series Editor

Raphael E. Pollock, M.D., Ph.D.
Head, Division of Surgery
Professor and Chairman, Department of Surgical Oncology
The University of Texas M. D. Anderson Cancer Center
1515 Holcombe Boulevard
Houston, TX 77030, USA

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This volume of the M. D. Anderson Solid Tumor Oncology Series is dedicated to The Lustgarten Foundation for Pancreatic Cancer Research and the Pancreatic Cancer Action Network.

The Lustgarten Foundation for Pancreatic Cancer Research (www.lustgarten-foundation.org) is a private, not-for-profit organization dedicated to advancing the diagnosis, treatment, cure, and prevention of pancreatic cancer. The foundation was established in the summer of 1998 by Marc Lustgarten, Vice Chairman of Cablevision Systems Corporation, who lost his battle with pancreatic cancer the following year at the age of 52. The Lustgarten Foundation continues the work that Marc started on behalf of the hundreds of thousands of people whose lives will be affected by pancreatic cancer. To date, the Lustgarten Foundation has funded nearly \$4.5 million in pancreatic cancer research.

The Pancreatic Cancer Action Network (PanCan; www.pancan.org) provides public and professional education that embraces the urgent need for more research on effective treatments, prevention, and early detection of pancreatic cancer. Pam Acosta, Paula Kim, and Terry Lierman, all of whom lost a parent to pancreatic cancer, launched PanCan in 1998 with a celebrity gala fundraiser called *An Evening with the Stars*—now a yearly tradition. Today, PanCan Team Hope groups are being formed throughout the United States as people come together to assist the scientific community in finding a cure for this disease.

Series Preface

The field of solid tumor oncology is changing at an astonishing rate. To learn about new developments, generate fresh research perspectives, and then integrate these advancements into clinical practice is a daunting challenge confronting all who work in the oncology arena. The onset and rapid deployment of Internet capacities worldwide has created a milieu of global and instantaneous information access. The task of staying current is becoming even more challenging, and in some ways, more difficult to accomplish.

Against this information pressure backdrop, how can yet more didactic material for the already overburdened oncology physician be justified? Based on the premise that we all must remain in a learning mode if we are to remain relevant, The University of Texas M. D. Anderson Annual of Solid Tumor Oncology is designed to focus on a single disease site in each volume. It is our belief that there is an information “gray zone” that lies between the peer reviewed (and increasingly electronically available) individual research report and the large comprehensive multiauthored textbook. Between these two loci there exists an information gap that will be best served by a succinct disease site-focused volume that provides an in-depth analysis of current multimodality care for a specific solid tumor system, as well as the areas of basic, translational, and clinical research that will emerge for future clinical application. Each volume in this series is authored by an academic surgical oncologist of national repute in practice at the Department of Surgical Oncology at the University of Texas M. D. Anderson Cancer Center. Under the leadership of these individuals, outstanding experts throughout the world have been tapped to contribute to this effort.

The target audience is physicians who are focusing on solid tumor oncology. However, it is our hope that medical students and physicians-in-training who aspire to a career in solid tumor oncology will also find these volumes to be of value. In this new era, we are now beginning to understand the molecular determinants driving solid tumor carcinogenesis, proliferation, and dissemination. These molecularly based insights are moving rapidly into the clinical armamentarium. This poses a tremendous challenge to those of us who are not yet fully conversant, yet these developments also give confidence that we are about to enter what will certainly be the most exciting era yet in solid tumor oncology. The tumors afflicting our patients compel us to be our best, as does our own dedication to fighting this disease cluster that will surpass cardiovascular illness as a cause of mortality worldwide early in the next millennium. On behalf of my faculty colleague authors at the University of Texas M. D. Anderson Cancer Center and our many contributing experts, I would like to thank you for your willingness to participate with us in this exciting new project.

RAPHAEL E. POLLOCK, M.D., Ph.D.
Houston, Texas

Preface

This volume of the M. D. Anderson Solid Tumor Oncology Series reports the state of the art and describes recent developments in the epidemiology, biology, diagnosis, and treatment of exocrine pancreatic cancer. The authors have attempted to provide the information necessary for clinicians to update their treatment algorithms while also providing insight into novel therapies and areas of basic research under active investigation.

Progress in the understanding of the biology of pancreatic tumorigenesis will be incremental and likely paralleled by small improvements in diagnosis and treatment. The importance of modest gains in the laboratory or the clinic must not be underestimated. Apparently small advances in diagnostic techniques (i.e., imaging and endoscopy) and therapy (i.e., surgery, radiation, and novel local-regional and systemic therapies) often result in clinically meaningful differences in the quality and duration of patient survival. In addition, such advances serve to generate further research by encouraging both young and established investigators in the laboratory, and by stimulating clinicians to support the conduct of clinical trials.

We greatly appreciate the time and effort of all authors who contributed to this text; their contributions reflect their dedication and commitment to the study of pancreatic cancer.

DOUGLAS B. EVANS, M.D.
PETER W.T. PISTERS, M.D., F.A.C.S.
JAMES L. ABBRUZZESE, M.D.

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Contributors

Gulam Abbas, M.D., Long Island Jewish Medical Center, New Hyde Park, NY 11040, USA

James Abbruzzese, M.D., Department of Gastrointestinal Oncology and Digestive Diseases, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Jennifer Almond, R.G.N., Pg.C.E., Department of Surgery, University of Liverpool, Liverpool, L69 3GA, UK

Claudio Bassi, M.D., Surgical and Gastroenterological Department, Verona University, Verona, Italy

David J. Bearss, Ph.D., Arizona Cancer Center, Tucson, AZ 85724, USA

Hans G. Beger, M.D., F.A.C.S., Department of General Surgery, University of Ulm, Ulm, Germany

David H. Berger, M.D., F.A.C.S., Michael E. DeBakey Department of Surgery, Baylor College of Medicine, and Surgical Service, Houston Veterans Affairs Medical Center, Houston, TX 77030, USA

John D. Birkmeyer, M.D., Dartmouth Medical School, Hanover, NH 03755, USA

A. William Blackstock, M.D., Department of Radiation Oncology, Wake Forest University Baptist Medical Center, Winston-Salem, NC 27157, USA

Murray F. Brennan, M.D., F.A.C.S., Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Christiane J. Bruns, M.D., Department of Visceral and Vascular Surgery, University of Cologne, Cologne, Germany

Markus Büchler, M.D., Surgical and Gastroenterological Department, Verona University, Verona, Italy

Elizabeth R. Campbell, R.N., Pancreatic Cancer Program, Arizona Cancer Center, Tucson, AZ 85724, USA

Chusilp Charmsangavej, M.D., Department of Diagnostic Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Paul J. Chiao, Ph.D., Departments of Surgical Oncology, Molecular and Cellular Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Eugene A. Choi, M.D., Department of Surgery, Division of Surgical Oncology, University of Pennsylvania, Philadelphia, PA 19104, USA

Bryan Clary, M.D., Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

K.C. Conlon, M.D., M.B.A., F.A.C.S., Endosurgical Program, Gastric and Mixed Tumor Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Adrienne D. Cox, Ph.D., Departments of Radiation Oncology and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Christopher H. Crane, M.D., Department of Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Jeffrey A. Drebin, M.D., Ph.D., F.A.C.S., Hepatobiliary Pancreatic and Gastrointestinal Surgery Section, Department of Surgery, Washington University School of Medicine, St. Louis, MO 63110, USA

Janet Dunn, B.Sc., M.Sc., CRC Clinical Trials Unit, Institute for Cancer Studies, The Medical School, University of Birmingham, Birmingham B15 2TT, UK

Lee M. Ellis, M.D., Departments of Surgical Oncology and Cancer Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Richard A. Erickson, M.D., F.A.C.P., F.A.C.G., Division of Gastroenterology, Department of Medicine, Scott & White Clinic, Texas A&M University Health Science Center, Temple, TX 76508, USA

Douglas B. Evans, M.D., Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Carlos Fernandez-del Castillo, M.D., Department of Surgery, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114, USA

William E. Fisher, M.D., Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Veterans Affairs Medical Center, Houston, TX 77030, USA

Jason B. Fleming, M.D., Division of Surgical Oncology, Department of Surgery, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Andrea Formentini, M.D., Department of General Surgery, University of Ulm, Ulm, Germany

Wayne A.I. Frederick, M.D., Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Helmut Freiss, M.D., Department of Visceral and Transplantation Surgery, University of Berne, Berne, Switzerland

Frank Gansauge, M.D., Department of General Surgery, University of Ulm, Ulm, Germany

Gary R. Gecelter, M.D., F.A.C.S., Division of General Surgery, Long Island Jewish Medical Center, New Hyde Park, NY 11040, USA

Paula Ghaneh, M.B., Ch.B., F.R.C.S., Department of Surgery, University of Liverpool, Liverpool, L69 3GA, UK

Johannes Görich, M.D., Department of Radiology, University of Ulm, Ulm, Germany

Mahmudul Haq, M.D., Department of Internal Medicine, University of South Florida, Tampa, FL 33601, USA

Masahiro Hiratsuka, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

James R. Howe, M.D., F.A.C.S., Department of Surgery, University of Iowa College of Medicine, Iowa City, IA 52242, USA

Shingi Imaoka, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

Osamu Ishikawa, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

Nora A. Janjan, M.D., F.A.C.P., F.A.C.R., Department of Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Marina E. Jean, M.D., Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Ramon E. Jimenez, M.D., Department of Surgery, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114, USA

Toshiyuki Kabuto, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

Masao Kameyama, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

Harmeet Kaur, M.D., Department of Diagnostic Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

H.J. Kim, M.D., Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Christos Kittas, M.D., Ph.D., Department of Histology, Medical Faculty, University of Athens, Athens, Greece

Sandeep Lahoti, M.D., Department of Gastrointestinal Oncology and Digestive Diseases, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Laura A. Lambert, M.D., Department of General Surgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756, USA

Elizabeth A. Lano, M.D., Department of Diagnostic Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Steven D. Leach, M.D., Division of Surgical Oncology, The Johns Hopkins University, Baltimore, MD 21287, USA

Jeffrey E. Lee, M.D., Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Donghui Li, Ph.D., Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Karl H. Link, M.D., Surgical and Gastroenterological Department, Verona University, Verona, Italy

Andrew M. Lowy, M.D., Division of Surgical Oncology, University of Cincinnati Hospital, Cincinnati, OH 45267, USA

Evelyn M. Loyer, M.D., Department of Diagnostic Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Nikolaos J. Lygidakis, M.D., Ph.D., F.A.C.S., Surgical Oncology Clinic, Athens Medical Center-Apollonion Hospital, Athens, Greece

Anna L. Means, Ph.D., Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Ingrid M. Meszoely, M.D., Department of General Surgery, The Vanderbilt-Ingram Cancer Center, Nashville, TN 37232, USA

Deborah Moffitt, B.Sc., M.Sc., CRC Clinical Trials Unit, Institute for Cancer Studies, The Medical School, University of Birmingham, Birmingham B15 2TT, UK

John P. Neoptolemos, M.S., M.B., Bchir., M.D., F.R.C.S., Department of Surgery, University of Liverpool, Liverpool L69 3GA, UK

Hiroaki Ohigashi, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan

Eileen M. O'Reilly, M.D., Hepatobiliary Disease Management Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

Theodore N. Pappas, M.D., Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

Kiranpreet S. Parmar, M.D., The University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA

A. Scott Pearson, M.D., Department of Surgery, School of Medicine, Division of Surgical Oncology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Paolo Pederzoli, M.D., Surgical and Gastroenterological Department, Verona University, Verona, Italy

Peter W.T. Pisters, M.D., F.A.C.S., Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Robert Radinsky, Ph.D., Pharmacology and Pathology, Amgen Inc., Thousand Oaks, CA 91320, USA

Sotirios Raptis, M.D., Department of Internal Medicine, Medical Faculty, University of Athens, Athens, Greece

George Rassidakis, M.D., Department of Histology, Medical Faculty, University of Athens, Athens, Greece

Howard A. Reber, M.D., Department of Gastrointestinal Surgery, Division of General Surgery, UCLA School of Medicine, Los Angeles, CA 90095, USA

Suresh K. Reddy, M.D., F.F.A.R.C.S., Department of Anesthesiology, Pain Management Section, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

William F. Regine, M.D., Markey Cancer Center and Department of Radiation Medicine, University of Kentucky Medical Center, Lexington, KY 40536, USA

Alexander S. Rosemurgy II, M.D., F.A.C.S., Department of Surgical Digestive Disorders, Division of General Surgery, University of Southern Florida College of Medicine, Tampa General Hospital, Tampa, FL 33601, USA

Erwin Röttinger, M.D., Department of Radiation Therapy, University of Ulm, Ulm, Germany

Yo Sasaki, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, 537-8511, Japan

Miriam Schatz, Dipl.Psych., Department of General Surgery, University of Ulm, Ulm, Germany

Richard D. Schulick, M.D., Departments of Surgery and Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

Charles R. Scoggins, M.D., Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

John Slavin, M.B., B.S., M.S., F.R.C.S., Department of Surgery, University of Liverpool, Liverpool L69 3GA, UK

David Smith, M.B., Ch.B., M.R.C.P., Clatterbridge Centre of Oncology, Wirral, Merseyside, UK

Francis R. Spitz, M.D., Department of Surgery, University of Pennsylvania, Philadelphia, PA 19104, USA

Steven M. Strasberg, M.D., F.R.C.S. (C), F.A.C.S., Department of Surgery, University of Liverpool, Liverpool L69 3GA, UK

Robert Sutton, Ph.D., Department of Surgery, University of Liverpool, Liverpool L69 3GA, UK

Karen E. Todd, M.D., Division of Surgical Oncology, Los Angeles, CA 90095, USA

L. Gerard Toussaint III, M.D., Department of Neurosurgery, Mayo Clinic, Rochester, MN 55905, USA

Douglas Tyler, M.D., Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA

Lobros Vlachos, M.D., Ph.D., Department of Radiology, University of Athens, Athens, Greece

Daniel D. Von Hoff, M.D., F.A.C.P., College of Medicine, Arizona Cancer Center, The University of Arizona Health Sciences Center, Tucson, AZ 85724, USA

Andrew L. Warshaw, M.D., Department of Surgery, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114, USA

Irving Waxman, M.D., Department of Clinical Medicine, Gastrointestinal Endoscopy, Gastroenterology Section, University of Chicago, Chicago, IL 60637, USA

Robert A. Wolff, M.D., Department of Gastrointestinal Oncology and Digestive Diseases, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Terumasa Yamada, M.D., Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, 537-8511, Japan

Charles J. Yeo, M.D., Departments of Surgery and Oncology, The Johns Hopkins Hospital, Baltimore, MD 21287, USA

Larry L. Zhou, M.D., Department of Anesthesiology, Pain Management Section, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

1

Molecular Epidemiology

Donghui Li

Epidemiology and Risk Factors

Pancreatic cancer ranks 13th in incidence but 8th as a cause of cancer death worldwide.^{1,2} In the United States, pancreatic cancer is the fourth leading cause of cancer death in both men and women.³ Every year, 28,000 Americans die of pancreatic cancer, which accounts for 22% of gastrointestinal cancer deaths and 5% of all cancer deaths.⁴ There are some geographic variations in the incidence of pancreatic carcinoma worldwide. The mortality rate for pancreatic cancer is high in Denmark, Sweden, Finland, Ireland, Austria, Czechoslovakia, Hungary, and other European countries, and among nonwhite populations in the United States.⁵ The mortality rate is low in Hong Kong, Spain, Greece, Portugal, Yugoslavia, India, Kuwait, and Singapore.⁵

The most reliable and important predictor of pancreatic cancer incidence is age.⁶ The age-specific incidence in white American women and men increases continuously, even through the late-age category of 85 and older. About 80% of cases of pancreatic cancer occur between ages 60 and 80. Those in the 8th decade of life experience a risk approximately 40 times that of those in the 4th decade. Similar to observations in animal models, pancreatic cancer is more common in men than in women.⁶ In the United States, incidence and mortality rates of pancreatic cancer are higher in blacks of both sexes than in whites. Mortality rates in African Americans are also higher than those of African blacks, suggesting a role for environmental factors.⁷

Hereditary genetic factors account for less than 10% of the total pancreatic cancer burden.^{8–11} Ex-

cess risk of pancreatic cancers has been associated with hereditary pancreatitis with a standardized incidence ratio of 53.¹² Recent evidence also suggests that pancreatic cancer susceptibility is linked to germline mutations in genes causing familial cancer syndromes, eg, hMSH2 and hMLH1 in hereditary nonpolyposis colon cancer,¹³ p16 in familial atypical multiple mole–melanoma, and BRCA1/BRCA2 in breast/ovary cancers.^{10,14,15}

The most prominent and consistent risk factor in pancreatic cancer is cigarette smoking,^{2,4,5,16–18} the relative risk of smokers being at least 1.5. The risk increases as the level of cigarette smoking increases: the highest risk ratio, 10-fold, has been seen in males who consume more than 40 cigarettes daily.² The excess risk levels off 10 to 15 years after smoking cessation. The positive association of smoking with pancreatic cancer has been demonstrated in at least 8 prospective studies^{19–26} and a number of case-control studies.^{16–18,27} In addition, hyperplastic changes in pancreatic duct cells, with atypical changes in their nuclei, were observed among smokers during autopsies, and the extent of such changes appears to increase with the amount smoked.²⁸ As has been suggested, smoking may account for the sex difference in incidence of pancreatic cancer in the United States, because no male-female difference in incidence is seen among nonsmokers.⁷ Another suggestive relationship is the increased risk of pancreatic cancer as a second malignancy in patients with a first smoking-related malignancy, eg, cancer of the lung, head and neck, and bladder.²⁹

The second most important risk factor associated with pancreatic cancer seems to be diet, although

the data for diet are not quite as consistent as those for smoking.^{2,6,30,31} Generally, increased risks have been associated with animal protein and fat consumption and decreased risks with intake of vegetables and fruit. It has been speculated that the increased risk of pancreatic cancer in Western countries and the recent rise of incidence in Japan may be related to dietary factors.³² Obesity is a risk factor for pancreatic cancer and appears to contribute to the higher risk of this disease among blacks than among whites in the United States.^{33,34} The significant interaction between body mass index and caloric intake suggests the importance of energy balance in pancreatic carcinogenesis.³³ Methods of food preparation evaluated in several studies^{31,35–37} showed an association of increased pancreatic cancer risk with high consumption of salt, smoked meat, dehydrated food, fried food, and refined sugar. An inverse association was found with the consumption of food containing no preservatives and additives, raw food, food prepared by high-pressure cooking, and food prepared in electric or microwave ovens.^{31,35–37} Associations with the consumption of cereals, carbohydrates, and coffee are unclear and inconclusive.^{2,6,7,16} Lower levels of serum lycopene, a carotenoid present in fruits, and of selenium were found in persons in whom pancreatic cancer subsequently developed.³⁸ A significant inverse dose-response relationship has been found between the levels of serum folate and pyridoxine and risk of pancreatic cancer.³⁹

The association between alcoholism and pancreatic cancer has been found fairly weak and inconsistent. A few studies have demonstrated an increased risk of pancreatic cancer in heavy drinkers,^{40–42} but more studies have found no significant associations.^{43–51} However, there is general agreement that chronic high intake of alcohol may contribute to increased risk in smokers.^{2,6,7}

Excessively high rates of pancreatic cancer have been reported among workers in certain occupations, such as chemists, coal and gas exploration workers, and those in metal industries, leather tanning, textiles, aluminum milling, and transportation.^{52–54} The available evidence is insufficient, however, to identify any common occupations as certifiable causes of pancreatic cancer. Suggestive findings exist in relation to the products of incomplete combustion,^{55,56} to certain pesticides,^{57–59} and to other chemicals, ie, formaldehyde,⁶⁰ styrene,⁶¹ and asbestos.⁶²

Several medical conditions have been associated with increased risk of pancreatic cancer, although neither the nature nor the sequence of the possible cause-effect relationship has been established.^{6,7,63,64} For example, diabetes has been seen repeatedly in connection with cancer of the pancreas, but it is not clear whether diabetics are really at higher subsequent risk of this cancer or whether the risks of cancer and diabetes are related in some other way. The hypothesis that chronic pancreatitis imparts a higher risk of pancreatic cancer has been examined in case-control studies,^{6,7,65–67} and evidence for both a positive association and for no association has been reported. Chronic pancreatitis most frequently is associated with alcoholism, biliary tract diseases, or both. The observation that the increased risk of developing pancreatic cancer is only for pancreatitis that occurs less than 10 years before the cancer diagnosis suggests either a common risk factor for both diseases or that some forms of pancreatitis are predisposing or early symptoms of the cancer.^{63,64,66} Other notable medical conditions associated with increased risk of pancreatic cancer include gastrectomy and cholecystectomy. It has been postulated that these conditions might predispose to pancreatic cancer through the enhanced formation of *N*-nitroso compounds by bacteria.⁶⁸

Overall, the primary etiology of pancreatic cancer is poorly understood. Known risk factors suggest a role for exposure to carcinogens present in cigarette smoke, diet, and the workplace. Oxidative stress and lipid peroxidation related to diet and some medical conditions may also be involved in the pathogenesis of the disease.

Mechanisms of Pancreatic Carcinogenesis

If the hypothesis that exposure to carcinogens contributes to human pancreatic cancers is true, two different mechanisms might be involved. First, carcinogen-containing bile may be refluxed into the pancreatic duct, causing pancreatic cancer.⁶⁹ Since most carcinogens excreted by the liver are converted into water-soluble noncarcinogenic compounds before being excreted in the bile, reversion to active carcinogens would be necessary before they could act in the pancreatic ductal system. The fact that Japanese men have the highest

rate for cancer of the liver and biliary system in the world but a fairly low rate of pancreatic cancer suggests that carcinogen-containing bile does not have the same effect on these organs.²⁴ This is true even though the pancreas may have some special tumor-promoting factors that allow the mutation-containing cells to gain growth advantage and eventually develop into tumors. Another possible mechanism is that pancreatic duct cells can metabolically activate carcinogens, or that ultimate carcinogens do present in the pancreas.⁷⁰ The metabolic machinery of the pancreatic cells in patients with pancreatitis or pancreatic carcinoma has been shown to be differently regulated.

Carcinogen Metabolism

Information regarding the presence and activity of carcinogen-activating enzymes in human pancreatic tissues is available from several studies.^{71–77} In the earliest one, an explant of human pancreatic duct was exposed *in vitro* to benzo[*a*]pyrene or 7,12-dimethylbenz[*a*]-anthracene to learn whether pancreatic tissues have the capacity to activate polycyclic aromatic hydrocarbon (PAH) into reactive intermediates that bind to DNA.⁷¹ A lower level of DNA binding was found in the pancreas than in the human bronchus. A later study by Foster et al⁷² showed that P450 enzymes 3A1, 2E, 1A2, and NADPH cytochrome P450 oxidoreductase, as well as phase II enzyme glutathione S-transferase (GST) 5-5, were detectable by immunohistochemical assay of normal pancreatic tissues from seven organ donors. The phase I enzymes, but not the phase II enzyme, were induced in tissue samples from 6 patients with chronic pancreatitis and 10 patients with pancreatic cancer. The induction of phase I enzymes in patients with pancreatitis or pancreatic cancer was also reported by investigators who used the same method in another study.⁷³ The expression of CPY 1A1 and 2E1 in pancreatic tissues was detectable by reverse transcription polymerase chain reaction but not by Northern blot in a study of 5 organ donors or surgically removed tissues,⁷⁴ suggesting a low level of expression of these enzymes. A more recent study involving pancreatic tissues from 29 organ donors showed that pancreatic microsomes displayed no activity of cytochrome P450 1A2, 3A4, 1A1, 2B6, 2E1, and 4A1, enzymes involved in activation of aromatic

amines.⁷⁵ Furthermore, immunoblots detected only epoxide hydroxylase at low levels but no expression of P450 1A2, 2A6, 2C8/9/18/19, 2E1, 2D6, and 3A3/4/5/7. The inconsistency of some of the observations in different studies may be partially explained by the various methods used. Overall, human pancreatic tissues seem to have a very low level of expression of cytochrome P450 enzymes for carcinogen activation. The metabolic activation of nitroaromatic hydrocarbons involves conversion to the *N*-hydroxy derivatives through reduction of the nitro group. In contrast to the absence of *N*-oxidation, nitroreductase activities were found in pancreatic cytosols and microsomes at levels comparable to those in the human liver.⁷⁵ These observations demonstrate the capacity of the pancreas to catalyze the reductive metabolism of carcinogenic nitroaromatic hydrocarbons to *N*-hydroxy derivatives, thus providing a critical pathway for their metabolic activation. Such capacity may underlie elevated risks associated with certain exposures, such as combustion products, jet fuel exhaust, and coke-oven emissions.

Activation of aromatic and heterocyclic amines and nitroaromatic hydrocarbons also involves esterification of their *N*-hydroxy derivatives, which can generate more reactive electrophiles. The *O*-acetyltransferase activity, which can be catalyzed by NAT1 and NAT2, was found in pancreatic tissue in which NAT1 appears to play a predominant role.⁷⁵ The presence of NAT1 activity in the pancreas suggests a pathway for metabolic activation of arylamines and nitroaromatics in the pancreas similar to that proposed for the human urinary bladder and lung.

The expression of phase II enzymes, eg, GST, in pancreatic tissues was examined by immunohistochemical analysis in 12 normal pancreatic tissues and 26 pancreatic adenocarcinomas.⁷⁶ Fifty-four percent of the malignant tissues expressed GST- π and 8% expressed GST- α , but none of the normal or malignant tissue samples expressed GST- μ . Although the biochemical characteristics of different classes of GST are not fully understood, absence of the GST- μ gene has been related to increased risk of smoking-induced human cancers.⁷⁷ Thus, it is plausible that lack of GST- μ may contribute to individual susceptibility to pancreatic cancer. Nevertheless, the effects of genetic polymorphisms on individual cancer susceptibility could be obscured

by the phenotypic variability.⁷⁷ This was suggested by findings from a recent study that demonstrated a 7-fold variation in total GST content and 6- to 30-fold variations in levels of expression of 5 GST subunits in 43 normal human pancreases.⁷⁸

Metabolic activation is a prerequisite for the carcinogenic effect of many carcinogens, and considerable interindividual variation exists in the metabolic capacity of carcinogen activation. If the hypothesis that carcinogen exposure is involved in human pancreatic cancers is correct, one would expect individual variation in carcinogen metabolism to affect cancer risk. Unfortunately, so far only two studies have explored the association between genetic polymorphisms of drug-metabolizing enzymes and the risk of pancreatic cancer.^{79,80} In a study of 45 cases and 53 controls, no association was found between susceptibility to pancreatic cancer and genetic polymorphisms of cytochrome P450 1A1, 2D6, and 2E1, enzymes that activate chemical carcinogens.⁷⁹ In another study of 81 pancreatic cancer cases, 78 controls and 119 patients with chronic pancreatitis, a nonsignificant excess of NAT1 slow acetylators was found in cancer cases versus controls.⁸⁰ A significant overexpression of GSTM1 AB or B genotype was found in all pancreatic disease cases.⁸⁰ These observations suggest that the polymorphism of GSTM1 and NAT1 enzymes may be associated with a modest increase in susceptibility to pancreatic diseases. Since cigarette smoking is an etiological factor in pancreatic cancer, individuals with an unfavorable genetic makeup of carcinogen metabolism may well be at increased risk of developing pancreatic cancer. Because the sample sizes of these studies were small, larger studies are definitely needed to explore this issue.

In summary, the presence of carcinogen-activating enzymes and the absence of detoxifying enzymes in the human pancreas may be partially responsible for the organ's susceptibility to carcinogen exposure. Although many other factors are involved in pancreatic carcinogenesis, the detection of a high level of DNA adducts further supports the hypothesis that carcinogens are involved in pancreatic carcinogenesis.

DNA Adducts in Pancreatic Tissues

Data from animal studies support the hypothesis that activation of carcinogens in the liver is suffi-

cient to lead to formation of DNA adducts in the pancreas. For example, in the rat and dog, oral administration of radiolabeled 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a food-borne carcinogen, resulted in high levels of radioactivity bound to DNA in many organs, the level of DNA adducts being highest in the pancreas.^{81,82} In addition, intravenous injection of radiolabeled reactive metabolites of PhIP, *N*-hydroxy-PhIP, or *N*-acetoxy-PhIP into rats resulted in high levels of DNA-PhIP adducts in the pancreas.⁶ These data and the evidence of carcinogen-metabolizing enzymes in the pancreas support the notion that organotropism of the pancreas may, to some degree, govern the susceptibility of this organ to carcinogenic agents.⁶

In support of this hypothesis, a number of studies have shown detection of DNA adducts in human pancreatic tissues.^{75,83,84} In the first,⁸³ 37 autopsy samples demonstrated a significantly lower level of adducts in the pancreas compared with that in the lung. No significant correlation was seen between smoking history and DNA adduct levels in the pancreas, average levels being, respectively, 3.35, 2.45, and 2.0 per 10⁸ nucleotides in the 14 persons who had smoked, 7 who had quit, and 16 who had never smoked. The second study⁷⁵ involved pancreatic tissues from 29 organ donors. In 5 of 13 smokers and 3 of 16 nonsmokers, a major DNA adduct was observed that was chromatographically identical to the predominant 4-aminobiphenyl (ABP)-DNA adduct, *N*-(deoxyguanosine-8-yl)-ABP. 4-ABP is a representative aromatic amine in cigarette smoke. Although overall adduct levels were relatively low, in the range of 0.2 to 1.1 adducts per 10⁸ nucleotides, detection of the putative ABP adduct in the pancreatic tissues suggests that human pancreas may be a target organ for carcinogenic aromatic amines.

To test the hypothesis that DNA damage derived from carcinogen exposure is involved in pancreatic carcinogenesis, my research group measured aromatic DNA adducts in 13 normal tissues adjacent to tumor and 20 tumors from pancreatic cancer patients by ³²P-postlabeling.⁸⁴ Normal pancreatic tissues from 5 nonpancreatic cancer patients and 19 healthy organ donors served as controls. To correlate the DNA adduct level with the patients' characteristics, we collected information on age, sex, body mass index (BMI), and smoking status from medical records. A significantly higher level of total DNA

adducts was detected in pancreatic cancer patients than in the controls. The mean level of total adducts per 10^8 nucleotides in adjacent tissues was 102 ± 21 , compared with 39 ± 6 and 13 ± 1 in tumor and control tissues, respectively. The apparently higher level of DNA adducts observed in our study than in the previous report⁸⁴ can be partially explained by the different versions of the ^{32}P -postlabeling assay and various chromatographic methods used.

Among the DNA adducts observed, one single aromatic adduct was present in 100%, 90%, and 0% of the adjacent, tumor, and control tissues, respectively. The identity of this adduct remains unknown. Two novel clusters of adducts were observed in 11 of 13, 12 of 20, and 2 of 24 adjacent, tumor, and control tissues, respectively; the presence of these adducts was correlated positively with smoking status. Seven of 10 smokers and 3 of 10 nonsmokers displayed these 2 adduct clusters, compared with 7 of 10 nonsmokers who did not have these adducts ($P < 0.05$ by χ^2 test). In addition, the previously defined smoking-related diagonal radioactive zone was detected in 3 adjacent tissues only, although 50% (10 of 20) of the patients with pancreatic cancers in this study were ever smokers. The level of total DNA adducts in smokers tended to be higher than that in nonsmokers ($P = 0.09$, t test). These observations suggested that smoking induces DNA adduct formation in the pancreas, but the adduct profiles may be different from those in the lung and other target tissues of smoking.

Oxidative Stress and Lipid Peroxidation

DNA damages can be also derived from endogenous process such as oxidative stress. Oxygen radicals are well known to play an instrumental role in the development of inflammatory tissue damage, degenerative diseases, and cancer.^{85,86} Oxygen radicals can be derived not only from cigarette smoking or other carcinogen exposure, but also from normal cellular functions, such as metabolism of nutrients and hormones. Free radicals react most readily with polyunsaturated fatty acid, resulting in a chain reaction of lipid peroxidation. In experimental models of acute pancreatitis (cerulein-, diet-, and sodium taurocholate-induced pancreatitis), lipid peroxidation products were found to increase in the pancreatic tissues before morphological changes could be detected.⁸⁷ In patients with acute

or chronic pancreatitis, increased serum and tissue levels of lipid peroxidation products and decreased levels of glutathione have also been reported,^{88–90} suggesting ongoing peroxidation of lipids caused by enhanced generation of oxygen radicals. Moreover, detection of lipid peroxidation products—induced DNA adducts, such as malondialdehyde adduct and etheno adducts, in human pancreatic tissues has been reported in several studies.^{89,91–93} These findings support the notion that the human pancreas is susceptible to oxidative stress- and lipid peroxidation-related damages that may play an important role in pancreatic carcinogenesis.

When we examined the DNA damage in pancreatic tissues induced by oxygen radicals and lipid peroxidation products, we found levels of oxidative DNA damage and lipid peroxidation-related DNA adducts to be significantly higher in patients with pancreatic cancer than in controls. Using the high-performance liquid chromatography electrochemical detection method,⁹⁴ we detected 8-hydroxyguanosine, a mutagenic oxidized DNA base, at levels of 13.0 ± 2.3 (mean $\pm$ SE), 11.9 ± 3.0 , and 7.1 ± 1.06 per 10^5 nucleotides in tumors ($n = 23$), adjacent tissues ($n = 11$), and controls ($n = 23$), respectively.⁹⁵ The difference between controls and adjacent tissues was at borderline significance ($P = 0.08$); between controls and tumors it was statistically significant ($P = 0.03$). In addition, putative DNA adducts derived from malondialdehyde, an end product of lipid peroxidation, were detected in all tissue samples examined, and the level was significantly higher in the adjacent tissues than in the tumors and control samples.⁸⁴ Multiple regression analyses showed that the BMI correlated positively with levels of aromatic adducts ($r = 0.58$, $P = 0.03$) and total adducts ($r = 0.51$, $P = 0.06$) in the tumors.⁸⁴

Overall, the detection of high levels of DNA adducts in pancreatic tissues was consistent with previous epidemiological findings and supports the hypothesis that DNA damage related to carcinogen exposure and oxidative stress is involved in human pancreatic carcinogenesis. Unfortunately, DNA adducts induced by nitrosamine and other alkylating agents have never been measured in human pancreas despite the fact that these DNA damages may be important in inducing *K-ras* mutation. Among the alkylation products of DNA, O^6 -methyl guanine (O^6 -MG) is a lesion of critical importance in the induction of mutations.⁹⁶ The formation and

persistence of O⁶-MG has long been identified as a key parameter in determining tumor incidence in animal models. O⁶-MG is a promutagenic lesion that results in guanine (G) to adenine (A) transition, the most common *K-ras* mutation seen in human pancreatic cancer. The presence of O⁶-MG in pancreatic tissues is under intensive investigation in our laboratory.

Mutation Spectrum

Studies of mutation spectra of the tumor suppressor gene p53 have shown that specific endogenous or exogenous mutagens may induce characteristic patterns of DNA alteration in the tumor as a fingerprint of exposure.^{97,98} For example, some carcinogen-related human cancers, ie, tumors of the lung and upper aerodigestive tract, displayed spectra of p53 mutations dominated by G to thymine (T) transversion on the nontranscribed strand. This mutation spectrum is consistent with data for several different types of carcinogens found in tobacco smoke, eg, benzo[*a*]pyrene and tobacco-specific nitrosamines. The frequency of p53 mutation and of G to T transversion on the nontranscribed strand has been positively correlated with lifetime cigarette consumption or the history of tobacco and alcohol use. The mutation frequency of the p53 gene in pancreatic cancer is about 44%. The mutational pattern is similar to that in bladder cancer, another smoking-related cancer, but not to that in lung cancer.^{97,99} Forty-one percent of the p53 mutations in pancreatic cancer were guanine cytosine (GC) to adenine thymine (AT) transitions, and 13% were GC to TA transversions. In contrast, 24% and 40% of the p53 mutations in lung cancers were GC to AT and GC to TA substitutions, respectively.⁹⁸

Pancreatic tumors have the highest frequency of *K-ras* mutation among all human cancers.¹⁰⁰ Eighty percent to 100% of pancreatic cancers have been reported to harbor activating point mutations in codon 12 of the *K-ras* gene.^{101–105} It is believed that *K-ras* mutation is an early event during pancreatic carcinogenesis.^{106,107} Mutant *K-ras* has been detected in tumor cells recovered from peripheral blood, pancreatic juice, stool, and fine-needle aspirates, the last showing promise as a tool for gene-based diagnosis.^{108–111} Several studies have shown, however, that the mutated genes can also be detected in patients with chronic pancreatitis and

other noninvasive pancreatic lesions.^{109,112} Thus, although mutations in *K-ras* may not have the specificity needed for population-based screening tests for pancreatic cancer, study of the frequency, timing, and mutational spectra of the *K-ras* gene may provide insight into the etiology and molecular pathogenesis of pancreatic cancers. In pancreatic cancer, a higher frequency of *K-ras* mutation has been associated with the patients' smoking or drinking status.¹¹³ In a study of 82 surgically resected or biopsied primary adenocarcinomas of the pancreas from patients in the United States, *K-ras* mutations were found in 89% of ex-smokers and 86% of current smokers, compared with 68% of never-smokers.¹¹⁴ The association between *K-ras* mutation and alcohol consumption was also examined in 51 pancreatic cancer cases,¹¹⁵ and the risk of mutation was found 3 times higher in alcohol drinkers than nondrinkers, with a linear trend. The risk of mutation was also found to be higher for smokers than nonsmokers.¹¹⁵ Interestingly, when mutations were found in both p53 and *K-ras* genes in a tumor, the same type of mutation (either transition or transversion) was detected in the same tumor, suggesting a common mechanism.¹¹⁶ These findings further support the hypothesis that exposure to carcinogens through cigarette smoke may increase the frequency of gene mutation, which in turn contributes to pancreatic carcinogenesis, although no specific carcinogens are suggested by the limited information.

K-ras mutation is common in various tumor models of animals exposed to different chemical carcinogens, eg, PAH, aromatic amine, nitrosamine, and other alkylating agents.¹¹⁷ The high frequency of *K-ras* mutation in human pancreatic cancers parallels that found in pancreatic tumors in hamsters induced by the alkylating carcinogen *N*-nitrosobis(2-oxopropyl) amine (BOP).¹¹⁸ In human pancreatic cancers, however, the mutation spectrum does not implicate a specific carcinogen but suggests exposure to multiple cancer-causing agents. According to a summary of 13 studies of pancreatic cancer, including 349 mutants of the *K-ras* gene, 62% of the mutations are G to A transitions, and 35% are G to T transversions.¹¹⁴ G to A transition at codon 12 of the *ras* gene is a frequent mutation found in mammary and lung carcinomas in animals treated with nitrosamines and other alkylating agents. The transition is also exclusively pres-

ent in BOP-induced pancreatic carcinoma in hamsters.^{105,118} G to T transversion at codon 12, on the other hand, has been seen in murine lung carcinoma induced by benzo[*a*]pyrene, a classical PAH carcinogen. The majority of K-*ras* mutations found in human lung cancers are also G to T transversions.¹¹⁹ Based on these observations, it is possible that the high frequency and the mutation spectra of the K-*ras* gene in human pancreatic cancers reflect a potential role of exposure to aromatic amines and nitrosamines, as well as PAH in the etiology and pathogenesis of this disease.

Summary

The severity of the pancreatic cancer problem and the scarcity of information on its etiology call for more research effort. Epidemiological studies have found that the incidence and mortality of pancreatic cancer increased for several decades earlier in this century but have tended to level off in recent years. Rates increase with age and are higher in males than females and higher in blacks than whites. Both genetic and environmental factors may play significant roles in the etiology of pancreatic cancer. Exposure to carcinogens through cigarette smoking, diet, and occupational contact may increase the risk of pancreatic cancer. This hypothesis is supported by the experimental evidence that (1) the pancreas has the capacity to activate carcinogens; (2) DNA adducts are detected in pancreas tissue; and (3) genotoxic compounds might be involved, as suggested by the mutation spectra in pancreatic tumors. We hope, with advances in molecular biology and new methods of molecular epidemiological approach, to develop the tools for identifying high-risk individuals in whom this deadly disease can be prevented. An understanding of the etiological and molecular events leading to the development of pancreatic carcinoma may provide a basis for the development of effective strategies for the prevention, early diagnosis, and treatment of this disease.

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2

The Molecular Biology of Pancreatic Cancer

Marina E. Jean, Andrew M. Lowy, Paul J. Chiao, and Douglas B. Evans

Introduction

Pancreatic cancer is a deadly disease with no effective therapy short of surgical resection. Unfortunately, only a minority of patients are candidates for potentially curative surgery as the tumor spreads early to extrapancreatic sites. Patients with metastatic pancreatic cancer survive less than 1 year following diagnosis. The current challenge for both clinicians and scientists is to translate the growing body of knowledge of the molecular basis of this disease into effective strategies for early diagnosis and systemic treatment.

Molecular studies of pancreatic duct carcinomas have revealed that this cancer is associated with several genetic mutations. These mutations include activation of *K-ras* and inactivation of *p53*, *p16*, and *DPC4*. Other alterations that occur in pancreatic cancer include dysregulation of growth factors and growth factor receptors and upregulation of matrix metalloproteinases (MMPs) and regulators of tumor angiogenesis. This chapter reviews the current state of knowledge of the molecular alterations that occur in pancreatic cancer, because they are potential targets for therapeutic intervention.

Analysis of the allelotype of pancreatic cancer suggests that this tumor conforms to the “multi-step” process of tumorigenesis.¹ In an analysis of 42 pancreatic adenocarcinomas, Rozenblum et al found that 38% had alterations of 4 genes and another 38% had alterations of 3 genes. These investigators also discovered that 83% of the carcinomas had a “distinctive genetic fingerprint” consisting of *K-ras* activation and inactivation of both the *p16* and *p53* tumor suppressor genes.²

The mutations of *K-ras*, *p53*, and *p16* that are associated with pancreatic carcinoma have also been identified in patients with preneoplastic pancreatic ductal lesions or chronic pancreatitis.^{3,4,5,6} Loss of cell cycle regulation appears to be vital to pancreatic tumorigenesis, as the most commonly altered genes (*K-ras*, *p53*, *p16*, and *DPC4*) are all linked to pathways critical to cell cycle regulation.^{3,7} The inactivation of multiple regulatory pathways of the cell cycle may account for the aggressiveness of pancreatic cancer.

Oncogenes: *K-ras* and *HER2/neu*

K-ras

The *K-ras* proto-oncogene, located on chromosome 12p13, is the most commonly mutated gene in pancreatic cancer. *K-ras*, a member of the *Ras* gene family, which also includes *H-ras* and *N-ras*, codes for a membrane-associated 21-kDa guanine-nucleotide binding protein with intrinsic guanosine triphosphatase (GTPase) activity. *K-ras* point mutations in codons 12, 13, and 61 have been identified in other cancers, but the *K-ras* mutations associated with pancreatic cancer occur almost exclusively at codon 12 and most commonly involve a second base transition of guanine to adenine (GGT → GAT).^{8,9} Mutations in *K-ras* alter the ability of the intrinsic GTPase to hydrolyze GTP to GDP, resulting in a constitutively active GTP-bound Ras protein.^{5,7,8} Ras proteins are essential to the transduction of growth-promoting signals from cell surface tyrosine kinase receptors to intracellular pathways involved in cellular differ-

entiation and proliferation.^{8,10,11} Therefore, constitutive activation of *Ras* results in uncontrolled cell growth. Before the *K-ras* protein can localize to the cell membrane, it must undergo posttranslational modification.¹² The obligatory first step in the processing of the *K-ras* protein is the addition of a farnesyl group to the cysteine residue located at the carboxy-terminal CAAX motif (C, cysteine; A, any aliphatic amino acid; X, any other amino acid). This reaction is catalyzed by the enzyme farnesyl-protein transferase (FPTase).¹² Since addition of the farnesyl group is critical for *Ras* function, this step in *Ras* protein processing is a logical molecular target for cancer therapy. Phase 1 and 2 clinical trials of various farnesyl transferase inhibitors are currently under way.

In several studies of both pancreatic cancer derived cell lines and primary tumors, the frequency of *K-ras* mutations at codon 12 has ranged from 71% to 100%.^{2,8,12–16} These frequencies are the highest reported for any tumor type.⁷ *K-ras* mutations have also been reported in 28% to 81% of noninvasive pancreatic intraductal lesions as well as in chronic pancreatitis.^{5,14,17–19} The high mutation frequency and the presence of *K-ras* mutations in lesions considered to be precursors of pancreatic adenocarcinoma suggest that *K-ras* mutations occur early and may be essential to pancreatic tumorigenesis.^{5,7,14,20} No correlation between *K-ras* mutation and patient survival or tumor size or stage has been found, which suggests that *K-ras* mutations may be more important in the initiation of tumorigenesis.^{8,9,21–23}

Because *K-ras* mutations are ubiquitous in pancreatic cancer, *K-ras* is currently being investigated as a possible tumor marker for pancreatic cancer. Techniques to detect *K-ras* in serum, stool, pancreatic juice, and tissue samples are currently being investigated.^{12,20,24}

HER2/neu (c-erbB-2)

The *HER2/neu* proto-oncogene, located on chromosome 17p11–q21, encodes a 185-kDa transmembrane glycoprotein receptor that is homologous to the epidermal growth factor (EGF) receptor and that has intrinsic tyrosine kinase activity.^{5,6,25} The *HER2/neu* receptor is thought to be involved in signal transduction pathways that culminate in cellular proliferation and differentiation of the exocrine pancreas.²⁶

In breast cancer cell lines, the expression of *HER2/neu* has been shown to confer a growth advantage and to contribute to cellular transformation. Other studies have shown that *HER2/neu* may regulate cell adhesion or invasive growth.²⁷ The role of this gene in pancreatic cancer may be mediated through interactions with other growth factor receptors, such as the EGF receptor.^{27–29} Transforming growth factor- α (TGF- α) and EGF have been found to modulate *HER2/neu* function; the EGF receptor and *HER2/neu* glycoprotein are capable of dimerizing with each other. Thus, their concomitant overexpression may lead to synergistic effects on cellular transformation and/or to tumor progression.²⁷

HER2/neu overexpression has been reported to occur in 17% to 58% of pancreatic cancers^{25–27,30–32} and is believed to occur primarily at the transcriptional level.^{23,26,33} Several studies have shown a correlation between *HER2/neu* expression and tumor histologic grade, with overexpression of this gene occurring more frequently in precursor lesions than in advanced, poorly differentiated cancers.^{6,27,30,31} Dugan et al found that even within a single tumor there was a higher level of *HER2/neu* expression in areas with glandular differentiation than in areas of poor differentiation.²⁷ Such findings suggest that *HER2/neu* overexpression is an early event in pancreatic carcinogenesis, perhaps initially conferring a growth advantage that becomes less important as tumors become less differentiated.^{6,31}

Although there are conflicting reports in pancreatic cancer, *HER2/neu* expression has been found to correlate with prognosis in other cancers (including breast, ovarian, and gastric).^{25,27,34} Consequently, the role of this gene in pancreatic cancer continues to be explored. Herceptin, a human monoclonal antibody to the *HER2/neu* receptor, is currently being studied in phase 2 clinical trials as a potential therapeutic agent for pancreatic cancer, and its effect on pancreatic tumors may provide additional clues to the biology of pancreatic cancer.²⁵

Tumor Suppressor Genes: *p53*, *p16*, and *DPC4* (Smad4)

p53

The *p53* tumor suppressor gene is the most commonly mutated gene in human cancer and is inactivated in

40% to 80% of pancreatic cancers,^{8,14,21,32,35–38} 58% to 100% of pancreatic cancer cell lines, and 75% of pancreatic cancer xenografts.⁷ *p53* is a transcription factor that acts primarily at the G1/S checkpoint to regulate cell growth and apoptosis via transactivation of genes such as *p21/WAF1/CIP1* and *bax*. The complex and unique spectrum of *p53* mutations found in pancreatic cancer may result from the action of various carcinogens.^{7,14} Inactivation of *p53* most commonly occurs via point mutations or intragenic deletions. Seventy percent of the point mutations are transitions, with the remainder being transversions. Interestingly, in pancreatic cancers associated with cigarette smoking, inactivation of *p53* is more likely to result from transversion point mutations than from transition mutations. Although point mutations account for most inactivations of the *p53* gene, intragenic deletions have been reported to account for up to 32% of *p53* genetic alterations.⁷ Unlike other cancers, *p53* gene mutations in pancreatic cancer are spread between exons 5–8 and not localized to a specific codon.^{8,38}

Because serum *p53* concentrations appear to correlate with *p53* mutations and have also been reported to parallel the progression of pancreatic cancer, *p53* is being investigated as a possible tumor marker for pancreatic cancer.^{8,23,39} Reports have conflicted, but the most recent data suggest an association between *p53* mutation and shortened survival in patients with pancreatic adenocarcinoma.^{6,23,40–43} The inconsistencies of past results may have resulted from technical differences in the methods used to detect *p53*.^{41,44–48} It has been suggested that analysis of *p21/WAF1/CIP1* and *mdm2* in addition to *p53* status may provide better prognostic information.⁴¹ *Mdm2* is a normal regulator of *p53* protein stability and function and inhibits *p53* by binding to its transactivation domain and by tagging *p53* protein for more rapid proteolysis.⁴⁹

Both in vivo and in vitro evidence suggest that the presence of wild-type *p53* is an important determinant of the sensitivity of tumor cells, including pancreatic cancer cells, to chemotherapeutic agents.^{5,50–54} Reintroduction of wild-type *p53* into pancreatic cancer cell lines has been shown to induce apoptosis.^{5,55} Restoration of normal *p53* function to tumor cells through gene therapy is currently being investigated with the hope that the sensitivity of these pancreatic tumors to chemotherapy and radiation therapy can be enhanced.^{5,56,57}

p16

p16 is a member of the INK4 family of cyclin-dependent kinase inhibitors (CKIs), which includes *p15* and *p19ARF*, whose overall function is to inhibit cell cycle progression. Alterations in *p16* have been found in several types of human cancers.^{5,58} The INK4 family members are all located within close proximity to each other on chromosome 19p21. *p15* is located only 30 kb downstream of *p16*, and *p19* shares a second exon with *p16*.⁵ Therefore, deletion of the *p16* gene is often also followed by loss of function of *p15* and *p19*, which can lead to a significant disruption of cell cycle regulation.⁵

p16 specifically binds cyclin-dependent kinase 4 (CDK4) and prevents the formation of the active cyclin D1/CDK4 complex.^{7,8} In cells that are quiescent, or in G₁ before the restriction point has been reached, the retinoblastoma (Rb) protein is in its unphosphorylated active state and binds the nuclear transcription factor E2F. Cyclin-dependent kinases (CDKs), such as cyclin D1/CDK4, phosphorylate Rb, thus causing the release of E2F and the subsequent transcription of genes necessary for cell cycle progression. CKIs such as *p16* and the above-mentioned *p21/WAF1/CIP1* prevent CDK activity and maintain Rb in its unphosphorylated state, thus halting cell cycle progression.⁸ Inactivation of *p16* thus results in the loss of the inhibitory effects on cell cycle progression and uncontrolled cellular proliferation. Mutation of *p16* has been associated with decreased survival in patients with pancreatic cancer.⁵⁹

Inactivation of the *RB1/p16* pathway has been reported in up to 98% of pancreatic carcinomas.^{3,5} Although the pathway may be abrogated by alterations of any member of the pathway, in pancreatic cancer, it is almost exclusively inactivated by *p16* alterations.^{3,5} The *RB1* tumor suppressor gene, which is located on chromosome 13q14 and which has been found to be mutated in numerous tumor types,^{60,61} is inactivated in fewer than 6% of pancreatic tumors.²² In contrast, *p16* inactivation has been reported to be present in 80% to 98% of pancreatic cancer xenografts and primary pancreatic carcinomas, which is the highest reported in any tumor type.^{2,3,7,8,18,62,63} Inactivation of *p16* requires mutations in both alleles. Deletions account for 85% of the alterations in one allele, with intra-

genic point mutations accounting for 30% to 38%, deletions accounting for 41% to 50%, and hypermethylation of the *p16* promoter accounting for approximately 15% of the alterations of the second allele.^{3,5,7,63} *p16* mutation has been associated with decreased survival in patients with pancreatic cancer.⁵⁹

DPC4 (Smad4)

The *DPC4* tumor suppressor gene is located on chromosome 18q21 and codes for a nuclear transcription factor (Smad4) that is thought to be activated via the TGF- β pathway to initiate transcription of the *p21* and *p15*.^{5,64,65} The TGF- β signaling pathway has been shown to be involved in the downregulation of epithelial cell growth and in the promotion of differentiation of certain cell types.^{8,66} TGF- β is believed to exert its effects on cellular proliferation and differentiation through the induction of CKIs, specifically *p21/WAF1/CIP1* and *p15INK4*, which is thought to be mediated through Smad4.^{64,67,68} The proposed pathway begins with ligand binding to the TGF- β cell surface receptors, thus activating the intrinsic serine-threonine kinase activity of the receptors. This results in phosphorylation of Smad1 and Smad2 proteins, which are found in the cytoplasm. The phosphorylated Smad proteins then form a complex with Smad4 and subsequently translocate to the nucleus and induce transcription of *p21/WAF1/CIP1* and *p15*, which then halt cell cycle progression at the G₁/S checkpoint.^{64,67,69–75}

Studies in pancreatic cancer cell lines have shown that the ability of TGF- β to induce expression of *p21/WAF1/CIP1* is dependent on the expression of wild-type *DPC4*.⁶⁴ Thus, inactivation of the *DPC4* gene would result in the loss of TGF- β -induced *p21/WAF1/CIP1* and *p15* expression and the loss of an important inhibitor of cellular proliferation in pancreatic carcinoma.^{64,65,71,76} Hunt et al demonstrated that overexpression of the *DPC4* gene in *DPC4*/Smad4-null cells could bypass the need for TGF- β receptor activation and restore inhibition of cell proliferation.^{65,75} These data provided further evidence that *p21/WAF1/CIP1* is a downstream target of *DPC4* in the TGF- β signaling pathway and that *DPC4* is a key downstream mediator of TGF- β signaling.

Biallelic inactivation of *DPC4*/Smad4 has been reported in 30% to 53% of pancreatic adenocarci-

nomas,^{2,62,77,78} with homozygous deletions accounting for 30% and intragenic mutations and loss of heterozygosity accounting for another 22% of the inactivations.^{7,77}

Other Tumor Suppressor Genes

The tumor suppressor gene *DCC* (deleted in colon carcinoma), which is located within 1 megabase of *DPC4* on chromosome 18q21, is unlikely to play a major role in pancreatic carcinogenesis. Several reports show no loss of *DCC* expression in pancreatic cancer.^{21,22}

The *BRCA2* gene, located on chromosome 13q12–13, has been implicated in both familial and sporadic pancreatic cancers. *BRCA2* gene mutations occur in approximately 7% of cases of pancreatic cancer.^{6,18} Although patients with a germline mutation of *BRCA2* have been reported to have an increased incidence of pancreatic cancer,^{5,6,8,44} the exact tumor suppressor function of the *BRCA2* gene is unknown.

The *FHIT* (fragile histidine triad) gene, located on chromosome 3p14, encodes an AP3A hydrolase whose actions are involved in regulating cell cycle progression at the G₁/S checkpoint.^{5,79} High frequencies of *FHIT* inactivation have been reported in several tumor types, with altered *FHIT* expression reported in 62% of pancreatic cancer.^{80,81} The recognition that 60% of pancreatic cancers showed allelic losses on chromosome 3p14, which coincides with the FRA3b fragile site, led to the proposition that *FHIT* may be a tumor suppressor gene in pancreatic cancer.^{82–85} However, the significance of *FHIT* mutations in pancreatic tumorigenesis is still unclear.

Growth Factors: EGF, FGF, TGF- β , IGF, HGF, and VEGF

It is widely recognized that perturbations in the functions and/or expression of growth factors and their receptors may lead to abnormal cell growth and the acquisition of features that are characteristic of the transformed phenotype. Several growth factors have been proposed to be involved in pancreatic cancer. Although these growth factors may

have a synergistic effect on pancreatic cancer cell growth, whether or not each growth factor plays a defined role in tumor progression and metastasis is unknown.⁸⁶ Growth factors are produced by many different cell types and exert their actions through a variety of mechanisms including autocrine, paracrine, intracrine, and juxtacrine. In the pancreas, growth factors also operate via a proxicrine mechanism, as the venous effluent from some of the hormone-producing islet cells flows into the intrapancreatic portal circulation and thus passes through the exocrine pancreas. The activation of most growth factor receptors enhances cell proliferation. However, some growth factors, such as TGF- β , may inhibit cell proliferation.⁸⁷

Epidermal Growth Factor (EGF)

The EGF receptor, a 170-kDa transmembrane glycoprotein with intrinsic tyrosine kinase activity, is activated by a family of ligands [EGF, TGF- α , heparin-binding (HB)-EGF, amphiregulin, and betacellulin], which all have homologous amino acid sequences with conserved positioning of 6 cysteine residues.^{6,87,88} Upon binding of the ligand, the EGF receptor dimerizes and its tyrosine residues are phosphorylated, thus allowing effector proteins to associate with the phosphorylated residues. SOS and GRB2, two key effector proteins, can then interact with activated Ras-GTP, resulting in activation of the mitogen-activated protein (MAP) kinase, PI3-kinase, and phospholipase C- γ pathways.^{87,88} Thus, the EGF receptor plays a key role in the activation of several pathways involved in cellular proliferation and differentiation; EGF overexpression would likely result in loss of control of cellular proliferation.

EGF and the EGF-like ligands are potent mitogens in many cell types, including pancreatic cells. The addition of EGF, TGF- α , amphiregulin, and HB-EGF to human pancreatic cancer cell line cultures has been shown to increase cellular proliferation.^{87,89} However, each ligand has a different pattern of cellular localization, suggesting that each plays a different specific role in the EGF signal transduction pathways.⁸⁷ Both EGF and TGF- α , the two principal ligands of the EGF receptor, can be found in normal acinar and pancreatic ductal cells. However, it is the overexpression of either EGF or TGF- α or of the EGF receptor that distin-

guishes pancreatic cancer from the normal pancreas. Overexpression of EGF, TGF- α , or the EGF receptor has been shown to result in the transformed phenotype in several tumor types.² TGF- α may play a special role in pancreatic tumorigenesis, as it has been shown to be a more potent stimulator of "anchorage-independent growth of cultured human pancreatic cancer cells."⁹⁰ The EGF receptor can heterodimerize with a family of homologous receptors that includes *HER2/neu*, *HER3*, and *HER4*, thus adding to the complexity of potential interactions between the EGF ligands and receptors that is involved in determining the overall output.^{86,87}

The importance of the EGF family in pancreatic tumorigenesis has been shown by several studies. The administration of anti-TGF- α antibodies and amphiregulin antisense oligonucleotides has been shown to suppress pancreatic cancer cell growth in vitro.⁹¹ More direct evidence for the involvement of the EGF receptor in pancreatic cancer comes from studies done with a truncated EGF receptor that lacks intrinsic tyrosine kinase activity, but retains its ability to heterodimerize with the EGF receptor. Transfection of the truncated EGF receptor into a pancreatic cancer cell line (Panc1) significantly decreased EGF receptor signaling and diminished cellular proliferation.⁹² Of note, the fact that EGF signaling still has an influence on Panc1 tumor cell growth, even though the cell line already has a p53 and *K-ras* mutation, suggests that excessive activation of the EGF signaling pathway confers some additional growth advantage.^{87,92}

The above-mentioned ligands and the EGF receptor are all overexpressed in pancreatic cancer, with overexpression resulting from increased gene transcription, as indicated by the observed increase in mRNA levels.⁹⁰ Overexpression of the EGF receptor has been reported in 30% to 50% of pancreatic cancers.^{26,90} Both *HER2/neu*, as previously mentioned, and *HER3* are overexpressed in pancreatic cancer.^{8,87} Overexpression of EGF and TGF- α has been reported to occur in 12% to 46% and in 50% to 95% of cases, respectively.^{8,93}

The concomitant overexpression of EGF receptor and EGF or TGF- α has been associated with shortened survival and increased tumor aggressiveness in patients with pancreatic cancer.^{28,87,93} Cytoplasmic amphiregulin immunoreactivity in pancreatic tumors has also been found to correlate

with advanced tumor stage and decreased postoperative survival.⁹³ Given the contribution of the EGF signaling pathways to pancreatic cancer, agents that can block the EGF signal transduction pathway or interfere with the EGF receptor ligands represent a promising therapeutic avenue.⁸⁷ Potential therapies that require further investigation include antisense oligonucleotides and TP-40, a fusion protein composed of exotoxin A and TGF- α that has been shown to have activity against EGF receptor-expressing cells.^{6,91}

Fibroblast Growth Factor (FGF)

The fibroblast growth factor family, currently with 9 identified members that include acidic(a)FGF/FGF1, basic(b)FGF/FGF2, and keratinocyte growth factor(KGF)/FGF-7, is mitogenic and motogenic in several cell types and is also believed to be involved in cellular differentiation, tissue repair, and angiogenesis.^{88,94} In pancreatic acinar cells, aFGF and bFGF have also been shown to stimulate amylase release.⁹⁰

The FGFs bind to 2 different classes of receptors: high-affinity and low-affinity. The high-affinity receptors (FGFR-1, FGFR-2, FGFR-3, and FGFR-4) are transmembrane glycoproteins with intrinsic tyrosine kinase activity that are the key mediators in FGF signaling. The low-affinity receptors, which alone have no signaling properties, enhance the presentation of FGF ligands to the high-affinity receptors.^{87,88,95,96} Several variants of the high-affinity receptors FGFR-1, FGFR-2, and FGFR-3 exist as a result of alternative splicing, with each of the variants being expressed to different degrees in different cell types. For example, the KGF receptor is a splice variant of FGFR-2.⁹⁵

The expression of aFGF and bFGF has been reported to be 8 to 11 times higher in human pancreatic cancer than in normal tissue.¹⁷ bFGF expression but not aFGF expression has been correlated with shortened patient survival.⁹⁷ The reason for this difference is unclear. Overexpression of the specific 2 Ig-like forms of FGFR-1 has also been reported in pancreatic cancer.⁹⁸ The finding of overexpression of FGFR-1 and of members of the FGF family raises the possibility that excessive autocrine and paracrine activation of FGF-dependent pathways contributes to pancreatic tumorigenesis.⁸⁷ Interestingly, transfection of Panc1

cells with truncated FGFR-1 caused significant growth inhibition. Use of the truncated FGFR-1, in addition to being a potential therapeutic option, also supports the suggestion that the FGF family contributes to pancreatic cancer.⁹²

Transforming Growth Factor- β (TGF- β)

As previously mentioned, the TGF- β family of growth factors is involved in the differentiation of certain cell types and in the inhibition of epithelial cell growth. TGF- β s have also been shown to enhance mesenchymal-derived cell proliferation, stimulate angiogenesis, modulate the composition of the extracellular matrix, and exert immunosuppressive effects.^{17,68,90} Thus, aberrant expression of members of the TGF- β family can contribute to tumorigenesis in several ways. Each of the 3 isoforms of TGF- β (TGF- β -1, TGF- β -2, TGF- β -3) is believed to play a different role in the control of cellular functions, as suggested by the temporal changes in TGF- β isoform expression that have been reported to occur during embryogenesis, carcinogenesis, and wound repair.^{68,99}

The TGF- β ligands bind to 3 main receptors (TBRI, TBRII, and TBRIII), all of which are found in the normal pancreas.¹⁰⁰ The formation of a heterotetrameric complex between TBRII and TBRI is crucial to the initiation of the TGF- β signaling pathway in response to ligand binding.¹⁰¹ TBRII, which is a transmembranous protein with intrinsic serine-threonine kinase activity, requires TBRI in order to initiate signaling, whereas TBRI requires TBRII in order to bind ligand.¹⁰⁰ Once TBRI and TBRII dimerize, Smad2 and Smad3 are activated and form a complex with Smad4, which can then translocate to the nucleus and activate gene transcription.⁷¹ TBRIII, also known as betaglycan, is not directly involved in TGF- β signal transmission but instead acts by enhancing ligand presentation to TBRI and TBRII.¹⁰²

All three TGF- β isoforms are overexpressed in pancreatic cancer. This overexpression has been found to correlate with shorter postoperative survival and increased tumor aggressiveness.^{100,103} Overexpression of TBRII has also been reported in pancreatic cancer.¹⁰⁰ Considering that TGF- β inhibits epithelial cell growth, the finding that overexpression of TGF- β isoforms and TBRII confers a growth advantage to pancreatic tumor cells seems

paradoxical and suggests that the role of TGF- β in pancreatic cancer is more complex. Resistance to TGF- β growth inhibition has been shown in several malignancies and occurs as a result of a variety of mechanisms, most involving mutations of the *TBR1* gene.^{88,104,105} It may be that in pancreatic cancer, tumor cell-derived, overexpressed TGF- β isoforms act via a paracrine mechanism to enhance tumor development and tumor aggressiveness by promoting angiogenesis and antitumor immune responses and by modulating the extracellular matrix, and that the pancreatic cancer cells themselves have lost their responsiveness to the growth inhibitory actions of TGF- β and thus are capable of continued growth.^{88,100}

Insulin-like Growth Factor (IGF)

Insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II) are structurally homologous polypeptides that exert mitogenic effects on several different cell types. Both growth factors are produced by numerous cell types, have been implicated in various malignancies, and have been shown to exert their effects via autocrine and paracrine mechanisms.^{106,107} Insulin, unlike IGF-I and IGF-II, is produced exclusively by the beta cells of the Islet of Langerhans and is primarily involved in glucose homeostasis and the regulation of metabolic pathways. The role of insulin in tumorigenesis is less clear; in pancreatic cancer, its effect appears to be its ability to activate the IGF-I receptor.⁸⁷

Both the IGF-I and the insulin receptors are heterotetrameric transmembranous glycoproteins with intrinsic tyrosine kinase activity.¹⁰⁶ Following ligand binding, both receptors undergo phosphorylation and thus activate insulin receptor substrate-I (IRS-I), which then initiates a cascade of events that have mitogenic and metabolic effects.¹⁰⁸ The IGF-II receptor does not have the signaling capabilities of the IGF-I and insulin receptors, consists of only 1 transmembranous glycoprotein chain, and exerts its effects through its ability to activate the IGF-I receptor. Although the insulin and IGF-I receptors are capable of binding all 3 ligands, the affinity of each receptor is greatest for its specific ligand. The IGF-II receptor binds only IGF-I and IGF-II.¹⁰⁶

Several members in the IGF family signaling pathway, including IGF-I, IGF-I receptor, IGF-II

receptor and IRS-I, are overexpressed in pancreatic cancer.^{109–111} Several studies support the significance of the IGF-I receptor-mediated mitogenic signal in pancreatic cancer. Both IGF-I receptor antisense oligonucleotide and anti-IGF-I receptor antibodies have been shown to inhibit the proliferation of cultured human pancreatic cancer cell lines.¹⁰⁹ The overexpression of IRS-I noted in pancreatic cancers contributes to excessive activation of the IGF-I receptor signaling pathway.¹¹⁰

Although IGF-II and insulin do not appear to be overexpressed in pancreatic cancer, both are believed to play a role in pancreatic tumorigenesis through their ability to activate the IGF-I receptor. IGF-II also can bind to and activate the IGF-II receptor, which subsequently further activates the IGF-I receptor.¹¹¹ The IGF-II receptor has also been shown to have mitogenic effects and to activate TGF- β 1, which is also overexpressed in pancreatic cancer. Thus, there is abundant evidence that the IGF family of ligands and receptors may contribute to pancreatic tumorigenesis.¹⁰³

Hepatocyte Growth Factor (HGF)

The hepatocyte growth factor (HGF) receptor, a c-met proto-oncogene encoded heterodimeric transmembranous protein with intrinsic tyrosine kinase activity, is mainly expressed by epithelial cells.^{86,104,112} HGF is produced by mesenchymal cells present in the stroma and exerts effects on epithelial cells in a paracrine fashion.⁸⁶ Ligand binding results in phosphorylation of the HGF/c-met receptor via its intrinsic tyrosine kinase activity, which then initiates a signal transduction cascade in a manner similar to other tyrosine kinase receptors.⁶

HGF has been shown to affect numerous cellular processes, including proliferation, differentiation, motility, angiogenesis, and invasiveness, with its specific effects determined by the target cell type.^{104,112} Thus, aberrant upregulation of the HGF signaling pathway can contribute to several aspects of tumor development and progression.

Although HGF and HGF/c-met receptor are found at barely detectable levels in normal pancreas, both are markedly overexpressed in pancreatic carcinoma, particularly in the ductal structures. HGF/c-met receptor and HGF expression levels in pancreatic cancer are 7 and 10 times higher than

levels found in normal pancreatic tissue.^{112,113} This upregulation of the HGF pathway may contribute to both pancreatic tumorigenesis and tumor progression. In vitro studies in pancreatic cancer cell lines show that addition of HGF to culture results in HGF/c-met receptor activation and increased cell motility, cell growth, and invasiveness.^{86,112} Overexpression of the HGF/c-met receptor has also been found to cause constitutive activation of the receptor in the absence of ligand binding.^{105,112} Use of a tyrosine kinase inhibitor, tyrphostin, in pancreatic cell lines has been shown to block HGF receptor-dependent signaling and thus has therapeutic potential via its ability to block HGF growth-promoting effects.⁸⁶

HGF/c-met receptor expression has been shown to correlate with the degree of tumor differentiation, with well-differentiated pancreatic cancer cell lines showing the highest and the poorly differentiated cell lines showing the lowest levels of receptor expression.¹¹² Increased expression of HGF and HGF/c-met have also been detected in precursor lesions, with more dysplastic lesions showing increased expression levels and well-differentiated carcinomas showing the highest expression levels.¹¹³ This association of loss of HGF/c-met receptor expression with dedifferentiation, which has also been observed with *HER2/neu*, suggests that the HGF signaling pathway contributes to the differentiated pancreatic cancer phenotype and may act as a promoting agent in the early stages of pancreatic ductal tumorigenesis.¹¹² A correlation between HGF/c-met receptor overexpression and increased patient survival has been reported in pancreatic cancer and supports this hypothesis.¹¹³ However, overexpression of HGF itself has not been shown to correlate with patient survival.

Vascular Endothelial Growth Factor (VEGF)

The vascular endothelial growth factor (VEGF) protein family consists of 4 isoforms, each with a distinct molecular size and secretion pattern, which are generated by alternative splicing of a single gene. VEGF functions as a mitogen for vascular endothelial cells, an inducer of vascular permeability, and as an important regulator of angiogenesis.^{114–117} VEGF signaling is mediated through 2 transmembranous receptors with intrinsic tyrosine

kinase activity (VEGF receptor-1 and VEGF receptor-2).

Upon comparison with normal pancreatic tissue, a 5-fold increase in VEGF mRNA transcripts has been reported and found to correlate with increased VEGF expression in pancreatic cancer samples. This increased VEGF expression was found to correlate with increased tumor size, increased blood vessel number, and a greater degree of local spread, but not with decreased patient survival.¹¹⁷ Thus, overexpression of VEGF in pancreatic carcinomas is believed to play a supportive role for tumor cell growth by stimulating angiogenesis.

Although it is not known why VEGF is overexpressed in pancreatic cancer, VEGF overexpression is probably the cumulative effect of the pattern of molecular alterations in pancreatic cancer and thus may be an epigenetic phenomenon. *K-ras* mutations and overexpression of EGF, PDGF, and TGF- β isoforms, molecular alterations commonly found in pancreatic cancer, have all been shown to up-regulate VEGF expression.¹¹⁷ The unique contribution of VEGF to pancreatic tumor cell growth is not clear as several other growth factors [bFGF, platelet-derived growth factor(PDGF)-BB, TGF- α , HGF, and TGF- β] that are overexpressed in pancreatic cancer have also been found to have angiogenic effects.¹¹⁷

Telomerase

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA onto chromosomal ends. The enzyme is active in germline cells and other immortal cells. Somatic cells normally lose their telomerase activity and subsequently lose telomere length in the process of cell division. Telomeric shortening appears to coincide with cell senescence.^{8,118,119} Telomerase activity appears to be reactivated in most human cancers and may be necessary for cancer cells to attain immortality. Telomerase activity was detected in 93% of colorectal cancers, 80% of lung cancers, 93% of breast cancers, and 94% of neuroblastomas.¹¹⁸ In addition, telomerase activity was detected in 95% to 100% of pancreatic cancers, whereas no activity was present in benign pancreatic tumor tissue samples.^{118,119} An analysis of pancreatic ductal brushing samples showed similar results, with all of the

pancreatic cancer specimens and none of the benign pancreatic specimens showing telomerase activity.¹¹⁸ Because telomerase reactivation may be a rate-limiting step in pancreatic carcinogenesis, the use of telomerase activity as a potential diagnostic tool for differentiating pancreatitis from pancreatic cancer has been proposed.^{118,119}

Tumor-Associated Proteinases: MMPs and uPA

Because degradation of the extracellular matrix (ECM) is critical to tumor invasion and metastasis, tumor-associated proteinases, such as MMP and urokinase-type plasminogen activator, are believed to contribute to the uniquely aggressive behavior of pancreatic cancer.⁸

Matrix Metalloproteinases (MMPs)

The MMP family of zinc-dependent, membrane-bound endopeptidase enzymes has a broad spectrum of proteolytic activity against several components of the extracellular matrix.^{120,121} On the basis of substrate specificity, the MMP family can be divided into 3 groups: collagenases, gelatinases, and stromelysins.^{120,121} MMPs are involved in both normal physiologic activities, such as wound healing and embryogenesis, and in pathologic activities, such as tumor invasion.

MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs), which include TIMP-1, TIMP-2, and TIMP-3. TIMPs irreversibly bind to and inhibit MMP activity. During tumor invasion, the critical balance between MMPs and TIMPs is altered, with MMPs outnumbering TIMPs, resulting in ECM degradation and tumor invasion. Although it is unclear which MMPs and TIMPs are associated with the metastatic potential of pancreatic cancer, MMP-2, MMP-3, and TIMP-1 appear to have the highest levels of immunoreactivity and expression in pancreatic cancer specimens when compared to normal pancreatic tissue.^{122,123} MMP-1 and MMP-2 have also been reported to be associated with the development of liver metastasis.⁸

Animal model and cell line studies have shown that MMP inhibition can delay tumor growth, promote encapsulation of the tumor, and inhibit an-

giogenesis.²⁵ Batimastat (BB-94), an MMP inhibitor, has been shown to inhibit proliferation of pancreatic cancer cells in vitro while showing no cytotoxic effects. In mice with orthotopically implanted pancreatic tumor cells, systemic treatment with batimastat resulted in decreased tumor weight and volume and prolonged survival.¹²¹ Marimastat (BB-2516), another MMP inhibitor with oral bioavailability, has showed no significant toxicity in phase 1/2 trials. Phase 3 trials are under way to determine the effectiveness of marimastat in patients with pancreatic cancer.²⁵

Urokinase-Type Plasminogen Activator (uPA)

uPA has a broad spectrum of effects on several physiologic and pathologic processes, including fibrinolysis, adhesion, migration, invasion, and remodeling.⁸ uPA is a serine proteinase that converts plasminogen to its active form, plasmin.¹²⁴ Plasmin can degrade several components of the extracellular matrix, including fibrin, fibronectin, laminin, and type IV collagen.⁸ uPA binds to the urokinase receptor (uPAR), a cysteine-rich cell surface receptor. The binding of uPA to uPAR increases the rate of uPA's enzymatic activity, resulting in accelerated generation of activated plasmin.¹²⁴ Receptor binding also localizes the activity of uPA to the cell surface, resulting in localized plasmin generation and focal proteolysis, thus facilitating the passage of tumor cells through tissue barriers.^{8,124}

Immunohistochemistry revealed increased uPA expression in 78% of 76 pancreatic cancer specimens studied.¹²⁵ uPA and uPAR mRNA levels were 6 and 4 times higher, respectively, in pancreatic cancer than in normal controls.¹²⁴ A study of patients with pancreatic carcinoma revealed that the postoperative survival time of patients with the concomitant overexpression of uPA and uPAR (median, 9 months) was significantly shorter than that of patients in whom only one or neither of these factors was overexpressed (median, 18 months).¹²⁴

Conclusion

Although our knowledge of the molecular alterations in pancreatic cancer has grown significantly

over the past 10 years, there is still much to be learned. It is clear that oncogenes, tumor suppressor genes, growth factors, and tumor-associated proteinases all play a role in pancreatic tumorigenesis. However, a better understanding of the relative contribution of each of these molecular alterations is necessary and will aid the development of more effective diagnostic and therapeutic strategies to deal with this deadly and aggressive cancer.

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3

Molecular Markers as a Tool for the Early Diagnosis of Pancreatic Cancer

James R. Howe

Introduction

There were 28,600 estimated cases of pancreatic cancer in the United States in 1999, and 28,600 deaths.¹ These are startling figures, and despite the significant efforts by clinicians and scientists to the contrary, pancreatic cancer remains an almost universally fatal disease. Although we might find occasional 5 year survivors reported in large series, many of these patients will still die of recurrent or metastatic disease,² highlighting the aggressive biologic nature of these neoplasms. Unfortunately, the majority of patients do not present until their tumors grow to obstruct the bile duct or cause pain, by which time they usually have already breached the capillary and lymphatic networks surrounding the organ. Although tests such as computed tomography (CT) scans and endoscopic retrograde cholangiopancreatography (ERCP) can help to make the diagnosis in symptomatic patients, there is currently no practical screening tool that would allow for these tumors to be discovered at an earlier, potentially curable stage.

Molecular biologic studies of pancreatic cancer have given us many insights into the cellular mechanisms leading to neoplastic transformation. The genetic changes that occur frequently in pancreatic cancer could be used to help differentiate benign from malignant pancreatic disease. As reviewed previously^{3,4} and discussed in Chapter 2, the most frequently identified genetic changes in pancreatic adenocarcinomas are *KRAS2* mutations. *KRAS2* (also known as *K-ras* or *c-K-ras*) is an oncogene that is frequently somatically mutated in a variety of human cancers, with the highest rate of muta-

tion being found in pancreatic adenocarcinoma, and most commonly reported at frequencies of 80% to 95%.^{5–11} The most frequently deleted tumor suppressor genes in pancreatic cancer are *CDKN2* (also known as *p16*), *TP53* (also known as *p53*), and *Smad4* (also known as *DPC4*). *CDKN2* is an inhibitor of cyclin-dependent kinases 4 and 6, which inhibit the function of the retinoblastoma protein, an important regulator of the cell cycle.¹² Mutations, homozygous deletions, or hypermethylation of the *CDKN2* gene have been reported in 85% to 98% of pancreatic adenocarcinomas.^{13,14} The p53 protein is a transcriptional regulator which can induce apoptosis or cause cell cycle inhibition through binding to double-stranded DNA, and mutant p53 can complex with wild-type protein to inhibit its tumor suppressive function.¹⁵ *TP53* mutations are found in approximately 50% of pancreatic adenocarcinomas.^{16–21} *Smad4* is a common mediator of the transforming growth factor β , activin, and bone morphogenetic protein pathways that binds to DNA in conjunction with other Smad and nuclear binding proteins, presumably regulating transcription of genes that induce apoptosis or suppress proliferation.^{22–25} Approximately 55% of pancreatic adenocarcinomas have homozygous deletions or mutations of *Smad4*,²⁶ but germline mutations have not been detected in patients with familial pancreatic carcinoma.²⁷ The localization of the *BRCA2* gene was helped by the identification of homozygous deletions of this genetic region in pancreatic cancers, and germline mutations have been described in approximately 7% of sporadic patients for *BRCA2*, making this the most common inherited genetic predisposition known for pancreatic

cancer.²⁸ The genetic pathways to pancreatic cancer are likely to involve alterations of several of these genes, as we have seen in the multistep pathway of colorectal carcinogenesis.²⁹ This has been confirmed by Rozenblum et al, who examined *KRAS2*, *CDKN2*, *TP53*, and *Smad4* in 42 pancreatic carcinomas and found that all 4 were altered in 38% of tumors, 3 genes in 38%, 2 genes in 15%, and 1 of these genes in 8%.²¹

This chapter will review the efforts that have been made toward the clinical application of our genetic knowledge to making the diagnosis of pancreatic cancer. We will see that the vast majority of studies have been limited to analysis of the *KRAS2* gene, because of the high incidence of mutation in pancreatic adenocarcinomas and the relative simplicity of looking for these changes. Most investigators have examined cells obtained during invasive procedures such as percutaneous fine needle aspiration of pancreatic masses or pancreatic juice collected at ERCP. Others have explored the utility of methods that could be used to screen the larger group of asymptomatic patients, by analysis of stool and blood samples. None of these techniques have yet become standard of care, but these tools currently hold the most promise for the early diagnosis of pancreatic cancer and therefore are our best hope to lower the death rate from this lethal disease.

KRAS2 Mutations and the Early Diagnosis of Pancreatic Cancer

At the present time, there is no serum marker with high sensitivity and specificity for pancreatic cancer, and therefore no good screening test for asymptomatic individuals. Most patients will ultimately present with symptoms of obstructive jaundice or upper abdominal pain, and the workup may not yield a cytologic diagnosis of pancreatic cancer. The latter group of patients are more likely to have earlier, potentially curable lesions, if only the diagnosis could be made. One method of early detection would be the identification of molecular genetic alterations in exfoliated cells that are specific for pancreatic cancer. Suitable material could be obtained from such sources as pancreatic juice or brushings collected at the time of ERCP, or CT-guided fine-needle aspiration of pancreatic masses.

Examination of Pancreatic Juice and Brushings

A large number of reports have studied the utility of screening samples of pancreatic juice, bile, duodenal juice, or brushings obtained at ERCP for *KRAS2* mutations. Most have examined pancreatic juice samples following the intravenous administration of secretin (usually 1 U/kg), which is collected by cannulation of the papilla of Vater using a fiberoptic duodenoscope. Cytologic analysis of pancreatic juice samples or pancreatic duct brushings may yield the diagnosis, but a negative result does not exclude carcinoma. Most studies have focused upon *KRAS2* mutations because of their high incidence in pancreatic cancer, and the relative ease of testing for them because they occur almost exclusively at codon 12.³ Pancreatic juice is centrifuged, DNA is extracted from cell pellets, and then amplified by the polymerase chain reaction (PCR) using primers for *KRAS2* exon 1. Technical difficulties encountered in such analyses may arise from the relatively small amount of DNA present, and the possibility of low numbers of mutant cells relative to normal ductal epithelium. Mutation rates of *KRAS2* in pancreatic adenocarcinoma have ranged from 55% to 100% in large series of resected specimens (averaging 80%³), and early studies suggested that these mutations were absent in benign conditions such as chronic pancreatitis or cholelithiasis.^{30–33} Based upon initial studies, it appeared that *KRAS2* mutations were not only sensitive for pancreatic adenocarcinoma, but also quite specific, and therefore had excellent potential to differentiate between benign and malignant pancreatic disease. Furthermore, *KRAS2* mutations were generally not found in pancreatic endocrine tumors.^{34–36} However, other periampullary malignancies may also have *KRAS2* mutations, with an approximately 34% incidence in ampullary carcinoma,^{6,37–40} 36% incidence in bile duct cancers,^{34,37,40,41} and 67% incidence in cholangiocarcinoma⁴² (reviewed in Howe and Conlon³). Therefore, in the not uncommon situation of a patient with obstructive jaundice or a pancreatic mass and negative cytology, the finding of a codon 12 *KRAS2* mutation would be highly suggestive of pancreatic or periampullary malignancy.

Table 3.1 lists the studies examining *KRAS2* mutations in patients suspected of having pancreatic

TABLE 3.1. Studies of *KRAS2* mutations in pancreatic adenocarcinoma from pancreatic juice, duodenal juice, and brush cytology.

Authors	Source* Method**	No. of mutations/ tumors	Percent mutations	Controls†
Tada et al ³⁰	PJ PCR-MASA	6/6	100%	0/3 CP
Watanabe et al ³¹	PJ ASO	11/20	55%	0/18 CP
Kondo et al ³²	PJ PCR-RFLP/SSCP	6/9	66.7%	0/10 CP
Trumper et al ³³	PJ PCR-RFLP	16/16	100%	0/5 CP 0/6 NI
Berthelemy et al ⁴³	PJ PCR-RFLP/Seq	17/22	77.3%	0/24 NI 0/29 AP/CP
Uehara et al ⁴⁴	PJ ASO	7/14	50.0%	4/13 CP
Watanabe et al ⁴⁵	PJ PCR-RFLP	21/26	80.8%	2/32 CP
Watanabe et al ⁴⁶	PJ HPA	11/20 (HPA) 15/20 (PCR-RFLP)	55.0% 75.0%	0/20 CP 4/20 CP
Kondo et al ⁴⁷	PJ PCR-RFLP/Seq	26/43	60.5%	4/22 CP 0/28 NI
Fukushima et al ⁴⁸	PJ/Bile PCR-RFLP	5/14	35.7%	
Kondoh et al ^{49††}	PJ SSCP	10/15	66.7%	0/8 CP 0/4 Cys/PD
Watanabe et al ⁵⁰	PJ HPA/PCR-RFLP	19/29 (HPA) 22/29 (PCR-RFLP)	65.5% 75.9%	1/26 CP 5/26
Nakaizumi et al ⁵¹	PJ SSCP/Seq	13/15	86.7%	6/13 CP 3/15 NI
Yamaguchi et al ⁵²	PJ PCR-RFLP/HPA	21/25 (PCR-RFLP) 17/26 (HPA)	84.0% 65.4%	
Iguchi et al ⁵³	DJ SSCP	12/19	63.2%	1/41 CP
Wilentz et al ⁵⁴	DJ ASO	8/34 22/34 (tissue)	23.5% 64.7%	0/9 CP
Nakamura et al ⁵⁵	DJ/PJ PCR-RFLP/ASO	14/23 (DJ) 14/20 (PJ)	60.9% 70.0%	1/12 CP 1/9 Cys
Abbruzzese et al ⁵⁶	Bile Seq.	14/17	82.4%	—
van Es et al ⁵⁷	BR PCR-RFLP/ASO	9/15	60.0%	
Van Laethem et al ⁵⁸	BR ASO	29/36	80.6%	19/76 CP

*PJ = pancreatic juice; DJ = duodenal juice after IV secretin; BR = brushings

**MASA = mutant allele specific amplification; SSCP = single strand conformational polymorphism; Seq = sequencing; ASO = allele-specific oligonucleotide hybridization; PCR-RFLP = PCR and *Bst*NI restriction enzyme digestion; HPA = hybridization protection assay.

†CP = chronic pancreatitis; NI = normal subjects; Cys = cyst/pseudocyst; PD = pancreas divisum; AP = acute pancreatitis

††1 cystic neoplasm, 4 intraductal papillary tumors

cancer, using cells obtained from pancreatic juice, bile, or brushings obtained at ERCP. The mutations rates range from as low as 24% to as high as 100%, depending on how samples are collected and how

mutations are examined. Most studies report mutation rates between 60% and 80%, slightly below that seen in studies reported from tissue sections. These studies demonstrate that it is feasible to de-

fect mutations from the relatively small number of cells obtained by these methods, which is made possible by the ability to greatly amplify specific DNA sequences by the PCR. Therefore, screening of such samples for mutations could be extremely helpful in the early diagnosis of patients with pancreatic cancer, which is clearly most useful for patients with negative or suspicious cytology. Unfortunately, the fact that someone is having a diagnostic ERCP performed means that he or she is not asymptomatic, and therefore that the tumor may have proceeded beyond a curable stage.

Further analysis of the studies listed in Table 3.1 demonstrates one of the problems with using *KRAS2* mutations for the early diagnosis of pancreatic cancer. These mutations are more common than originally thought in benign conditions, depending upon the methods used for PCR enrichment of mutant alleles and their detection. The methods used for analysis of *KRAS2* mutations deserve some discussion. Allele-specific oligonucleotide hybridization is perhaps the least sensitive and can be technically demanding. Here, cells are used as template for PCR of exon 1, and the amplification products are spotted onto nylon membranes.⁵⁹ Oligonucleotide probes specific for various point mutations and the wild-type sequence at codon 12 are then hybridized to the membranes. This method yields information regarding the specific mutations present, but optimal hybridization conditions can be difficult using oligonucleotides. PCR-MASA (mutant allele-specific amplification) is another method that uses different forward primers for *KRAS2* exon 1 specific for the wild-type sequence and 1 of 3 different codon 12 mutations commonly observed in the Japanese population.³⁰ Successful amplification with a primer specific for one of these mutations provides a sensitive means of detecting these changes, where as little as 0.1 to 0.01 ng of mutant DNA can be amplified in a background of 1 μ g of wild-type sequences.³⁰ Nested PCR is a technique employing two successive rounds of amplification (the second using primers internal to the first), which greatly increases the copy number from relatively small amounts of DNA. This is then followed by direct sequencing to determine the specific mutation present, and can detect mutations present in both codons 12 and 13, which these other methods do not.^{39,60} Perhaps the most widely used method is the PCR-RFLP (restriction fragment

length polymorphism), where initial amplification is employed using primers flanking codon 12 that have one basepair mismatches with the the wild-type *KRAS2* sequence. These slight mismatches do not inhibit amplification and create 2 sites for digestion with the restriction endonuclease *BstNI* in wild-type samples (which yields PCR products of 114, 29, and 14 basepairs after *BstNI* digestion), and 1 site in codon 12 mutant samples (yielding 143 and 14 bp products).⁴¹ The mutant allele is then enriched by using the same 5' primer (this sequence is removed from wild-type alleles by *BstNI* digestion) and a 3' primer upstream of the second *BstNI* site. This method is uninformative with respect to the specific mutations present, so either sequencing or hybridization with mutation-specific oligonucleotides is necessary to determine the base changes.

The increasing recognition that *KRAS2* mutations may be present in benign conditions potentially limits their usefulness in the early diagnosis of pancreatic malignancy. Several studies have shown that these mutations are relatively common in hyperplastic pancreatic ductal epithelium (Table 3.2), and therefore that *KRAS2* mutation may be an early event in neoplastic transformation. The first study to examine this was by Lemoine et al, and they found no mutations in either ductal papillary hyperplasia or intraductal papillary neoplasms.⁶¹ This study differs from the others in the table because the ductal epithelium was not microdissected out and therefore there was probably admixture of normal and hyperplastic cells. Yanagisawa et al looked at patients diagnosed with mucous cell hyperplasia after surgical resection and found a 63% incidence of *KRAS2* mutation,⁶² while Caldas et al found a 65% incidence of mutations in microdissected areas of mucous cell hyperplasia in patients having pancreatic resection for pancreatic cancer, chronic pancreatitis, or cholangiocarcinoma.⁶³ Tada et al found a lower incidence of mutation (32%) in microdissected hyperplastic ducts from patients with pancreatic cancer or chronic pancreatitis,⁶⁴ and Rivera et al lower still (18%) in hyperplastic ducts from patients with chronic pancreatitis.⁶⁵ Z'graggen et al microdissected separate areas from patients with intraductal papillary mucinous tumors and demonstrated an increase in *KRAS2* mutations as the epithelium changed through the stages of hyperplasia, dysplasia, and carcinoma.⁶⁶ Moskaluk et al microdissected out areas of pancreatic intraduc-

TABLE 3.2. Rate of *KRAS2* mutations in noninvasive pancreatic lesions.

Authors	Source* Method**	Tumor type†	No. of mutations/ tumors	Percent mutations
Lemoine et al ⁶¹	PB	DPH	0/5	0%
	ASO	IPN	0/5	0%
Yanagisawa et al ⁶²	MPB	MCH	10/16	62.5%
	ASO			
Caldas et al ⁶³	MPB	MCH	11/17	64.7%
	PCR/Seq			
Tada et al ⁶⁴	MPB	MCH	12/38	31.6%
	PCR/Seq			
Rivera et al ⁶⁵	MPB	HCP	2/11	18.2%
	ASO/Seq			
Z'graggen et al ⁶⁶	MPB	MCH	1/5	20.0%
	PCR/Seq	MCH-LGD	2/6	33.3%
		MCH-HD	8/12	75.0%
		CA	13/16	81.3%
Moskaluk et al ⁶⁷	MPB	FH	2/7	28.6%
	PCR/Seq	PH	12/12	100%
Terhune et al ⁶⁸	MPB	FH	8/17	47.0%
	PCR/RFLP	PH	27/44	61.4%
		ADH	1/2	50.0%
		ADH/CA	5/7	71.4%

*PB = paraffin blocks; MPB = microdissected paraffin blocks

**ASO = allele-specific hybridization; PCR/Seq = PCR/sequencing; PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism

†DPH = ductal papillary hyperplasia; IPN = intraductal papillary neoplasms; MCH = mucous cell hyperplasia; HCP = hyperplastic areas in patients with chronic pancreatitis; LGD = low-grade dysplasia; HGD = high grade dysplasia; CA = carcinoma; FH = flat hyperplasia; PH = papillary hyperplasia; ADH = atypical ductal hyperplasia; ADH/CA = atypical ductal hyperplasia in patients with adjacent ductal cancer

tal lesions (tall columnar epithelial cells confined to the pancreatic duct) from surgical specimens with pancreatic adenocarcinoma and found that in these precursor lesions there was a 29% incidence of *KRAS2* mutations in areas of flat hyperplasia and 100% in papillary hyperplasia, once again confirming that these mutations occur at a premalignant stage.⁶⁷ Terhune et al microdissected ducts from patients with pancreatic cancer and chronic pancreatitis and also found a significant incidence of these mutations at various stages of hyperplasia.⁶⁸ These studies suggest that *KRAS2* mutation is an early event in neoplastic progression, and although they are not specific for carcinoma, these mutations may predict for precursor lesions that will develop into cancer. This may apply even to chronic pancreatitis, which is known to increase the risk for pancreatic cancer and is associated with ductal hyperplasia.

A few reports illustrate the potential clinical value of *KRAS2* mutations in the early diagnosis of

pancreatic adenocarcinoma. Berthelemy et al found codon 12 *KRAS2* mutations in 2 patients with narrowing of the pancreatic duct by ERCP, but no clear evidence of pancreatic cancer on the initial workup. Both patients were later diagnosed with pancreatic cancer, at 18 and 40 months following the finding of *KRAS2* mutation.⁴³ Wakabayashi et al described a patient with pancreatic cancer whose frozen pancreatic juice from 42 months earlier was retrospectively found to have a *KRAS2* mutation.⁶⁹ No mass was found on diagnostic imaging at the earlier presentation, but the patient did have a localized stenosis of the pancreatic duct on ERCP. Cytology was negative at this time, and the patient was followed for the next 3½ years until he was found to have a significant increase in his serum CA19-9 level and a mass on CT scan. At the time of operation, the patient was found to have a moderately differentiated carcinoma of the pancreatic body with liver metastases. Ochi et al described an-

other case, where a 74-year-old woman presenting with symptoms of abdominal fullness was worked up by ERCP and CT, and no definitive lesion was found in the pancreas. Pancreatic juice cytology was negative, but a codon 12 mutation was found in *KRAS2*. The patient was followed by ERCP every 3 months until cytology was finally positive 19 months later. A T1_aN0M0 adenocarcinoma of the body of the pancreas was identified after distal pancreatectomy and splenectomy.⁷⁰

Another technique for the detection of *KRAS2* mutations has been developed to help differentiate *KRAS2* mutations arising from malignant versus benign disorders, called the hybridization protection assay (HPA). This begins with PCR amplification of *KRAS2* exon 1, followed by hybridization using oligonucleotide probes specific for different base changes in codon 12. These oligonucleotides are labeled with an acridinium ester, allowing for chemiluminescent detection. The probes are separately hybridized to the PCR products from each patient sample, and then a hydrolysis buffer is added to destroy all the nonbound probe. The chemiluminescence can then be quantitated, and cutoff values have been determined that allow for distinguishing the higher number of changes seen in pancreatic cancer patients and exclusion of lower

copy number changes as seen in chronic pancreatitis patients.^{46,50} These authors have demonstrated that 15/20 pancreatic adenocarcinoma and 4/20 chronic pancreatitis patients had *KRAS2* mutations by PCR-RFLP analysis, but only 11/20 adenocarcinomas and none of the 20 chronic pancreatitis patients had mutations with the HPA assay.⁴⁶ Although the method is less sensitive than PCR-RFLP, it can be positive in the presence of as low as 5% mutant cells, gives information regarding the specific mutations, and can better differentiate between pancreatic cancer and chronic pancreatitis.

Assessing *KRAS2* Mutations from Fine-Needle Aspirates

Another modality widely used to make the cytologic diagnosis of pancreatic adenocarcinoma is fine needle aspiration (FNA). This is generally performed under CT guidance using a 22-gauge spinal needle; cellular aspirates are smeared onto slides, and additional material is fixed, centrifuged, and embedded in paraffin blocks.

Shibata et al retrospectively examined 36 paraffin-embedded FNA specimens from patients with pancreatic cancer for *KRAS2* mutations (Table 3.3).⁷¹

TABLE 3.3. *KRAS2* mutations from fine needle aspirates of pancreatic tumors.

Authors	Method*	No. of mutations/ tumors (%)**	Cytology
Shibata et al ⁷¹	PCR/RNase mismatch	18/25 (72.0%)	Malignant
		2/8 (25.0%)	Atypical
		0/3 (0%)	Benign
Urban et al ⁷²	PCR-RFLP	8/9 (90.9%)	Malignant
		2/5 (40.0%)	Benign
		1/2 (50.0%)	Endocrine/Mixed
Villanueva et al ⁷³	PCR-RFLP/Seq	28/42 (66.7%)	Malignant
		8/12 (75.0%)	Suspicious
		3/6 (50.0%)	Inadequate
		2/12 (16.7%)	Normal
Evans et al ⁷⁴	PCR-RFLP	19/23 (82.6%)	Positive
		2/2 (100%)	Nondiagnostic
Apple et al ⁷⁵	PCR-RFLP	39/41 (95.1%)	Positive
		1/1 (100%)	Atypical
		4/4 (100%)	Benign
Pabst et al ⁷⁶	PCR-RFLP	13/26 (50.0%) Ca.	—
		1/11 (9.1%) CP	
		0/5 (0%) NI	

*PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism; seq = sequencing

**Ca = cancer; CP = chronic pancreatitis; NI = normal

Mutations were found in 72% (18/25) of those with malignant cytology, 25% (2/8) with atypical cytology, and in none (0/3) with benign cytology. The lower than expected rate of *KRAS2* mutations was suspected to be due either to sampling error, insufficient numbers of cells, or errors in diagnosis (all tumors were unresectable, but patients had death within a year compatible with pancreatic adenocarcinoma). In this study, the addition of *KRAS2* mutation information would have helped in the management of only 2 patients with atypical cytology results. An even lower percentage of *KRAS2* mutations was reported from FNA specimens by Pabst et al.⁷⁶ They found mutations in only 13 of 26 (50%) cases, again pointing out the potential for nondiagnostic results using this technique. These authors did not correlate their results with the cytology findings, so it is very difficult to determine to what extent the low mutation rate may have been due to inadequate sampling of the tumors.

Urban et al found *KRAS2* mutations in 10 of 11 (91%) FNAs from patients with pancreatic cancer, 1 cystadenocarcinoma, and no mutations in 1 islet cell tumor and 3 cases of chronic pancreatitis.⁷² Two patients with *KRAS2* mutations and negative cytology had pancreatic adenocarcinoma, and 8 of 9 tumors with positive cytology had mutations. Overall, the cytologic diagnosis was correct in 13 of 16 cases, but when pathology and *KRAS2* mutations were combined, 16 of 16 patients had the correct diagnosis. The authors concluded that *KRAS2* mutation and cytology together are of diagnostic utility in pancreatic carcinomas. Villanueva et al performed FNA on patients with pancreatic tumors, of whom 75 were ultimately diagnosed with pancreatic adenocarcinoma.⁷³ They found that 67% (28/42) of FNAs with malignant cytology, 75% (8/12) with suspicious cytology, 50% (3/6) of specimens deemed inadequate, and 17% of normal aspirates (2/12) had *KRAS2* mutations. In 11 FNA samples that were compared to resection specimens, 8 had the same *KRAS2* mutation status (5 positive, 3 negative), while 3 others had mutations found in the resected specimen only. The authors believed this was due either to tumor heterogeneity or to the needle not reaching the tumor. The presence of *KRAS2* mutations would have been of diagnostic help in 11 patients, and the authors suggested that molecular analysis be limited

to specimens with suspicious, inadequate, or normal cytology.

Evans et al examined FNA samples by cytology and PCR-RFLP for *KRAS2* mutations from 25 patients with pancreatic adenocarcinoma.⁷⁴ Cytology slides were prepared first; then the syringe was rinsed with media and centrifuged, and cell pellets were embedded in paraffin. The supernatant remaining was then used for the analysis of *KRAS2* mutations. They found that 19 of 23 (83%) specimens with positive cytology had mutations, and 2 nondiagnostic samples by cytology also had mutations. This study showed that *KRAS2* mutation analysis could be performed accurately with materials usually discarded, and thereby not interfering with cytologic analysis.

Apple et al examined FNA specimens for *KRAS2* mutations retrospectively from 46 patients with a pathologic diagnosis of pancreatic cancer.⁷⁵ They found that cytology was positive in only 41 of 46 (89%) of cases, whereas molecular analysis was positive in 44 of 46 cases (96%). This study included 34 primary and 12 metastatic tumors (liver, lung, bone, duodenum, nodes, omentum), and mutations were detected in samples with as few as 5 cells, and with tumor to normal cell ratios of as low as 1:1000. These studies suggest that the addition of mutation testing for *KRAS2* could be of great usefulness in making the diagnosis of pancreatic cancer, but should probably be reserved for those cases where cytology is nondiagnostic.

Early Diagnosis from *KRAS2* Mutations or p53 Protein in Peripheral Blood Samples

The problem with fine-needle aspirates, brushings, and pancreatic juice specimens is that they all require clinical suspicion and invasive testing. Therefore, these procedures may be helpful for diagnosis of individual patients, but are not likely to significantly reduce the overall death rate from pancreatic cancer. For this, a true screening tool applicable to asymptomatic patients will be necessary, and to this end several investigators have examined peripheral blood samples for *KRAS2* mutations from patients with known pancreatic cancer (Table 3.4). Tada et al analyzed peripheral blood samples for *KRAS2* mutations from 6 patients known to have pancreatic adenocarcinoma, and

TABLE 3.4. Studies analyzing plasma or blood for molecular genetic changes in pancreatic cancer.

Authors	Source Method* Gene	No. of mutations/ patients	Percent mutations	Controls**
Tada et al ³⁰	Blood PCR-MASA <i>KRAS2</i>	2/6	33.3%	
Nomoto et al ⁷⁷	Blood PCR-RFLP <i>KRAS2</i>	10/10 blood 13/17 liver 2/10 washings	100% 76.5% 20.0%	0/13
Shibata et al ⁷⁸	Blood—Buffy coat Anti-CD45 Ab PCR-MASA <i>KRAS2</i>	8/13 nodes 5/7 tissue 6/8 blood	61.5% 71.4% 75.0%	0/10 N1
Mulcahy et al ⁷⁹	Plasma PCR-RFLP/Seq <i>KRAS2</i>	17/21	81.0%	0/5 N1 0/3 CP
Yamada et al ⁸⁰	Plasma/Tissue PCR-MASA	15/21 tissue 9/21 plasma	71.4% 42.9%	0.5 N1 0/4 CP
Castells et al ⁸¹	Plasma PCR-RFLP <i>KRAS2</i>	12/44 plasma 28/39 Tissue	27.3% 71.8%	2/37 CP 0/4 N1 0/9 AP
Suwa et al ⁸²	Serum ELISA p53	23/104	22.1%	1/9 islet 2/35 N1 0/15 CP
Soeth et al ⁸³	Blood/BM RT-PCR for CK20	5/27 bone marrow 2/22 blood	18.5% 9.1%	2/20 BM 2/58 blood

*MASA = mutant allele-specific amplification; PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism; BM = bone marrow
**N1 = Normal; CP = chronic pancreatitis; AP = acute pancreatitis; BM = bone marrow

found that 2 had *KRAS2* mutations detected in peripheral blood samples, 1 who had distant metastases and the other without known metastases. They extrapolated that there might have been as many as 2 million metastatic cells circulating in the bloodstream, and that this might be a factor in the poor overall prognosis of pancreatic cancer patients.³⁰

Increased amounts of circulating plasma DNA have been described in cancer patients, with the highest levels seen in those with pancreatic cancer (646 ng/ml in pancreatic cancer, 270 ng/ml in colorectal cancer, and 14 ng/ml in healthy subjects).⁸⁴ Mulcahy et al extracted DNA from the plasma of 21 patients with pancreatic adenocarcinoma and searched for *KRAS2* mutations.⁷⁹ They found mutations in 17 of 21 (81%) patients, and the same mutation was found in both tissue and blood specimens in the 10 patients where they were available. Four patients had *KRAS2* mutations detectable in plasma collected between 5 and 14 months prior to

their diagnosis. This study demonstrated that early diagnosis of pancreatic cancer may indeed be possible through a relatively noninvasive method.

In a similar study, Yamada et al found *KRAS2* mutations in 15 of 21 (71%) resected pancreatic cancer resection specimens, and in 9 of 21 plasma samples from the same patients.⁸⁰ All 9 positive plasma samples came from patients with positive tissue samples. They also extracted DNA from the buffy coat (mononuclear cell layer), and no mutations were detected in DNA extracted from these cells, suggesting that examination of the plasma fraction was more sensitive than extracting DNA from circulating metastatic cells. None of 5 healthy patients or 4 with chronic pancreatitis had plasma *KRAS2* mutations. Interestingly, these mutations cleared in the plasma of 3 patients 1 to 2 months posttreatment after palliative surgery, 1 after potentially curative surgery, and 2 after intensive chemotherapy and radiotherapy. Two patients had

persistently detectable mutations after chemotherapy and radiotherapy, and another after potentially curative resection. The latter patient recurred within 6 months and had died by 1 year. Larger tumors were more likely to have mutations found in the plasma than smaller tumors ($P = 0.04$). This study suggested that plasma *KRAS2* mutations might be useful not only for the diagnosis of pancreatic adenocarcinoma, but may also be helpful in predicting tumor recurrence.

The time course of *KRAS2* mutations appearing in the blood of 10 patients with pancreatic cancer was studied by Nomoto et al, who extracted DNA from peripheral blood at various times before and after laparotomy.⁷⁷ Five patients had *KRAS2* mutations detectable upon opening the abdomen, and all 10 patients were positive at the time of resection. Most then decreased to undetectable levels over 2 weeks, and only 1 remained positive on post-operative day 21. Liver biopsies were also performed at the time of laparotomy, and 13 of 17 (77%) cases were found with *KRAS2* mutations, suggesting a very high rate of occult liver metastases. Based on these provocative findings, release of tumor cells into the circulation must be relatively common, and increases with manipulation of the tumor. Presumably, much of this micrometastatic disease does not become clinically problematic, perhaps due to host immune surveillance mechanisms.

Shibata et al devised a method to attempt to improve the chances of detecting circulating metastatic cells in the blood.⁷⁸ They extracted the buffy coat from whole blood, then incubated it with microbeads bound to CD45 antibody (which recognizes leukocyte common antigen) followed by antihuman CD45 antibody and magnetic separation. The unbound fraction, enriched for nonmononuclear cells, was then used as the substrate for PCR-MASA. They found *KRAS2* mutations in the blood of 6 of 8 patients with pancreatic cancer, and the same *KRAS2* sequence was found in the 7 tissue samples available. In 1 patient, an additional mutation was found in the blood. Ten normal controls were negative for mutations. The authors felt that this technique could be practical in patients, and that since it looked at the buffy coat fraction, it was specific for viable circulating cancer cells, unlike methods looking at plasma. Whether this initial enrichment step is truly necessary with the sensitivity of PCR techniques is debatable, as are claims

that this method is better due to its specificity to viable cancer cells in the blood, for the detection of viable cells may be less sensitive than free DNA for early detection of pancreatic cancer.

Castells et al found *KRAS2* mutations in the plasma collected from 12 of 44 (27%) patients prior to any intervention, and in 28 of 39 (72%) tissue or pancreatic juice specimens from these tumors.⁸¹ No mutations were detected in DNA extracted from the buffy coat layer from these 44 patients' blood, suggesting that the mutant *KRAS2* in blood came not from intact circulating metastatic cells, but rather from DNA free in the plasma. This was also the first study in which mutations were found in the plasma of patients with chronic pancreatitis (2 of 37 patients, 5%). Plasma *KRAS2* mutation was significantly associated with reduced survival, with a 17% 6-month survival in mutation-positive patients and 41% in mutation-negative patients ($P < 0.005$).

Other markers have been examined in the blood of patients with pancreatic cancer, such as CK20 (an intermediate filament), whose presence was found to be too infrequent (9%) to be useful.⁸³ Suwa et al examined serum p53 protein levels by enzyme-linked immunoabsorbent assay (ELISA) from 104 patients with pancreatic adenocarcinoma, and found a significantly higher mean level ($0.27 \text{ ng/ml} \pm 0.02$) versus that seen in healthy volunteers (0.15 ± 0.02) or in patients with chronic pancreatitis (0.15 ± 0.02 ; $P < 0.05$). When a cutoff 2 standard deviations above that of mean healthy volunteers was chosen (0.37 ng/ml), 22% of patients with adenocarcinoma, 11% with islet cell tumors, 6% of healthy volunteers, and none with chronic pancreatitis would have been considered positive. Higher levels were seen in patients with distant metastases than without (mean of 0.31 ng/ml vs 0.21 , respectively; $P < 0.05$). When compared to CA19-9 (elevated in 72% of cases) and CEA (elevated in 51% of cases), the serum p53 level was found to be a much less sensitive indicator of pancreatic adenocarcinoma.

Mutations in Stool Samples

Since exfoliated pancreatic cancer cells are shed into the pancreatic juice, stool is a potentially good source material to screen for these tumors (Table 3.5). Caldas and colleagues described a procedure

TABLE 3.5. Studies screening stool samples for *KRAS2* mutations in pancreatic adenocarcinoma.

Authors	Method*	No. of mutations/ patients	Percent mutations	Controls**
Caldas et al ¹³	ASO	11/11 tissue	100%	1/3 CP
		6/11 stool	54.5%	
Berndt et al ⁸⁵	PCR-RFLP/ASO	32/35 tissue	91.4%	2/6 CP
		10/25 stool	40.0%	
Wenger et al ⁸⁶	PCR-RFLP/ASO	28/36 tissue	77.8%	2/5
		7/36 stool	19.4%	

*ASO = allele-specific hybridization; PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism

**CP = chronic pancreatitis; NI = Normal

for testing stool samples for *KRAS2* mutations in 11 patients with pancreatic adenocarcinoma and 3 with chronic pancreatitis.¹³ One gram of frozen stool samples were suspended in buffer and centrifuged, phenol-chloroform extraction was performed, the samples were treated with proteinase K, phenol-chloroform extracted twice more, and then the DNA was precipitated using glass beads. PCR amplification for *KRAS2* exon 1 was performed, the products subcloned, and then plaque hybridizations carried out using specific oligonucleotide probes for different *KRAS2* mutations. They found mutations in the stool of 6 of 11 pancreatic adenocarcinoma patients, all of whom had mutations in paraffin-embedded tumor sections (and in 5 of 6 cases the same substitution was found as in the paraffin-embedded sections). One of 3 chronic pancreatitis patients had mutations found in the stool, and all were negative on paraffin sections. *KRAS2* mutations were presumed to have come from exfoliated cells from abnormal pancreatic ducts and not just limited to neoplasms, because they were also seen in chronic pancreatitis patients. Berndt et al followed a similar procedure and found mutations in 10 of 25 (40%) patients with pancreatic adenocarcinoma, and 2 of 6 (33%) with chronic pancreatitis.⁸⁵ Wenger (a coauthor of Berndt) and colleagues published a similar report, which described mutations in 7 of 36 (19%) patients with pancreatic adenocarcinoma, and 2 of 5 (40%) with chronic pancreatitis. They suggested that since screening of stool is noninvasive and that 20% to 40% of pancreatic cancers could potentially be identified in this fashion, this type of screening could be warranted if it results in potentially curative surgery being performed on an additional 20%

of patients.⁸⁶ Berndt et al suggested that this alone was not an appropriate screening test, for it had a low diagnostic sensitivity of 40%, it could be positive due to colorectal neoplasms, and mutations result from benign pancreatic disease. They suggested that a stool test based upon molecular analysis of several genes (*KRAS2*, *p53*, and *CDKN2*) would be preferable.⁸⁵

Other Sources for Molecular Analysis

Ando et al examined paraaortic lymph nodes for *KRAS2* mutations; the nodes had been removed at the time of extended pancreaticoduodenectomy.⁸⁷ All 13 primary pancreatic cancers examined were *KRAS2* mutation-positive, and their associated nodes were negative by histopathology. They found that 8 of 13 patients had mutations detected in their lymph nodes, and a total of 42 of 101 nodes analyzed had mutations (Table 3.6). In this study, the sensitivity of detection was shown to be 100 pg of mutant DNA in 1 μ g of total DNA (0.01%), and there was no clear correlation between nodal *KRAS2* mutations and survival. These findings were reminiscent of those by Nomoto et al for mutations found in liver biopsy samples.⁷⁷ This study confirmed that the rate of occult micrometastases is higher than usually recognized, and that routine histologic examination probably misses significant numbers of occult metastases.

Nomoto et al collected peritoneal washings at the time of laparotomy from 20 patients with pancreatic adenocarcinoma.⁸⁸ They examined the cells by routine cytology, immunostaining for CA19-9 and CEA, and *KRAS2* mutations. Although all primary tumors were found to have *KRAS2* mutations, mu-

TABLE 3.6. *KRAS2* mutation detection in lymph nodes and peritoneal washings of patients with pancreatic adenocarcinoma.

Authors	Source Method*	No. of mutations/ patients	Percent mutations	Controls**
Ando et al ⁸⁷	Nodes	13/15 tissue	86.7%	0/5 IS
	PCR-RFLP	8/13 nodal groups	61.5%	
		42/101 total nodes	41.6%	
Nomoto et al ⁸⁸	Washings	20/20, tissue	100%	
	PCR-RFLP <i>KRAS2</i>	2/20 cytology	10.0%	
	CA19-9/CEA IS	2/20 mutation	10.0%	
		4/20 IS	20.0%	

*PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism; IS = immunostaining
**IS = immunostaining

tations were found in the peritoneal washings of only 2 patients (Table 3.6). These same 2 patients were also the only ones found to have positive cytology, and these cells were also positive by immunostaining. Two additional patients had positive immunostaining, both with only 5 to 10 positive cells, which the authors felt was below the limit of detection for PCR (1 in 10⁴ cells). All 4 patients with positive immunostaining (including 2 with *KRAS2* mutations) later died with peritoneal carcinomatosis. Of these 3 methods for evaluating peritoneal disease, immunostaining was the most sensitive and *KRAS2* mutation analysis was found to be no better than cytology.

Usefulness of Other Genes in the Diagnosis of Pancreatic Cancer

The *TP53* Gene

Previous studies have demonstrated an incidence of *TP53* mutations of approximately 50% in pancreatic adenocarcinoma,^{16–20,49,89,90} with most reports examining exons 5–8, although a few have also included exons 3, 4, and 9. Others have looked for p53 staining by immunohistochemistry, which takes advantage of the fact that mutant p53 protein is relatively stable and builds up, while wild-type protein generally does not accumulate in normal cells. Baas et al examined 19 colorectal tumors (10 adenomas, 9 carcinomas) to determine the correlation between p53 immunostaining and DNA mutations.⁹¹ Six of 7 tumors with high p53 expression

(nuclear labeling >30%) also had mutations detected in exons 5–9, and in 5 tumors with low expression (1% to 30% nuclear labeling), 2 had mutations while 3 did not. In 7 tumors with negative expression by immunohistochemistry, 1 tumor had a truncating mutation that presumably did not allow for nuclear localization of the protein. Overall, the authors found a 67% sensitivity of high p53 expression with mutations and a 90% specificity, indicating significant discrepancies between the 2 methods. Suwa et al found that 3 pancreatic cancer cell lines with known *TP53* missense mutations were positive by immunostaining, 3 with the wild-type p53 sequence were negative by immunostaining, and 2 of 4 with other gene alterations (2 splice site mutations, 1 with deletion of exons 2–9, and 1 frameshift mutation) were positive.⁸² Causes of false-negative immunostaining might include truncating mutations, gene deletions abolishing the p53 gene product, and missense mutations that might not lead to enough protein stabilization to lead to detectable nuclear accumulation of p53. Other potential causes of p53 overexpression besides missense mutations include alterations in the promoter sequence, interactions with other proteins that stabilize wild-type p53 protein (such as the SV40 large T antigen and the product of the *mdm2* gene), and “genotoxic damage.” In the latter case, p53 overexpression is induced by cellular injury (such as radiation) with accumulation of the normal protein in the nucleus, leading to cell cycle arrest.^{91,92} Clearly, the correlation between immunostaining and *TP53* mutation is only fair to good, and the former does not guarantee the latter. However, immuno-staining

of p53 is technically simpler to perform and can be performed in many immunopathology labs. Mutation detection requires several different PCR reactions from sample material that is usually limited, followed by sequencing or single-strand conformational polymorphism (SSCP).

Early evidence suggested that *TP53* mutation or overexpression was more specific for pancreatic adenocarcinoma than *KRAS2*, for these changes were initially only seen in invasive or in situ lesions. Casey et al found that 16 of 34 (47%) pancreatic cancers overexpressed p53, while none of 24 chronic pancreatitis specimens did.¹⁷ DiGiuseppe et al found that 2 of 17 in situ carcinomas demonstrated diffuse nuclear immunostaining for p53.⁹³ However, Boschman et al serially sectioned pancreatic adenocarcinoma specimens and examined precursor lesions, nodal metastases, and primary tumors for p53 expression by immunohistochem-

istry.⁹⁴ They found that 10 of 25 (40%) primary tumors, 2 of 7 nodal metastases (29%), 5 of 14 (36%) carcinomata in situ, and 6 of 17 (35%) hyperplastic lesions had p53 overexpression. Therefore, p53 mutation may also be an early event in cancer progression, like *KRAS2*, and is probably not as specific for pancreatic cancer as previously thought.

van Es et al examined p53 immunostaining in both cytology and surgical specimens from patients with pancreatic adenocarcinoma.⁵⁷ In surgical resection specimens, they found that 11 of 17 tumors (65%) overexpressed p53, while only 6 of 17 (35%) cytology specimens from the same patients showed overexpression (Table 3.7). They ascribed the discrepancy in 5 cases to Giemsa staining for the cytology (7 were Giemsa stained, 10 were fixed in ethanol), which may have interfered with p53 immunostaining. Nine of 15 cytology specimens that could be amplified by PCR had *KRAS2* mutations,

TABLE 3.7. Studies examining p53 mutations or overexpression in pancreatic juice and brush cytology specimens; telomerase activity in pancreatic juice.

Authors	Source* Method**	Exon—no. of mutations	No. of mutations/ tumors†	Percent mutations
van Es et al ⁵⁷	BR	—	6/17 BR	35.3%
	IC		11/17 resected	64.7%
Sturm et al ⁹⁵	BR		15/36 BR	41.7%
	IC		20/36 resected	55.6%
Ishimaru et al ⁹⁶	BR		12/20 Cytol	60.0%
	IC		18/20 BR	90.0%
			0/8 CP	0%
Iwao et al ⁹⁷	BR	5—2	27/44 Cytol	61.4%
	IC	6—1	36/44 IC	81.8%
	PCR/Seq	7—1	12/14 PCR/Seq	81.8%
		8—9 (1 pt. 6,8)		
Yamaguchi et al ⁵²	PJ	5—3	11/26	42.3%
	PCR/SSCP	6—2		
		7—3		
		8—3		
Kaino et al ^{98††}	PJ	5—1	3/8 Carcinoma	37.5%
	PCR/SSCP/Seq	6—1	2/4 Adenoma	50.0%
		7—1	0/8 N1	0%
		8—2		
Uehara et al ⁹⁹	PJ	—	8/10 <i>KRAS2</i>	80.0%
	PCR-RFLP		8/10 Telomerase	80.0%
	TRAP		0/3 CP	0%
			0/3 N1	0%

*BR = brush cytology; IC = immunohistochemistry; SSCP = single-strand conformational polymorphism; Seq = sequencing; N1 = normal; TRAP = telomeric repeat amplification protocol

**IC = immunohistochemistry; PCR/RFLP = PCR/*Bst*NI restriction fragment length polymorphism

†BR = brush cytology; Cytol = cytology; CP = chronic pancreatitis; IC = immunohistochemistry; Seq = sequencing; N1 = normal

††Intraductal papillary mucinous tumors

and 4 of 17 cases were negative for p53 overexpression and *KRAS2* mutations. Sturm et al performed brushings through the ampulla immediately after the surgical resection of pancreatic adenocarcinomas, and then performed immunohistochemistry for p53 on these cytology specimens.⁹⁵ They found p53 overexpression (defined as nuclear staining in $\geq 10\%$ of epithelial cells) in 20 of 36 (56%) resected specimens, 5 of which were negative for p53 overexpression in the brush cytology specimens. These authors noted that intratumor heterogeneity was one source of discrepancy between resected specimens and cytology, because they found p53-negative areas in tumors that were otherwise p53-positive. Ishimaru et al found that 18 of 20 (90%) of brushings from patients with pancreatic adenocarcinoma were positive for p53 immunostaining, while none of 8 patients with chronic pancreatitis were positive.⁹⁶ Interestingly, cytologic examination by the Papanicolaou technique was positive for malignancy in only 12 (60%) patients. Six of 8 cytology-negative carcinomas were positive for p53 immunostaining, suggesting that p53 immunostaining is a valuable adjunct to cytology in making the preoperative diagnosis of pancreatic cancer.

This same group later examined p53 immunostaining in 44 pancreatic adenocarcinomas, and found 36 (82%) to be p53-positive (defined as any cells showing nuclear accumulation), while cytology was positive only in 27 (62%).⁹⁷ The authors attributed the high rate of p53 overexpression in tumors to fixation in ethanol rather than formalin, presumably better preserving the protein in these specimens. p53 immunostaining was negative in 30 cases of chronic pancreatitis and in 9 papillary adenomas. p53-positive cells were microdissected from the slides in 14 cases, and then amplified by PCR for *TP53* exons 5–8. *TP53* mutations were found in 12 of 14 cases (86%), demonstrating an excellent correlation between p53 immunostaining positivity and mutations of the p53 gene.

Yamaguchi et al examined pancreatic juice collected from patients with pancreatic cancer for mutations in *TP53* exons 5–8 by SSCP and found a mutation rate of 42% (very similar to results seen in tissue sections).⁵² No mutations were found in 16 cases of chronic pancreatitis or 4 mucin-producing adenomas, and the p53 mutation status was the same in both pancreatic juice and resected

tissue in the 5 cases where both were examined. They also found *KRAS2* mutations in 21 of 26 patients (81%). Nine tumors were *KRAS2*+/*p53*+, 12 were *KRAS2*+/*p53*–, 2 were *KRAS2*–/*p53*+, and 2 were *KRAS2*–/*p53*–. One patient who was *KRAS2*–/*p53*+ with subtle findings on CT and ERCP suspicious for a pancreatic head mass was taken to surgery based upon p53 overexpression, and the diagnosis of pancreatic cancer was confirmed at surgery. Overall, 23 of 25 patients had mutations of one or both genes, and the authors suggested that analysis of both genes would enhance screening and diagnosis of pancreatic cancer.

Kaino et al analyzed pancreatic juice for both *KRAS2* and *p53* mutations from 12 patients with intraductal papillary mucinous tumors (IPMT; 8 carcinomas, 4 adenomas).⁹⁸ They found *KRAS2* mutations in all 12 tumors, and *p53* mutations in 3 of 8 (38%) carcinomas and 2 of 4 (50%) adenomas. The same mutations were found both in pancreatic juice and the resection specimens. p53 immunohistochemistry performed on 11 tumors revealed overexpression in 4 of the 5 samples with known p53 mutations, the 1 exception being in a tumor with a codon 212 substitution (Phe>Leu). One of 6 p53 mutation-negative cells showed p53 overexpression. These authors also concluded that analysis of *KRAS2* and *p53* in pancreatic juice would be useful for the early diagnosis of IPMT.

Telomerase

Telomerase is an enzyme that synthesizes the tandem repeats normally present at the ends of human chromosomes, which are successively depleted with each cycle of DNA replication. This enzyme is inactive in normal cells, which predetermines a finite number of cell divisions prior to senescence. However, telomerase may be activated in a variety of cancers and can be an important factor contributing to their immortalization.¹⁰⁰ Uehara et al compared the telomerase activity in 10 pancreatic cancers to their *KRAS2* mutation status (Table 3.7).⁹⁹ Telomerase activity >5.0 was detected in 8 of 10 (80%) of tumors, in 0 of 3 chronic pancreatitis patients, and in 0 of 3 normal patients. *KRAS2* mutations were found in 8 of 10 tumors, but also in 2 of 3 patients with chronic pancreatitis, and 1 of 3 patients with normal pancreases. The specificity and the positive predictive value of telom-

erase were better than those of *KRAS2*, but this assay requires RNA from cellular extracts, and therefore the template of interest must be immediately frozen upon collection.

Conclusions

Despite the large number of studies that have been reviewed here expressing enthusiasm about using molecular genetic techniques for the early diagnosis of pancreatic cancer, none have prospectively incorporated these tools into clinical decision making. *KRAS2* has clearly been the most popular gene examined because mutations are usually present in pancreatic adenocarcinomas, and they can be easily detected using a variety of sensitive, PCR-based techniques and only small amounts of template DNA. The problem with *KRAS2* mutations is that they may also be present in hyperplastic lesions of the pancreas and chronic pancreatitis, and therefore the specificity of these mutations is less than desirable. Although early reports of p53 would suggest that this gene is more specific for pancreatic cancer, p53 overexpression may also be found in premalignant lesions as well. Other genes have not been extensively analyzed for mutations using tissue or fluid obtained during the diagnostic workup of patients suspected of having pancreatic cancer, including *CDKN2* and *DPC4*, which have mutation frequencies of approximately 80% and 50%, respectively, in these tumors.²¹ These tumor suppressor genes (including *TP53*) are more difficult to examine in that they can be inactivated by deletion, point mutation, and tissue-specific changes such as promoter methylation. Detection of all such potential changes might require techniques less feasible from limited amounts of DNA, such as Southern blotting or amplification and sequencing (or SSCP) of several exons. Furthermore, development of pancreatic cancer appears to involve defects in several different tumor suppressive pathways, and analysis of only one gene may be overly simplistic.

Despite these shortcomings, there is still great potential for these techniques. Even though *KRAS2* mutations occur with some frequency in hyperplastic lesions of the pancreas, these lesions may ultimately progress to malignancy, as seen in the subset of chronic pancreatitis patients who develop pancreatic cancer. Three studies have described the

early diagnosis of pancreatic cancer by testing for *KRAS2* mutations, while most others performed just retrospective analyses. p53 immunostaining and PCR-RFLP *KRAS2* analysis together of pancreatic juice would be perhaps the most useful routine studies to perform on patients being worked up for pancreatic cancer, especially in those with negative cytology. A significant reduction in pancreatic cancer deaths, however, will only come from improved methods of screening. Testing of stool samples from populations at greatest risk is one provocative possibility, although the need to extract DNA from this complex source material (with admixtures of bacteria, sloughed intestinal cells, and rare pancreatic ductal epithelial cells) limits its practicality. Analysis of plasma samples for *KRAS2* mutations may represent the simplest method that presently could be applied to screening populations at risk. If a mutation were found, then one could envision proceeding next to a CT scan and/or ERCP. In these and other ways yet to be determined (such as new drug development), molecular biologic techniques will lead to earlier diagnosis and improved survival in patients with pancreatic cancer. The studies we have reviewed in this chapter have opened the door for future prospective trials to confirm the usefulness of these techniques in bringing advances from the laboratory to the bedside.

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4

Cell Signaling Pathways in Pancreatic Cancer

Jason B. Fleming

Introduction

Given that most patients with pancreatic cancer will develop metastases and die secondary to the metabolic effects of their growth,¹ effective systemic therapy is critical to reversing the aggressive biology of this disease; unfortunately, traditional treatment strategies such as chemotherapy and radiotherapy have failed to significantly improve the prognosis of patients with advanced pancreatic cancer.^{2,3} A proposed approach is to counteract one or more of the molecular abnormalities that contribute to tumor proliferation and metastasis, and recent studies have identified a host of genetic alterations in these tumors that may be suitable targets for selective intervention. Using human pancreatic tumor specimens and cell lines, numerous laboratories have identified a number of characteristic genetic abnormalities including the following: point mutations at codon 12 of the *K-ras* proto-oncogene⁴⁻⁷; inactivation of the cyclin-dependent kinase (CDK) inhibitory protein p16⁸; inactivating *p53* mutations⁹; and loss of *p15^{ink4a}* and *Smad4/DPC4* gene expression.^{8,10} Kern and colleagues have examined pancreatic adenocarcinomas for abnormalities of the *K-ras* oncogene and the *p16^{ink4b}*, *p53*, and *Smad4/DPC4* tumor suppressor genes. All of the tumors have *K-ras* gene mutations. Thirty-eight percent of the tumors have 4 altered genes, another 38% have 3 altered genes, 15% have 2 altered genes, and 8% of the tumors have 1 altered gene. As each of these genes is involved in different signaling mechanisms within the cell, these data demonstrate that inactivation of multiple pathways occurs in the development of invasive pancreatic

cancer¹¹ (Table 4.1). Investigators have also demonstrated that circulating growth factors and their receptors normally involved in cell growth are overexpressed in human pancreatic cancer specimens or cell lines. While the described genetic or biochemical abnormalities all contribute to the aggressive biology of pancreatic cancer, it has been difficult to develop unifying hypotheses that can relate these changes to the critical features of pancreatic carcinoma (rapid growth, drug and radiation resistance, and the early emergence of metastases). The purpose of this chapter is to place the established genetic abnormalities within the context of the growing knowledge regarding cell signaling pathways.

Membrane Interactions

Receptor Tyrosine Kinases

The cellular response to growth factors and other stimulatory molecules is mediated by a host of cell-surface receptors; these typically contain an extracellular domain that interacts with a ligand, a transmembrane domain that anchors the receptor to the cell membrane, and an intracytoplasmic domain that interacts with downstream signaling components. Binding of a ligand to its receptor induces conformational changes within the receptor and/or cross-linking of receptors to initiate downstream events. Many of the mitogenic (growth-stimulatory) receptors that have been implicated in pancreatic adenocarcinoma, such as epidermal growth factor (EGF) and its receptor (EGF-R), are receptor tyrosine kinases (RTKs), which undergo autophosphorylation of tyrosine residues within their

cytoplasmic domains upon ligand binding.^{12–15} Phosphorylation culminates in the activation of the Ras protein, the product of the frequently mutated *ras* proto-oncogene.

Constitutive activation of RTKs, either by structural alteration of the receptor itself or by unregulated presentation of the ligand, can induce mouse fibroblasts to acquire a fully transformed phenotype,¹⁶ and it has been suggested that unrestricted activation of proliferative pathways via RTKs could contribute to malignant transformation. Additionally, one of the hallmarks of tumor cells is the overexpression of RTK growth factor receptors. For example, in most cancers EGF-R is not mutated; however, overexpression of EGF-R occurs often and stimulates proliferation in many tumors. Thus, the overexpression of EGF-R creates an autocrine loop in which the mitogenic potential of EGF is selectively enhanced in neoplastic as compared to normal cells.

EGF *HER2/neu*

EGF-R belongs to the type 1 growth factor receptor family. As in the other RTKs, EGF-R contains an extracellular ligand-binding domain, a trans-membrane domain, and an intracellular domain.¹⁷ The intracellular domain possesses tyrosine kinase activity, and activation of the receptors by specific ligands leads to the phosphorylation of various intracellular substrates. The EGF receptor, also known as human EGF receptor 1 (HER1), is a member of a family of several receptors including

EGF receptor type 2 (HER2 or c-erb-B2), type 3 (HER3 or c-erb-B3), and type 4 (HER4), which has recently been cloned.^{12–18} When specific ligands, which include EGF and transforming growth factor alpha (TGF- α), bind to EGF-R, the tyrosine kinase activity of the receptor is increased, and the resulting intracellular signals stimulate cell growth.¹⁹ In epithelial cells, overexpression of EGF or EGF-R type 2 (HER2 or c-erb-B2) leads to malignant transformation, and the presence of EGF and TGF- α enhances proliferation of the newly transformed cells.¹⁷

Primary pancreatic cancers exhibit increased levels of EGF-R, TGF- α , and EGF by immunohistochemical and in situ hybridization analysis, while low levels persist in the normal pancreas.^{17,18,20} Expression of the EGF receptors is intense in all grades of pancreatic cancer, and approximately 40% to 50% of pancreatic cancers exhibited increased EGF type 2 receptor (c-erb-B2) immunoreactivity.²¹ Furthermore, overexpression of the EGF type 3 receptor (HER3 or c-erb-B3) protein is associated with advanced pancreatic tumor stage and shorter postoperative survival.¹⁸ Correlation analysis has demonstrated that combined expression of EGF-R and either TGF- α or EGF in pancreatic tumors is associated with more aggressive disease and a significantly shorter survival time postoperatively.^{19,21} From these studies it appears that the overexpression of EGF receptors plays a role in pancreatic cancer progression and negatively influences patient prognosis after tumor resection. These findings also support an autocrine

TABLE 4.1. Genetic abnormalities in pancreatic adenocarcinoma, known position in cell signaling pathways, and general dysfunction associated with each component in pancreatic cancer.

Cellular site	Description	Abnormality in pancreatic cancer
Membrane Elements		
EGF/HER2-neu	Receptor tyrosine kinase	Increased activity (autocrine)
HGF/c-met	Receptor tyrosine kinase	Increased activity (paracrine)
TGF- β /TR β -II	Serine/Threonine receptor	Decreased anti-proliferative Increased invasive signaling (?autocrine)
Cytosolic Intermediates		
Ras	G-protein	Activation
Smad4/DPC4	Transcriptional activator	Inactivation
Nuclear Factors		
p53	Cell cycle/apoptotic response to cell stress	Inactivated
p16/RB	Cell cycle control	Inactivated
p15	TGF- β -induced cell cycle control	Inactivated

method of stimulation in which EGF, produced by pancreatic cancer cells, binds to an upregulated EGF receptor on the same cells.

The *HER2/neu* proto-oncogene encodes a 185kDa transmembrane glycoprotein with tyrosine kinase activity, which is closely related to the EGF-R.²² Increased *HER2/neu* expression is observed in 30% of infiltrating breast cancers and 60% of in situ breast carcinomas.²³ Furthermore, increased *HER2/neu* expression is predictive of a poor clinical outcome.²⁴ Immunohistochemical evaluation has also demonstrated a significantly increased *HER2/neu* expression in up to 50% of pancreatic intraductal lesions and infiltrating carcinomas as compared with normal ducts.²⁵ As in other solid malignancies, there appears to be a relationship between pancreatic tumor *HER2/neu* overexpression and a poor clinical prognosis.²⁶

HGF/*c-met*

Hepatocyte growth factor, also known as scatter factor, is an important mediator of epithelial-stromal reactions and is the mesenchymal ligand of the epithelial cell-surface tyrosine kinase receptor hepatocyte growth factor receptor (HGF-R) encoded by the *c-met* oncogene.²⁷ First identified as a potent stimulator for hepatocytes in primary culture, HGF was subsequently established to stimulate the proliferation of epithelial cells, act as a morphogen during embryogenesis, and promote cellular motility.²⁸ The presence of HGF promotes the growth of carcinoma cells,^{27,28} and the binding of HGF to HGF-R stimulates invasive activity in carcinoma cells²⁹ and is a potent angiogenic agent.^{29,30} Receptor stimulation activates intrinsic receptor tyrosine kinase activity, and intracellular signaling is mediated, at least in part, by activation of the Ras pathway³¹ with subsequent stimulation of the growth-promoting MAP kinase cascade. HGF is secreted by cells of mesenchymal origin whereas expression of the HGF receptor is confined to epithelial cells; therefore, HGF, produced by surrounding stroma, normally acts in a paracrine manner to stimulate epithelial cell growth.

Hepatocyte growth factor is present in the stromal cells of the normal pancreas; however, by immunohistochemistry and Northern blot analysis a 10-fold elevation is found in pancreatic carcinoma, presumably derived from the stroma and not the

malignant cells.³² Overexpression of the *c-met* gene (and the HGF receptor) occurs in pancreatic cancer,³³ while only mild expression is identified in normal pancreas. Furthermore, increased *c-met* and HGF expression in hyperplastic, dysplastic, and malignant ductal epithelial cells of the pancreas suggests that overexpression of this receptor-ligand system is an important early event in pancreatic cancer development.³⁴ In summary, increased local production of HGF by surrounding stroma binds to its receptor on pancreatic cancer cells and stimulates pancreatic cancer cell growth, motility, and angiogenesis.

TGF- β /TGF- β Receptors

The transforming growth factor (TGF- β) family consists of a large number of structurally related polypeptide growth factors that play crucial roles in the regulation of cellular processes such as cell division, differentiation, motility, adhesion, and apoptosis. Members of the TGF- β superfamily, including the TGF- β s, the activins, and the bone morphogenetic proteins (BMPs), control development and homeostasis in many organisms, including humans.³⁵ Mammalian cells express 3 isotypes of TGF β (TGF- β 1, TGF- β 2, and TGF- β 3) and 3 TGF- β receptors: type I (T β R-I), type II (T β R-II), and type III (T β R-III).³⁶ TGF- β initiates signaling by binding to specific sets of 2 transmembrane serine/threonine kinases called the type I and type II receptors. The type I receptor plays a central role as the kinase that phosphorylates Smad proteins, whereas the type II receptor activates the type I receptor. The role of the ligand is to bring together these 2 kinases, forming a receptor complex and allowing the phosphorylation of the type I receptor by the type II receptor.³⁶ This phosphorylation is essential to activate the type I receptor and subsequently phosphorylate Smad proteins, thereby inducing their translocation into the nucleus and activation of target genes (Figure 4.1).

TGF- β was first identified as a promoter of the transformed phenotype in fibroblasts³⁷; however, it was quickly discovered to have antiproliferative activity for the majority of mammalian epithelial cells tested.³⁸ Initial observations of the frequent loss of antiproliferative responses to TGF- β in tumor-derived cell lines led to the notion that disruption of TGF- β signaling might predispose to, or cause,

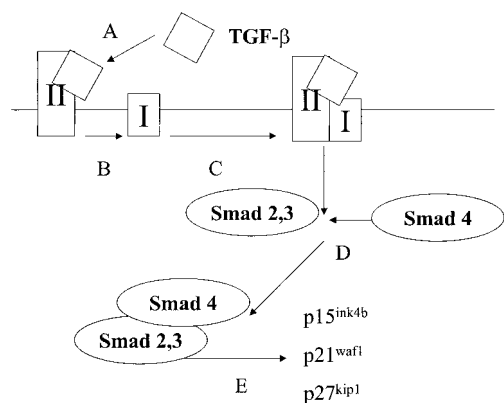


FIGURE 4.1. The proposed TGF- β antiproliferative signaling pathway. TGF- β (A) binds as a ligand to the type II TGF- β receptor. This allows the formation of a heteromeric complex of the type I and type II receptors (B). This complex formation results in phosphorylation of the Smad 2 and 3 proteins (C). Smad 4 binds to this complex in the cytosol (D) and permits DNA binding and transcription activation of target genes to occur in the nucleus (E). These genes act to control cell-cycle progression.

cancer. The first major validation of this hypothesis came with the finding that T β R-II is mutated in the vast majority of hereditary or somatic forms of nonpolyposis colorectal cancer with microsatellite instability, a form of colorectal cancer accounting for approximately 10% of cases.³⁹

The effect of TGF- β s in human pancreatic carcinoma has also been characterized; pancreatic cancer samples, in comparison with normal pancreatic samples, exhibit an increased expression of TGF- β ; however, TGF- β 1 fails to inhibit cell proliferation in many pancreatic cancer cell lines tested.⁴⁰ By in situ hybridization all 3 isotypes are highly expressed in approximately 30% of primary tumors; furthermore, this overexpression is associated with more aggressive tumor growth and reduced patient survival after resection. These findings suggest that the TGF- β s act as growth stimulators in human pancreatic tumors in vivo, while the growth-inhibiting effects of TGF- β s on pancreatic cancer cells are lost.⁴⁰ All three TGF- β receptors have been found in the normal human pancreas.⁴¹ In pancreatic cancer, T β R-I and T β R-II receptor proteins are markedly overexpressed, whereas the levels of T β R-III remain unchanged.⁴¹ Type I and II receptors are concomitantly expressed

in 54% of pancreatic cancers⁴²; furthermore, the presence of both T β R-I and T β R-II is associated with advanced tumor stage. These data demonstrate that the normal growth-inhibitory signal via TGF- β s is lost in pancreatic cancers, at least in part due to inactivation of signal pathway elements. However, the above findings also suggest that TGF- β stimulation through the T β Rs persists and contributes to the neoplastic process in pancreatic cancer.

Intermediate Elements

Ras

Many growth factors are known to bind and activate 2 types of surface receptors: those possessing an intrinsic tyrosine kinase activity (RTKs), or those that transmit signals to the cytoplasm through the interaction with heterodimeric GTP-binding proteins (G proteins).⁴³ The latter are collectively known as G protein-coupled receptors (GPCRs) and comprise the largest group of cell surface receptors; these are activated by a diverse array of external stimuli. Activation of the GPCRs elicits a change in protein conformation that uncovers previously masked G protein binding sites. This results in the exchange of GDP for GTP bound to the G protein subunits and the initiation of intracellular signaling responses.⁴⁴ P21ras (Ras) is a prototype for the G protein family and is encoded for by the cellular proto-oncogene *ras*.⁴⁵ The binding of Ras to GTP is controlled by surface protein-ligands that modulate Ras interaction with its bound nucleotide.⁴⁶ Similarly, activation of RTKs, which include the EGF and HGF receptors, leads to stimulation of a number of intracellular signaling cascades, including the Ras/MAP kinase proliferative cascade. Thus, both GPCRs and tyrosine kinase receptors converge at the level of Ras to initiate activity leading to MAP kinase activation and transcriptional regulation. GPCR activation of the Ras superfamily also stimulates a variety of second messenger generating systems. These may, in turn, stimulate a number of highly interconnected cytoplasmic signaling pathways, which ultimately reach the nucleus and thus control gene expression. Therefore, the final biologic outcome of many ligand-receptor binding and cellular stimulation path-

ways centers on the Ras protein and most likely results from a network of responses, rather than from a single series of sequential events.

The Ras-dependent pathway plays a major role in normal cellular proliferation, and the membrane-bound Ras proteins appear to be involved in tumor development by altering the signal transduction pathway across the membrane. The *ras* genes, consisting of three families (*K-ras*, *H-ras*, and *N-ras*), were first identified in the 1960s as viral oncogenes that caused cancer in mice.⁴⁷ Human homologues of these genes were detected in the early 1980s and, during the ensuing decade, mutations of *ras* were noted to be among the most common genetic abnormalities associated with human cancer. Mutations of the *ras* genes modulate the interaction of their protein product with GTP.⁴⁶ In particular, mutations of the *ras* gene lead to diminished triphosphatase activity, locking Ras in the “switched on” state. Mutations that lead to constitutive association of Ras with GT are believed to represent an important step in the progression of cells toward unchecked proliferation and malignant transformation.⁴⁸ A landmark of Ras transformation consists of the ability of the transformed cells to proliferate even in conditions of restricted supply of growth factors and nutrients.⁴⁹ In addition, constitutively activated isoforms of Ras are known for their strong impact on the organization of the cytoskeleton of transformed cells.⁵⁰

Excessive activation of the Ras protein is associated with nearly all pancreatic cancers,⁵¹ and the *K-ras* member of the *ras* gene family is mutated in pancreatic adenocarcinomas at a frequency unprecedented among other tumors. As with other *ras* genes, the *K-ras* proto-oncogene encodes a guanine nucleotide-binding protein normally involved in the signal transduction pathways of cell growth and differentiation. Point mutations, occurring in either codon 12, 13, or 61 of a *K-ras* gene, convert the Ras protein product to a continuously active state.⁵² Several recent studies have reported that more than 95% of pancreatic cancers contain *K-ras* genes with a mutation at codon 12, although codon 13 and 61 mutations have also been detected.⁵³ It also appears that mutations in *K-ras* occur in the early stage of pancreatic carcinogenesis.⁵⁴ The high incidence of *K-ras* mutations in early and invasive pancreatic carcinoma suggests that oncogenic Ras activation is a principal molecular event in the

pathogenesis of pancreatic cancer development. After treatment with radiation and surgery, the survival is lower in patients with *K-ras* mutant pancreatic cancer than patients with wild-type *K-ras* tumors.⁵⁵ The high frequency of activating mutations and the central position of Ras problems in cell signaling pathways underscores the biologic importance of oncogenic Ras in pancreatic cancer cell behavior and response to therapy.

Smads

TGF- β regulates growth of epithelial cells by the activation of downstream genes, which induce G1 cell cycle arrest. It would therefore be expected that loss of the components of this TGF- β pathway would be advantageous in the clonal evolution of neoplasms, and it is resistance to the antiproliferative effects of TGF- β that characterizes aggressive neoplasms.⁵⁶ Recently, the Smad proteins have been identified as an often-disrupted link between TGF- β and its downstream target genes. The Smad family of receptor substrates was discovered through studies on Dpp (decapentaplegic), a *Drosophila* member of the TGF- β family.⁵⁷ A search for genes involved in dpp function led to the identification of a novel gene, named *Mad* (mothers against dpp). Shortly afterwards, three *Mad* homologues, called *sma-2*, *3*, and *-4*, were identified in the nematode *Caenorhabditis elegans*⁵⁸ and up to 8 homologues, named Smads (for *Sma*- and *Mad*-homologues), were identified in humans and other vertebrates.⁵⁹

Based on their structural and functional properties, Smads fall into three subclasses. The first class comprises Smads that act as direct substrates of TGF- β family receptors and are commonly referred to as “receptor-related Smads.” These Smads fall into groups depending on the receptor they recognize: the substrates and mediators of BMP receptor signaling, Smads 1, 5, and 8^{60–64}; and the substrates and mediators of the TGF- β and activin receptors, Smads 2 and 3.^{65,66} In this particular subclass of Smads, the receptor phosphorylation sites are serine amino acids in the C-terminal region known as the “Mad homology domain” number 2 (MH2).⁶⁷

The second class includes Smads that are not direct receptor substrates but whose function is essential for signaling by the receptor regulating Smads. To this second class, sometimes referred to

as “co-Smads,” belongs Smad4 in vertebrates. Studies with Smad4 have shown that this protein forms a complex with receptor-phosphorylated Smads,⁶⁸ which take it to the nucleus where it binds to DNA at the MH1 domain.⁶⁹ Formation of this complex is required for optimal binding and transcriptional activation of target genes in BMP, TGF- β and activin responses⁷⁰ (Figure 4.1).

The third class of Smads includes the antagonistic Smads, Smads 6^{71,72} and 7⁷³ in vertebrates. When overexpressed at high levels Smads 6 and 7 can block TGF- β , activin, and BMP signaling non-selectively.⁷⁴ The expression of antagonistic Smads is induced in response to the TGF- β agonists that they counteract,⁷⁵ suggesting that antagonistic Smads might participate in negative feedback loops to regulate the intensity and duration of TGF- β responses. Alterations leading to a gain of TGF- β activity are thought to play a central role in fibrotic disorders characterized by excessive accumulation of interstitial matrix material in several visceral organs⁷⁶ and in the genesis of certain human cancers including pancreatic cancer.

The gene encoding Smad4 was originally cloned as a tumor suppressor gene on chromosome 18q21, which is frequently deleted or mutated in pancreatic adenocarcinomas; hence its original name *DPC4* (deleted in pancreatic cancer locus 4).^{77,78} Besides large deletions affecting the entire Smad4-encoding locus, the mutations inactivating Smad4 in tumors include small deletions and frameshift mutations mostly affecting the MH2 domain. These mutations prevent the formation of Smad2-Smad4 complexes in response to TGF- β signaling.⁷⁹ Genetic analysis of pancreatic cancers has demonstrated homozygous deletions of the *Smad4/DPC4* gene in 30% of pancreatic cancers tested and inactivating point mutations in an additional 22% so that approximately 55% of pancreatic adenocarcinomas lack Smad4 protein expression.⁷⁷ Recent in vitro studies have also demonstrated that the presence of *Smad4/DPC4* is necessary for the growth inhibitory effects of TGF- β signaling and *p21^{waf1}* induction.⁸⁰ Furthermore, utilizing an adenoviral vector containing the *Smad4/DPC4* open reading frame, this gene can be restored in pancreatic cell lines that lack its expression.⁸¹ In so doing this gene is expressed at supranormal levels and the growth inhibition induced in pancreatic adenocarcinoma cells is coincident with upregulation of target genes

p21^{waf1} and *p15^{ink4b}*.⁸⁰ Taken together, these studies support the central role of Smad4 in TGF- β signaling and suggest that the inactivating *Smad4/DPC4* mutations in pancreatic cancers are at least partly responsible for the disruption of the TGF- β signaling pathway observed in pancreatic adenocarcinomas.

Target Genes

Cell/Cycle–Apoptotic Pathways

A coordination and balance between cell proliferation and apoptosis is crucial for normal development and tissue size in the adult. Cancer results when clones of mutated cells survive and proliferate inappropriately, disrupting this balance. One mechanism for maintaining growth homeostasis is cellular dependence upon extracellular growth factors for survival.⁸² Cells that exhaust local supplies of these factors or that move to new locations away from the source will die; however, pancreatic cancer cells often remain stimulated at the membrane level by autocrine/paracrine loops or Ras activation. The loss of this mechanism of growth control is certainly part of the story, but a high percentage of pancreatic cancers possess proliferative or survival-promoting mutations in tumor suppressor genes that normally function downstream at the level of nuclear transcription. Either kind of mutation would be expected to cause an increase in the number of mutant cells regardless of the upstream messages and allow for growth of the cell in hostile surroundings—a requirement for metastasis. It follows that mutations of the downstream elements of the signal transduction pathways often occur concomitantly with upstream abnormalities.

RB/p16

Growth factor signaling results in Cyclin D synthesis and assembly with catalytic Cyclin-dependent kinase (CDK) partners⁸³; however, CDK inhibitors, such as p15 and p16, normally prevent cell-cycle progression when they bind to CDK and interfere with CDK phosphorylation of the RB (retinoblastoma) family members (Figure 4.2A). After signaling, the increase in Cyclin D levels produces Cyclin D-CDK complexes that sequester p15

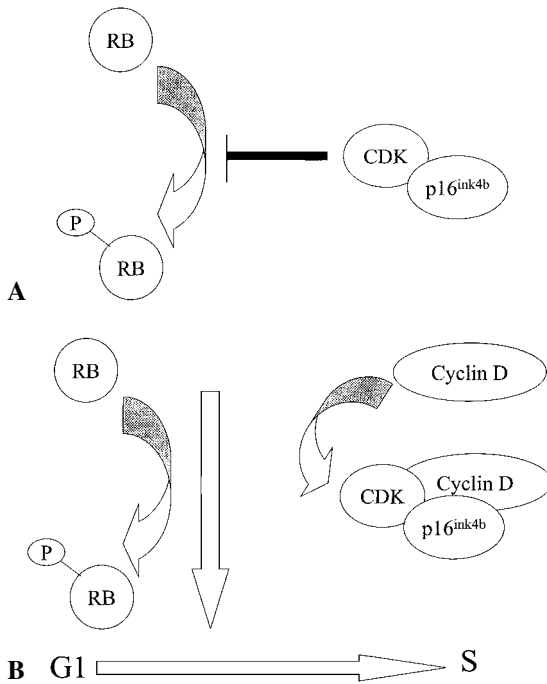


FIGURE 4.2. Cyclin D/Rb/p16 proliferation signaling pathways. In quiescent cells (A), p16 normally acts as a CDK inhibitor, preventing the CDK-induced phosphorylation (inactivation) of the retinoblastoma (RB) protein. After signaling (B), increasing levels of Cyclin D will complex with the CDK and sequester p16 and allow the phosphorylation of RB. The phosphorylated RB is inactivated, which permits transcription factors such as E2F to stimulate cell cycle progression from G1 to S phase.

and p16 and permit RB inactivation (by phosphorylation) and the transcription of molecules that drive cells into S phase (Figure 4.2B). The p16/Cyclin D/RB pathway is critical in the development of many human cancers, and genetic evidence has accumulated that these components are all involved in tumorigenesis. A consequence of deregulation of this pathway is uncontrolled activation of E2F family members, resulting in the inappropriate transcription of genes required for S phase.⁸⁴ *Rb* was first described as the tumor suppressor gene that is inactivated in the development of retinoblastoma tumors. Loss of function of RB is generally associated with increased cellular proliferation⁸⁵; however, it appears that p16 is the major negative regulator of this pathway. Encoded for by the *p16^{ink4b}* gene, located at a chromosomal site (9p21) fre-

quently damaged in epithelial neoplasms, the 16 kDa product is itself a CDK inhibitor.⁸⁶ A non-functional p16 protein produces inactivation of RB, resulting in unregulated cell-cycle progression and cellular proliferation.

Although mutations of the *Rb* gene are infrequent in pancreatic cancers, the *p16^{ink4b}* gene is inactivated by mutations in up to 82% of pancreatic adenocarcinomas.^{87–90} Nearly half of these inactivations are intragenic mutations of *p16^{ink4b}*, and the remainder are homozygous deletions of the gene. Schutte et al identified an additional mechanism by which the RB/p16 pathway might be inactivated. In addition to structural mutations, transcriptional silencing of the *p16^{ink4b}* gene occurs by hypermethylation of 5'-CpG-rich regions of the DNA in the promoter region of the gene. The hypermethylation prevents gene transcription from occurring even with a structurally intact *p16^{ink4b}* gene, and this prevents p16 protein expression in the remaining 20% of pancreatic tumors. Furthermore, no abnormalities are present in the other components (RB or Cyclin D) of the pathway. In total, the RB/Cyclin D/p16 pathway is abrogated in 98% of pancreatic adenocarcinomas, all through inactivation of the *p16^{ink4b}* gene. These data demonstrate the central role of the Rb/p16 pathway, and the *p16^{ink4b}* gene, in the development of pancreatic carcinoma.⁹¹

p53

Ras-transformed cells are more sensitive than their normal counterparts to treatments that result in cellular damage and apoptotic (programmed cell death) signaling. This increased sensitivity is one explanation for why radiation and chemotherapy, which induce apoptotic signaling by damaging DNA and disrupting the cell cycle, work well in tumors. The likelihood that tumor cells will be resistant to these treatments increases greatly when other mutations occur that block steps in death signaling and execution. Since its discovery, the *p53* gene has been known as a tumor suppressor gene that is inactivated in a high percentage of malignancies. Its normal role in cell function has been poorly understood until recently, but it is now evident that the p53 protein functions within the framework of a signal transduction pathway. Signals arising from various forms of cellular stress, the most well studied of which is DNA damage,

are transmitted by p53 to a large number of genes and factors controlling various cellular responses, including G1 and G2 arrest, differentiation, and apoptosis.⁹² However, there are at least 2 genetically distinct pathways involved in the regulation of p53 function.

In the normal p53 pathway (Figure 4.3A) a tonic loop exists in which the basal level of p53 activates a basal level of downstream target genes including *mdm2*.^{93,94} Mdm2 protein binds p53, inactivating the ability of p53 to function as a transcription factor by blocking access to the basal transcription apparatus and also targeting p53 for ubiquitin-mediated degradation.^{95,96} A range of insults can induce a set of signaling pathways that alter the level and state of p53, disrupting the tonic inhibitory loop to allow for DNA repair and other activities. It is likely that the kinetics and spectrum of downstream targets activated (or repressed) are modified by cell type-specific factors.

In neoplasia (Figure 4.3B), loss of function of the p53 protein occurs as a consequence of mis-

sense mutation, allelic loss, or inactivation by viral proteins such as HPV E6. As a consequence, the downstream targets of p53 are not appropriately regulated and there is loss of their normal function—for example, loss of p53-activated cell death, growth arrest, and/or control of genomic stability. Loss of these important p53-dependent processes may occur early in the genesis of some tumors or, alternatively, may be steps in tumor progression. One of the commonest features of mutant p53 is its accumulation within cells, a phenotype seen frequently in tumors and detectable by immunohistochemistry. While there are multiple mechanisms for this clinical phenotype, one important mechanism is the loss of activation of *mdm2* mRNA and hence mdm2 protein. Consequently, the mdm2-dependent degradation pathway of p53 does not operate and thus p53 protein accumulates at high levels, although the protein is inactive.

Notable in advancing the molecular pathology of pancreatic cancer have been a series of reports in recent years describing alterations in the *p53* gene/protein,^{97–100} which occur at a frequency similar to that reported in other common human malignancies, such as colorectal, breast, lung, and hepatocellular cancer.¹⁰⁰ Detailed analysis has identified that 40% to 70% of pancreatic adenocarcinomas harbor *p53* mutations.^{97–100} Importantly, *p53* alterations appear to be a relatively early event in the development of pancreatic ductal cancer as suggested by the occurrence of pancreatic cancer in subjects harboring germline mutations of the *p53* gene⁹⁹ and by the presence of p53-immunoreactive cells in the intraductal component of pancreatic cancer.⁹⁸ However, some evidence suggests that p53 protein abnormalities are a late event seen mainly in advanced pancreatic cancer.⁹⁹ The presence in both *K-ras* and *p53* gene mutations suggests that a cooperative effect of *K-ras* and *p53* alterations may contribute to the malignant phenotype in ductal pancreatic carcinogenesis.¹¹ In culture, human pancreatic adenocarcinoma cells with mutant *p53* have reduced radiosensitivity,¹⁰¹ and the presence of wild-type p53 protein by immunohistochemical staining of primary tumors predicts an increased length of survival for patients with pancreatic adenocarcinoma.^{102,103} The p53 protein appears central in the cross talk between cell-cycle control mechanisms and cell death sig-

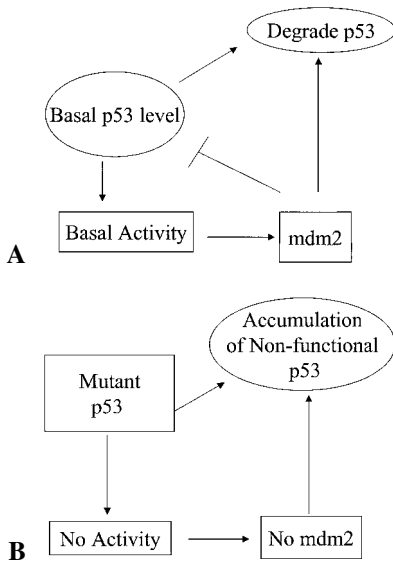


FIGURE 4.3. Regulatory pathways for p53. Normally (A), a basal level of the p53 protein will act to stimulate mdm2 production, which binds to and degrades p53. In neoplasia (B), when a nonfunctional p53 protein is produced secondary to *p53* gene mutations, no mdm2 protein is present. This allows for accumulation of nonfunctional p53 protein to occur within the cytosol of the cell.

nals; consequently, the loss of p53 function greatly favors pancreatic cancer cell survival and growth.

p15

Downstream cell-cycle inhibitors (p15, p21 and p27) are believed to mediate the antiproliferative effects of TGF- β signaling, and, since TGF- β inhibits epithelial cell growth, disruption of its signaling pathways could initiate neoplastic growth. Increase in the concentration of the CDK4/6-inhibitor p15 and the resulting cell cycle arrest is one mechanism by which TGF- β inhibits cellular growth.¹⁰⁴ The association of p15 to Cyclin D1-CDK4/6 complexes then releases p27 that subsequently binds to, and inhibits, Cyclin-CDK complexes and promotes cell cycle arrest at G1.^{105,106} It has been suggested that p15 expression is under the transcriptional control of Smad2/4 complexes formed after the activation of TGF- β receptors.^{107,108,114} Genotypic evaluation of human pancreatic tumors has identified *Smad4/DPC4* mutations in 62% of primary tumors studied,¹⁰⁶ and inactivation of *p15^{ink4a}* occurs in up to 60% of pancreatic cancers. This suggests that alterations in the *p15^{ink4a}* gene might be in part responsible for pancreatic cancer cell resistance to TGF- β inhibitory effects.⁸ When the p15, Smad4/DPC4, and TGF- β receptor abnormalities are evaluated together, nearly all pancreatic adenocarcinomas have disruption of the TGF- β signaling pathway.⁸ The relevance to the clinical approach to therapy is potentially great as a primary tumoricidal effect of radiotherapy is thought to be the induction of TGF- β ; consequently, those cell clones with an impaired TGF- β pathway would have a survival advantage. Interestingly, the *p15^{ink4a}* and *p16^{ink4b}* genes are located in the 9p21 chromosomal region, and losses of DNA in this region occur at high frequency in pancreatic carcinomas¹⁰⁶; both individual and combined deletions of *p15^{ink4a}* and *p16^{ink4b}* have been described in pancreatic cancer. Consequently, the TGF- β antiproliferative signal pathway is dysfunctional in nearly all invasive pancreatic cancers tested, although the clinical significance of these findings remains to be seen.

In summary, TGF- β is believed to represent a highly conserved mechanism of response to inflammation or other proliferative signals; however, in the

setting of progression from normal epithelium to invasive carcinoma, cellular sensitivity diminishes. Simultaneously, local tissue TGF- β levels (produced from both malignant cells and surrounding stromal elements) increase during neoplastic progression. Therefore, TGF- β appears to have a multifactorial role in tumorigenesis; in early stages when cells still respond to its antimitogenic effect, TGF- β may act as a tumor suppressor. After cells become insensitive to growth inhibition by TGF- β during malignant progression, it may function as a tumor promotor by stimulating angiogenesis, immunosuppression, and the synthesis of extracellular matrix; these effects provide a favorable microenvironment for rapid tumor growth and metastasis.

Common Themes

Tumors arise through the stepwise mutation of proto-oncogenes and tumor suppressor genes. The initial identification of these genes and their functions suggested that mutations of them would affect discrete pathways, with each making discrete contributions to the full malignant phenotype. As more is learned about the complexities of these pathways, it has become increasingly difficult to ascribe distinct biological functions to each. There are, however, central themes that must be remembered if biological-based therapy can be applied to this disease.

Cross Talk

In a broad sense, these pathways are essential for modulating cell proliferation and maintaining cellular homeostasis by regulating protein synthesis, cytoskeletal integrity, hormone secretion, and cell death. Many steps in the various signaling cascades involve members of protein families in which different family members overlap partially with each other in terms of their functional capabilities but differ in terms of others. In addition, the different signaling cascades themselves can interact significantly; via this “cross talk,” one signaling cascade can actually stimulate the function of alternate cascades. In summary, the pathways presented schematically in this chapter cannot be considered in isolation.

The signaling that occurs between the proliferation and cell death machinery is one example of cross talk between pathways. The normal function of communication between the proliferation and death pathways is to prevent the survival and expansion of clones of aberrant cells. Because of this connection, mutations that promote inappropriate entry into the cell cycle in cancer cells often also promote apoptosis (programmed cell death). Conversely, mutations that block cell death are associated with inappropriate proliferation and greatly promote cancer development. One of the most important links between the proliferation and cell death machinery is the tumor suppressor p53, which promotes cell-cycle arrest or apoptosis in response to DNA damage or a strong oncogenic stimulus to proliferate (eg, activated Ras). Most tumors have disruption of either p53 or an upstream activator of this pathway, the *p19^{ARF}* tumor suppressor, a gene located at the same genetic locus (and subject to the same DNA mutations) as the *p15^{ink4a}* and *p16^{ink4b}* genes. Expression of oncogenes, such as *ras*, result in the accumulation of p19 protein, which acts to promote p53 stabilization and function^{110–116} (Figure 4.4). Furthermore, recent evidence demonstrates that E2F plays an important role in limiting the oncogenic consequences of loss of the RB/p16 pathway by promoting apoptosis through activation of several pathways, including p19-p53. Thus, a common pathway through which p53 and p16 regulate cell growth and mediate tu-

mor-suppressor action is partly their ability to control the cellular division process. Such controls allow cells to halt DNA replication, repair damaged DNA, or turn on the apoptotic pathway. Thus, mutation in any of these genes would allow the proliferative signals of activated Ras to dominate.

Centrality of Activated Ras

From the membrane receptor to downstream target genes, the interaction between these pathways appears to consistently revolve around the Ras protein. Oncogenic Ras proteins are persistently switched on, leading to the expression of downstream genes of multiple pathways that are not normally expressed in pancreatic ductal epithelial cells. Thus, oncogenic Ras allows for the maintenance of the transformed cells and expression of the aggressive tumorigenic phenotypes demonstrated in pancreatic adenocarcinomas. It is known that Ras normally acts to receive transmembrane signals from both tyrosine kinase receptors and G protein receptors; however, activated Ras will promote the production of growth factors and the expression of their receptors (EGF-R/HGF-R) maintaining the cell in a growth stimulatory state. Furthermore, activated Ras is intimately involved in downstream transcription activation. The expression of Cyclin D1 is regulated by activated Ras, forging a potential link from growth-factor signaling to cell-cycle progression through G1 to S phase. The elevated Cyclin D1 levels coupled with the nearly universal disruption of the RB/p16 pathway sustain the proliferative drive of pancreatic cancer cells. Additionally, Ras-transformed cells demonstrate a limited growth inhibitory response to TGF- β but a paradoxical increase in invasive activity and metastatic behavior.^{117–119} Genetic analysis identifies activating mutations of the *K-ras* gene as necessary and very early events in the development of nearly all pancreatic adenocarcinomas. Once activated, Ras promotes growth factor stimulation and cell proliferation. As genetic mutations occur in tumor suppressor genes, sequential clones of cells become refractory to growth regulatory/cell-death signals and gain an increasing ability to survive in harsh conditions and metastasize.

The complexity and cross talk demonstrated in these various pathways combined with the redundancy of the genetic mutations serve to make pan-

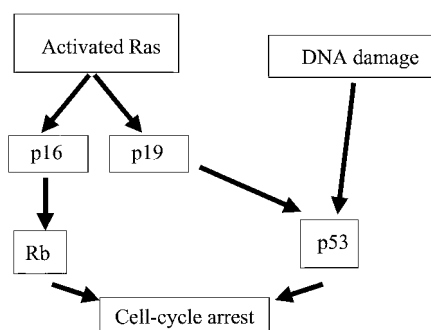


FIGURE 4.4. Cross-talk that occurs after activation of the Ras protein. Through these interactions, cell-cycle arrest would normally occur after Ras activation to allow for DNA repair and other cellular functions. In neoplasia, inactivation of downstream pathway components prevents cell-cycle arrest and allows the proliferative and growth-stimulatory signals of activated Ras to dominate.

creatic cancer the lethal malignancy seen in clinical practice. Improvements in therapy must address these issues, and it is not likely that any single agent or method will achieve meaningful results. The centrality of the *ras* gene does, however, offer treatment possibilities that are currently being pursued.

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5

Epithelial Stem Cells in Pancreatic Regeneration and Neoplasia

Ingrid M. Meszoely, Anna L. Means, Charles R. Scoggins, and Steven D. Leach

Introduction

The resistance of human pancreatic ductal adenocarcinoma to traditional antineoplastic therapy emphasizes the need for effective chemoprevention and screening strategies for this disease. However, the cell of origin for human pancreatic cancer remains unknown. Recent studies suggest that pluripotent epithelial stem cells exist within the adult pancreas and may be responsible for production of preneoplastic lesions that ultimately result in pancreatic cancer.

With respect to stem cells in general, the past year has witnessed a rapid expansion in this field.^{1–5} Much of this effort has been focused on the ability to isolate and maintain undifferentiated stem cells in culture and manipulate them toward a defined lineage. These developments provide a foundation for the potential utilization of stem cells for the purposes of replacing dysfunctional, injured, or degenerating cells in human disease. In addition, these advances may provide insight into the normal process of tissue self-renewal, as well as into the aberrant growth processes characteristic of carcinogenesis.

As in other organs, the application of stem cell technology to pancreatic disease holds great promise. This is readily apparent in patients with diabetes mellitus. The identification and selective expansion of a population of pancreatic stem cells capable of generating differentiated insulin-producing cells has the potential to restore glucose homeostasis to affected individuals. Research efforts directed toward this goal have provided the bulk of the information currently available regard-

ing the existence of stem cells in the mature pancreas and their potential role in ongoing cellular proliferation and differentiation during tissue regeneration and in different pathologic states.^{6,7} Among these states, pancreatic ductal adenocarcinoma may represent a condition in which aberrant expansion of a stem cell population results in tumorigenesis. A principal characteristic of pancreatic adenocarcinoma is unregulated ductal epithelial proliferation. Although these tumors usually exhibit a ductal morphology, pancreatic cancers often express additional nonductal markers. Chromogranin A expression is found in 45% of all cases, suggesting a potential endocrine lineage.⁸ Acinar cell markers, such as elastase and lipase, are found in up to 20% of cases. These findings suggest that pancreatic cancer may originate from a population of undifferentiated cells bearing pluripotential capacity, implying the participation of epithelial stem cells.

Epithelial Stem Cells

Stem cells play an important role both in embryonic development and in tissue regeneration of the adult. While the characterization of stem cell populations has been best described in the hematopoietic system, the presence of epithelial stem cells has become increasingly accepted in a number of tissues, including the central nervous system, skin, skeletal muscle, liver, and intestine. Although the precise definition of what constitutes a stem cell remains controversial, several characteristics are generally accepted.⁹ Stem cells may therefore be de-

defined as undifferentiated cells with the capacity to proliferate, to self-renew, to give rise to the principal differentiated cell types within a given tissue, and to repopulate a tissue following injury. Many stem cells exhibit these characteristics invariably, while others are quiescent and have the potential to exhibit these characteristics only under the appropriate stimuli (ie, facultative stem cells). Adult stem cells are generally felt to be pluripotent, as defined by their capacity to produce progeny with potential to differentiate along any one of several different lineage pathways. The cellular milieu and the subsequent expression of lineage-specific genes influence commitment toward a defined lineage.

Epithelial Stem Cells in Pancreatic Development

The three main epithelial compartments of the pancreas share a common endodermal origin.¹⁰ The mammalian pancreas forms from discrete evaginations or buds on the dorsal and ventral aspects of

the embryonic foregut (Figure 5.1). During the early stages of embryogenesis these buds ultra-structurally appear to consist of elaborately folded epithelial sheets. As the embryo approaches mid-gestation, these epithelial sheets become progressively more branch-like. Structures resembling acini and ducts become histologically apparent. Organized islets do not appear until the end of gestation, although cells expressing endocrine markers can be found scattered throughout the developing pancreas as well as budding from the emerging ductal epithelium during early gestation and prior to the expression of differentiated exocrine markers.^{10,11}

The folded epithelial evaginations of the early pancreas that give rise to mature ductal epithelium consist of pluripotential progenitor cells. These cells are considered to be the cells of origin for both endocrine and exocrine pancreas. Under the appropriate stimuli, these pluripotential cells have the capacity to proliferate and differentiate along several lineage pathways in order to give rise to definitive islet, acinar, and ductal cell types^{12,13} (Figure 5.2).

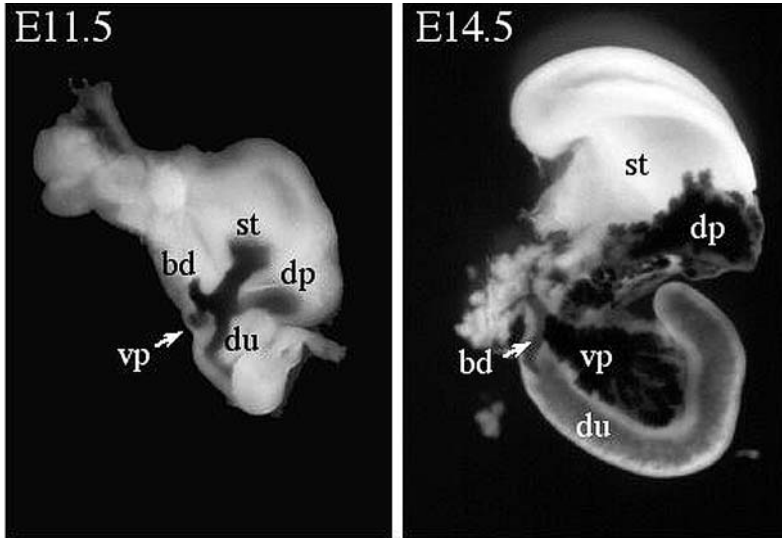


FIGURE 5.1. Morphogenetic events in the developing foregut. Images represent isolated foregut tissue from *Pdx1^{lacZ/wt}* mouse embryos sacrificed at embryonic day (E) 11.5 and 14.5. Expression of the *Pdx1* homeobox gene is indicated by dark Xgal reaction product. On E11.5, the dorsal and ventral pancreatic buds are seen as well-developed evaginations originating from foregut endoderm. *Pdx1* gene expression is noted in both the dorsal and ventral bud as well as the antral stomach and rostral duodenum. By E14.5, the developing pancreas has undergone extensive branching morphogenesis. The ventral bud has now rotated to fill the duodenal loop. *Pdx1* gene expression remains strong throughout the developing pancreatic epithelium (dp = dorsal pancreas; vp = ventral pancreas; st = antral stomach; du = duodenum; bd = bile duct.)

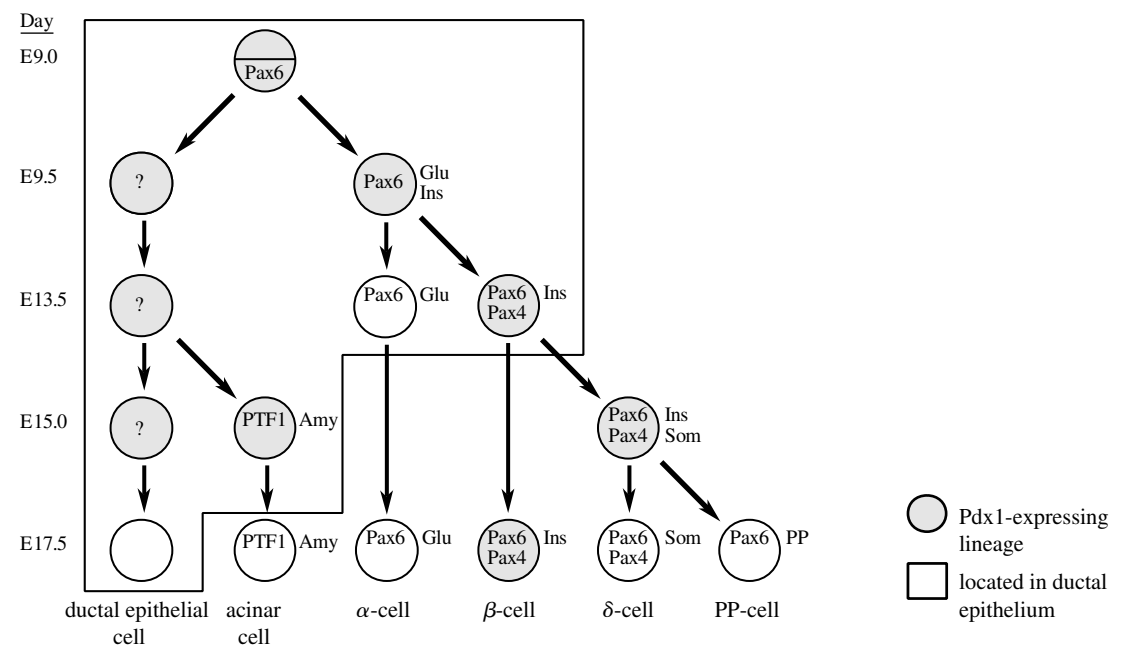


FIGURE 5.2. A model for cell lineage differentiation in embryonic pancreas. Time axis on left indicates day of embryonic (E) life. Shaded circles indicate cell lineages expressing Pdx1; hatched area indicates cell lineages located in embryonic ductal epithelium. Proteins depicted inside circles represent additional nuclear transcription factors known to regulate pancreatic development. Proteins outside circles represent differentiated secretory products. Note that all mature cell types arise from Pdx1-expressing epithelial stem cells. Also note expression of Pax6 by a subset of Pdx1-expressing epithelial cells prior to the onset of insulin and glucagon expression on E9.5. Later in development, both Pdx1 and Pax6 are restricted to islet lineages.

The endoderm-derived progenitor cells of the early pancreas express a number of lineage-specific transcription factors known to be important for normal pancreatic development. These include Pdx1, Pax6, Pax4, Nkx6.1, Nkx2.2, Prox1, Isl1, NeuroD, Hlxb9, and Ptf1.^{6,7,14,15} The homeodomain gene Pdx1 (*Pancreatic/Duodenal Homeobox gene 1*) plays a critical role in normal pancreatic development,^{16–18} as evidenced by the fact that homozygous deletion of this gene results in pancreatic agenesis.^{19,20}

Pdx1-expressing pancreatic precursor cells differentiate along three divergent lineage pathways (islet, acinar, and ductal) to give rise to the definitive cell types of the endocrine and exocrine pancreas. The early embryonic pancreas exhibits widespread Pdx1 expression. As the pancreas continues to develop, Pdx1 expression becomes downregulated initially in the differentiating acinar cells and then subsequently in ductal epithelium. Postnatally, Pdx1 expression is nearly undetectable in the terminally differentiated

exocrine cells, but is maintained at high levels in mature endocrine cells.^{16,18,20}

Epithelial Stem Cells in the Adult Pancreas

The majority of endocrine cells in the adult pancreas are derived from precursor cells of embryonic ductal epithelium. It is generally accepted that islet cell neogenesis is relatively complete in the neonate, with limited appreciable proliferation in the adult under normal conditions. In contrast, an increasing body of evidence suggests that under the appropriate and, perhaps, pathologic stimuli, new islets may be generated from (1) division of terminally differentiated islet cells, (2) transdifferentiation of mature pancreatic ductal epithelium, or (3) stem cells located within ductal epithelium in a manner that recapitulates embryonic development. This last concept is supported by a variety of mod-

els discussed below in which the induction of pancreatic ductal proliferation results in the generation of differentiated nonductal cell types.

The initial supporting evidence for this concept arose from the selective destruction of pancreatic insulin-producing cells in neonatal rats. Portha and colleagues have studied the effects of streptozotocin, a known beta-cell toxin, in 1- to 2-day-old rats.²¹ Following islet injury, both histologic and ultrastructural evidence demonstrated regeneration of insulin-producing cells from small pancreatic ducts.^{22,23} High levels of mitosis were evident within the exocrine pancreas, whereas low levels were present in the surviving beta cells. These findings suggest that adult ductal epithelium has the capacity to proliferate and generate functional insulin-producing cells. These observations are further supported by studies utilizing isolated pancreatic ductal epithelium.^{24,25} In these studies, ductal epithelium isolated from adult rats treated with alloxan to minimize beta cell contamination was recombined with fetal mesenchyme *in vitro* to recapitulate the embryonic milieu. The implantation of these recombinants into nude mice resulted in the formation of islet-like structures. This phenotype was not observed with either implanted epithelial cells or mesenchyme alone. Immunocytochemical analysis of these structures demonstrated both glucagon and insulin immunopositivity, suggesting the presence of pluripotent stem cells within adult ductal epithelium.

Models involving the induction of pancreatic ductal proliferation also substantiate the ability of existing or emerging ductal epithelium to generate differentiated endocrine cells. Obstruction of the pancreatic duct by ligation is a well-documented model of pancreatic injury resulting in ductal proliferation.^{26–29} In addition to acinar cell loss and ductal epithelial expansion, islet cell neogenesis has been described in this model. These newly formed ducts are highly proliferative and exhibit classic ductal markers by immunohistochemistry. In addition, some of these ductal cells coexpress insulin or glucagon and exhibit immunoreactivity with antibody to glucose transporter 2 (Glut2).³⁰ This protein has been shown to be widely expressed in the undifferentiated epithelium of the embryonic pancreas but is subsequently restricted to beta cells in the adult.³¹ Differentiated beta cells, as indicated by expression of insulin, are also present throughout the pancreatic parenchyma as small islets, islet-cell clusters, or single cells.³⁰ Intermediate cell

types, or transitional cells coexpressing amylase and insulin, are commonly observed in this model, suggesting the potential for progression of one cell type to another.³² Similar results have been observed using other forms of ductal obstruction, including celophane wrapping of the pancreatic head.^{33–35}

Ductal proliferation and islet cell neogenesis have also been examined in an animal model that exhibits selective beta cell destruction induced by autoimmunity. The nonobese diabetic mouse is an animal model commonly used for the study of human insulin-dependent diabetes mellitus. These mice exhibit an almost 3-fold increase in ductal proliferation over control mice as measured by bromodeoxyuridine (BrdU) incorporation, a marker of DNA synthesis.³⁶ Glucagon-positive cells are increased in the ductal epithelium, suggesting initiation of islet cell neogenesis. Like embryonic pancreatic epithelium, this proliferating ductal epithelium also expressed the embryonic marker Pdx1. These findings suggest that pancreatic ductal epithelial proliferation may represent an initial event prior to regeneration of definitive endocrine cells following islet injury. This process may involve the expression of embryonic genes resulting in a program of cellular differentiation similar to that seen in embryonic pancreas.

Transgenic overexpression of the lymphokine interferon- γ (IFN- γ) in pancreatic beta cells represents another model of pancreatic injury resulting in the generation of differentiated islet cells from proliferating ductal epithelium. In this model, progressive destruction of pancreatic islets and widespread inflammation is mediated by activated T cells.³⁷ As the inflammatory response progresses, there is loss of acinar cells and an increase in the number and size of ducts. Ultimately, these ductal structures coalesce to form an elaborate ductal network involving the majority of the pancreatic parenchyma. Intraluminal islet budding from this proliferating ductal epithelium is commonly observed. Larger buds morphologically resemble islets. Immunohistochemical analysis demonstrates the presence of the 4 major endocrine cell types within these islet-like structures. The newly formed endocrine cells are capable of proliferation as indicated by BrdU incorporation. Double-labeling studies suggest the presence of transitional cell types involving duct/acinar and acinar/endocrine cells in the progression from precursor ductal epithelium to definitive endocrine cells.³⁸ The stem

cell nature of this ductal epithelium is further supported by the observation that these duct cells also express the embryonic marker Pdx1.³⁹

Transgenic overexpression of IFN- γ was noted to be associated with the upregulation of keratinocyte growth factor (KGF). KGF, a member of the fibroblast growth factor family, is a known cytokine with both mitogenic and morphogenic properties in the developing embryo. It also has been shown to induce proliferation of adult pancreatic ductal epithelium.⁴⁰ This background prompted the study of the transgenic overexpression of KGF under regulation of an insulin promoter. Unexpectedly, in addition to ductal proliferation, these mice exhibit the emergence of ductal epithelium within the islets themselves. Clusters of differentiated endocrine cells surround these CA II-expressing ducts. At the periphery of some islets, large atypical cells appeared that resembled mature hepatocytes. All of these hepatocyte-like cells express alpha feto protein, an embryonic hepatic marker, and the majority express albumin, suggesting these cells may be undergoing a program of hepatocellular differentiation.⁴¹ Considering the common embryonic origin of the liver and the pancreas, it is entirely plausible that pluripotential stem cells could differentiate along either of these lineage pathways.

The emergence of hepatocytes within the pancreas has been observed in several other models, including the administration of exogenous agents such as ciprofibrate, TCDD, and cadmium.⁴²⁻⁴⁴ Detailed analysis of pancreatic hepatocyte differentiation has been best characterized in a model utilizing rats recovering from a copper-deficient diet.^{45,46} Following 8 weeks of copper deficiency, these animals exhibit a loss of over 90% of the pancreatic acinar cells. The pancreatic parenchyma is replaced with atypical interstitial cells or oval cells, adipocytes, and proliferating ductules. On the rats return to a normal diet, microscopic and ultrastructural analysis suggests that these albumin-expressing oval cells are arising from albumin-expressing ductal epithelium. Six to 8 weeks later, there is little acinar cell regeneration; instead 60% of the pancreatic parenchyma consists of islands of differentiated hepatocytes. These hepatocytes express albumin as well as several other classic hepatocyte markers. Their histologic and ultrastructural appearance is similar to that of liver parenchymal cells. These observations further suggest that cells present in proliferating pancreatic ductal epithelium retain the capacity to gen-

erate both differentiated hepatic and pancreatic cells types and may therefore represent pluripotential stem cells.

A model of pancreatic regeneration following partial pancreatectomy further validates the stem cell theory of pancreatic ductal proliferation. Eight to 10 weeks following 90% pancreatectomy, the remnant rat pancreas has been shown to undergo partial regeneration, with an increase in weight over the sham controls and a 3- to 4-fold increase in mitotic indices in all cell compartments.^{47,48} Between 40% and 50% of both endocrine and exocrine cell mass is restored, demonstrating significant regeneration. Further investigation demonstrates that shortly after pancreatectomy, in addition to proliferation of the existing ductal epithelium, there are new focal areas of proliferating ductules. These ductules differentiate into both pancreatic islets and exocrine tissue and are capable of forming lobules indistinct from the original pancreatic parenchyma.⁴⁹ This ductal epithelium also expresses the embryonic gene Pdx1, suggesting that this epithelium shares both morphologic and molecular features in common with the stem cells of the embryonic pancreas.⁵⁰

Together, these studies of pancreatic regeneration and proliferation suggest that a population of cells with stem cell activity persists in the adult pancreas. This stem cell population appears fully capable of initiating tissue renewal following either selective injury to individual cell types or more global tissue loss following partial pancreatectomy. Among these different models, several common principles have emerged. First, most models of pancreatic regeneration involve a component of ductal epithelial proliferation, suggesting the possible presence of epithelial stem cells within the adult pancreatic duct. Second, regenerative epithelial proliferation in the pancreas is frequently associated with reactivation of embryonic markers, including the Pdx1 homeodomain protein. These findings suggest that pancreatic epithelial regeneration may recapitulate a developmental program active during embryogenesis.

Epithelial Stem Cells and Pancreatic Cancer

The cell of origin of pancreatic cancer remains uncertain. Although animal models are currently available to allow accurate assessment of the pro-

gression of pancreatic adenocarcinoma,⁵¹ a definitive precursor cell has not yet been identified. The literature contains very few definitive studies addressing the role of stem cells in pancreatic adenocarcinoma, although many authors advocate the concept that carcinoma originates from the aberrant expansion of a stem cell population. Evaluation of this hypothesis has recently been facilitated by data suggesting that pancreatic ductal metaplasia induced by overexpression of transforming growth factor (TGF- α) in transgenic mice results in the development of malignant pancreatic ductal carcinoma with low frequency and long latency.⁵² While the inefficient generation of frank carcinoma in these mice limits the ability to study malignant epithelium, the long latency period provides a unique opportunity to investigate premalignant events, including cellular precursors.

Overexpression of TGF- α in the murine pancreas results in progressive acinar cell loss, both perilobular and intralobular fibrosis, and widespread ductal metaplasia.^{53,54} The duct-like epithelial cells express CA II and also have the capacity to produce mucin. Transitional structures exhibiting acinar morphology, zymogen granules, and faint mucin-positivity are observed, with the level of mucin-positivity becoming more intense as cells progress toward a ductal phenotype.⁵⁵ A number of epithelial cells within the metaplastic ducts exhibit classic endocrine markers including insulin, glucagon, and chromogranin A in a manner resembling islet cell development in embryonic pancreas.^{56,57} In addition, there is focal expression of the Pax6 homeobox gene and widespread activation of Pdx1 within this premalignant metaplastic ductal epithelium.⁵⁷ In the early embryonic pancreas, Pax6 appears to be expressed in a subset of ductal epithelial cells destined toward an endocrine-specific lineage. In the normal adult pancreas, Pax6 expression is absent in the ductal epithelium and is limited to differentiated endocrine cells.⁵⁸

Evidence documenting the premalignant nature of this metaplastic epithelium is provided by the observation that 50% of these animals develop dysplastic duct lesions, and 10% develop multifocal adenocarcinoma by 1 year of age.⁵² These tumors originate from metaplastic ducts, and express the ductal markers CA II and Duct-1. Weak amylase expression is detected in discrete cellular clusters,

suggesting a pluripotent differentiation capacity. The expression of these embryonic genes in premalignant ductal epithelium lends further credence to the hypothesis that an epithelial stem cell population resembling cells in the embryonic pancreas may play a role in the development of pancreatic adenocarcinoma.

This concept is further substantiated by studies describing the histological changes observed in the progression of nitrosamine-induced pancreatic tumors in the Syrian golden hamster.⁵¹ This is currently the best available animal model of human pancreatic adenocarcinoma by virtue of its ductal phenotype, biologic behavior, loss of *DCC* and *Rb-1* tumor suppressor genes, and mutations of the *K-ras* proto-oncogene.⁵⁹ These tumors arise through sequential stages of ductal hyperplasia, atypical hyperplasia, intraductal carcinoma, and ultimately, invasive ductal carcinoma.⁵¹ When hamster pancreatic organogenesis is compared to early stages of nitrosamine-induced carcinogenesis, both are characterized by similar undifferentiated ductal epithelium.^{60,61} Dispersed throughout this epithelium are individual endocrine cells, similar to those observed in premalignant epithelium from transgenic mice overexpressing TGF- α . As carcinogenesis progresses, rudimentary islets appear to be generated from both periinsular and intrainsular ductal epithelium. Ductal adenocarcinoma is generated from this same epithelium. These findings suggest an intimate relationship between ductal epithelial expansion, stem cells capable of generating new islet tissue, and pancreatic ductal adenocarcinoma.

This relationship is further supported by the studies documenting in vitro generation of ductal adenocarcinomas from isolated juvenile murine pancreatic islets. Pancreatic islets infected with a retrovirus-carrying polyoma middle T oncogene generate cells that have both neuroendocrine features and features of pancreatic ductal epithelium.⁶² When these cells are injected into syngeneic mice, well-differentiated ductal adenocarcinomas are formed. These islet-derived tumors do not express any classic endocrine markers, but do express cytokeratin, a marker of ductal differentiation. The susceptible progenitor cells responsible for generating these tumors therefore appear to reside within or in intimate approximation to isolated islets. These findings further suggest that these cells may have pluripotential differentiation capacity and

may serve as potential targets in the initiation of carcinogenesis.

These studies suggest that epithelial stem cells of the adult pancreas may be the cells of origin of pancreatic cancer. These cells are potentially the most vulnerable to oncogenic agents by virtue of their increased mitotic activity, capacity for self-renewal, and ability to differentiate along multiple pathways. These properties may distinguish this population as susceptible targets for the initiation of carcinogenesis. The progression to pancreatic cancer may be attributable to the inability of these epithelial stem cells to fully differentiate in an attempt to regenerate a differentiated cell population, a concept referred to as maturation arrest or blocked ontogeny.⁶³ The loss of growth regulatory mechanisms or lack of a negative feedback signal from a renewed differentiated cell population may result in the continuous expansion of a stem cell epithelium, ultimately leading to carcinogenesis.

Stem Cells Versus Transdifferentiation

Despite the extensive recent progress in this area, the identity and origin of the precursor cells responsible for generating newly differentiated cell types within the adult pancreas remain uncertain. The two principal hypothesis addressed in the literature are (1) the expansion of a subset of undifferentiated stem cells with the capacity to generate a population of differentiated progeny (stem cell hypothesis), and (2) the transformation of one differentiated cell type to another (transdifferentiation hypothesis).⁶⁴ The distinction between these 2 hypotheses becomes relevant when considering potential targets for chemoprevention and/or novel antineoplastic therapy.

The mechanism traditionally thought to be responsible for the process of tissue regeneration is transdifferentiation. In response to the need for cellular renewal, differentiated cells undergo a process of dedifferentiation. This transitional stage involves a loss of a differentiated phenotype and acquisition of the potential to initiate an alternative differentiation pathway. This would imply downregulation of one set of lineage-specific genes and the upregulation of others, resulting in a phenotypic

switch.⁶⁴ Many animal and in vitro models have supported this hypothesis. Known carcinogens induce tumors with an acinar phenotype in mice and rats instead of the ductal tumors commonly seen in humans.⁶⁵ Histologic studies of the progression of nitrosamine-induced pancreatic adenocarcinoma in the hamster suggest a pseudoductular transformation of resident acini.⁶⁶ Others, however, utilizing the same model, advocate a stem cell or ductal progenitor,⁶⁷ or both ductal and acinar progenitors.⁶⁸ Acini from transgenic mice expressing SV-40 large T antigen under regulation of an elastase promoter develop acinar cell tumors with focal areas of ductal differentiation.⁶⁹ *c-myc* expression targeted to acini of transgenic mice promotes the development of mixed tumors with both acinar and ductal phenotypes.⁷⁰ In vitro models utilizing cultures of isolated acini from mouse, hamster, and human pancreas have also demonstrated the loss of an acinar phenotype and the gain of a ductal phenotype.^{71–73}

In addition, many of the studies described previously support the concept that islet cell neogenesis and the emergence of pancreatic hepatocytes may occur through a transdifferentiation event. These studies demonstrate the loss and acquisition of multiple lineage-specific proteins, and describe multiple transitional cell types that coexpress divergent lineage-specific markers. These findings may fulfill the definition of transdifferentiation. To account for the expression of embryonic genes in this process, one would have to maintain that dedifferentiation involves regression to an undifferentiated cell type similar to that observed during embryogenesis. Definitive proof of this concept will require the development of methodologies for in vivo lineage tracing in mammalian tissues.

Clinical Correlates

Within this chapter, we have presented numerous studies to support the presence of a stem cell function in the adult pancreas. Although these data suggest that stem cells may indeed reside within the adult pancreas, the identity and location of these cells remains unknown. Under normal conditions, these cells appear to remain quiescent, awaiting the appropriate stimulus for the initiation of tissue regeneration. These events generally occur under

pathologic conditions, such as injury to either the exocrine or endocrine pancreas. These features may in fact represent the underlying mechanism responsible for the frequent histologic association between chronic pancreatitis and pancreatic cancer, as well as the 16-fold increase in pancreatic cancer risk observed in patients with clinical evidence of chronic pancreatitis.⁷⁴

Future studies will continue to characterize the possible role of epithelial stem cells in pancreatic tumorigenesis, and may ultimately allow identification of the "cell of origin" for human pancreatic cancer. Based upon the inherent biologic aggressiveness of pancreatic cancer as well as its widely recognized resistance to antineoplastic therapy, measurable progress in the management of this disease may only be made through successful development of strategies for early detection and eradication of premalignant precursor lesions. This goal will not likely be achieved until successful characterization of relevant precursor cells is accomplished. Following identification of specific epithelial stem cell populations as the "cell of origin" for this disease, directed studies of gene expression and growth regulation are likely to generate new approaches for molecular screening in the form of "pretumor" markers. In addition, identification of these cells may lead to specific strategies for reduction of this precursor population within intact pancreas. Recent data suggesting that premalignant pancreatic epithelium exhibits a unique pattern of gene expression resembling embryonic pancreas raises the possibility of targeting cell-specific therapies that would, it is hoped, involve diminished toxicity for normal adult tissue.⁵²

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6

Inherited Pancreatic Cancer Syndromes

David H. Berger and William E. Fisher

Introduction

It is estimated that up to 10% of cases of pancreatic cancer occur as a result of an inherited genetic predisposition. Although this incidence of genetic susceptibility is higher than reported for all other inherited cancer syndromes, it has been difficult to identify the specific genetic defect or defects that contribute to this susceptibility. Unlike other inherited cancer syndromes, such as familial adenomatous polyposis (FAP), and Li-Fraumeni syndrome, familial pancreatic cancer does not often occur in association with a unique, recognizable clinical syndrome. Additionally, whereas the hallmark of other inherited cancer syndromes is an early age of onset of malignant tumors, the median age of onset for most patients with inherited pancreatic cancer is similar to that seen in sporadic cases. For no other common adult cancer are there as many known pathways whose defects give rise to inherited susceptibility, nor is there any other organ for which the fraction of cancers having an inherited basis is higher than for the pancreas.¹

Extensive evidence exists for an inherited genetic basis for pancreatic adenocarcinoma. Several case series report the clustering of multiple cases of pancreatic cancer in the same family.^{2–4} Epidemiologic studies have demonstrated that a family history of pancreatic cancer is an important risk factor for the disease. Ghadirian et al, in a population-based, case-control study of pancreatic cancer in Quebec, Canada, found that 7.8% of cases but only 0.6% of controls reported a family history of

pancreatic cancer.⁵ Falk et al, in another case-control study, demonstrated an increased risk of pancreatic cancer in people who reported a history of cancer in a close relative (odds ratio = 1.86; 95% CI, 1.42–2.44). In this study the risk was highest for patients who reported a history of pancreatic adenocarcinoma in a close relative (odds ratio = 5.25; 95% CI, 2.08–13.21).⁶ A study of 362 cases of pancreatic adenocarcinoma and 1408 hospital controls from northern Italy showed a relative risk of 2.8 (95% CI, 1.3–6.3) for pancreatic cancer in association with a family history of this cancer in a first-degree relative.⁷

Further evidence for the existence of an inherited susceptibility for pancreatic cancer comes from the observation that there is an excess of expected cases in several familial cancer syndromes. These syndromes are associated with germline mutations in various cancer predisposing genes.⁸ An increase in incidence of pancreatic cancer has been found in patients with the familial melanoma syndrome,⁹ breast-ovarian cancer syndrome secondary to BRCA2 mutations,⁸ Peutz-Jeghers syndrome,^{10,11} hereditary nonpolyposis colorectal cancer syndrome,^{12,13} familial adenomatous polyposis syndrome,¹⁴ and ataxia-telangiectasia.¹⁵

In this chapter we will discuss the currently known inherited syndromes where an excess of pancreatic cancer exists. We will briefly discuss each specific clinical syndrome, highlight the risk of pancreatic cancer within each syndrome, and review what is known about the genetic abnormality contributing to the syndrome.

BRCA2

Syndrome

The majority of families with a dominant predisposition to breast and/or ovarian cancer have been shown to harbor germline mutations in either the BRCA1 or BRCA2 genes.^{16,17} BRCA2 mutations are known to predispose individuals to a high lifetime risk of breast cancer, together with a lower, but significant, risk of ovarian cancer.^{16,17} Patients with mutations in the BRCA2 gene also appear to have an increased risk of malignancy at other sites including the oropharynx, stomach, prostate, liver, and pancreas.¹⁶

Risk of Pancreatic Cancer

The presence of somatic deletions of the BRCA2 chromosomal region in sporadic pancreatic cancer provided the initial evidence of a link between BRCA2 and pancreatic cancer.^{18,19} A family history of pancreatic cancer has been noted to predict the presence of a BRCA2 mutation.^{20,21} Furthermore, pancreatic cancers are more frequent in breast cancer families with BRCA2 mutations than in families without mutations.²¹ In the largest study of BRCA2 carriers published to date, the Breast Cancer Linkage Consortium found the relative risk of developing pancreatic adenocarcinoma for carriers of a BRCA2 mutation to be 3.51 (95% CI, 1.87–6.58). The risk was even higher for younger patients with a mutation (relative risk 5.54, 95% CI, 2.72–11.32).¹⁶ It appears that germline BRCA2 mutations represent the most common inherited predisposition to pancreatic carcinoma identified to date. Goggins et al found a germline BRCA2 mutation in 7.3% of patients with pancreatic cancer. This may actually be a low estimate given the methods of mutation detection used in this study.²² Other studies have shown that up to 10% of pancreatic cancers may be associated with BRCA2 mutations.⁸ Another factor that may lead to underestimating the importance of BRCA2 mutations in pancreatic cancer is that most of these patients have no family history of pancreatic cancer.¹ A low penetrance and a rather high but variable age of onset of pancreatic cancer in BRCA2 mutation carriers suggests that other factors modify the risk of developing pancreatic carcinoma.²²

Genetic Abnormality

The BRCA2 gene has more than 20 exons, with a very large exon 11. The gene encodes a highly charged protein with a putative granin domain. The preponderance of mutations detected in the BRCA2 gene are microdeletions, which result in a frame-shift. These mutations result in the production of a truncated nonfunctional protein. Similar to BRCA1, founder mutations have been identified in the BRCA2 gene in members of defined ethnic groups. A recurrent germline mutation, 6147delT, has been detected in 8% of Ashkenazi Jewish women diagnosed with early breast cancer (reviewed in van Golen et al²³). The function of the BRCA2 protein has not been clearly defined. Use of transgenic models has demonstrated that at least one normal copy of BRCA2 is necessary for embryogenesis.²⁴ It appears BRCA2 plays a role in DNA repair. In vitro work documents a cell cycle-dependent regulation of BRCA2 corresponding to an up-regulation of mRNA during S-phase and mitosis.²⁵

Familial Melanoma

Syndrome

A positive family history of melanoma has been reported in 8% to 14% of melanoma patients. Familial cases tend to be younger, to have more moles, and to develop multiple primary melanomas. There is a melanoma relative risk of 2.2 for people who report at least one affected first-degree relative (reviewed in Greene²⁶). Within large genetically defined pedigrees, transmission of susceptibility to melanoma is inherited as an autosomal dominant trait, with a calculated penetrance of 53% by age 80.²⁷ At least three separate genetic loci have been linked to an increased melanoma risk. However, only abnormalities at chromosome 9p21 have been associated with an increased risk of pancreatic cancer in patients with a history of melanoma. This site has been found to be the location of the p16 gene (also called CDKN2, MTS-1, INK4a).²⁶ Up to 50% of melanoma pedigrees harbor p16 mutations.²⁸

Risk of Pancreatic Cancer

A population-based study of second malignancies in patients diagnosed first with malignant melanoma

revealed nearly a two-fold excess in the risk of pancreatic cancers. This was most dramatic in patients with melanoma prior to age 50 years.²⁹ Goldstein et al compared the incidence of pancreatic cancer in 10 melanoma-prone kindreds with mutations in the p16 gene to 9 melanoma-prone kindreds without p16 mutations. The relative risk of pancreatic cancer in those melanoma families with p16 mutations was 22 (7 observed versus 0.32 expected). There were no pancreatic cancer cases in melanoma families without p16 mutations.³⁰ Mutations in p16 also appear to play a significant role in the development of sporadic pancreatic cancer. The p16 gene product is expressed in the normal pancreas. Pancreatic adenocarcinoma develops from histologically identifiable intraductal lesions that undergo architectural and cytological changes as they progress toward cancer. Examination of these “duct lesions” has shown a loss of intranuclear expression of p16.³¹ In one study, allelic deletions of the p16 gene were found in 22 (85%) of 26 pancreatic adenocarcinomas.³² Other studies have confirmed deletions in exons 1 and 2 of p16 in a high proportion of pancreatic cancers.^{33–35} An immunohistochemical study of 62 pancreatic cancer specimens demonstrated that 42% failed to express this tumor-suppressor gene.³⁶

Genetic Abnormality

Cannon-Albright et al, using multipoint linkage analysis to study 11 melanoma families in Utah, documented the presence of a partially penetrant, dominant melanoma susceptibility locus on 9p21 termed CMM2.^{37,38} The CMM2 gene has been identified as CDKN2A, also known as MTS1. This gene encodes a protein designated p16^{INK4a}. p16 is a low-molecular-weight protein that inhibits the activity of the cyclin D1-cyclin-dependent kinases CDK4 and CDK6. When active, these kinases phosphorylate the retinoblastoma protein, allowing the cell to pass through the G1 cell-cycle checkpoint. Therefore p16 is a natural tumor suppressor that slows cell growth by arresting cells at G1. The inactivation of p16 by deletion or mutation of the gene leads to unchecked cell growth.³⁹ Interestingly, the CDKN2a gene can encode two distinct proteins depending on which of two alternative first exons (E1 α or E1 β) is transcribed. When E1 α is transcribed the resulting protein is p16^{INK4a}. When

E1 β is transcribed, p19^{ARF} is encoded.⁴⁰ The role, if any, of p19 in melanoma pathogenesis has yet to be determined.

Hereditary Pancreatitis

Syndrome

Hereditary pancreatitis is a chronic, idiopathic inflammatory disorder of the pancreas affecting multiple family members over two or more generations.⁴¹ Hereditary pancreatitis is inherited in an autosomal dominant fashion with an estimated penetrance of 80%.⁴² Clinically, the disease is characterized by a history of episodic abdominal pain and acute pancreatitis beginning in early childhood, an equal sex distribution, a family history of pancreatitis, a high frequency of large calcified stones in the pancreatic duct, and an exclusion of other causes of pancreatitis.⁴³ The initial attack of pancreatitis usually occurs between 1 and 13 years of age, with a median age of onset at about 5 years.⁴¹ There is development of chronic pancreatitis in most individuals as early as the teenage years.⁴⁴ The gene for this disorder, PRSS1, is located on chromosome 7q35 and encodes for the cationic trypsinogen gene.^{41,45}

Risk of Pancreatic Cancer

Lowenfels et al performed a case-control study of 246 patients with hereditary pancreatitis to determine the risk of developing pancreatic cancer in this group of patients. Thirty members of the cohort had testing for PRSS1 mutations, and in all 30 a mutated copy of the PRSS1 gene has been identified. In this study the strength of the association between pancreatitis and pancreatic cancer was estimated by using the standardized incidence ratio (SIR), which is the ratio of the observed cases to the expected cases in the background population. These authors found a SIR for pancreatic adenocarcinoma of 53 (95% CI, 23–105) in patients with a history of hereditary pancreatitis. There was no increased risk for other malignancies. The mean age of diagnosis of pancreatic cancer in the cohort was 56.9 $\pm$ 11.2 years. The estimated cumulative risk of developing pancreatic cancer to age 70 years for the cohort was nearly 40%. Interestingly, for

patients with a paternal inheritance pattern the cumulative risk for pancreatic cancer approached 75%.⁴⁶

Genetic Abnormality

Based on the hypothesis that hereditary pancreatitis was due to an abnormal pancreatic enzyme leading to autodigestion of the pancreas and knowing the probable site of the gene through linkage analysis, Whitcomb et al sequenced two genes, cationic and anionic trypsinogen. Mutational screening analyses for each of the exons from these two genes were performed for multiple affected and unaffected members of families with hereditary pancreatitis. A single G to A transition mutation was identified in the third exon of cationic trypsinogen. This mutation results in an Arg(CGC) to His(CAC) substitution at amino acid residue 105 of the protein. This mutant protein is then resistant to cleavage and inactivation, leading to autodigestion of the pancreas.⁴⁵ The tumor susceptibility seen is presumably due to mitogenic stimulation and clonal outgrowth of pancreatic ductal cells as part of the normal healing responses that occur subsequent to repeated rounds of tissue destruction. Since the cationic trypsinogen gene does not fit into the classical definition of either a tumor suppressor, oncogene, or a mismatch repair gene, it appears to be the first of what may be considered a new class of cancer susceptibility genes.¹

Familial Adenomatous Polyposis

Syndrome

Familial adenomatous polyposis (FAP) is characterized by the presence of hundreds to thousands of colorectal adenomas that often progress to carcinomas. The polyps first appear in adolescence, usually around age 16 years. Polyps in the stomach and small intestine develop in about 90% of patients with FAP. The polyps in the small bowel are adenomas; however, the gastric polyps are hyperplastic without malignant potential. Small intestinal neoplasia occurs in the ampulla of Vater and periampullary region, and carcinoma develops in up to 5% of these patients. Classically, patients with FAP develop cancer at a median age of 40

years. However, the age at which cancer develops is variable. Colon cancer is rare before 30 years of age, and cancer may not develop in some patients until they are in their 50s or 60s. FAP syndrome is also characterized by extraintestinal features such as osteomas of the mandible, skull, and long bones and a variety of other benign soft tissue tumors, such as fibromas and lipomas. Desmoid tumors develop in 10% to 15% of patients with FAP, often as a complication of laparotomy. Desmoids are benign but aggressive tumors of mesenteric fibroblasts that can envelop and obstruct the gastrointestinal tract, blood vessels, and ureters, sometimes causing death.^{14,47-51}

Risk of Pancreatic Cancer

The adenomatous polyps seen in FAP disease occur in the duodenum as well as the colon. This occurrence predisposes these patients to an increased risk of periampullary adenocarcinomas. Periampullary tumors are usually seen in the 6th and 7th decades of life in patients who have previously undergone colectomy.^{14,47-51} The risk of pancreatic adenocarcinoma is also significantly increased. Giardiello et al, in a case-control study of 197 FAP pedigrees, found a relative risk of 4.46 (95% CI, 1.2-11.4) for pancreatic adenocarcinoma in patients with the syndrome.¹⁴

Genetic Abnormality

Linkage analysis has demonstrated that mutations of the adenomatous polyposis coli (APC) gene are the cause of the FAP syndrome. The APC gene is located on chromosome 5q. A germline mutation in this gene inactivates the function of the APC gene product. Usually, the mutation creates a premature stop codon in the APC gene, which in turn leads to the translation of a truncated APC protein. The APC gene encodes for a protein that binds to a cytoskeletal element called β -catenin. Normally, APC binds to β -catenin, preventing its activation of downstream transcription factors. In the absence of APC β -catenin accumulates, leading to activation of transcription. The location of the mutation in the APC gene in part determines the age of onset, number of polyps, and age at which cancer develops in different FAP pedigrees (reviewed in Polakis et al⁵²).

The APC gene may have a critical function in regulation of cell growth in digestive tissues. Studies have shown that inactivation of the APC gene plays a significant role in development of up to 85% of sporadic cases of colorectal carcinoma. Somatic mutations in the APC gene have also been detected in gastric and pancreatic carcinomas.⁵⁰ Horii et al reported somatic mutations of the tumor-suppressor gene APC in 40% of cases of sporadic pancreatic cancers.⁴⁷

Hereditary Nonpolyposis Colorectal Cancer

Syndrome

Hereditary nonpolyposis colorectal cancer (HNPCC), or Lynch syndrome II, is characterized by adenocarcinoma of the colon associated with adenocarcinoma of other organs, primarily of the breast, endometrium, and ovary, but also of the pancreas. Cancers arise in discrete adenomas but there are not hundreds to thousands of polyps as in FAP. HNPCC is defined clinically by the Amsterdam criteria: families must have at least three relatives with colorectal cancer, one who is a first-degree relative of the other two; colorectal cancer must involve at least two generations; and at least one cancer case must occur before 50 years of age. Carcinoma can occur at a very early age (20s to 30s) in patients with HNPCC. However, the mean age at cancer diagnosis is approximately 40 years. HNPCC may account for as much as 4% to 6% of colorectal cancer in the general population.^{12,13,53}

Risk of Pancreatic Cancer

In a study of 40 Finnish HNPCC kindreds, 6 of 293 putative gene carriers with clinically or histologically documented cancer had pancreatic carcinoma.¹² Conversely, an investigation of 22 Dutch HNPCC families identified no cases of pancreatic cancer among 148 cancer patients.⁵³ Lynch et al have described a number of HNPCC kindreds with at least one person diagnosed with pancreatic cancer. In one of these kindreds, one case of pancreatic cancer was seen in three of the five affected generations.¹³

Genetic Abnormality

Pedigree studies have clearly documented the transmission of an autosomal dominant factor that predisposes the affected individuals to colonic cancer in association with other forms of cancer. Affected individuals with HNPCC have widespread genetic alterations within dinucleotide repeats, which are called replication errors or microsatellite instability. This increase in microsatellite instability in HNPCC families was shown to be due to germline mutations in one of four different DNA mismatch repair genes. A germline mutation in one of four genes that play a role in DNA mismatch repair—hMSH1 and hMSH2 (human Mut L homologue 1 and human Mut S homologue 2), hPMS1 and hPMS2 (human postmeiotic segregation 1 and 2)—leads to a mutator phenotype that results in an increased incidence of mutations in other genes. This propensity to pass on mutations in oncogenes and tumor-suppressor genes is responsible for the increased cancer incidence seen (reviewed in Schmutte and Fishel⁵⁴).

Peutz-Jeghers Syndrome

Syndrome

The clinical hallmarks of Peutz-Jeghers syndrome (PJS) are intestinal hamartomatous polyposis and melanin pigmentation of the skin and mucous membranes. Polyps can arise anywhere in the gastrointestinal tract, but are most common in the jejunum. The syndrome is inherited in an autosomal dominant fashion. The true incidence of this syndrome is unknown because of the lack of accurate registries. The relative risk of cancer in PJS patients may be as high as 18 times that of the general population. Patients with PJS who develop cancer appear to have a worse prognosis than patients with similar sporadic tumors. Recently the gene that causes PJS was identified on chromosome 19p13.3.⁵⁵

Risk of Pancreatic Cancer

The risk of death from gastrointestinal cancer in patients with PJS is 13- to 30-fold greater than in the general population. The average age at which cancer is diagnosed in PJS patients ranges from 38 to 50 years.⁵⁶ Several cases of pancreatic cancer in

extremely young patients have been reported. Bowlby described a 16-year-old male with PJS who died of pancreatic adenocarcinoma.⁵⁷ Thatcher et al reported a 38-year-old man and Walpole reported a 19-year-old male with PJS who died from adenocarcinoma of the pancreas.^{58,59} The risk of developing pancreatic cancer in patients with PJS appears to be increased; however, the true relative risk is unknown. Giardiello et al reported that 48% of 31 patients from unrelated PJS kindreds developed cancer and four of these were adenocarcinoma of the pancreas.¹¹ In another review, 22% of PJS patients developed cancer and one of these was a case of pancreatic cancer.¹⁰

Genetic Abnormality

Attempts at using linkage analysis to identify the gene responsible for PJS were unsuccessful. Therefore comparative genomic hybridization of 16 polyps from a PJS patient was attempted. By this technique it was identified that 6 of the 16 polyps had deletion of 19p13.3 as the only change. Subsequent analysis of 12 PJS families for loss of heterozygosity (LOH) confirmed the presence of the gene for PJS at 19p13.3. Subsequent DNA analysis identified the *STK11/LKB1* gene as being responsible. Sequencing of the *STK11/LKB1* gene from the 12 PJS families identified mutations in 11. The gene encodes a serine/threonine kinase with unknown function. Most mutations in the gene result in the production of a truncated protein. It appears that this gene behaves as a typical tumor suppressor with LOH, loss of function mutation, and early onset of cancer.⁵⁵ Furthermore, it appears that mutations in this gene are also important in the pathogenesis of sporadic pancreatic adenocarcinoma. Su et al have demonstrated mutations in the *STK11/LKB1* gene in 6% of specimens of sporadic pancreatic carcinomas.⁵⁶

Ataxia-Telangiectasia

Syndrome

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder with a population incidence of approximately 1 in 100,000. The clinical hallmark of the disease is progressive neuromotor dysfunction resulting

from several neuropathological processes including cerebellar cortical atrophy. Ataxia is the presenting symptom in this syndrome and is manifested when the child begins to walk. The ataxia is progressive. Speech becomes increasingly dysarthric, and the child is immobilized by the end of the first decade of life. Dilated blood vessels (telangiectasias) in the eyes and occasionally on the facial skin are common. The telangiectasias usually are not observed until after the second year of life. Additional features of A-T include thymic degeneration, immune deficiency, recurrent sinus and pulmonary infection, retarded somatic growth, premature aging, gonadal dysgenesis, extreme predisposition to lymphoreticular malignancies, and acute sensitivity to ionizing radiation. A-T carriers are prone to develop malignancies of the breast, stomach, and pancreas.^{60,61}

Risk of Pancreatic Cancer

There appears to be an increase in cancer incidence in A-T homozygotes and probably in heterozygotes as well.⁶² Additionally, mutations in the *ATM* gene (the gene mutated in A-T) may play a role in sporadic cancers. The most common cancers seen in A-T homozygotes are lymphoreticular. *ATM* appears to play the role of a classic tumor suppressor in T-prolymphocytic leukemia.⁶¹ Among A-T carriers there appears to be an increase in incidence of some solid tumors, especially adenocarcinoma of the breast.¹⁵ A number of studies investigating the incidence of cancer in the relatives of patients with A-T report an association of pancreatic cancer with the syndrome. Among close relatives in 25 patients with A-T probands, there were 7 cases of pancreatic cancer. This incidence was significantly higher than the 1.4 cases expected ($P < 0.02$).⁶³ In a study of 110 families with A-T, 7 cases of pancreatic cancer were observed in blood relatives of patients with A-T (3.3 cases were expected). Among the spousal controls, only 1 case was observed (1.3 expected).¹⁵ Now that *ATM* has been identified, further studies should clarify the cancer risk in A-T carriers and the role of *ATM* in sporadic cancers.

Genetic Abnormality

ATM has been mapped to chromosome 11q22-23. The gene contains 66 exons encoding a 13-kb tran-

script. The predicted ATM protein contains 3056 residues. Its C-terminal region of 400 amino acids is highly similar to the catalytic subunit of phosphatidylinositol 3-kinases (PI 3-kinases).⁶¹ ATM is most similar to the subset of PI 3-kinases that are involved in cell-cycle control and/or in the detection of DNA damage. Defects in these proteins lead to genomic instability, hypersensitivity to DNA-damaging agents, and defects in DNA damage-induced cell-cycle checkpoint controls.⁶⁴ Over 250 mutations in the ATM gene have been identified. The mutation sites span the entire open reading frame of the transcript. The large majority of mutations truncate the protein or leave large deletions in it. The truncated ATM derivatives appear to be very unstable, leaving no detectable protein product.^{60,61} It is interesting to note that ATM and BRCA2 may have overlapping function. In fact, BRCA2 deficient mice exhibit a wide range of defects; most of them show a striking resemblance to those of ATM-deficient mice.⁶¹ This observation may be in part responsible for increased risk of breast cancer seen in A-T carriers.

Summary

As we have documented, up to 10% of cases of pancreatic cancer occur as a result of an inherited genetic predisposition. This incidence of genetic susceptibility is higher than reported for all other inherited cancer syndromes. Furthermore, given that the penetrance for the genes identified to date is low and the development of pancreatic cancer in affected individuals occurs later in life, the 10% estimate for an inherited basis of pancreatic cancer is low. In considering the true frequency for an inherited predisposition to pancreatic cancer it is important to take into account the fact that all the genetic changes associated with pancreatic adenocarcinoma detailed in this review have been identified within the past 10 years.

Knowing that the incidence of an inherited basis for pancreatic cancer is high and that results for standard treatment are poor, the question arises of whether we can use our current knowledge to screen and prophylactically treat patients at risk. Unlike with colorectal cancer and breast cancer, there is currently no simple, reliable test to screen patients for pancreatic cancer. Additionally, the re-

quired prophylactic surgery for pancreatic cancer is associated with a significantly higher morbidity and mortality than that required for patients with a genetic predisposition for breast and colorectal cancer. At this time it would be unwise to recommend intensive screening of any single group for pancreatic cancer, with the possible exception of patients with hereditary pancreatitis who have developed chronic changes in their pancreas. In this small subset of patients a relative risk of 40 to 75 times the general population may justify screening.

Further research into the pathogenesis of pancreatic cancer will undoubtedly uncover other genes that may confer an increased risk for this disease. When this research is coupled with work aimed at understanding the mechanism by which the currently known genes contribute to pancreatic carcinogenesis, we hope to make a significant impact on the mortality of this disease.

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Pancreatic Cancer: Radiologic Staging

Harmeet Kaur, Evelyn M. Loyer, Elizabeth A. Lano,
and Chusilp Charnsangavej

Introduction

The pancreas is a retroperitoneal organ that lies deep in the abdomen behind the stomach, transverse colon, and small bowel. The organ is not easily palpated, or easily examined by routine clinical examination and conventional radiography. Not until the late 1970s, after the discovery and use of cross-sectional imaging techniques such as computed tomography (CT), ultrasound (US), and magnetic resonance imaging (MRI), could the organ be clearly defined and examined noninvasively. In this chapter, we describe imaging anatomy of the pancreas, and imaging findings of pancreatic tumors with the differential diagnosis based on morphologic and hemodynamic changes of the tumors. In addition, we discuss staging of pancreatic tumors, using state-of-the-art CT technique, with special attention on peripancreatic vascular anatomy, and use this information for planning surgery.

Anatomy of the Pancreas

The pancreas lies transversely along its long axis in the anterior pararenal space of the retroperitoneum. The size of the pancreas varies depending upon the age; it is thicker in younger individuals and becomes atrophied in older ages. The pancreas develops from the dorsal and ventral diverticuli branching from the duodenum to form the head, body, and tail. However, no clear anatomic landmarks within the pancreas separate these segments, except external landmarks such as the superior

mesenteric vein (SMV) used to separate the head from the body and tail of the pancreas.

The head of the pancreas lies within the C-loop of the second portion of the duodenum. The lateral surface of the head is up against the serosa of the duodenum. Between the posterior surface of the head and the inferior vena cava are retroperitoneal fat and small posterior peripancreatic nodes. Medially, the head of the pancreas is closely related to the superior mesenteric artery (SMA) and SMV. At the caudal portion of the head, a small portion of the pancreas extends behind the SMA and SMV, forming an uncinate process. It is connected to the SMA and SMV by small twigs of vessels that are branches of the jejunal artery and vein, or arise directly from the posterior wall of the SMA and SMV. The cranial portion of the head of the pancreas is located on the right side of the SMA and SMV. As the SMV joins the splenic vein to form the portal vein, it runs behind the head of the pancreas, and from that point extending to the left retroperitoneum, the head of the pancreas becomes the body.

The body and tail of the pancreas course transversely to the left side of the retroperitoneum toward the splenic hilum. After it passes in front of the abdominal aorta and celiac axis, it curves posteriorly and in the cranial direction. In most cases, it follows the course of the splenic artery and vein, staying anterior and slightly caudal to those vessels.

The pancreas is covered by the posterior peritoneal layer that forms the posterior wall of the lesser sac and the inframesocolic compartment of the peritoneal cavity. Just at the caudal margin

along the body and tail of the pancreas, the root of the transverse mesocolon that is formed by those two posterior peritoneal layers merges anteriorly to suspend the transverse colon into the peritoneal cavity. The root of the transverse mesocolon extends to the right, traversing across the head of the pancreas and second portion of the duodenum. The transverse mesocolon forms the inferior boundary of the lesser sac.

Pancreatic Duct Anatomy

The pancreatic duct from the body and tail of the pancreas develops from the dorsal bud. It joins the duct from the ventral bud that develops into the head of the pancreas to form the main pancreatic duct of Wirsung. This duct then joins the common bile duct at the distal 1 to 2 cm of the bile duct before entering into the duodenum at the major papilla. If the dorsal duct in the cranial portion of the head of the pancreas persists and drains into a smaller papilla slightly cranial to the opening of the major papilla, this duct is known as an accessory duct of Santorini.

Imaging Techniques

CT is currently the imaging of choice for the evaluation of patients with suspected pancreatic masses. The technique of scanning continues to evolve toward a faster scan speed and thinner collimation to allow imaging of the pancreas and screening for metastatic disease at a proper time interval after the delivery of intravenous (IV) contrast enhancement.

When used to evaluate patients with suspected pancreatic tumors, CT requires IV contrast enhancement because the difference between the density of the normal pancreas and that of the tumor is otherwise insufficient to help distinguish the tissues. Studies on pancreatic and liver enhancement using various scanners and rates of IV contrast enhancement have shown that peak enhancement of the pancreas occurs before peak enhancement of the liver.^{1,2} This is because blood supply to the pancreas derives exclusively from the celiac axis and the SMA, while the blood supply to the liver derives from the hepatic artery and portal vein. Lesion conspicuity of the pancreatic tumor—particu-

larly for a ductal adenocarcinoma, which is a hypovascular tumor—is best detected during the pancreatic parenchymal phase. The current CT protocols are designed to optimize enhancement of the pancreas, the peripancreatic vessels, and the liver for lesion detection and staging evaluation.^{3–7} In the current state-of-the-art CT technique, multiphasic scanning with a helical CT scanner using slice thicknesses of 3 to 5 mm and pitch factors of 1.5 to 2 is performed to cover the entire pancreas during the pancreatic parenchymal phase. The delay time is 35 to 50 seconds after the delivery of IV contrast material at the rate of 5 ml/sec, or 40 to 70 seconds for the injection rate of 3 ml/sec. The liver and pancreas are then scanned during the portal dominant phase (60–70 sec) to detect hepatic metastases. When a multislice helical CT scanner is used, scanning the liver and pancreas can be accomplished in 10 seconds. We prefer scanning during the arterial phase at 20 to 30 seconds after the delivery of IV contrast material at 5 ml/sec for evaluation of arterial anatomy, followed by the pancreatic parenchymal phase at 40 to 50 seconds and the portal dominant phase at 60 to 70 seconds.

Transabdominal US is not widely used for the evaluation of pancreatic masses because of a limited field of view and body habitus. The presence of gas in the gastrointestinal tract and fat in the peritoneum limits the ability to examine the entire pancreas. However, US is excellent for the evaluation of patients with obstructive jaundice because of the ability to follow the dilated bile duct and to define the causes of obstruction, whether due to a pancreatic mass or benign conditions such as a stone. Endoscopic US is an emerging technology that has been used to define and diagnose pancreatic masses as well as for local staging of the mass,^{8–10} particularly in conjunction with aspiration biopsy.^{11–13} The technique, however, requires significant expertise in endoscopy and ultrasonography and may not be widely available in clinical practice. Intraoperative US is also a useful technique that can be performed in the operating room.¹⁴ It is a proven technique to detect small lesions such as an islet cell tumor or to define the relationship between a pancreatic mass and the adjacent vessels to determine the resectability of the tumor. However, it must be emphasized that it is performed intraoperatively and not as a preoperative procedure.

MRI has become more available for clinical uses in the evaluation of pancreatic disease, particularly when fast scanning techniques and dynamic IV contrast enhancement are used in conjunction with fat-suppressed imaging sequences.^{15–19} The technique, known as MR cholangiopancreatography (MRCP), uses fast scanning technique with heavily weighted T2 imaging sequence to produce high-quality images of the bile duct and pancreatic duct. MRCP is a noninvasive imaging technique that can demonstrate the bile duct and pancreatic duct without endoscopy, catheterization, and contrast injection of the ducts.^{20,21}

MRI provides several benefits over CT, including superior contrast between various tissues, the ability to modify various pulse sequences to both improve lesion conspicuity and limit artifacts, imaging without exposing the patient to ionizing radiation, and the ability to visualize the vessel without the use of IV iodinated contrast material. With a state-of-the-art system and advanced software, MRI produces high-quality images of the liver and pancreas and may improve lesion detection over CT.^{18,19} Current MRI of the pancreas is usually performed at high field strength (1.5 T or higher) with the following sequences: T1-weighted, with or without fat-suppressed spin-echo; T2-weighted multi-echo (fast spin-echo); and T1-weighted gradient-recalled echo (fast low-angle-shot [FLASH] or spoiled gradient recalled acquisition [or spoiled grass; SPGR]), without and with dynamic bolus IV injection of gadopentetate dimeglumine.

Angiography has very limited use in the diagnosis and staging of pancreatic tumors in current practice. High-quality helical CT has replaced angiography in the evaluation of vascular involvement and prediction of resectability. The technique also provides adequate anatomic information for surgical planning. On rare occasion, we may use angiography to sort out complicated vascular anatomy prior to surgery or use it in conjunction with CT arterial portography to detect hepatic metastasis.

Imaging Strategies in Pancreatic Tumors

The goals of imaging studies in patients with pancreatic masses or suspected pancreatic carcinoma are to make an accurate diagnosis of the pancreatic masses, and to provide accurate staging of the disease for surgical planning. Imaging of the pancreas

should be designed to help determine whether there is a mass in the pancreas, what the diagnosis is, and whether the lesion is resectable. Currently, there is no screening program for pancreatic cancer because there is no simple screening technique and few patients are known to be at high risk for developing the disease.

The discussion will focus on common pancreatic tumors in three categories: (1) pancreatic tumors originated from ductal epithelium, (2) cystic tumors and cysts, and (3) islet cell tumors. Ductal adenocarcinoma is by far the most common tumor of the pancreas, accounting for approximately 80% of pancreatic tumors. Cystic tumors and islet cell tumors are much less common but they have characteristic features that can be recognized. Because they are quite rare, other tumors such as acinar cell carcinoma, anaplastic carcinoma, pleomorphic giant cell carcinoma, pancreatic lymphoma, and pancreatic tumors of mesenchymal origin will not be described here.

Pancreatic Ductal Adenocarcinoma

The pathologic features of the tumor define the changes in the density and the pattern of enhancement of pancreatic tumors. Most ductal adenocarcinomas have an abundant, dense fibroblastic stroma, with a decrease in number of vessels within the tumor. This fibrotic process was thought to be a result of chronic pancreatitis secondary to pancreatic duct obstruction. Most recent studies, however, suggest that fibrosis and invasiveness of the tumor are secondary to the high production of growth factors (such as fibroblast growth factor and platelet-derived growth factor), cytokines (such as TNF- α), and metallic metalloproteinases produced by the tumor cells.²²

US Findings

The US appearances of pancreatic ductal adenocarcinoma are nonspecific. The tumors are hypoechoic on both transabdominal and endoscopic US (Figure 7.1). The sensitivity in detecting the pancreatic mass is limited by the body habitus when using transabdominal US but is significantly improved with endoscopic US. The experience of endoscopists and the availability of the technology, however, are rather limited at present. The findings of hypoechoic masses on US are nonspecific, but the use of fine-needle biopsy via an endoscope improves the specificity of the diagnosis.



FIGURE 7.1. Pancreatic ductal adenocarcinoma at the head of the pancreas demonstrated on endoscopic sonography. The mass (arrows) is hypoechoic.

CT Findings

CT appearances of ductal adenocarcinoma correlate well with the scirrhous and fibrotic changes in the tumor. The tumor appears as a poorly defined, hypodense mass during the pancreatic parenchymal phase of IV contrast enhancement (Figures 7.2 and 7.3) but in about 40% of the cases becomes isodense relative to the normal pancreas during the delayed phase. The density of tumors that contain a mucin pool or have a pseudocyst is usually lower than that of the pancreas, and these tumors do not enhance after IV contrast enhancement. Ductal adenocarcinomas have a hypodense appearance on CT in 75% to 90% of cases.^{4,5,7} In most cases of advanced disease, the hypodense area in the tumors is apparent during both (pancreatic parenchyma and portal dominant) phases of IV contrast enhancement. In cases of small tumors that do not change the contour of the pancreas and can likely be resected, however, scanning during the pancreatic parenchymal phase is crucial. This is because the difference in density between the tumor and the pancreatic parenchyma is then at its maximum, allowing the lesions to be best recognized during this phase. About 80% of small tumors appear isodense during the delayed phase.

MR Findings

The findings on MR images are similar to CT, particularly when IV contrast enhancement is used. On T1-weighted images, ductal adenocarcinoma is hypointense compared with the normal pancreas, and

the lesion is better seen when a fat-suppressed sequence is used. On T2-weighted images, the signal intensity of ductal adenocarcinoma varies depending on the degree of desmoplastic reaction, necrosis, and inflammatory changes. Immediately after a bolus injection of gadolinium contrast material, the tumor remains hypointense and the normal pancreas becomes hyperintense;^{18,19} therefore, the lesion is better recognized during this phase. The lesion can become isointense relative to the normal pancreas during the delayed phase.

Staging of Pancreatic Ductal Adenocarcinoma

The staging of pancreatic adenocarcinoma using conventional CT and MRI techniques is highly accurate for diagnosing advanced disease, ie, hepatic metastases, vascular involvement, and peritoneal carcinomatosis, any of which would preclude surgery, but these staging techniques are not sensitive enough to predict resectability.^{23,24} New imaging strategies using helical CT and dynamic MRI with proper phases of scanning help to assess the relationship between the tumor and the adjacent vessels and more accurately predict resectability.^{7,25–33} Using the CT findings described below, Evans et al³³ have established criteria for unresectability, particularly for arterial involvement, that are currently used at our institution (Table 7.1).

Vascular Involvement

Current CT technique accurately defines the extent of vascular involvement and correctly predicts resectability of the tumor in 80% to 85% of cases.^{29–31} Loyer et al³⁰ and Lu et al³¹ categorized vascular involvement in pancreatic ductal adenocarcinoma based on the relationship between the hypodense tumor and the adjacent vessels. In our experience with these criteria,³⁰ in cases in which a fat plane (type A) or normal pancreatic parenchyma (type B) separated the tumor from adjacent vessels (Figure 7.2), the tumor could be resected without venous resection in 21 (95%) of 22 patients. In cases in which the tumor was inseparable from the vessels but the points of contact formed a convexity against the vessel (type C), it could not reliably be predicted whether the tumor was fixed against the vessel. In most cases in which the tumor was partially encircling the vessel (type D) (Figure 7.3), the tu-

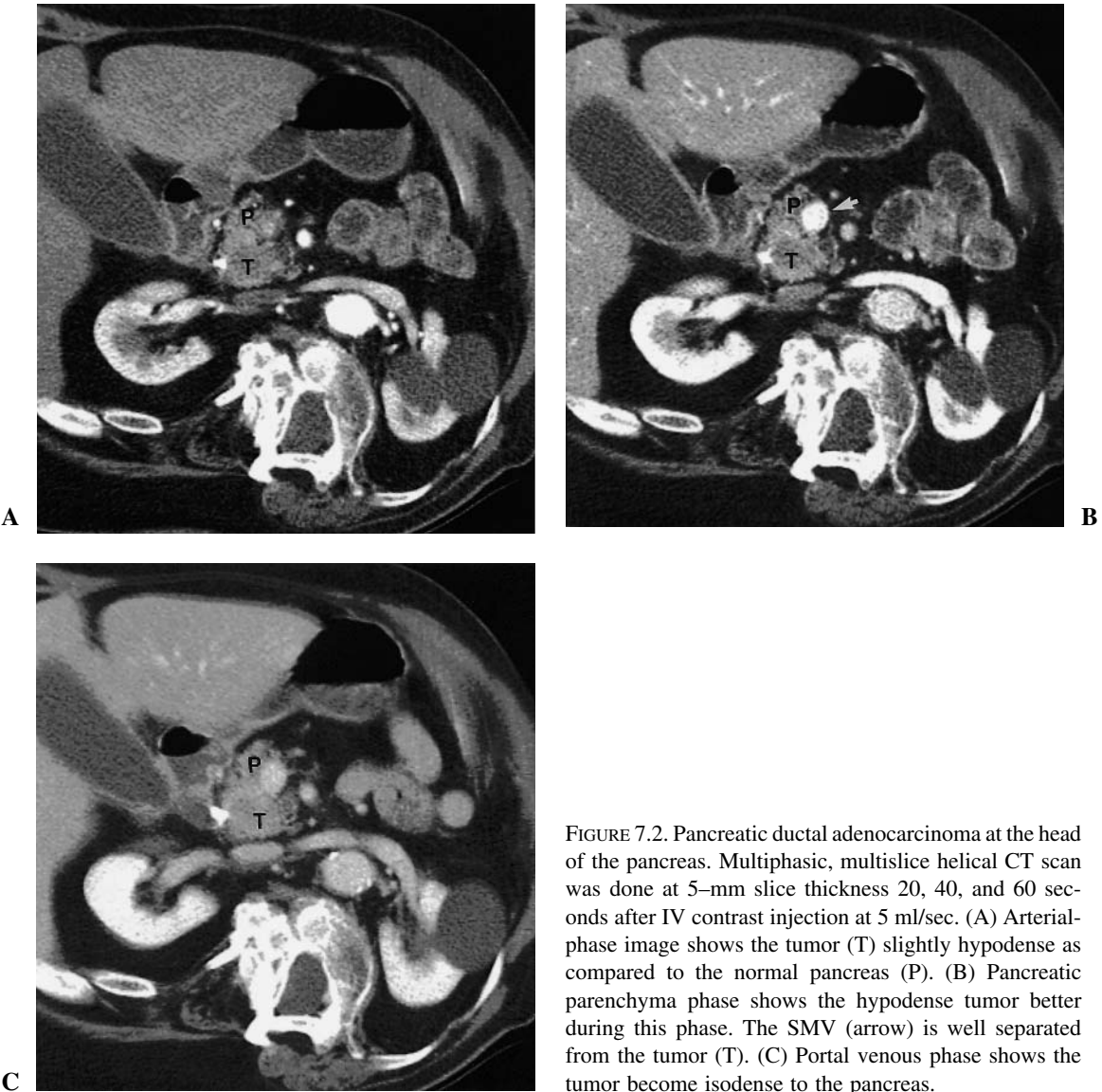


FIGURE 7.2. Pancreatic ductal adenocarcinoma at the head of the pancreas. Multiphasic, multislice helical CT scan was done at 5-mm slice thickness 20, 40, and 60 seconds after IV contrast injection at 5 ml/sec. (A) Arterial-phase image shows the tumor (T) slightly hypodense as compared to the normal pancreas (P). (B) Pancreatic parenchyma phase shows the hypodense tumor better during this phase. The SMV (arrow) is well separated from the tumor (T). (C) Portal venous phase shows the tumor become isodense to the pancreas.

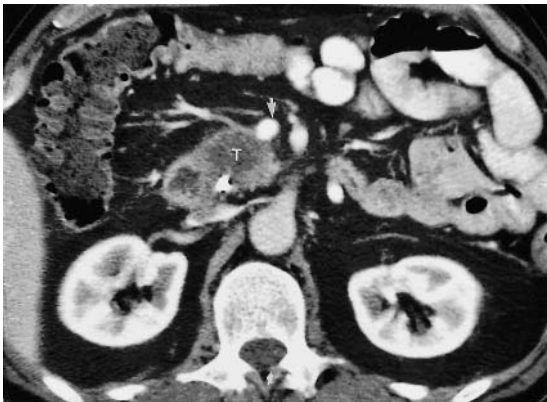


FIGURE 7.3. Pancreatic ductal adenocarcinoma in the head of the pancreas with involvement of the SMV. CT scan at the level of the head of the pancreas shows hypodense tumor (T) inseparable from the SMV (arrow).

TABLE 7.1. Clinical and radiologic staging of pancreatic cancer.

Stage	Clinical/radiologic criteria
I Resectable (T1-2, selected T3, NX, M0)	No encasement of the celiac axis or SMA Patent SMV and portal vein (PV) No extrapancreatic disease
II Locally advanced (some T3 and T4, NX-1, M0)	Arterial encasement (celiac or SMA) or venous occlusion (SMV, PV) No extrapancreatic disease
III Metastatic (any T, any N, M1)	Hepatic, peritoneal metastasis, occasionally lung

mor was fixed against the vessel and venous resection was required to remove the tumor. In all cases in which the tumor completely encircled (type E) or occluded (type F) the vessel (Figure 7.4), the tumors were not resectable with a negative margin.

Pathways of Tumor Infiltration

Identification of the pathways by which tumors spread is important for surgical planning to achieve a negative-margin resection. These pathways are particularly important in cases in which the tumor spreads beyond the usual extent of a pancreaticoduodenectomy or the tumor is located in the retroperitoneum behind the SMA, where direct intraoperative assessment is not possible until the final stage of resection. Tumor invasion of the duodenum is usually not a contraindication for

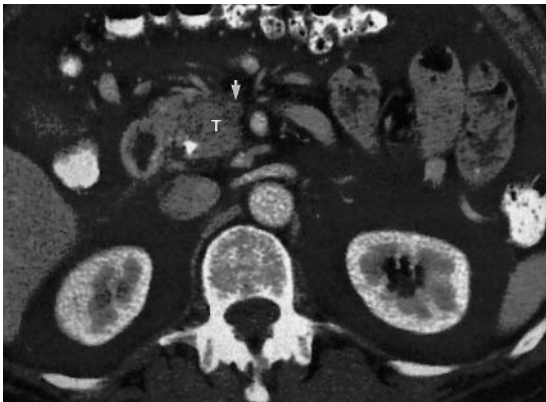


FIGURE 7.4. Pancreatic ductal adenocarcinoma (T) in the head of the pancreas with occlusion of the SMV (arrow).

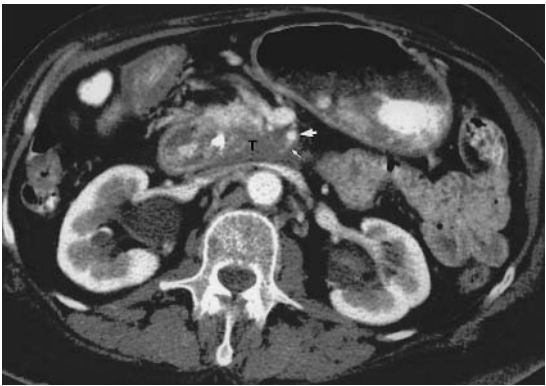


FIGURE 7.5. Pancreatic ductal adenocarcinoma (T) with involvement along the inferior pancreaticoduodenal artery (small arrow) toward the posterior wall of the SMA (large arrow) makes this tumor unresectable.

resection because the tumor and the duodenum are removed together in the resected specimen. Tumors arising from the pancreas adjacent to the duodenum tend to infiltrate (1) along the anterior pancreaticoduodenal arcade toward the gastroduodenal artery behind the pylorus and toward the proper hepatic artery in the hepatoduodenal ligament, or (2) along the posterior pancreaticoduodenal vein toward the inferior surface of the portal vein. Tumors arising in the cephalad portion of the pancreatic head near the pancreatic neck may infiltrate superiorly toward the common hepatic artery or infiltrate inferiorly into the mesentery and transverse mesocolon and along the SMV. Tumors arising from the uncinate process of the pancreas may infiltrate along the inferior pancreaticoduodenal arcade toward the posterior surface of the SMA (Figure 7.5) or into the jejunal mesentery from which the inferior pancreaticoduodenal arcade originates. Tumors arising from the head of the pancreas near the confluence of the gastroduodenal trunk, where the gastroduodenal trunk drains into the SMV, may infiltrate into the base of the transverse mesocolon along the middle colic artery or vein. Knowledge of this anatomy and the potential pathways of local tumor invasion is important for planning aggressive surgery.

Hepatic Metastasis

Hepatic metastasis is common, particularly in patients with tumors arising from the body and tail of

the pancreas. Detection of hepatic metastasis in patients with advanced disease is not difficult with CT or MRI. In patients with limited disease, however, CT and MRI have a 26% to 42% sensitivity for detecting hepatic metastases smaller than 2 cm or metastases at the surface of the liver.^{23,24} Laparoscopic examination and intraoperative US, used in conjunction with preoperative CT or MRI, may improve the ability to detect hepatic metastasis.

Comparison of Imaging Modalities

Comparison studies among CT, MRI, and endoscopic US suggest that fine-needle aspiration biopsy guided by endoscopic US is probably more sensitive and more specific for lesion detection and diagnosis than are CT and MRI.^{9,12,26} Lesion detection with high-quality MRI may be slightly better than with CT, but comparison studies are limited by patient-selection criteria, interpretation criteria, the consistency of the equipment, and the experience of the radiologists.^{25,27–28} For staging of advanced disease, however, MR and CT yielded equally accurate results, which are better than those of endoscopic US, because endoscopic US has a limited field of view. For potentially resectable disease, current data and our experience favor helical CT, which uses better criteria of vascular involvement.^{28–31}

Differential Diagnosis

The finding of a hypodense mass on contrast-enhanced CT or hypointense mass on contrast-enhanced MRI is, in our experience and that of others, indicative of ductal adenocarcinoma in 90% of cases. Other tumors and tumor-like lesions, such as lymphoma, an unusual islet cell tumor, and chronic pancreatitis, could be hypodense, but these conditions rarely appear as isolated hypodense masses. Therefore, other associated findings must be taken into account in the differential diagnosis. Moreover, the finding of an isodense mass does not entirely exclude the diagnosis of pancreatic ductal adenocarcinoma or other surgically treated lesions. In a case in which the patient has painless jaundice and an isodense mass is seen on CT, chronic pancreatitis, a bile duct tumor, or an ampullary tumor should be considered more likely than a ductal adenocarcinoma. Findings on endoscopic retrograde

cholangiopancreatography are needed to combine with CT findings to establish the diagnosis.

Islet Cell Tumor

An islet cell tumor consists of solid sheets of tumor cells with numerous small vessels interspersed throughout. This tumor has no significant scirrhous or fibrotic components. A small islet cell tumor, particularly a functioning tumor such as an insulinoma, poses less of a diagnostic problem because it is hypervascular and is easily recognized on CT as a hyperdense enhancing mass during the early phase of IV contrast enhancement. This enhancement pattern is similar to MR with dynamic IV contrast enhancement. On US, a functioning islet cell tumor can be seen as a well-defined hypoechoic mass easily distinguishable from the normal pancreas. When an islet cell tumor is large with areas of necrosis or cystic degeneration, the periphery of the tumor remains hypervascular,^{34,35} and this peripheral enhancement can be used to distinguish it from a necrotic or mucin-producing pancreatic ductal adenocarcinoma. Calcification is seen more frequently on CT scans in islet cell tumors than in ductal adenocarcinomas; only about 50% to 60% of large, nonfunctioning islet cell tumors contain calcification.

Patients with a functioning islet cell tumor such as an insulinoma or gastrinoma frequently present early because the tumor produces early symptoms. The tumor is generally small and difficult to detect preoperatively. On the other hand, patients with nonfunctioning islet cell tumors frequently present late because of the lack of symptoms and in many cases with large masses and hepatic metastases.

Chronic Pancreatitis

A common problem encountered by diagnostic radiologists is differentiating a pancreatic carcinoma from chronic pancreatitis. The diagnosis of chronic pancreatitis that is characterized by parenchyma atrophy, diffuse ductal dilatation, and diffuse parenchymal calcification is not difficult, and these appearances are seen in 60% to 70% of patients with chronic pancreatitis. However, chronic pancreatitis presenting as a focal mass, which is seen in 30% of cases of chronic pancreatitis, is more difficult to diagnose. Although a hypodense mass is likely to be a ductal adenocarcinoma, a hypodense

mass in a small number of patients may be caused by chronic pancreatitis and an isodense mass in a small number of patients may be caused by a ductal adenocarcinoma. Endoscopic US with fine-needle aspiration biopsy is our current diagnostic approach before planning treatment.

Cystic Neoplasms and Simple Cysts

Benign congenital cysts of the pancreas are rare. Some of the patients with benign pancreatic cysts may be associated with syndromes in which cysts are found in many organs, such as von Hippel Lindau syndrome, and polycystic disease. Other types of benign cysts that may be acquired include lymphoepithelium cysts, posttraumatic cysts, and pseudocyst.

Cystic tumors of the pancreas are rare tumors originating from the ductal component of the gland, and accounting for only 1% of pancreatic neoplasms. They can be divided into three groups: microcystic cystadenoma, mucin-producing cystic tumors, and solid papillary cystic tumors.³⁶⁻⁴¹

Microcystic cystadenoma is considered a benign tumor without malignant potential. It is frequently found incidentally in elderly women. When the mass is large, it can produce pressure symptoms and may require surgical intervention. The most important characteristic of this tumor is the presence of numerous small cysts with interspersed fibrovascular septae conglomerating in a single well-circumscribed mass. The tumor is generally hypervascular and can be easily recognized on CT

(Figure 7.6).^{38,39} Central scar can be seen in 13% to 20%. Calcification was reported in 38% of these tumors. The tumor is generally well circumscribed and not invasive. Microcystic cystadenoma has been shown to be an irregular anechoic mass with a central hyperechoic area with possible acoustic shadowing if the lesion is calcified. Lesions with numerous tiny cysts may have a hypoechoic appearance without well-defined anechoic area.

Mucin-Producing Cystic Tumors

Mucin-producing cystic tumor is a group of tumors that may have various malignant potentials, from a benign tumor such as a mucinous cystadenoma to a malignant tumor such as a cystadenocarcinoma that can be invasive and metastatic.³⁶⁻³⁹ Many pathologists believe a benign cystadenoma can transform to a cystadenocarcinoma. Therefore, it is important to distinguish a mucin-producing cystic tumor from a microcystic cystadenoma. The tumor typically is a unilocular or multilocular cystic mass with a dense fibrous wall that may contain tumor nodule or dystrophic calcification. The tumor vascularity in mucin-producing cystic tumors is quite variable. One of the most important features is the content of the cyst; mucin-producing cystic tumors produce mucous fluid, while the content of fluid in microcystic cystadenoma is serous. The tumor is more commonly seen in middle-aged women, and the tumors generally produce symptoms from pressure symptoms to weight loss.

Another variant of mucin-producing cystic tu-

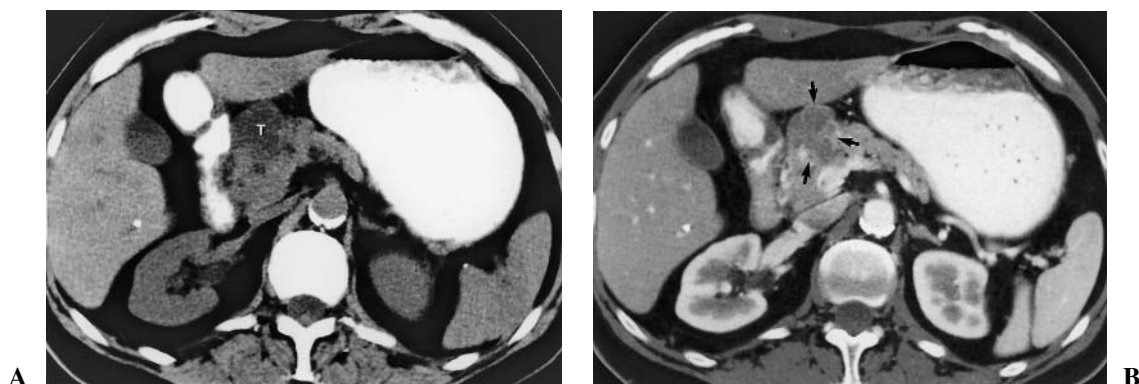


FIGURE 7.6. Microcystic cystadenoma at the head of the pancreas. (A) Unenhanced CT scan at the level of the head of the pancreas shows a well-defined, hypodense, fluid-density mass (T). (B) Enhanced CT scan shows enhancing lesion (arrows) with small locules of cysts. This was confirmed to be a microcystic cystadenoma.



FIGURE 7.7. Mucin-producing cystadenoma involving the body and tail of the pancreas with large cystic mass (arrows) and multiple septation and daughter cystic nodules (open arrow) in the mass.

mors is ductectatic mucinous cystadenoma and cystadenocarcinoma.⁴⁰ This lesion can occur in a side branch of the pancreatic duct, or the entire pancreatic duct can be involved. The patient can present with symptoms of recurrent episodes of pancreatitis because the mucin causes obstruction of the pancreatic duct. If the tumor is benign, these episodes can last for years, particularly when the lesion is not recognized. However, in many instances, metastatic disease may develop if the tumor is malignant.

CT appearances of a mucin-producing cystic tumor are those of a loculated fluid-filled cystic mass

with septation and nodules in the wall (Figure 7.7). Imaging studies generally cannot distinguish cystadenoma from cystadenocarcinoma unless there is evidence of tumor invasion and metastasis. In a ductectatic type, repeated episodes of pancreatitis in a patient who did not have history of alcohol abuse and gallstones but with progressive dilatation of the pancreatic duct should lead to further investigation to find the cause of pancreatic duct obstruction (Figure 7.8). Typically, endoscopic retrograde cholangiopancreatography is most useful to establish the diagnosis because the endoscopist can observe mucin protruding from the ampulla and multiple filling defects in the dilated pancreatic duct.

Solid Papillary Epithelial Neoplasm

This is another rare cystic tumor with low malignant potential. It is frequently seen in young females. The tumors usually have a well-defined margin and a thick capsule. However, the internal architecture of the tumor is rather complex because it may contain a solid component, hemorrhagic areas, and cystic degeneration.⁴¹ The patient may present because of pressure symptoms or mass effect or acute abdominal pain because of hemorrhage in the mass.

CTs of these lesions show various features depending upon the content of the tumor from cystic, hemorrhagic, and solid mass. The key features to

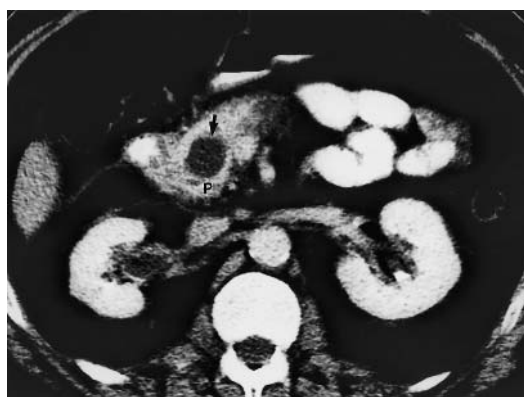
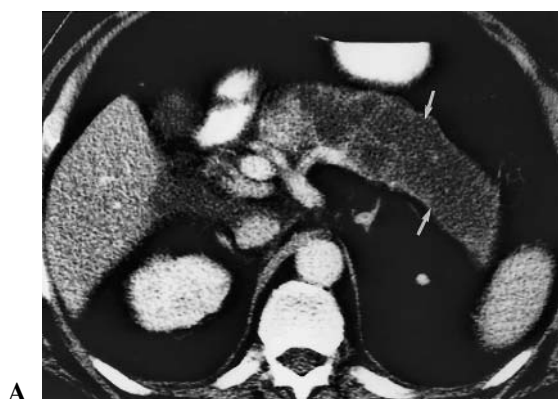


FIGURE 7.8. “Ductectatic” mucinous cystadenoma involving the entire body of the pancreas. This was a patient with lymphoma who had multiple episodes of pancreatitis. (A) CT at the level of the body of the pancreas shows diffuse low-density changes (arrows) throughout the body. This is due to a diffusely dilated pancreatic duct with pancreatic parenchyma atrophy. (B) CT at the level of the head of the pancreas shows dilated pancreatic duct (arrow) sparing the parenchyma of the pancreatic head (P).

suggest the diagnosis are the patients' ages and the appearances of mixed solid and cystic mass in a well-circumscribed mass.

Conclusion

Diagnosis of pancreatic tumors can be accurately made on imaging studies such as CT, MRI, and US based on pathologic, morphologic, and hemodynamic features of the tumors. The techniques of examination can be properly designed to answer clinical questions.

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Pancreatic Cancer: Radiologic Staging

Harmeet Kaur, Evelyne M. Loyer, Elizabeth A. Lano,
and Chusilp Charnsangavej

Introduction

The pancreas is a retroperitoneal organ that lies deep in the abdomen behind the stomach, transverse colon, and small bowel. The organ is not easily palpated, or easily examined by routine clinical examination and conventional radiography. Not until the late 1970s, after the discovery and use of cross-sectional imaging techniques such as computed tomography (CT), ultrasound (US), and magnetic resonance imaging (MRI), could the organ be clearly defined and examined noninvasively. In this chapter, we describe imaging anatomy of the pancreas, and imaging findings of pancreatic tumors with the differential diagnosis based on morphologic and hemodynamic changes of the tumors. In addition, we discuss staging of pancreatic tumors, using state-of-the-art CT technique, with special attention on peripancreatic vascular anatomy, and use this information for planning surgery.

Anatomy of the Pancreas

The pancreas lies transversely along its long axis in the anterior pararenal space of the retroperitoneum. The size of the pancreas varies depending upon the age; it is thicker in younger individuals and becomes atrophied in older ages. The pancreas develops from the dorsal and ventral diverticuli branching from the duodenum to form the head, body, and tail. However, no clear anatomic landmarks within the pancreas separate these segments, except external landmarks such as the superior

mesenteric vein (SMV) used to separate the head from the body and tail of the pancreas.

The head of the pancreas lies within the C-loop of the second portion of the duodenum. The lateral surface of the head is up against the serosa of the duodenum. Between the posterior surface of the head and the inferior vena cava are retroperitoneal fat and small posterior peripancreatic nodes. Medially, the head of the pancreas is closely related to the superior mesenteric artery (SMA) and SMV. At the caudal portion of the head, a small portion of the pancreas extends behind the SMA and SMV, forming an uncinate process. It is connected to the SMA and SMV by small twigs of vessels that are branches of the jejunal artery and vein, or arise directly from the posterior wall of the SMA and SMV. The cranial portion of the head of the pancreas is located on the right side of the SMA and SMV. As the SMV joins the splenic vein to form the portal vein, it runs behind the head of the pancreas, and from that point extending to the left retroperitoneum, the head of the pancreas becomes the body.

The body and tail of the pancreas course transversely to the left side of the retroperitoneum toward the splenic hilum. After it passes in front of the abdominal aorta and celiac axis, it curves posteriorly and in the cranial direction. In most cases, it follows the course of the splenic artery and vein, staying anterior and slightly caudal to those vessels.

The pancreas is covered by the posterior peritoneal layer that forms the posterior wall of the lesser sac and the inframesocolic compartment of the peritoneal cavity. Just at the caudal margin

along the body and tail of the pancreas, the root of the transverse mesocolon that is formed by those two posterior peritoneal layers merges anteriorly to suspend the transverse colon into the peritoneal cavity. The root of the transverse mesocolon extends to the right, traversing across the head of the pancreas and second portion of the duodenum. The transverse mesocolon forms the inferior boundary of the lesser sac.

Pancreatic Duct Anatomy

The pancreatic duct from the body and tail of the pancreas develops from the dorsal bud. It joins the duct from the ventral bud that develops into the head of the pancreas to form the main pancreatic duct of Wirsung. This duct then joins the common bile duct at the distal 1 to 2 cm of the bile duct before entering into the duodenum at the major papilla. If the dorsal duct in the cranial portion of the head of the pancreas persists and drains into a smaller papilla slightly cranial to the opening of the major papilla, this duct is known as an accessory duct of Santorini.

Imaging Techniques

CT is currently the imaging of choice for the evaluation of patients with suspected pancreatic masses. The technique of scanning continues to evolve toward a faster scan speed and thinner collimation to allow imaging of the pancreas and screening for metastatic disease at a proper time interval after the delivery of intravenous (IV) contrast enhancement.

When used to evaluate patients with suspected pancreatic tumors, CT requires IV contrast enhancement because the difference between the density of the normal pancreas and that of the tumor is otherwise insufficient to help distinguish the tissues. Studies on pancreatic and liver enhancement using various scanners and rates of IV contrast enhancement have shown that peak enhancement of the pancreas occurs before peak enhancement of the liver.^{1,2} This is because blood supply to the pancreas derives exclusively from the celiac axis and the SMA, while the blood supply to the liver derives from the hepatic artery and portal vein. Lesion conspicuity of the pancreatic tumor—particu-

larly for a ductal adenocarcinoma, which is a hypovascular tumor—is best detected during the pancreatic parenchymal phase. The current CT protocols are designed to optimize enhancement of the pancreas, the peripancreatic vessels, and the liver for lesion detection and staging evaluation.^{3–7} In the current state-of-the-art CT technique, multiphasic scanning with a helical CT scanner using slice thicknesses of 3 to 5 mm and pitch factors of 1.5 to 2 is performed to cover the entire pancreas during the pancreatic parenchymal phase. The delay time is 35 to 50 seconds after the delivery of IV contrast material at the rate of 5 ml/sec, or 40 to 70 seconds for the injection rate of 3 ml/sec. The liver and pancreas are then scanned during the portal dominant phase (60–70 sec) to detect hepatic metastases. When a multislice helical CT scanner is used, scanning the liver and pancreas can be accomplished in 10 seconds. We prefer scanning during the arterial phase at 20 to 30 seconds after the delivery of IV contrast material at 5 ml/sec for evaluation of arterial anatomy, followed by the pancreatic parenchymal phase at 40 to 50 seconds and the portal dominant phase at 60 to 70 seconds.

Transabdominal US is not widely used for the evaluation of pancreatic masses because of a limited field of view and body habitus. The presence of gas in the gastrointestinal tract and fat in the peritoneum limits the ability to examine the entire pancreas. However, US is excellent for the evaluation of patients with obstructive jaundice because of the ability to follow the dilated bile duct and to define the causes of obstruction, whether due to a pancreatic mass or benign conditions such as a stone. Endoscopic US is an emerging technology that has been used to define and diagnose pancreatic masses as well as for local staging of the mass,^{8–10} particularly in conjunction with aspiration biopsy.^{11–13} The technique, however, requires significant expertise in endoscopy and ultrasonography and may not be widely available in clinical practice. Intraoperative US is also a useful technique that can be performed in the operating room.¹⁴ It is a proven technique to detect small lesions such as an islet cell tumor or to define the relationship between a pancreatic mass and the adjacent vessels to determine the resectability of the tumor. However, it must be emphasized that it is performed intraoperatively and not as a preoperative procedure.

MRI has become more available for clinical uses in the evaluation of pancreatic disease, particularly when fast scanning techniques and dynamic IV contrast enhancement are used in conjunction with fat-suppressed imaging sequences.^{15–19} The technique, known as MR cholangiopancreatography (MRCP), uses fast scanning technique with heavily weighted T2 imaging sequence to produce high-quality images of the bile duct and pancreatic duct. MRCP is a noninvasive imaging technique that can demonstrate the bile duct and pancreatic duct without endoscopy, catheterization, and contrast injection of the ducts.^{20,21}

MRI provides several benefits over CT, including superior contrast between various tissues, the ability to modify various pulse sequences to both improve lesion conspicuity and limit artifacts, imaging without exposing the patient to ionizing radiation, and the ability to visualize the vessel without the use of IV iodinated contrast material. With a state-of-the-art system and advanced software, MRI produces high-quality images of the liver and pancreas and may improve lesion detection over CT.^{18,19} Current MRI of the pancreas is usually performed at high field strength (1.5 T or higher) with the following sequences: T1-weighted, with or without fat-suppressed spin-echo; T2-weighted multi-echo (fast spin-echo); and T1-weighted gradient-recalled echo (fast low-angle-shot [FLASH] or spoiled gradient recalled acquisition [or spoiled grass; SPGR]), without and with dynamic bolus IV injection of gadopentetate dimeglumine.

Angiography has very limited use in the diagnosis and staging of pancreatic tumors in current practice. High-quality helical CT has replaced angiography in the evaluation of vascular involvement and prediction of resectability. The technique also provides adequate anatomic information for surgical planning. On rare occasion, we may use angiography to sort out complicated vascular anatomy prior to surgery or use it in conjunction with CT arterial portography to detect hepatic metastasis.

Imaging Strategies in Pancreatic Tumors

The goals of imaging studies in patients with pancreatic masses or suspected pancreatic carcinoma are to make an accurate diagnosis of the pancreatic masses, and to provide accurate staging of the disease for surgical planning. Imaging of the pancreas

should be designed to help determine whether there is a mass in the pancreas, what the diagnosis is, and whether the lesion is resectable. Currently, there is no screening program for pancreatic cancer because there is no simple screening technique and few patients are known to be at high risk for developing the disease.

The discussion will focus on common pancreatic tumors in three categories: (1) pancreatic tumors originated from ductal epithelium, (2) cystic tumors and cysts, and (3) islet cell tumors. Ductal adenocarcinoma is by far the most common tumor of the pancreas, accounting for approximately 80% of pancreatic tumors. Cystic tumors and islet cell tumors are much less common but they have characteristic features that can be recognized. Because they are quite rare, other tumors such as acinar cell carcinoma, anaplastic carcinoma, pleomorphic giant cell carcinoma, pancreatic lymphoma, and pancreatic tumors of mesenchymal origin will not be described here.

Pancreatic Ductal Adenocarcinoma

The pathologic features of the tumor define the changes in the density and the pattern of enhancement of pancreatic tumors. Most ductal adenocarcinomas have an abundant, dense fibroblastic stroma, with a decrease in number of vessels within the tumor. This fibrotic process was thought to be a result of chronic pancreatitis secondary to pancreatic duct obstruction. Most recent studies, however, suggest that fibrosis and invasiveness of the tumor are secondary to the high production of growth factors (such as fibroblast growth factor and platelet-derived growth factor), cytokines (such as TNF- α), and metallic metalloproteinases produced by the tumor cells.²²

US Findings

The US appearances of pancreatic ductal adenocarcinoma are nonspecific. The tumors are hypoechoic on both transabdominal and endoscopic US (Figure 7.1). The sensitivity in detecting the pancreatic mass is limited by the body habitus when using transabdominal US but is significantly improved with endoscopic US. The experience of endoscopists and the availability of the technology, however, are rather limited at present. The findings of hypoechoic masses on US are nonspecific, but the use of fine-needle biopsy via an endoscope improves the specificity of the diagnosis.



FIGURE 7.1. Pancreatic ductal adenocarcinoma at the head of the pancreas demonstrated on endoscopic sonography. The mass (arrows) is hypochoic.

CT Findings

CT appearances of ductal adenocarcinoma correlate well with the scirrhous and fibrotic changes in the tumor. The tumor appears as a poorly defined, hypodense mass during the pancreatic parenchymal phase of IV contrast enhancement (Figures 7.2 and 7.3) but in about 40% of the cases becomes isodense relative to the normal pancreas during the delayed phase. The density of tumors that contain a mucin pool or have a pseudocyst is usually lower than that of the pancreas, and these tumors do not enhance after IV contrast enhancement. Ductal adenocarcinomas have a hypodense appearance on CT in 75% to 90% of cases.^{4,5,7} In most cases of advanced disease, the hypodense area in the tumors is apparent during both (pancreatic parenchyma and portal dominant) phases of IV contrast enhancement. In cases of small tumors that do not change the contour of the pancreas and can likely be resected, however, scanning during the pancreatic parenchymal phase is crucial. This is because the difference in density between the tumor and the pancreatic parenchyma is then at its maximum, allowing the lesions to be best recognized during this phase. About 80% of small tumors appear isodense during the delayed phase.

MR Findings

The findings on MR images are similar to CT, particularly when IV contrast enhancement is used. On T1-weighted images, ductal adenocarcinoma is hypointense compared with the normal pancreas, and

the lesion is better seen when a fat-suppressed sequence is used. On T2-weighted images, the signal intensity of ductal adenocarcinoma varies depending on the degree of desmoplastic reaction, necrosis, and inflammatory changes. Immediately after a bolus injection of gadolinium contrast material, the tumor remains hypointense and the normal pancreas becomes hyperintense;^{18,19} therefore, the lesion is better recognized during this phase. The lesion can become isointense relative to the normal pancreas during the delayed phase.

Staging of Pancreatic Ductal Adenocarcinoma

The staging of pancreatic adenocarcinoma using conventional CT and MRI techniques is highly accurate for diagnosing advanced disease, ie, hepatic metastases, vascular involvement, and peritoneal carcinomatosis, any of which would preclude surgery, but these staging techniques are not sensitive enough to predict resectability.^{23,24} New imaging strategies using helical CT and dynamic MRI with proper phases of scanning help to assess the relationship between the tumor and the adjacent vessels and more accurately predict resectability.^{7,25–33} Using the CT findings described below, Evans et al³³ have established criteria for unresectability, particularly for arterial involvement, that are currently used at our institution (Table 7.1).

Vascular Involvement

Current CT technique accurately defines the extent of vascular involvement and correctly predicts resectability of the tumor in 80% to 85% of cases.^{29–31} Loyer et al³⁰ and Lu et al³¹ categorized vascular involvement in pancreatic ductal adenocarcinoma based on the relationship between the hypodense tumor and the adjacent vessels. In our experience with these criteria,³⁰ in cases in which a fat plane (type A) or normal pancreatic parenchyma (type B) separated the tumor from adjacent vessels (Figure 7.2), the tumor could be resected without venous resection in 21 (95%) of 22 patients. In cases in which the tumor was inseparable from the vessels but the points of contact formed a convexity against the vessel (type C), it could not reliably be predicted whether the tumor was fixed against the vessel. In most cases in which the tumor was partially encircling the vessel (type D) (Figure 7.3), the tu-

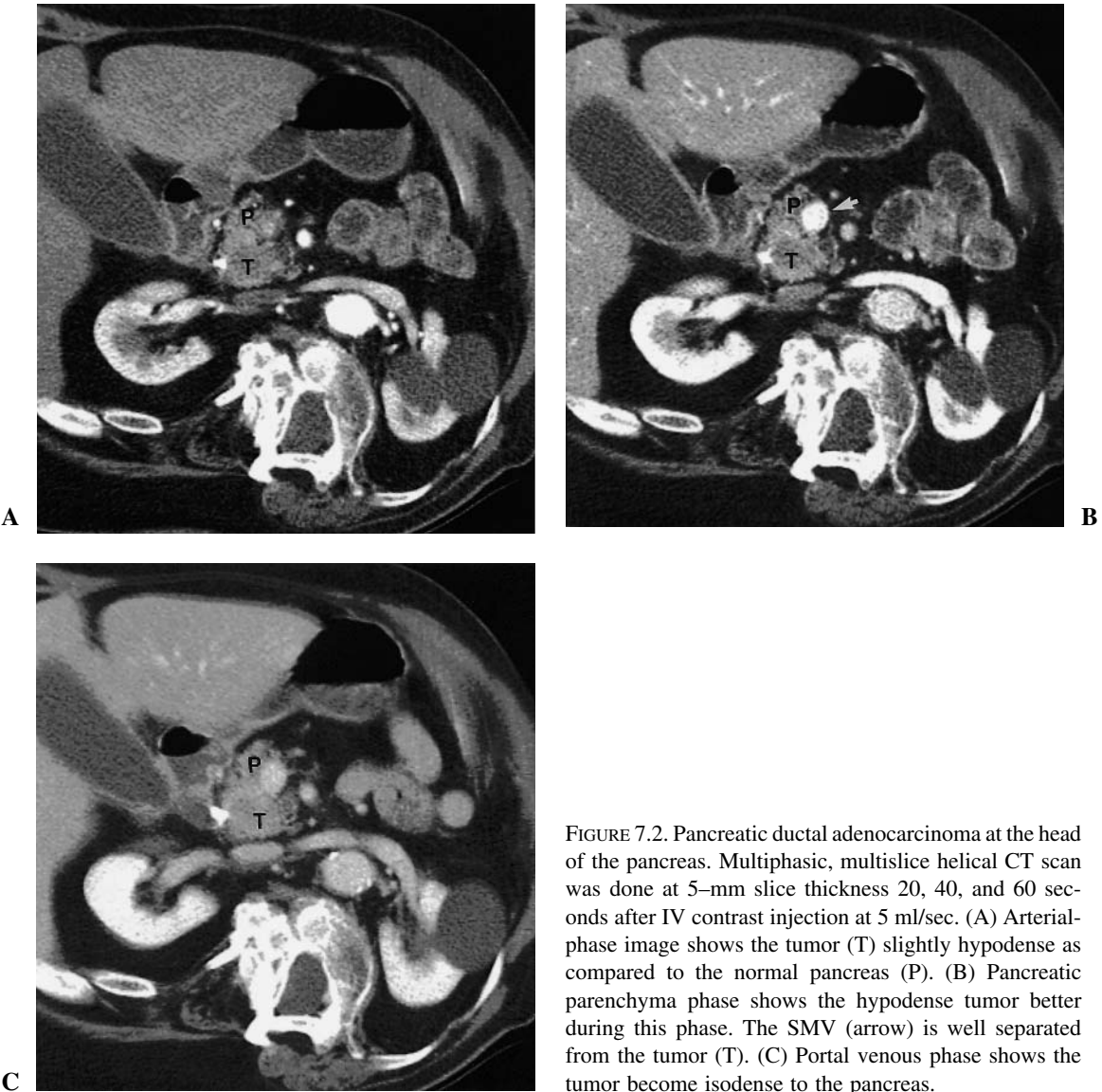


FIGURE 7.2. Pancreatic ductal adenocarcinoma at the head of the pancreas. Multiphasic, multislice helical CT scan was done at 5-mm slice thickness 20, 40, and 60 seconds after IV contrast injection at 5 ml/sec. (A) Arterial-phase image shows the tumor (T) slightly hypodense as compared to the normal pancreas (P). (B) Pancreatic parenchyma phase shows the hypodense tumor better during this phase. The SMV (arrow) is well separated from the tumor (T). (C) Portal venous phase shows the tumor become isodense to the pancreas.

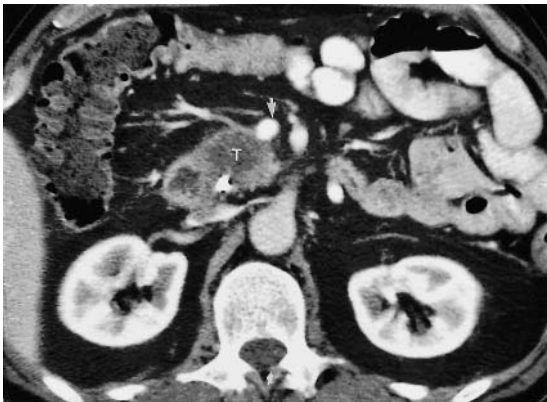


FIGURE 7.3. Pancreatic ductal adenocarcinoma in the head of the pancreas with involvement of the SMV. CT scan at the level of the head of the pancreas shows hypodense tumor (T) inseparable from the SMV (arrow).

TABLE 7.1. Clinical and radiologic staging of pancreatic cancer.

Stage	Clinical/radiologic criteria
I Resectable (T1-2, selected T3, NX, M0)	No encasement of the celiac axis or SMA Patent SMV and portal vein (PV) No extrapancreatic disease
II Locally advanced (some T3 and T4, NX-1, M0)	Arterial encasement (celiac or SMA) or venous occlusion (SMV, PV) No extrapancreatic disease
III Metastatic (any T, any N, M1)	Hepatic, peritoneal metastasis, occasionally lung

mor was fixed against the vessel and venous resection was required to remove the tumor. In all cases in which the tumor completely encircled (type E) or occluded (type F) the vessel (Figure 7.4), the tumors were not resectable with a negative margin.

Pathways of Tumor Infiltration

Identification of the pathways by which tumors spread is important for surgical planning to achieve a negative-margin resection. These pathways are particularly important in cases in which the tumor spreads beyond the usual extent of a pancreaticoduodenectomy or the tumor is located in the retroperitoneum behind the SMA, where direct intraoperative assessment is not possible until the final stage of resection. Tumor invasion of the duodenum is usually not a contraindication for

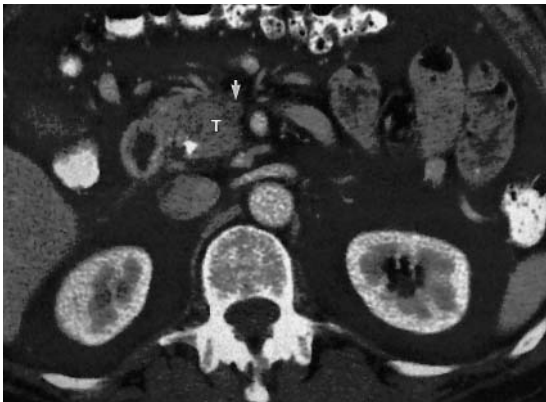


FIGURE 7.4. Pancreatic ductal adenocarcinoma (T) in the head of the pancreas with occlusion of the SMV (arrow).

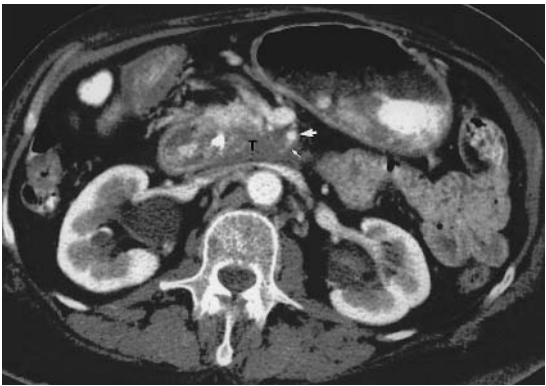


FIGURE 7.5. Pancreatic ductal adenocarcinoma (T) with involvement along the inferior pancreaticoduodenal artery (small arrow) toward the posterior wall of the SMA (large arrow) makes this tumor unresectable.

resection because the tumor and the duodenum are removed together in the resected specimen. Tumors arising from the pancreas adjacent to the duodenum tend to infiltrate (1) along the anterior pancreaticoduodenal arcade toward the gastroduodenal artery behind the pylorus and toward the proper hepatic artery in the hepatoduodenal ligament, or (2) along the posterior pancreaticoduodenal vein toward the inferior surface of the portal vein. Tumors arising in the cephalad portion of the pancreatic head near the pancreatic neck may infiltrate superiorly toward the common hepatic artery or infiltrate inferiorly into the mesentery and transverse mesocolon and along the SMV. Tumors arising from the uncinate process of the pancreas may infiltrate along the inferior pancreaticoduodenal arcade toward the posterior surface of the SMA (Figure 7.5) or into the jejunal mesentery from which the inferior pancreaticoduodenal arcade originates. Tumors arising from the head of the pancreas near the confluence of the gastroduodenal trunk, where the gastroduodenal trunk drains into the SMV, may infiltrate into the base of the transverse mesocolon along the middle colic artery or vein. Knowledge of this anatomy and the potential pathways of local tumor invasion is important for planning aggressive surgery.

Hepatic Metastasis

Hepatic metastasis is common, particularly in patients with tumors arising from the body and tail of

the pancreas. Detection of hepatic metastasis in patients with advanced disease is not difficult with CT or MRI. In patients with limited disease, however, CT and MRI have a 26% to 42% sensitivity for detecting hepatic metastases smaller than 2 cm or metastases at the surface of the liver.^{23,24} Laparoscopic examination and intraoperative US, used in conjunction with preoperative CT or MRI, may improve the ability to detect hepatic metastasis.

Comparison of Imaging Modalities

Comparison studies among CT, MRI, and endoscopic US suggest that fine-needle aspiration biopsy guided by endoscopic US is probably more sensitive and more specific for lesion detection and diagnosis than are CT and MRI.^{9,12,26} Lesion detection with high-quality MRI may be slightly better than with CT, but comparison studies are limited by patient-selection criteria, interpretation criteria, the consistency of the equipment, and the experience of the radiologists.^{25,27–28} For staging of advanced disease, however, MR and CT yielded equally accurate results, which are better than those of endoscopic US, because endoscopic US has a limited field of view. For potentially resectable disease, current data and our experience favor helical CT, which uses better criteria of vascular involvement.^{28–31}

Differential Diagnosis

The finding of a hypodense mass on contrast-enhanced CT or hypointense mass on contrast-enhanced MRI is, in our experience and that of others, indicative of ductal adenocarcinoma in 90% of cases. Other tumors and tumor-like lesions, such as lymphoma, an unusual islet cell tumor, and chronic pancreatitis, could be hypodense, but these conditions rarely appear as isolated hypodense masses. Therefore, other associated findings must be taken into account in the differential diagnosis. Moreover, the finding of an isodense mass does not entirely exclude the diagnosis of pancreatic ductal adenocarcinoma or other surgically treated lesions. In a case in which the patient has painless jaundice and an isodense mass is seen on CT, chronic pancreatitis, a bile duct tumor, or an ampullary tumor should be considered more likely than a ductal adenocarcinoma. Findings on endoscopic retrograde

cholangiopancreatography are needed to combine with CT findings to establish the diagnosis.

Islet Cell Tumor

An islet cell tumor consists of solid sheets of tumor cells with numerous small vessels interspersed throughout. This tumor has no significant scirrhous or fibrotic components. A small islet cell tumor, particularly a functioning tumor such as an insulinoma, poses less of a diagnostic problem because it is hypervascular and is easily recognized on CT as a hyperdense enhancing mass during the early phase of IV contrast enhancement. This enhancement pattern is similar to MR with dynamic IV contrast enhancement. On US, a functioning islet cell tumor can be seen as a well-defined hypoechoic mass easily distinguishable from the normal pancreas. When an islet cell tumor is large with areas of necrosis or cystic degeneration, the periphery of the tumor remains hypervascular,^{34,35} and this peripheral enhancement can be used to distinguish it from a necrotic or mucin-producing pancreatic ductal adenocarcinoma. Calcification is seen more frequently on CT scans in islet cell tumors than in ductal adenocarcinomas; only about 50% to 60% of large, nonfunctioning islet cell tumors contain calcification.

Patients with a functioning islet cell tumor such as an insulinoma or gastrinoma frequently present early because the tumor produces early symptoms. The tumor is generally small and difficult to detect preoperatively. On the other hand, patients with nonfunctioning islet cell tumors frequently present late because of the lack of symptoms and in many cases with large masses and hepatic metastases.

Chronic Pancreatitis

A common problem encountered by diagnostic radiologists is differentiating a pancreatic carcinoma from chronic pancreatitis. The diagnosis of chronic pancreatitis that is characterized by parenchyma atrophy, diffuse ductal dilatation, and diffuse parenchymal calcification is not difficult, and these appearances are seen in 60% to 70% of patients with chronic pancreatitis. However, chronic pancreatitis presenting as a focal mass, which is seen in 30% of cases of chronic pancreatitis, is more difficult to diagnose. Although a hypodense mass is likely to be a ductal adenocarcinoma, a hypodense

mass in a small number of patients may be caused by chronic pancreatitis and an isodense mass in a small number of patients may be caused by a ductal adenocarcinoma. Endoscopic US with fine-needle aspiration biopsy is our current diagnostic approach before planning treatment.

Cystic Neoplasms and Simple Cysts

Benign congenital cysts of the pancreas are rare. Some of the patients with benign pancreatic cysts may be associated with syndromes in which cysts are found in many organs, such as von Hippel Lindau syndrome, and polycystic disease. Other types of benign cysts that may be acquired include lymphoepithelium cysts, posttraumatic cysts, and pseudocyst.

Cystic tumors of the pancreas are rare tumors originating from the ductal component of the gland, and accounting for only 1% of pancreatic neoplasms. They can be divided into three groups: microcystic cystadenoma, mucin-producing cystic tumors, and solid papillary cystic tumors.^{36–41}

Microcystic cystadenoma is considered a benign tumor without malignant potential. It is frequently found incidentally in elderly women. When the mass is large, it can produce pressure symptoms and may require surgical intervention. The most important characteristic of this tumor is the presence of numerous small cysts with interspersed fibrovascular septae conglomerating in a single well-circumscribed mass. The tumor is generally hypervascular and can be easily recognized on CT

(Figure 7.6).^{38,39} Central scar can be seen in 13% to 20%. Calcification was reported in 38% of these tumors. The tumor is generally well circumscribed and not invasive. Microcystic cystadenoma has been shown to be an irregular anechoic mass with a central hyperechoic area with possible acoustic shadowing if the lesion is calcified. Lesions with numerous tiny cysts may have a hypoechoic appearance without well-defined anechoic area.

Mucin-Producing Cystic Tumors

Mucin-producing cystic tumor is a group of tumors that may have various malignant potentials, from a benign tumor such as a mucinous cystadenoma to a malignant tumor such as a cystadenocarcinoma that can be invasive and metastatic.^{36–39} Many pathologists believe a benign cystadenoma can transform to a cystadenocarcinoma. Therefore, it is important to distinguish a mucin-producing cystic tumor from a microcystic cystadenoma. The tumor typically is a unilocular or multilocular cystic mass with a dense fibrous wall that may contain tumor nodule or dystrophic calcification. The tumor vascularity in mucin-producing cystic tumors is quite variable. One of the most important features is the content of the cyst; mucin-producing cystic tumors produce mucous fluid, while the content of fluid in microcystic cystadenoma is serous. The tumor is more commonly seen in middle-aged women, and the tumors generally produce symptoms from pressure symptoms to weight loss.

Another variant of mucin-producing cystic tu-

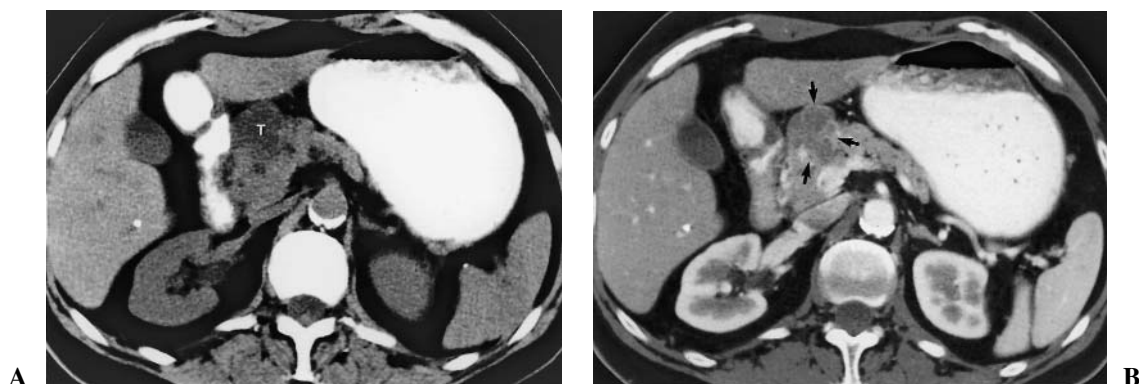


FIGURE 7.6. Microcystic cystadenoma at the head of the pancreas. (A) Unenhanced CT scan at the level of the head of the pancreas shows a well-defined, hypodense, fluid-density mass (T). (B) Enhanced CT scan shows enhancing lesion (arrows) with small locules of cysts. This was confirmed to be a microcystic cystadenoma.



FIGURE 7.7. Mucin-producing cystadenoma involving the body and tail of the pancreas with large cystic mass (arrows) and multiple septation and daughter cystic nodules (open arrow) in the mass.

mors is ductectatic mucinous cystadenoma and cystadenocarcinoma.⁴⁰ This lesion can occur in a side branch of the pancreatic duct, or the entire pancreatic duct can be involved. The patient can present with symptoms of recurrent episodes of pancreatitis because the mucin causes obstruction of the pancreatic duct. If the tumor is benign, these episodes can last for years, particularly when the lesion is not recognized. However, in many instances, metastatic disease may develop if the tumor is malignant.

CT appearances of a mucin-producing cystic tumor are those of a loculated fluid-filled cystic mass

with septation and nodules in the wall (Figure 7.7). Imaging studies generally cannot distinguish cystadenoma from cystadenocarcinoma unless there is evidence of tumor invasion and metastasis. In a ductectatic type, repeated episodes of pancreatitis in a patient who did not have history of alcohol abuse and gallstones but with progressive dilatation of the pancreatic duct should lead to further investigation to find the cause of pancreatic duct obstruction (Figure 7.8). Typically, endoscopic retrograde cholangiopancreatography is most useful to establish the diagnosis because the endoscopist can observe mucin protruding from the ampulla and multiple filling defects in the dilated pancreatic duct.

Solid Papillary Epithelial Neoplasm

This is another rare cystic tumor with low malignant potential. It is frequently seen in young females. The tumors usually have a well-defined margin and a thick capsule. However, the internal architecture of the tumor is rather complex because it may contain a solid component, hemorrhagic areas, and cystic degeneration.⁴¹ The patient may present because of pressure symptoms or mass effect or acute abdominal pain because of hemorrhage in the mass.

CTs of these lesions show various features depending upon the content of the tumor from cystic, hemorrhagic, and solid mass. The key features to

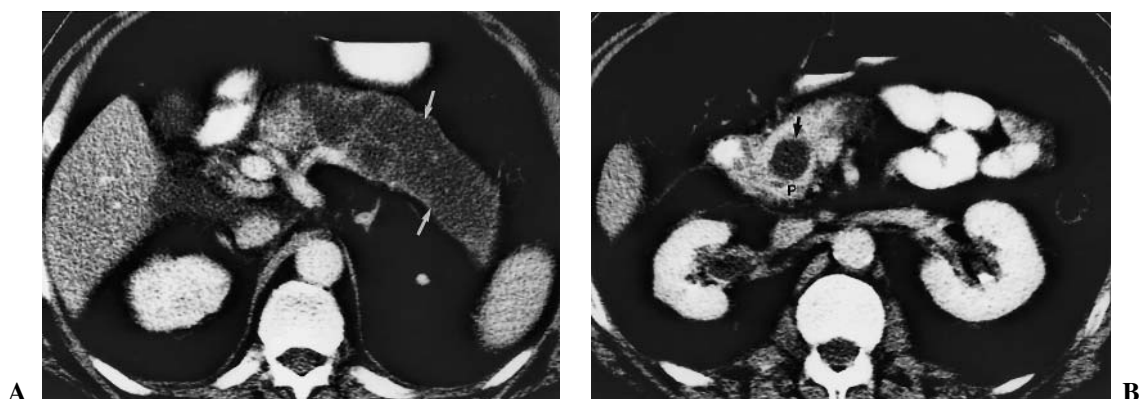


FIGURE 7.8. “Ductectatic” mucinous cystadenoma involving the entire body of the pancreas. This was a patient with lymphoma who had multiple episodes of pancreatitis. (A) CT at the level of the body of the pancreas shows diffuse low-density changes (arrows) throughout the body. This is due to a diffusely dilated pancreatic duct with pancreatic parenchyma atrophy. (B) CT at the level of the head of the pancreas shows dilated pancreatic duct (arrow) sparing the parenchyma of the pancreatic head (P).

suggest the diagnosis are the patients' ages and the appearances of mixed solid and cystic mass in a well-circumscribed mass.

Conclusion

Diagnosis of pancreatic tumors can be accurately made on imaging studies such as CT, MRI, and US based on pathologic, morphologic, and hemodynamic features of the tumors. The techniques of examination can be properly designed to answer clinical questions.

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8

Endoscopic Diagnosis and Staging: Endoscopic Ultrasound, Endoscopic Retrograde Cholangiopancreatography

Richard A. Erickson

Introduction

As the medical and operative therapeutic options for the management of pancreatic cancer increase, so does the importance of early diagnosis and accurate staging. Prior to the last decade, the “gold standard” for detecting small pancreatic lesions poorly seen by other imaging techniques was endoscopic retrograde cholangiopancreatography (ERCP).^{1,2} However, ERCP, as a diagnostic modality for pancreatic tumors, has now been largely superseded by impressive technologic advances in cross-sectional and three-dimensional imaging with spiral computed tomography (CT) and magnetic resonance imaging (MRI) and by another endoscopic procedure, endoscopic ultrasound (EUS).³ Assessing the most appropriate roles for CT, MRI, EUS, and ERCP in the diagnosis, staging, and therapy of pancreatic cancer is difficult because the capabilities of each of these procedures are continuously evolving and comparative studies of these procedures done at similar levels of cutting-edge expertise are rare. Which combinations of these imaging modalities are used for the patient with suspected pancreatic cancer at any given institution often depends less on published comparative data than on the institutional therapeutic approach to managing pancreatic cancer and the local availability, expertise, and interest in CT, MRI, EUS, or ERCP.

Endoscopic Ultrasound

Technologic Considerations

The basic principle of EUS involves placing a small, high-frequency ultrasound transducer on the

tip of a flexible fiberoptic or video-endoscope.⁴ The whole of the pancreas can be brought into close proximity to this transducer, since the neck, body, and tail of the pancreas lie within a couple of centimeters of the posterior wall of the body of the stomach and the pancreatic head is just adjacent to the duodenal bulb and second portion of the duodenum. The primary advantage of EUS over transcutaneous ultrasound is that this close proximity of an ultrasound transducer to the pancreatic parenchyma allows imaging with high-frequency (7.5 to 12 MHz) ultrasound. Since ultrasound resolution is directly dependent on transducer frequency, EUS results in pancreatic images with resolutions well below 1 mm, far better than the current capabilities of CT or MRI. Being a “live” procedure, EUS also offers the advantage of an interactive examination of the pancreas and surrounding tissues where subtle abnormalities can be aggressively pursued from different perspectives and at different frequencies. Finally, being an endoscopic procedure, EUS now allows combining its invasive biopsy (EUS-guided fine-needle aspiration) and therapeutic capabilities (eg, tumor injection therapy, celiac neurolysis) with the diagnostic procedure. Additionally, when EUS is combined sequentially under the same sedation with its sister endoscopic procedure, therapeutic ERCP, it produces an efficient combination of diagnostic, staging, and therapeutic capabilities that are very difficult to match with any other set of procedures.

The first use of echoendoscopes was reported 20 years ago.^{5,6} Commercially produced EUS units became available in the mid to late 1980s, with rotating transducers oriented perpendicular to the

shaft of the endoscopes. These “radial echoendoscopes” result in 360-degree views of the gastrointestinal tract wall and surrounding structures with imaging depths of up to 12 cm from the endoscope tip. In the early 1990s, echoendoscopes with curved-linear array ultrasonic transducers were produced.⁷ These “linear echoendoscopes” have the ultrasound transducer imaging parallel to the shaft of the endoscope. This modification was very important to the evolution of EUS because with these instruments, objects exiting the biopsy channel of the endoscope (eg, a needle) could be followed and guided into lesions in “real time.” Soon after the introduction of linear echoendoscopes, the first reports^{8–10} of EUS-guided fine-needle aspiration (FNA) appeared, a technique that has solidified the importance of EUS in oncologic evaluations.

As an endoscopic technique, EUS is obviously by its very nature more invasive than CT or MRI. Conscious sedation with a benzodiazepine (eg, midazolam) and a narcotic (eg, meperidine or fentanyl) is routinely used. EUS carries the same small major morbidity rate (0.05%) of diagnostic endoscopy.⁴ EUS is much less morbid than its counterpart endoscopic procedure, ERCP, which has a 3% to 7% risk of major morbidity, primarily pancreatitis (see below). In the United States, the charges for EUS are generally at the level of a diagnostic colonoscopy.

EUS in the Diagnosis of Pancreatic Neoplasms

Soon after its clinical introduction in the mid-1980s in Japan¹¹ and Germany,¹² it was recognized that EUS provided a major advance in the visualization of pancreatic neoplasms. Since that time, numerous series have demonstrated that EUS is superior to CT and MRI in the diagnosis of pancreatic diseases and especially neoplasms.^{13–16} Detection rates for pancreatic cancer using EUS, even lesions less than 3 cm, have been consistently in the range of 95% to 100%. EUS is also superior to ERCP in the diagnosis of small pancreatic neoplasms.³

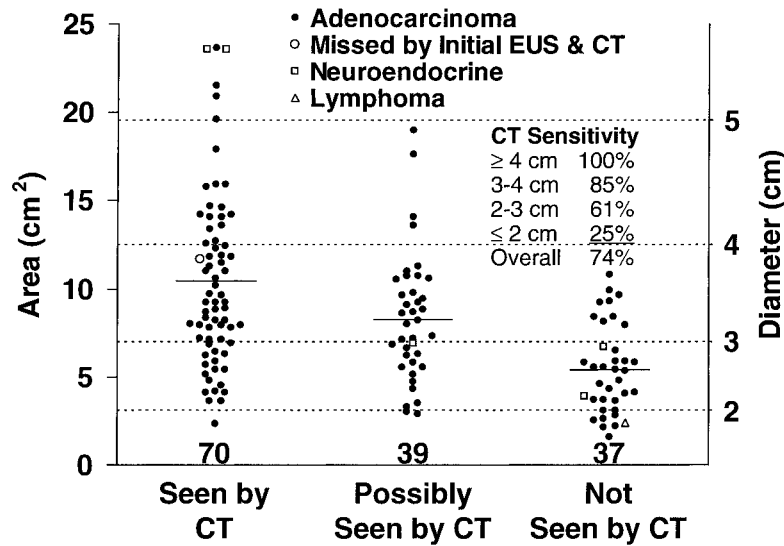
The superiority of EUS in diagnosing pancreatic neoplasms is being challenged by the recent technologic advances in spiral (helical) CT combined with much more powerful imaging computers that allow for rapid dual-phase scanning techniques,

minimization of volume-averaging artifacts, and three-dimensional reconstructions.¹⁷ Series comparing state-of-the-art helical CT with EUS are just now being published. Spiral CT has detection rates above 90%; however, EUS is still superior at detecting small pancreatic carcinomas.¹⁸ This superiority is illustrated by the data from the last four years at our own institution (Figure 8.1), where EUS has assumed a central role in the primary diagnosis of pancreatic cancer. In our series, most of these CTs were spiral, although usually not with dedicated dual-phase pancreatic imaging. Only 50% of pancreatic cancers were definitively detected by CT, and some 25% of the pancreatic masses were not visible at all on initial CT imaging. Even with the complete transition to spiral CT in the last 3 years, approximately 20% of pancreatic cancers diagnosed at our institution by EUS are still fully undetectable by CT. These data are very similar to those recently reported from Indiana University, where 26% of patients diagnosed with pancreatic carcinoma by EUS had no mass visible at all on CT.¹⁹

It must be remembered that in series comparing EUS to CT for pancreatic cancer, patients presenting with metastatic liver disease (about one third of patients at our own institution) are generally not included because there is usually little indication for an EUS either for staging or primary diagnosis. These unfortunate patients almost always have a large pancreatic mass easily detectable by CT. However, patients presenting with metastatic disease are usually included in series assessing the overall diagnostic accuracy of CT.²⁰ Including these patients can increase the overall diagnostic sensitivity of CT in pancreatic cancer detection by 5% to 15% compared to series focused only on those patients undergoing both EUS and CT. For example, in our own institution's series, the overall sensitivity of CT versus EUS was 74% versus 99% (Figure 8.1) in patients having both CT and EUS. However, if all patients diagnosed with pancreatic cancer are included, the overall sensitivity of CT increases to 82%.

The most difficult clinical situation for diagnosing pancreatic carcinoma with EUS is when the patient has underlying chronic pancreatitis. Most of the missed lesions with EUS and EUS-FNA occur in this setting.^{8,13,15,21–24} Unfortunately, detecting pancreatic malignancy in this clinical scenario is

FIGURE 8.1. Comparative diagnostic success of EUS versus abdominal CT for pancreatic malignancies of various sizes at Scott & White (Texas A&M Science Center), 1995–1999.



also more difficult for other imaging modalities such as CT, MRI, and ERCP.

Despite the superior diagnostic capabilities of EUS, the frequency of its use in patients with suspected pancreatic neoplasms in the United States is still surprisingly low. This primarily stems from a general lack of availability of high-quality EUS. The reasons for this are multifactorial,²⁵ but the relative dearth of resources for practicing gastroenterologists to become expertly trained in EUS is one of the primary problems.

EUS-Guided Fine-Needle Aspiration

Technical Considerations

With the development of curved-linear array echoendoscopes in the early 1990s, EUS-guided FNA was soon added to the diagnostic capabilities of EUS. The first EUS-FNA of a pancreatic cancer was reported in 1994¹⁰ and multiple series soon followed.^{14,15,21,23,26–33}

The technique of EUS-guided FNA has been described in detail elsewhere,^{23,31} but basically involves passing a 22-gauge stainless steel needle through the biopsy port of an echoendoscope under real-time guidance into an endosonographically visualized pancreatic mass, lymph node, liver metastasis, or fluid collection (Figure 8.2A–F). The needle is then moved back and forth through the lesion with varying degrees of suction applied to it. The specimen is then deposited on one or more

cytology slides) for immediate staining and cytopathologic examination.

Yield of EUS-Guided FNA

Multiple series^{14,15,21,23,26–33} have demonstrated that EUS-guided FNA can provide a cytologic diagnosis in 80% to 93% or more of pancreatic malignancies. The diagnostic yield of EUS-FNA is dependent on technique, especially the active involvement of a cytopathologist. In the United States, a cytopathologist is usually available in the room or nearby to provide immediate feedback on the adequacy and preliminary cytologic diagnosis.^{21,23,26,27,30–33} Having a cytopathologist available for live feedback results in about a 10% increase in the yield of a positive diagnosis.^{23,29,33} The importance of having a cytopathologist present for increasing FNA yields has been well demonstrated in other clinical settings such as FNA of breast masses.³⁴ In most series, an average of 3 to 4 passes are usually necessary to provide a firm cytologic diagnosis of a pancreatic malignancy; however, malignant lymph nodes and liver metastases generally require only 1 or 2 EUS-guided FNA passes for a definitive diagnosis.^{23,29,35–37} There are no clinical or endosonographic features that predict when a patient’s lesion may take more FNA passes to make a diagnosis. The major determinant of FNA pass number is the differentiation of the tumor, with some masses taking up to 10 passes or more to make a diagnosis.²³ If a cytopathologist is

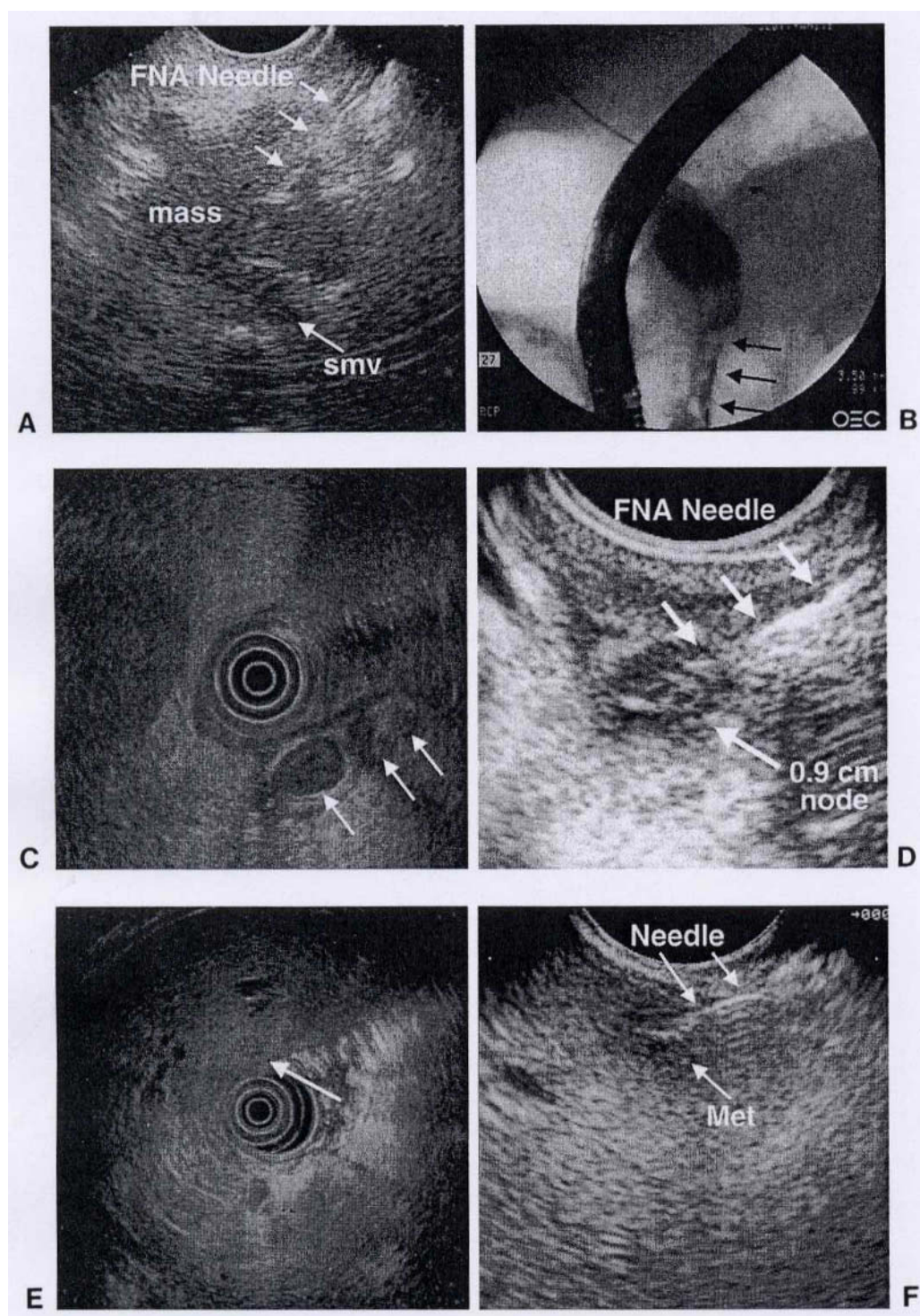


FIGURE 8.2. (A) Linear EUS image of EUS-guided FNA of a 3.5-cm pancreatic head adenocarcinoma invading into the superior mesenteric vein (smv). (B) Cholangiogram of the same lesion showing distal common bile duct stricture (arrows). (C) Multiple malignant periduodenal nodes (arrows) seen by radial EUS and EUS-guided FNA of one of these nodes (D). None of these nodes were seen by spiral CT. (E) Radial EUS demonstrating a 7-mm isolated liver metastasis from a pancreatic head adenocarcinoma. This lesion was not seen by CT and was sampled successfully by EUS-guided FNA (F). All radial images were produced using the Olympus UM20 echoendoscope at 7.5 MHz and all linear images using the Pentax FG-36UX echoendoscope at 7.5 MHz.

not immediately available, generally 5 to 6 passes into the lesion are recommended, realizing that this approach may result in a nondiagnostic specimen 15% to 20% of the time.²³ Recent technical modifications using larger needles to obtain actual microbiopsies have been made^{37–39}; however, whether these larger needles will significantly improve diagnostic accuracy is unclear^{39,40} except perhaps in the case of anaplastic neuroendocrine tumors.⁴¹

Similar yields of cytologic diagnosis are possible with transcutaneous ultrasound or CT-guided FNA of pancreatic masses, especially if a cytopathologist participates in the procedure, as is the routine at our institution. However, since EUS will visualize at least 20% more tumors than spiral CT, the net yield of a positive tissue diagnosis will be higher with EUS-guided FNA. An additional advantage of EUS-guided FNA is that, since it is a real-time procedure, when an abnormality is found in the pancreas, one can proceed directly on to FNA at that time. Typically, cross-sectional images are examined in detail after the patient has left the exam area, and if a lesion is found needing FNA, it is scheduled on a separate day. This may matter little in an ambulatory setting, but may cost an additional day or two of hospitalization if the patient is an inpatient.

The Importance of Obtaining a Preoperative Tissue Diagnosis in Pancreatic Cancer

A very important issue affecting whether one uses the superior diagnostic and FNA capabilities of EUS and EUS-guided FNA in evaluating pancreatic malignancies is the necessity of routinely trying to obtain a cytologic diagnosis of pancreatic cancer. Some investigators feel that attempts at obtaining a tissue diagnosis of pancreatic masses should be limited to only those patients who are not clearly operative candidates,^{42,43} while others argue that all patients warrant a preoperative attempt at tissue diagnosis.⁴⁴

Arguments Against Routine Attempts at Preoperative Tissue Diagnosis

- (1) There may be a significant risk of intraperitoneal or needle tract seeding with attempts at percutaneous biopsy.^{42,45}
- (2) A negative biopsy result does not rule out a pan-

creatic cancer and should therefore not dissuade against operation if the patient's symptoms, signs, and imaging suggest a pancreatic neoplasm is likely.^{42,43,46}

- (3) Pancreatic resection is the only treatment that has been shown to improve survival, so every patient possible should be given a chance at operation, regardless of a positive, equivocal or nondiagnostic tissue sample.^{42,43,45,46}

A conservative approach to seeking a preoperative tissue diagnosis may be reasonable in centers with a large volume of pancreatic resections that aggressively operate on patients with pancreatic masses with a very low mortality and morbidity and no protocols involving neoadjuvant therapy.⁴⁷ However, in most medical centers, routinely attempting to obtain a tissue diagnosis of pancreatic carcinoma by transcutaneous ultrasound or CT-guided FNA/biopsy or EUS-guided FNA in all patients has a number of equally strong rationales.

Arguments for Routine Attempts at Preoperative Tissue Diagnosis

- (1) The majority (80%) of patients with pancreatic cancer will eventually be found to be not resectable. A tissue diagnosis will be necessary in such patients before chemotherapy or radiation can be used and ideally should be available before inserting a permanent and expensive palliative device such as a metallic biliary or enteric stent.
- (2) Many surgeons are hesitant to perform a morbid operation such as a pancreaticoduodenectomy without confirmation of an underlying malignancy. If a tissue diagnosis has not been obtained preoperatively, the surgeon may then attempt an intraoperative biopsy to confirm malignancy prior to proceeding with resection. While having had a prior CT-guided biopsy of pancreatic cancer has not been shown to negatively affect survival, having had an attempt at operative biopsy of a pancreatic neoplasm has been reported to increase the risk of local-regional recurrence.⁴⁴
- (3) It has been argued that attempts at percutaneous biopsy of pancreatic carcinomas increases the risk of peritoneal seeding.^{45,48} However, patients having CT biopsies in these studies also had a much higher rate of unresectability, sug-

gesting that the positive peritoneal washings were more a reflection of larger, advanced tumors rather than of cells being spilled by CT biopsy.⁴⁹ In support of this is a recent review stating that no patient with a positive peritoneal cytology has been reported to have a potentially curative pancreaticoduodenectomy.⁴⁴ On the other hand, examination of mostly resectable patients showed no difference in positive peritoneal washings with (6%) or without (9%) a prior CT-guided FNA.⁴⁴ The concern has also been raised that attempts at needle aspiration may seed tumor cells in the needle tract, increasing the risk of an early local recurrence.⁴⁵ However, the frequency of this event after CT-guided biopsy appears to be very low, on the order of only 1 in 10,000 to 20,000,⁴⁹ although it may be more common after aspiration of pancreatic masses.^{50,51} There is little reason to expect that the risk of needle tract seeding should be any higher with EUS-guided FNA than the low rate seen in CT-guided FNA/biopsy. To date, I am aware of only 1, as yet unpublished, case of FNA tract seeding after EUS-guided FNA. EUS-guided FNA has additional theoretical advantages over CT-guided biopsy in that the FNA needle tract will usually be in the resective field of any pancreaticoduodenectomy, if it is eventually performed. The aspiration tract is also much shorter than that used by CT and usually does not cross the peritoneum.

- (4) Patients who appear to be resectable at the time of initial radiologic or endosonographic assessment may subsequently be found to be medically unsuitable candidates for resection. If EUS-guided FNA is not done at the time of the initial EUS, such patients may require additional procedures in order to obtain the tissue diagnosis necessary to be considered for non-resective therapies.
- (5) The future of pancreatic cancer management may also involve the more frequent use of neoadjuvant chemoradiation prior to operation,⁵² which mandates a preoperative tissue diagnosis.
- (6) The cytologic diagnosis and tumor differentiation may carry significant prognostic and management implications. Patient survival is poorer in patients with poorly differentiated and, interestingly, also with well-differentiated adenocarcinomas.⁵³ Additionally, as shown in our series²³

and others,^{54,55} about 1% to 2% of patients with pancreatic masses will have a primary pancreatic lymphoma that is managed very differently than pancreatic adenocarcinoma. Pancreatic neuroendocrine tumors also account for a small percentage of malignant-appearing pancreatic masses but are managed differently than pancreatic adenocarcinomas. These malignancies have a better prognosis than pancreatic adenocarcinoma and should be more aggressively approached operatively⁵⁶ and may do better with different chemotherapeutic approaches.⁵⁷ Good immunocytologic stains exist to help in the cytologic diagnosis of neuroendocrine tumors.⁵⁸

- (7) Finally, a perhaps more intangible, but no less important, rationale for obtaining a tissue diagnosis is letting patients and their families know their definitive diagnosis as soon as possible. Diagnosis ends the patient's and family's anguish of uncertainty over the disease and allows them to make the most informed decisions possible about future potentially very morbid therapeutic options.

Complications of EUS-Guided FNA

The overall complication rate of EUS-guided FNA appears to be about 1% to 2%,^{14,15,21,23,26–33,59} similar to that reported with CT or ultrasound-guided FNA or biopsy.^{50,60} The major complications reported with EUS-FNA are pancreatitis and bleeding. There has been only 1 death reported to date with EUS-guided FNA.³⁰ In that situation, a radial echoendoscope was used to perform pancreatic FNA, a technique that is no longer recommended because the needle tip cannot be followed into the tissue. In our own series of over 150 pancreatic EUS-guided FNAs we have had 2 major complications (1.5%), an episode of pancreatitis and a duodenal hematoma. EUS-guided FNA of cystic lesions may have a higher risk complication due to the risk of infection.⁵⁹ Because of this, intravenous antibiotics with oral antibiotics are routinely used for a few days for EUS-guided FNA of pancreatic cystic lesions.

EUS for Pancreatic Cancer Staging

General Principles

Not only does EUS provide superior diagnostic and cytologic information for patients with pancreatic

cancer, but it is an accurate staging tool for pancreatic malignancies to help decide the most appropriate therapeutic approach. There are a number of staging systems for pancreatic cancer,⁶¹ but the most frequently used in the United States is the recently modified⁶² American Joint Committee on Cancer (AJCC) criteria TNM-based staging system. When assessing the TNM staging of pancreatic malignancies, a systematic endosonographic examination of the upper gastrointestinal tract must be made as well as close examination of the tumor itself.

T Staging

Overall, EUS T staging accuracy for pancreatic cancer is about 80% to 85% at all stages,^{19,63–65} which is similar to other currently available imaging modalities. T1 lesions, tumors less than 2 cm in size and confined to the pancreatic parenchyma, are difficult to see by any other technique than EUS (see above) and it is therefore by default the most accurate study for T staging these fortunately rare tumors. When patients present with jaundice secondary to bile duct invasion or encasement they generally have at least a T3 lesion by the 1997 AJCC criteria. Since the pancreatic head and ampullary region is well visualized by EUS, assessing that a lesion is at least T3 is also very accurate by endosonography (Figures 8.3A, 8.3D). Duodenal wall invasion also is a T3 criterion and this is easily visualized both endoscopically and endosonographically at EUS.

However, the major T staging criterion most frequently affecting a patient's operability is invasion of the portal and/or superior mesenteric vein,⁶⁶ a T4 lesion by the 1997 AJCC criteria. Although some major oncologic centers will resect a partially invaded portal vein with results equally good to patients not having a portal vein resection,^{67,68} many surgeons still consider this a relative or absolute contraindication to an attempt at curative resection because of poor survival outcomes.^{69,70} The interface between the portal and superior mesenteric vein and a tumor of the pancreatic head is usually well visualized with EUS (Figures 8.2A, 8.3E, 8.3F). The overall accuracy of various endosonographic criteria for invasion have been assessed in detail⁷¹ and include an irregular venous wall (87%) (Figure 8.3F), loss of acoustic interface (78%) (Fig-

ure 8.3E), proximity of mass to the portal vein (73%), and absolute tumor size (39%). Using these criteria, EUS was more accurate (78%) than angiography (60%) at assessing portal vein involvement.⁷¹ A recent study reported an overall accuracy of 93% for vascular invasion.¹⁹ However, this study also showed that this degree of accuracy was highly dependent on the experience of the operator, with staging of about 100 pancreatic cancers being necessary to produce such high staging accuracy. If the patient is found to have endosonographic collaterals or portal vein encasement by tumor, the patient invariably has extensive portal vein involvement with tumor. Recent studies with dual-phase spiral CT and gadolinium-contrasted MRI show similar or better accuracies at assessing major vascular invasion.^{72,73}

Some T staging areas are difficult to assess by EUS. The superior mesenteric vein and especially the artery are often too deep to visualize adequately by EUS (Figure 8.3A). Additionally, assessing colonic invasion is usually out of range of the echoendoscope. Peritumor edema or pancreatitis can result in overstaging by EUS¹⁹; however, this problem is not unique to ultrasound. Thus in summary, T staging of pancreatic cancer by EUS is correct about 80% to 85% of the time, similar to other available procedures. It has unique staging advantages in small tumors and lesions of the pancreatic head but has the disadvantage of being highly operator-dependent and unable to see deep areas of mesenteric root and pancreatocolonic interface. EUS T staging is complementary to other techniques such as CT, MRI, and angiography.

N Staging

Because of the high resolution of EUS, detection of nodes as small as a few millimeters in and around the pancreas is routinely possible (Figure 8.2C). The celiac axis and periportal regions are particularly well visualized. On the other hand, the root of the small bowel mesentery and subduodenal periaortic regions are often poorly seen. Of course, just seeing a lymph node by EUS does not make it malignant. Nonspecific adenopathy around the portahepatis is a common finding, especially in the setting of previous inflammatory processes such as cholecystitis or pancreatitis. Endosonographic criteria for malignant adenopathy have been estab-

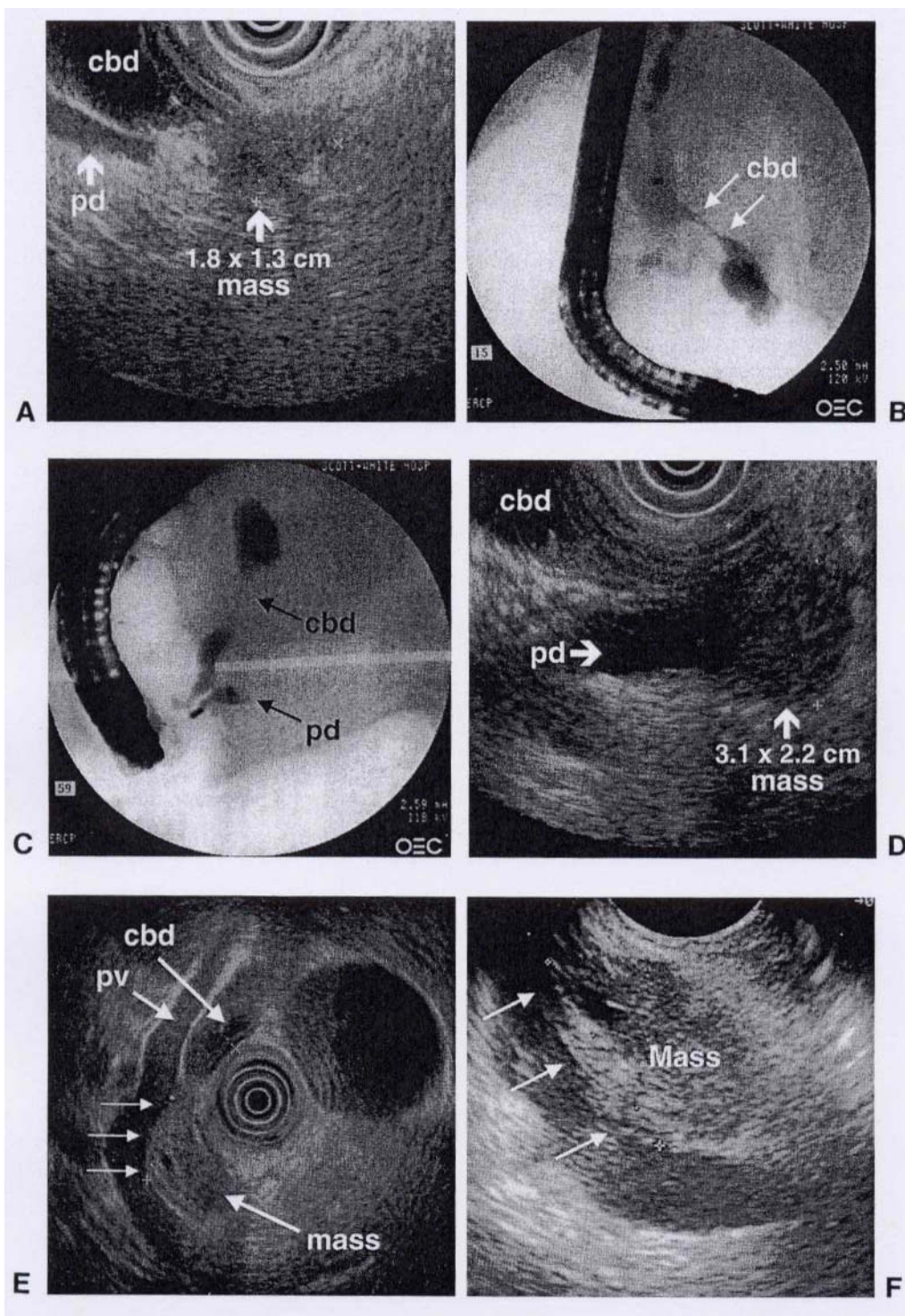


FIGURE 8.3. (A) Radial EUS image and (B) cholangiogram of a 1.8×1.3 -cm T3 adenocarcinoma of the pancreatic head. cbd = common bile duct, pd = pancreatic duct. (C) Demonstrates a classic "double-duct cut-off" ERCP sign of pancreatic cancer of the head. (D) Shows the corresponding radial EUS image clearly demonstrating a T3 lesion. (E) Shows a radial EUS view through the duodenal bulb with a pancreatic head mass demonstrating loss of acoustic interface (small arrows) with the portal vein (pv) suggesting portal vein invasion, a T4 lesion. (F) Shows a linear view of clear portal vein invasion (arrows) indicated by an irregular portal vein wall.

lished^{74,75} and include lymph node size greater than 1 cm, echolucency, homogeneity, round shape, and sharp edges. However, even if all these criteria are present, they have an overall accuracy of only about 80% to 90%.

Multiple series have shown the N stage accuracy of EUS in pancreatic cancer to be only about 65% to 70%.^{19,63–65} While this is far superior to the current capabilities of either spiral CT or MRI, it is still low enough to make surgical decisions based just on EUS imaging criteria suspect. The low accuracy of N staging by EUS is due to the combination of lack of specificity of endosonographic criteria for malignant adenopathy, micrometastatic nodal disease, and the inability to visualize some areas of metastatic nodes, especially the small bowel mesenteric root.

The addition of EUS-guided FNA has greatly enhanced the specificity of EUS N staging for pancreatic and other cancers.^{64,75} Nodes as small as 5 mm can be aspirated by EUS-guided FNA (Figure 8.2D), providing cytologic confirmation of metastatic disease to regional or distant nodes. Most surgeons consider cytologically documented nodal metastases a contraindication to resection because of the poor survivals in such patients.^{76–78} However, others argue that although survivals are poorer with nodal metastases, they are still better than with no resection at all.^{78,79} Thus, the significance of EUS-guided FNA documentation of nodal spread will depend on the institutional approach to such patients. However, if malignant adenopathy is considered a contraindication to resection, EUS and EUS-guided FNA can result in significant cost savings when used for pancreatic cancer assessment.³³ In our own series of EUS-guided FNA for pancreatic cancer, approximately 8% of patients undergoing diagnostic and staging EUS will be found to have nodal spread by EUS-guided FNA.²³

M Staging

Obviously EUS is limited in the areas it can examine for metastatic spread of pancreatic cancer and should not be considered a definitive imaging modality for M staging. About 80% of the liver is visible to EUS (Figure 8.2E), but the far right lobe and high dome of the liver are usually inaccessible. Because ultrasound does not penetrate into air, the

lungs cannot be seen, although small right pleural effusions are very visible and easily tapped endosonographically by EUS-guided FNA.⁸⁰ However, small amounts of peritoneal fluid⁸⁰ and liver metastases as small as 5 mm can be seen by EUS and sampled by EUS-guided FNA^{23,28,64,81} (Figure 8.2F). If cytologically positive, these findings would obviate any attempt at curative resection^{64,80,81} and therefore dramatically change the approach to managing that patient. Again, in our series,²³ we found occult metastatic disease in about 7% of our patients, primarily small liver metastases (Figures 8.2E, 8.2F).

Summary

EUS is comparable to other imaging modalities such as CT and MRI in T staging accuracy and superior in detecting malignant adenopathy. It can only be considered a supplementary procedure for M staging. EUS-guided FNA adds the capability of cytologically documenting the accuracy of N staging and M staging and can dramatically and cost-effectively affect the future management of the patient with pancreatic cancer, if positive. If EUS were used purely as a staging procedure, it would have to be considered complementary, not superior, to findings on CT and MRI. However, if EUS is being used primarily for its diagnostic, biopsy, and/or therapeutic capabilities, then the staging information obtained is an added bonus of the procedure.

Therapeutic EUS in Pancreatic Cancer

In addition to its use as a diagnostic tool, staging procedure, and method for obtaining a tissue diagnosis, EUS is now being used therapeutically in pancreatic carcinoma. This has been most well developed in the area of celiac neurolysis for control of pancreatic pain.⁸² By using EUS-guided fine needle injection (FNI), bupivacaine and absolute alcohol are injected on either side of the celiac artery through the posterior wall of the stomach. This is a much simpler and more precise technique than the radiologic transabdominal or anesthesiologic transthoracic approaches to celiac neurolysis. EUS celiac neurolysis takes about 10 minutes and can be done under the same sedation after a diagnostic and staging EUS has shown that the patient with severe pancreatic malignancy pain is not a surgical candidate. EUS-guided FNI is now also

being used for tumor injection therapy as discussed in Chapter 31.

The Role of EUS in Pancreatic Cancer

When high-quality EUS and EUS-guided FNA is readily available, EUS can and should take a central and early role in the evaluation of the patient with suspected pancreatic cancer.^{3,83-85} Its superiority as a diagnostic tool and very high specificity make it an ideal definitive test for patients suspected to have a pancreatic tumor by CT, percutaneous ultrasound, or clinical evaluation. Since cytologic diagnosis and staging information can also be obtained at the same time, EUS with EUS-guided FNA usually provides most, if not all, of the data needed to go on to definitive stage-specific therapy. Staging CT or MRI can also be used before or after EUS to complement the staging information provided, especially in equivocal operative cases and to rule out occult liver metastases. In the rare situation where EUS and EUS-guided FNA have failed to provide a definitive diagnosis, other modalities such as carbohydrate antigen (CA) 19-9,⁸⁶ ERCP, repeat EUS, or laparoscopy can be used depending on the clinical situation. Our own approach to incorporating EUS and EUS-guided FNA into the management of pancreatic cancer is summarized in Figure 8.4. Unfortunately, in most institutions, EUS is still relegated to being performed only as an imaging procedure in the relatively rare circumstance where CT, US, MRI, and

often ERCP have not provided definitive diagnostic information. Although this is a valid use of EUS, it minimizes the clinical impact of cost effectiveness of this powerful procedure by placing it at the end of the evaluation instead of near the beginning.

Endoscopic Retrograde Cholangiopancreatography

Technologic Considerations and Complications of ERCP

ERCP was first introduced in the late 1960s,⁸⁷ with the initial therapeutic sphincterotomies reported in 1974 from Japan and Germany.^{88,89} It is a technically demanding procedure,⁹⁰ but in the hands of a skilled endoscopist should have success rates in excess of 90% for visualizing the duct(s) of interest. In addition to the skill required to perform successful ERCP, it has one of the highest complication rates of any endoscopic procedure. Overall, major complications occur in about 5% to 10% of diagnostic and therapeutic ERCPs.⁹¹ Over half of these complications are related to ERCP-induced pancreatitis, and most of the rest are complications specific to endoscopic sphincterotomy, such as perforation and bleeding. Bacterial cholangitis is also a risk when cannulation of an obstructed biliary system is attempted. Performing ERCP in this setting usually mandates placing a biliary stent and using prophylactic antibiotics for the procedure.^{91,92}

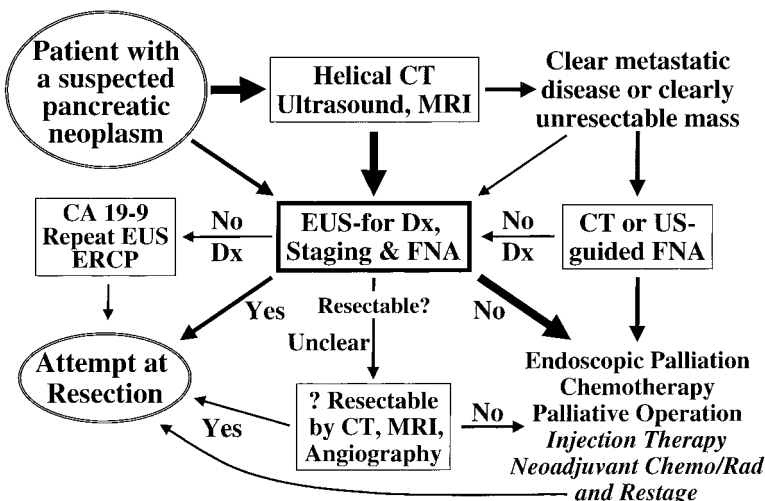


FIGURE 8.4. Algorithm used for incorporating EUS and EUS-guided FNA into the management of the patient with suspected pancreatic cancer at Scott & White (Texas A&M Science Center).

ERCP in the Diagnosis of Pancreatic Neoplasms

Soon after the introduction of ERCP, it was recognized that morphologic changes in the bile and/or pancreatic duct were seen in 90% to 100% of patients with pancreatic adenocarcinoma (Figures 8.2B, 8.3B, 8.3C).^{93–96} Especially important is the fact that ERCP can detect ductal abnormalities even in small pancreatic carcinomas.^{97,98} However, it was also evident in these studies that these ductal changes were nonspecific, leading to an overall accuracy in the range of 60% to 80% for diagnosing pancreatic cancer by ERCP alone. Even the classic ductographic appearances such as the “double duct cut-off” sign (Figure 8.3C) were not specific for just pancreatic carcinoma and could also be seen in chronic pancreatitis.^{99–101} Additionally, a normal pancreatogram does not exclude the possibility of an underlying pancreatic carcinoma, as this can be seen in about 7% of patients.¹⁰² Half of these normal pancreatograms may have missed the cancer because Santorini’s duct was not visualized.

ERCP for the Tissue Diagnosis of Pancreatic Neoplasms

The lack of specificity of the ductal abnormalities seen by ERCP with pancreatic cancer led to an early interest in trying to obtain brush cytologic specimens during ERCP to enhance the diagnostic accuracy of the procedure.^{93,94,103–109} However, success rates were extremely variable, running from 20% to 80%, with most series having yields of diagnostic specimens in the range of 40% to 50% for pancreatic carcinomas and 60% to 80% for cholangiocarcinoma. Many variations for obtaining cytologic or tissue specimens at the time of ERCP have been reported to try to improve the poor diagnostic yield of the procedure in pancreatic cancer.^{110–115} These include transpapillary biopsy,^{109,116,117} new brush devices,^{110,118,119} transductal fine-needle aspiration,¹²⁰ dilating strictures before brush cytology,¹²¹ or the addition of flow cytometry¹²² or *K-ras* analysis of pancreatic juice.^{123–125} However, none of these have dramatically improved the definitive tissue diagnosis of pancreatic cancer ERCP.

A problem with many of these ERCP cytologic series is that they somewhat artificially increase the

reported cytologic yield with ERCP by counting a “suspicious” diagnosis as positive for cancer. While it is true that a suspicious cytology usually means there is an underlying pancreaticobiliary malignancy,^{126,127} it is usually not sufficiently definitive information to proceed on with nonsurgical therapy such as chemotherapy, radiation therapy, or palliations such as placing a permanent metallic biliary or enteral stent.

Finally, although the complication rate for CT, US, or EUS-guided FNA of pancreatic masses is about 1% to 2% (see above), the complication rate for ERCP cytologic brushing is as high as 11% for the biliary tree and 21% for pancreatic strictures.¹²⁸ Thus, if ERCP is being performed for another indication (eg, stent placement for obstructive jaundice) and no tissue diagnosis is yet available, attempt at transductal brush cytology or biopsy is worthwhile. However, performing ERCP just for tissue diagnosis in suspected pancreatic malignancy can no longer be recommended when US, CT, and especially EUS-guided FNA have much higher yields of a definitive diagnosis and with much lower morbidities.

ERCP in the Staging of Pancreatic Neoplasms

There is very little information on whether ERCP provides any useful staging information for patients with pancreatic cancer. Certainly, in patients presenting with obstructive jaundice and having a biliary stricture by ERCP (Figures 8.2B, 8.3B, 8.3C), one can surmise that it is likely the patient has at least a T3 lesion (by 1997 AJCC criteria). However, there is no way to discern by ERCP whether the tumor extends into the portal or superior mesenteric vein by the appearance of a cholangiogram or pancreatogram. The length of either a pancreatic or biliary duct stricture does correlate with tumor as measured by other imaging modalities.^{129,130} Since tumor size does correlate with prognosis and tumor stage,¹³¹ stricture length at ERCP does give some crude staging, resectability, and prognostic information. However, this information is at best complementary to the much more accurate staging information provided by US, CT, MRI, or EUS. Somewhat surprisingly, even a normal pancreatogram with a pancreatic cancer does not imply a better prognosis or smaller tumor.¹⁰²

Therapeutic ERCP for Pancreatic Neoplasms

Like EUS, ERCP is not only a powerful diagnostic tool in pancreatic cancer, but since the 1980s has become a primary means for palliation of obstructive jaundice^{132,133} using endoscopically placed plastic or expandable metallic stents (see Chapter 19). There has also been some limited interest in stenting the obstructed pancreatic duct for pain relief in selected patients with pancreatic cancer.^{134–136} However, this invasive technique often requires repeated ERCPs, and the pain relief provided appears to be more temporary than that reported with the better-studied approach of celiac neurolysis (see Chapter 20).

The Role of ERCP in Pancreatic Cancer and Interface with EUS

ERCP developed in parallel with computed tomography and ultrasound. Despite the high sensitivity of ERCP for detecting ductal abnormalities in pancreatic cancer, because of its higher cost, invasiveness, and morbidity, it evolved into a diagnostic procedure used primarily when CT or US was nondiagnostic in a patient with suspected pancreatic pathology.^{137–140} With the advent of spiral CT, MRI, and now EUS, the role for purely diagnostic ERCP in pancreatic cancer should be limited to the occasional patient where pancreatic cancer is still suspected despite a negative EUS/EUS-guided FNA, CT, and/or MRI (Figure 8.4), or where these procedures may not be readily available at a nearby referral center.

In patients presenting with a suspected pancreatico-biliary malignancy and obstructive jaundice, ERCP has traditionally been considered the endoscopic procedure of choice.^{132,133} However, EUS can accurately diagnose almost all the causes of obstructive jaundice, including pancreatico-biliary malignancies^{141–144} and choledocholithiasis,^{145–147} with the accuracy equal to or better than ERCP. As described above, EUS-guided FNA provides a tissue diagnosis of underlying pancreatico-biliary malignancy with a much higher success rate (80% to 93%) than ERCP (30% to 60%). Additionally, unlike ERCP, EUS provides valuable staging information if a pancreatico-biliary malignancy is found. Most importantly, the diagnostic, cytologic, and staging information provided by EUS and

EUS-guided FNA does not carry ERCP's significant risk of pancreatitis or cholangitis, nor does performing EUS/EUS-guided FNA mandate placing a biliary stent^{91,92} or using prophylactic antibiotics^{148,149} in the obstructed patient as does performing a diagnostic ERCP. In fact, the only limitation of EUS compared to ERCP in obstructive jaundice is its inability to perform therapeutic endoscopic procedures on the biliary tree, such as removal of common bile duct stones or placing biliary stents in patients who are not going to be treated operatively.

The diagnostic capabilities and lower morbidity of EUS/EUS-guided FNA have led our institution^{150,151} and others^{152,153} to propose that the ideal endoscopic approach to obstructive jaundice of unknown origin is the combination of EUS/EUS-guided FNA followed directly by ERCP if necessary for therapy. This may be especially true for patients with possible malignant obstructive jaundice, since pancreatic cancer is the most common cause of malignant biliary obstruction and EUS/EUS-guided FNA has already been shown to be highly cost-effective in the management of pancreatic carcinoma.³³ We recently reported a study of the cost-effectiveness of this approach.¹⁵⁴ Using EUS/EUS-guided FNA before ERCP in obstructive jaundice saved \$1,000 to \$2,500 in medical costs per patient.

Conclusion

Although it is an endoscopic procedure, high-quality EUS with EUS-guided FNA capabilities can play a central early role in the diagnosis, cytologic assessment, staging, and even therapy of the patient with pancreatic cancer. The primary limiting factor in EUS assuming this preeminent position at most institutions has been the lack of well-trained endosonographers. This need is being aggressively addressed by the various gastroenterologic professional societies and gastroenterology training programs. With EUS, spiral CT, and MRI available, diagnostic ERCP has little role in the initial evaluation of patients with suspected pancreatic cancer unless they are presenting with obstructive jaundice. Even in this clinical setting, EUS and EUS-guided FNA may be the preferable first endoscopic procedure, with ERCP reserved for those patients definitely needing endoscopic stenting.

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9

Laparoscopic Staging

H. J. Kim and K. C. Conlon

Introduction

In 1999, approximately 28,600 new cases of pancreatic cancer were diagnosed in the United States, and 28,600 deaths estimated, making pancreatic cancer the fourth leading cause of cancer-related deaths.¹ The majority of these patients have advanced disease at the time of diagnosis, with over 50% of the patients presenting with metastatic disease. In spite of improvements in diagnosis, only 10% to 15% of the patients present with resectable tumors, with 5-year survival rates of 15% to 20% reported following potentially “curative” surgical resections.^{2,3}

Rationale/Implications for Therapy

Despite these dismal results it must be emphasized that currently, complete surgical resection with negative margins offers the only chance for long-term survival.²⁻⁶ Thus, identification of the subgroup of patients that could potentially benefit from a resection is critical. As in other settings, the goals of clinical staging should be directed not only at defining the extent of disease and directing appropriate therapy, but also at avoiding unnecessary intervention, in a safe and cost-effective manner.² In recent years nonoperative management of biliary and gastric outlet obstruction has improved, questioning the traditional approach regarding surgical exploration for palliation in this patient population.^{2,7,8} Patients with disseminated pancreatic cancer have a very limited survival and would have the most to gain from a selective surgical approach.^{9,10}

Preoperative Staging Modalities

Notwithstanding the rapid development of increasingly sophisticated diagnostic imaging modalities, the true extent of disease in pancreatic cancer remains difficult to fully ascertain nonoperatively.^{3,11-16} Adenocarcinoma of the pancreas has the propensity to be locally aggressive and metastasize to the liver and peritoneal cavity. Size itself does not predict whether metastatic disease will be present. Dynamic, contrast-enhanced computed tomography (CT) is the radiological study of choice in the preoperative evaluation of pancreatic malignancies. However, CT evaluation alone often underestimates the extent of disease, as small-volume (1–3 mm) liver/peritoneal/omental metastases are often outside the resolution of the technique. Several contemporary studies have examined the accuracy of various preoperative staging modalities. Warshaw et al.¹⁷ compared the results of dynamic contrast-enhanced CT, magnetic resonance imaging (MRI), angiography, and laparoscopy in patients considered to have potentially resectable disease. The accuracy of CT was 92% in determining unresectability (only 56% sensitive), but only 45% in predicting resectability. CT scans failed to identify small-volume liver metastases. Similarly, angiography was 95% accurate in determining unresectability (66% sensitive), but again only 54% in predicting resectability. MRI findings were similar to those of CT scanning and offered no advantages. The overall accuracy of laparoscopy in predicting unresectability was 98% (96% sensitive); however, in 19 cases in which no metastases were seen at laparoscopy, only 8 patients underwent subsequent resection.

While advances in CT scan imaging techniques have led to significant improvements in the diagnosis and staging of pancreatic malignancies, as suggested above, the resolutions of even the latest CT techniques are limited to lesions larger than 2 to 3 mm. Contrast-enhanced CT has demonstrated a high sensitivity in determining locoregional extension and vascular encasement, predicting unresectability in up to 100% of patients. However, the ability of CT to predict resectability still ranges from 57% to 88% in reported series.¹³⁻¹⁶ It must be said that differences in resectability rates may be due to variability in the technical performance and interpretations of CT among institutions as well as the philosophy and aggressiveness of the surgeon. Nonetheless, even at tertiary referral centers upwards of 15% to 20% of patients are found to have unresectable disease at laparotomy that was not apparent by preoperative imaging modalities. At the M. D. Anderson Cancer Center, in a specialized practice, the prospective use of helical, thin-section CT combined with objective CT criteria for resectability has resulted in a resectability rate of 80%.¹⁸

The improvements in CT technology have reduced the use of selective visceral angiography in staging. However, some groups continue to utilize it routinely and thus its role in the preoperative workup of pancreatic malignancies remains controversial.¹⁹⁻²¹ Dooley et al.¹⁹ assessed the role of angiography in 90 patients considered resectable by CT criteria; of the 68 patients with normal angiograms, 48 underwent resection (77% resectability rate). However, 21% of the patients that had angiographic criteria of unresectability ultimately underwent pancreaticoduodenectomy; despite this high false-positive rate, the authors concluded that angiography has a role in preoperative staging. Warshaw et al.¹⁷ also reported that selective angiography was a significant independent factor in determining resectability, but suggested that neither CT nor angiography sufficed alone. It is our feeling that the improvements in CT have obviated the need for angiography and we no longer routinely use this modality.

Recently, improvements in MRI have led some authors to suggest that it may replace high-resolution CT scans in the future as the primary diagnostic modality. Trede and colleagues from Germany reported that ultrafast MRI was more accurate

in evaluating extrapancreatic extension, lymph node involvement, and vascular invasion, when compared to CT, endoscopic retrograde cholangiopancreatography, or ultrasonography.^{22,23} Other studies have examined the evolving role of magnetic resonance cholangiopancreatography in the preoperative evaluation of pancreaticobiliary neoplasms.^{24,25} None of these reports have demonstrated a clear advantage of MRI over CT; however, as the technology continues to evolve and improve it may be that MRI will replace CT as the methodology of choice.

Potential Role of Laparoscopy

Recently, we and others have suggested that in combination with CT or MRI patients with localized, potentially resectable pancreatic cancer should undergo a laparoscopic procedure prior to open exploration. The aim would be to detect subradiological disease and prevent unnecessary exploration. This concept is not new, having been described in the early 20th century.^{26,27} However, poor instrumentation and lack of alternative therapeutic options limited its applicability. Recent improvements in the instrumentation²⁸ coupled with the development of a multidisciplinary approach to palliation have rekindled interest in the modality. Laparoscopy, with the addition of laparoscopic ultrasound, allows the surgeon to visualize the primary tumor, accurately determine vascular involvement, identify regional nodal metastases, and detect small-volume peritoneal/liver metastases, all of which can be biopsied if required.

In early studies, Cuschieri²⁹ and Warshaw et al.³⁰ demonstrated that small hepatic or peritoneal implants could be detected with considerable accuracy using standard laparoscopic techniques. In 114 patients with presumed localized pancreatic cancer treated at the Massachusetts General Hospital,³⁰ metastases were identified in 27 patients with laparoscopy, none of which required further open exploration. Forty-two patients were excluded from further surgery by angiography, and 40 patients were explored, with resections performed in 30 patients. Similar results were reported by John et al.,³¹ demonstrating unsuspected metastatic disease in 14 of 40 patients considered to have resectable disease prior to laparoscopy. However, in this study, laparoscopy was only 50% sensitive in predicting tu-

mor resectability, with failure in identifying intra-abdominal dissemination in 3 patients and locoregional disease in 12 patients.

In order to improve the sensitivity of laparoscopy in determining resectability, several groups have examined the addition of laparoscopic ultrasound (LUS). This modality partially overcomes the lack of tactile sensation present in standard laparoscopy.^{31–35} John et al³¹ reported an accuracy in predicting tumor resectability of 89% with the addition of LUS. Bemelman and colleagues³⁵ used LUS in combination with diagnostic laparoscopy in the staging of pancreatic head tumors, resulting in a sensitivity and specificity in determining resectability of 67% and 96%; 21 of 22 patients considered to have resectable disease after LUS underwent subsequent resection. Merchant and Conlon² from Memorial Sloan-Kettering Cancer Center (MSKCC) reported a series of 442 patients presenting with pancreatic cancer between 1992 and 1996. After dynamic CT scanning, 339 patients were deemed potentially resectable and 303 patients underwent extended staging laparoscopy; 199 patients were deemed resectable and 181 were ultimately resected, with a 9% false-negative rate. In our hands, laparoscopic assessment provided a positive predictive index of 100%, a negative predictive index of 91%, and an overall accuracy of 94%. The addition of LUS improved the accuracy of determining resectability from 94% to 98%. In an earlier report we showed that the improved resectability rate translated into a reduction in the hospital stay for patients who underwent laparoscopic staging.¹³ Patients with unresectable disease who underwent laparoscopy with biopsy alone had a significantly reduced median hospital stay (2 days) when compared to those undergoing open exploration (7 days), biliary bypass (9.5 days), or gastric and biliary bypass (12 days).

Technical Aspects of Laparoscopic Staging

A multiport laparoscopic technique, which aims to mimic the standard assessment of resectability, is recommended. To avoid visceral or vascular injury with a veres type needle we routinely use an open technique for creation of the “pneumoperitoneum.”

TABLE 9.1. Sequence of the examination.

• Port placement
• General peritoneal assessment/aspiration of peritoneal washings for cytology
• Examination of the liver/porta hepatis/foramen of Winslow
• Retraction of the transverse colon/visualization of ligament of Treitz and mesocolon
• Incision of gastrohepatic omentum/examination of lesser sac
• Laparoscopic ultrasonography

A 30-degree angled scope is used to perform the laparoscopic examination. Secondary ports are placed in the right (10-mm) and left (5-mm) upper quadrants in the line of a planned skin incision for laparotomy. A systemic examination is then performed (Table 9.1).

Initially, any ascites is aspirated and peritoneal washings performed. Washings are collected from the right and left upper quadrants of the abdomen prior to any manipulation of the primary tumor.

A systematic four-quadrant examination is performed with biopsy of any suspicious lesions. The liver is then examined. Placing the patient in a slight reverse Trendelenburg position facilitates this. Using a two-handed technique, we examined the liver with the aid of blunt dissecting instruments. Using this technique, small subcapsular lesions can be appreciated.

The hepatoduodenal ligament is examined for adenopathy or gross encroachment by tumor. The porta hepatis is examined along with the foramen of Winslow. If indicated, suspicious nodes or nodules can be biopsied.

The duodenum is assessed. In contrast to open exploration, the duodenum is not mobilized. We have found that this maneuver is tedious and has little yield.

The patient is then positioned in 10-degree reverse Trendelenburg, and the omentum retracted to the left upper quadrant. This facilitates the retraction of the transverse colon, which allows visualization of the ligament of Treitz and the transverse mesocolon.

Following examination of the colonic mesocolon the patient is returned to the supine position and the left lobe of the liver elevated by an atraumatic retractor. The gastrohepatic omentum is incised, allowing entry into the lesser sac. The neck, body,

and tail of the pancreas can be seen in most cases. The relationship of the tumor to the celiac axis and hepatic artery can be assessed. Suspicious portal, perigastric, or celiac lymph nodes can be biopsied. In very obese patients moving the camera port from the periumbilical to right-upper quadrant is required to allow these maneuvers to be performed.

In cases in which no contraindications to resection have been found, LUS is then performed. A description of our technique was previously published and is detailed below.³⁶ In brief, LUS evaluates the liver and pancreas, and assesses the relationship of the primary tumor to the portal vein and mesenteric vessels.

We believe that confirmed hepatic, serosal, peritoneal, or omental metastases or invasion or encasement of the perihilar portal vein, celiac axis, hepatic artery, or superior mesenteric artery constitute absolute contraindications to resection. Tumor extension outside the pancreas (ie, mesocolic involvement) or nodal disease represents a relative contraindication.

Laparoscopic Ultrasound

The addition of LUS to the staging procedure has been shown to increase the accuracy, positive predictive index, and negative predictive index of laparoscopic staging.³⁶ Ultrasound identifies small intraparenchymal hepatic lesions and extends our ability to delineate resectability in patients with equivocal findings at laparoscopy.

The ultrasound system employed at MSKCC has simultaneous imaging capability and employs pulsed and color-flow Doppler for vascular assessment. We use a two-way flexible, 10-mm, 7.5-MHz laparoscopic probe (Aloka, Japan). The ultrasound probe is inserted via the right upper quadrant 10-mm port. The liver is examined using Couinaud's anatomy as a guide. Initially, segments 1 to 3 are scanned. The vena cava is then visualized at the back of the dome of the right lobe; moving the probe anteriorly to the porta allows identification of the hepatic veins. Each hepatic segment is examined by gradual sweeping and rotational movements of the probe. When this is completed the probe is placed transversely across the hepatoduodenal ligament, a maneuver that allows examination of the bile duct, portal vein, and common hepatic artery. The transducer is then placed transversely on the gastrocolic omentum, the confluence of the portal vein with the superior mesenteric vein identified, and the relationship of the tumor to these structures assessed. The pancreas is then examined. Particular attention is paid to the tumor and any peripancreatic adenopathy. Rotation of the transducer at this point allows visualization of the celiac axis and the proximal hepatic artery. Alternatively, the left lateral segment of the liver can be used to provide for a tissue interface in order to examine the celiac axis. Figure 9.1 outlines our algorithm for staging peripancreatic malignancies.

Morbidity following laparoscopic staging remains low, and major complications (hemorrhage, visceral perforation, intra-abdominal infection, and shock) are uncommon, occurring in less than 2%

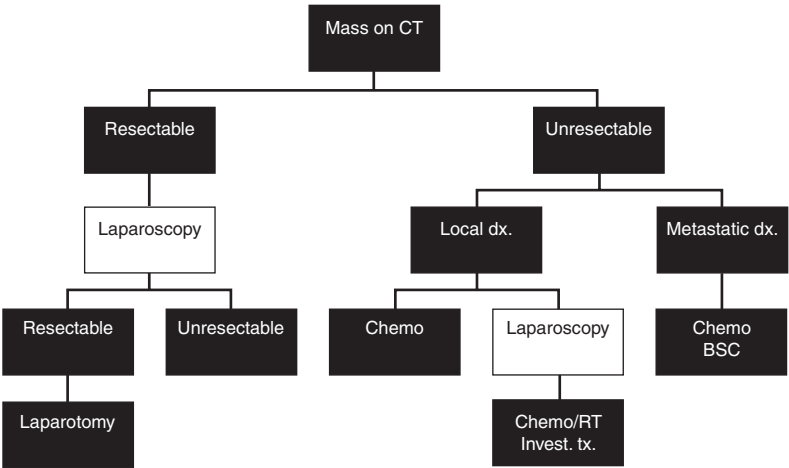


FIGURE 9.1. Placement of laparoscopy in the diagnostic algorithm for patients with suspected pancreatic cancer.

of cases. Minor complications such as wound infection, port herniation, or urinary retention are also uncommon. At our institution, minor complications have been noted in 1% of patients following laparoscopic staging procedures for pancreatic malignancies.^{13,37} Several clinical and basic research studies have also raised the concern regarding dissemination of disease during laparoscopy. Clinical data are sparse.^{38,39} Barrat et al examined 109 patients in a prospective study and suggested that peritoneal spread and tumor manipulation are important factors in port-site metastasis.³⁸ In contrast, Feig's group from the M. D. Anderson Cancer Center noted that in only 4 patients out of 533 cases (0.8%) did recurrence at the port site occur following laparoscopy. Three of these patients had advanced disease at the time of laparoscopy, and none of the 109 patients with pancreatic cancer developed a port-site recurrence.³⁹

Criticisms of Laparoscopic Staging

As was mentioned above, the utilization of laparoscopic staging for patients with pancreatic cancer is somewhat controversial. Some authors argue that it should be used in the majority of cases, others suggest a more selective approach, while some have stated that it has no role to play in the current management of peripancreatic malignancy. Critics argue that only exploratory laparotomy allows for accurate assessment of resectability and that most unresectable patients eventually require palliative procedures to relieve biliary or gastric obstruction.⁴⁰ Others have suggested that laparoscopy should be considered only for patients with demonstrable metastatic disease, as their life expectancy is short and nonoperative palliative methods adequate. The true role probably lies somewhere in between these views.

Espat et al.⁴¹ reported our prospective, nonrandomized experience with 155 patients with pancreatic adenocarcinoma undergoing laparoscopic staging alone in order to study the subsequent need for surgical palliation. In these patients with unresectable pancreatic cancer, Stage II/III (n = 40) and Stage IV (n = 115) disease, only 4 patients (3%) required an additional operation for palliation on follow-up; this result is similar to reported "failure" rates after prophylactic open biliary or gastric bypass procedures (Table 9.2).^{5,41} This study sug-

gested that operative palliation is not required for the majority of patients. As a result, we are aggressive in utilizing endoscopic or percutaneous biliary stenting in patients with obstructive jaundice secondary to unresectable pancreatic tumors. Operative management is mainly reserved for patients with gastroduodenal obstruction or patients with biliary obstruction that either fail conservative attempts or have expectations of relative extended survival. It must be emphasized that this study was not randomized and did not measure any quality-of-life parameters. The latter is required before this question is answered, and studies with this aim in mind are currently under way at our institution.

Critics of minimal access approaches to pancreatic cancer have also argued that if laparoscopy is unnecessary in a large percentage of patients, it therefore is not cost-effective.^{18,42,43} It has been suggested that the added benefit of laparoscopy after a high-quality, contrast-enhanced, current generation, helical CT scan is limited.¹⁸ However, our experience is that despite the use of state-of-the-art radiology, the added benefit of laparoscopy is approximately 20%. In the decade prior to the introduction of laparoscopy at MSKCC (1983–1992), only 35% of 1135 patients that were explored with curative intent were actually resected.⁴⁴ Over the next four years, 243 patients with radiologically resectable disease were evaluated by laparoscopy and 185 (74%) were ultimately resected. Currently, at MSKCC if a patient with pancreatic cancer undergoes an open operation the probability is >80% that he or she will be undergoing a resection. If the patient has been considered to have resectable disease

TABLE 9.2. Impact of laparoscopic versus open staging.

	MSKCC ^{41*}	Johns Hopkins ^{5**}
Years	1993–1997	1991–1997
Form of staging	Laparoscopic only	Open
Number of patients	155	256
Mean age	65	64
Median length of stay (days)	2	10.1
Mortality (%)	0	3.1
Morbidity (%)	<1%	22
Immediate reoperation (%)	0	1
Total reoperation (%)	3	4 / 2

*Pancreatic adenocarcinoma only

**94% pancreatic adenocarcinoma

after staging laparoscopy the figure is >92% (KC Conlon, unpublished data, 2000). Our overall resectability rate for patients remains similar to those reported from other tertiary referral centers who do not utilize routine laparoscopic staging, emphasizing our belief that staging laparoscopy does not affect the number of patients resected but rather reduces open procedures in those who would not be resected.⁴⁵

Conclusions and Future Considerations

Despite major advances in preoperative staging modalities and surgical techniques/technologies, the biological course and overall prognosis for patients with pancreas cancer remains dismal. It is clear that prospective trials of novel therapies are required for patients with potentially resectable, locally advanced or metastatic disease. In our experience, minimal-access surgical techniques in combination with dynamic, contrast-enhanced CT appears to be an efficient, cost-effective, and safe means of staging this disease. Further studies are ongoing to determine the quality-of-life implications of laparoscopic staging and palliative procedures.

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10

Whipple Procedure: 1935 to Present

Richard D. Schulick and Charles J. Yeo

Early Experience

Over a century has passed since the first successful treatment of a periampullary carcinoma (Table 10.1). William S. Halsted (Figure 10.1) of The Johns Hopkins Hospital reported the first successful resection of an ampullary cancer in 1899. He described a transduodenal local ampullary resection with reanastomosis of the pancreatic and bile ducts to the duodenum in a patient presenting with obstructive jaundice.¹ The patient redeveloped obstructive jaundice 3 months after the initial operation and required a second operation in which a cholecystoduodenostomy was performed to decompress the biliary tree. The patient died 6 months later and an autopsy revealed recurrent ampullary carcinoma invading into the head of the pancreas and duodenum.

In the early part of the 20th century, most ampullary cancers were managed by a transduodenal approach similar to that first reported by Halsted. In 1935, a review by Hunt and Budd described 76 patients with periampullary tumors managed by such a transduodenal approach.² The overall operative mortality in this group of patients was 49%. During this period, however, there were sporadic reports from several surgeons who performed en bloc pancreaticoduodenectomy. The first en bloc resection of the head of the pancreas and duodenum for periampullary carcinoma is credited to Codivilla, but unfortunately the patient did not survive the postoperative period.³ Kausch, a German surgeon from Berlin, performed the first successful partial two-stage pancreaticoduodenectomy in 1912.⁴ The neck of the pancreatic remnant was im-

planted into the distal end of the partially resected duodenum in the second stage of his procedure. In 1914, Hirschel reported the first successful one-stage partial pancreaticoduodenectomy.⁵ He removed part of the duodenum, the ampulla, part of the head of the pancreas, and the lower half of the common bile duct. The patient was reconstructed by implantation of the pancreatic duct into the duodenorrhaphy, as well as by gastrojejunostomy and interposition of a rubber tube between the common duct and duodenum. The patient was relieved of obstructive jaundice postoperatively and died 1 year later. No autopsy was performed on this patient so the status of the cancer and the fate of the rubber tube are unknown.

In 1935, Allen Oldfather Whipple (Figure 10.2) and colleagues reported 3 patients with ampullary cancer managed by a two-stage pancreaticoduodenectomy to the American Surgical Association at its annual meeting.⁶ The first stage involved ligation and division of the common bile duct, a cholecystogastrostomy, and a gastrojejunostomy (Figure 10.3). The second stage involved a pancreaticoduodenectomy, ligation of the pancreatic duct, closure of the pancreatic capsule over the neck of the pancreas, and retroperitoneal drainage. Whipple is generally credited with popularizing the operation that bears his name. Whipple was the Chairman of Surgery at Presbyterian Hospital at the time he developed this operation and later became the Clinical Director at Memorial Sloan-Kettering Cancer Center, both in New York City. In a reminiscence published posthumously, Whipple reported that he performed 37 pancreaticoduodenectomies during his career, with the operation evolving from a two-

TABLE 10.1. Milestones in the development of the Whipple procedure.

Individual	Year	Contribution
Halsted	1899	First successful local resection of ampullary cancer
Codivilla	1908	First published attempt at en bloc resection of head of pancreas and duodenum
Kausch	1912	First successful partial pancreaticoduodenectomy in two stages
Hirschel	1914	First successful partial pancreaticoduodenectomy in one stage
Whipple	1935	Reported three patients with ampullary cancer managed by pancreaticoduodenectomy in two stages
Brunschwig	1937	First successful pancreaticoduodenectomy in two stages for pancreatic cancer

stage to a one-stage procedure by the early 1940s.^{7,8} Whipple stressed the importance of treating patients with vitamin K and bile salts for several days before attempting a one-stage procedure in jaundiced patients. His one-stage operation involved reconstruction with an end-to-end choledochojejunostomy, an end-to-side pancreaticojejunostomy, and an end-to-side gastrojejunostomy, all anastomosed to the proximal jejunum, which was constructed in retrocolic fashion (Figure 10.4).

Brunschwig (from the University of Chicago and later from Memorial Sloan-Kettering Cancer Cen-

ter) reported extending the indication for pancreaticoduodenectomy to include cancer of the head of the pancreas in 1937.⁹ He resected a 4-cm adenocarcinoma of the head of the pancreas from a 69-year-old male in a two-stage procedure. In the first stage, he performed a cholecystojejunostomy, a gastroenterostomy, and an enteroenterostomy (Figure 10.5). In the second stage, he performed a partial pancreaticoduodenectomy and oversewed the pylorus, distal duodenum, and pancreatic neck.

During the 1940s and 1950s, pancreaticoduodenectomy was performed in limited numbers, with



FIGURE 10.1. William Stewart Halsted of The Johns Hopkins Hospital in Baltimore reported the first successful resection of an ampullary cancer in 1899.

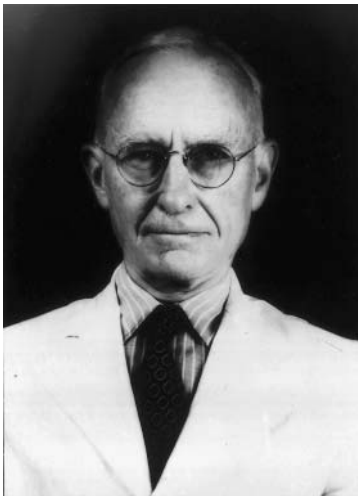


FIGURE 10.2. Allen Oldfather Whipple of The Presbyterian Hospital in New York City popularized the pancreaticoduodenectomy operation that bears his name.

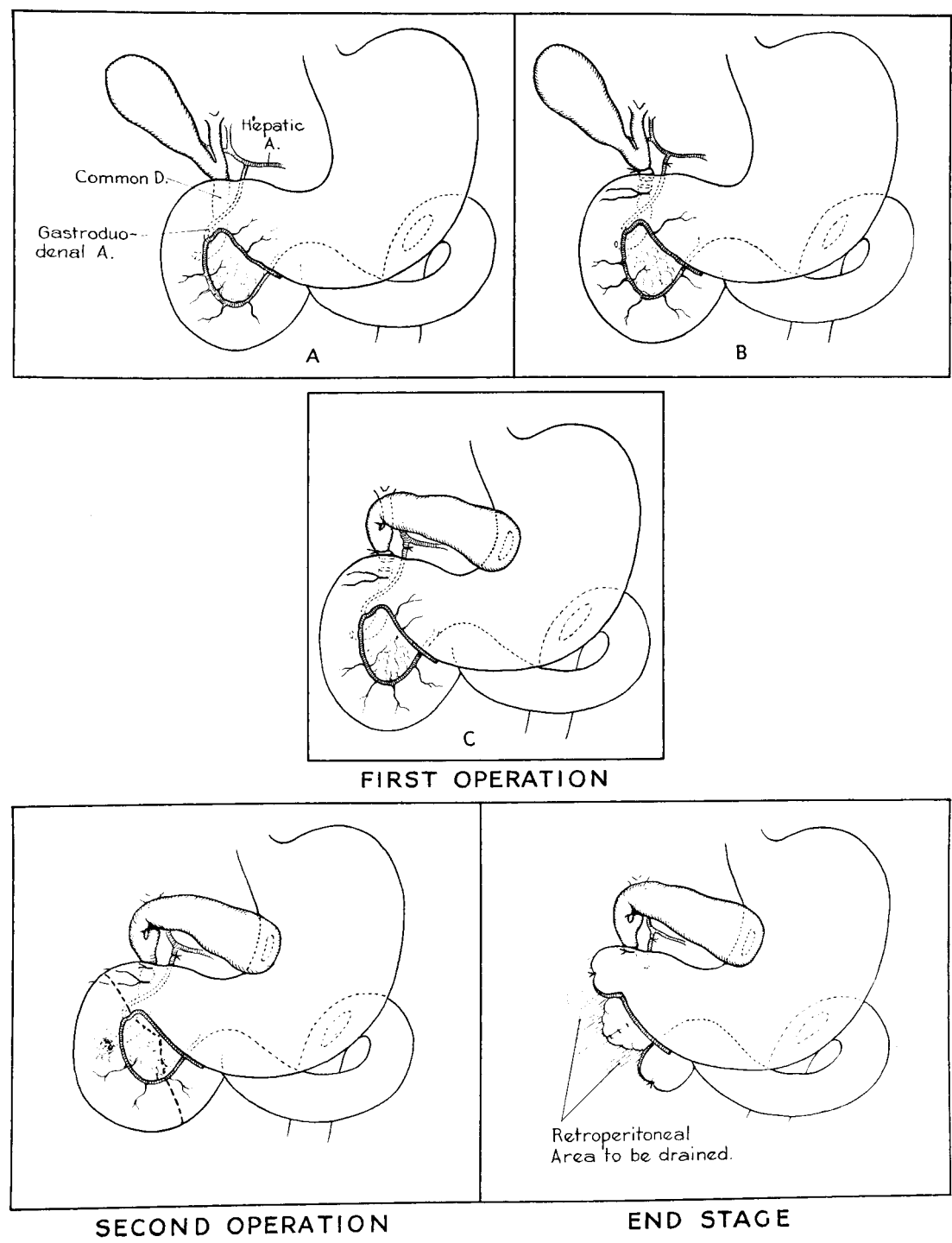


FIGURE 10.3. Steps in performing two-stage pancreaticoduodenectomy performed by Whipple. (From Whipple et al,⁶ by permission of *Ann Surg*.)

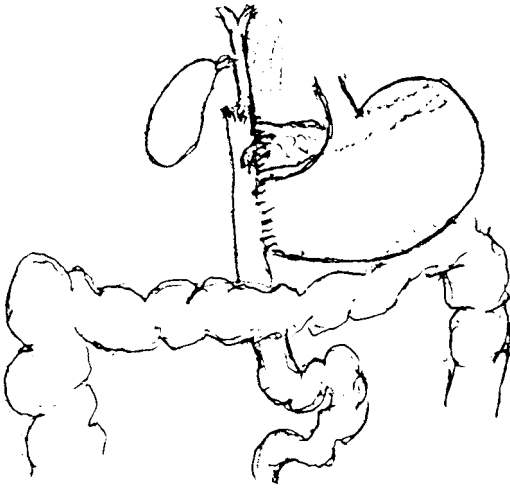


FIGURE 10.4. Freehand drawing by Whipple showing reconstruction after one-stage pancreaticoduodenectomy in his patients operated on in 1942 and after. (From Whipple,⁷ by permission of *Rev Surg*.)

variable results. It was applied to patients with periampullary neoplasms and was typically performed as a one-stage procedure. Even as late as the 1960s and 1970s, pancreaticoduodenectomy carried a high hospital mortality that approached 25% in some series, leading some authorities to suggest that its use be abandoned.^{10,11} A notable exception to this high mortality rate was a report by Howard in 1968, describing 41 consecutive patients treated by pancreaticoduodenectomy without a hospital

mortality.¹² More recently, improved hospital morbidity, mortality, and survival after pancreaticoduodenectomy have been reported.^{13–16} Trede et al, in 1990, reviewed 118 consecutive resections without an operative death.¹⁷ A report from Johns Hopkins in 1993 described 145 consecutive pancreaticoduodenectomies without mortality.¹⁸ A recent series from Johns Hopkins reporting on patients operated on in the 1990s noted that 190 consecutive resections had been performed without mortality out of a series of 650 patients undergoing the Whipple procedure from 1990 to 1996.¹⁹ Many centers now report operative mortalities of less than 4%.

Current Technique

Exposure for the Whipple procedure is performed usually through a vertical midline incision from the xiphoid process to several centimeters below the umbilicus. A bilateral costal margin retractor is then placed to elevate and separate the rib cage, and a second retractor is placed to separate the wound edges.

The first portion of the Whipple procedure is devoted to assessing the extent of disease and resectability. There is debate as to the benefits of a staging laparoscopy versus open staging in anticipation of surgical resection or palliation. These issues are discussed in separate chapters in this text. At open exploration, the entire liver is assessed for the presence of metastases not seen by preopera-

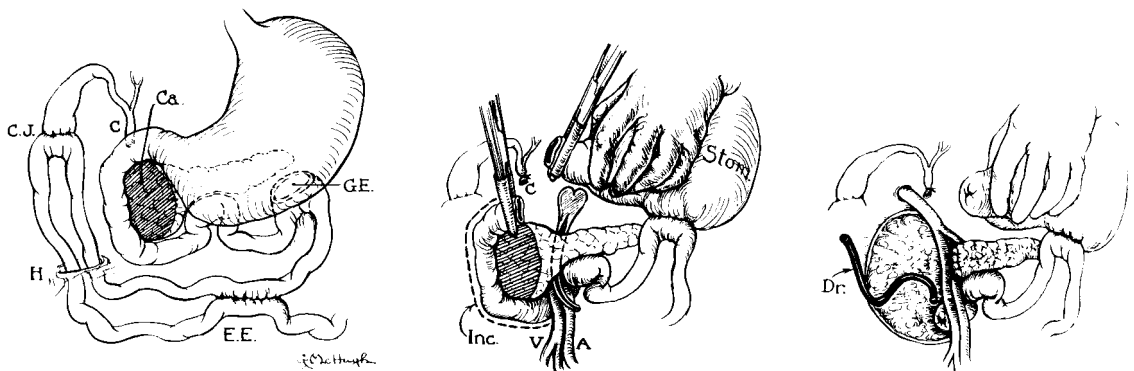


FIGURE 10.5. Three steps in performing two-stage pancreaticoduodenectomy performed by Brunschwig for adenocarcinoma of the head of the pancreas. (CJ = cholecystojejunostomy, C = common bile duct, Ca = pancreatic cancer, GE = gastroenterostomy, EE = enteroenterostomy, V = superior mesenteric vein, A = superior mesenteric artery, Dr = drain). (From Brunschwig,⁹ by permission of *Surg Gynecol Obstet*.)

tive imaging studies. The celiac axis is inspected for lymph node involvement. Tumor-bearing lymph nodes within the resection zone do not contraindicate resection because long-term survival is possible with peripancreatic nodal involvement. The parietal and visceral peritoneal surfaces, the omentum, the ligament of Treitz and the entire small and intra-abdominal large intestine are carefully examined. An extensive Kocher maneuver is performed by elevating the duodenum and head of the pancreas out of the retroperitoneum and into the midline, enabling visualization of the superior mesenteric vein and palpation of the superior mesenteric artery. The porta hepatis is assessed by mobilizing the gallbladder out of the gallbladder fossa, and dissecting the cystic duct down to the junction of the common hepatic duct and common bile duct.

If the intraoperative assessment reveals localized disease without tumor encroachment upon resection margins, the resection is performed in relatively standard fashion. If the intraoperative assessment reveals evidence of local tumor extension giving the early impression of unresectability, the normal sequence for performing the pancreaticoduodenectomy should be modified. The easiest and safest portions of the resection should be performed first, and the more difficult portions later. Many tumors that appear unresectable at initial evaluation can be successfully resected by working where it is easiest first and saving the hardest part for last.

Dissection of the gallbladder out of its fossa and division of the distal common hepatic duct early (close to the level of the cystic duct entry site) allows caudal retraction of the common bile duct. This nicely opens the dissection plane on the anterior surface of the portal vein, allowing caudal dissection behind the neck of the pancreas. During these maneuvers, the portal structures should be assessed for a replaced right hepatic artery originating from the superior mesenteric artery. If found, a replaced right hepatic artery should be dissected and protected from damage. The gastroduodenal artery is next identified and clamped atraumatically. This maneuver confirms its identity and confirms that the hepatic artery is not being supplied solely retrograde through superior mesenteric artery collaterals (in the setting of celiac axis occlusion).

The benefit of pylorus preservation versus classic pancreaticoduodenectomy with hemigastrec-

tomy is discussed in a separate chapter of this text. For a pylorus-preserving pancreaticoduodenectomy, the proximal gastrointestinal (GI) tract is divided 2 to 3 cm distal to the pylorus with a linear stapling device. The right gastric artery can often be spared, but may be taken if it allows better mobilization of the duodenum. For a classic Whipple procedure, a 30% to 40% distal gastrectomy is performed. The GI tract is divided distally at a point of mobile jejunum, typically 10 to 20 cm distal to the ligament of Treitz. The mesenteric vessels to the jejunum to be taken are carefully divided over clamps and tied to avoid bleeding. Once the proximal jejunum is separated from its mesentery, it can be delivered dorsal to the superior mesenteric vessels from the left to the right side.

The superior mesenteric vein caudal to the neck of the pancreas is most easily identified by performing an extensive Kocher maneuver. The superior mesenteric vein is identified running anterior to the third portion of the duodenum and is frequently surrounded by adipose tissue, as it receives tributaries from the uncinate process and neck of the pancreas, the greater curve of the stomach, and from the transverse mesocolon. In this location, the superior mesenteric vein is identified by dissecting the fatty tissue of the transverse mesocolon away from the uncinate process of the pancreas. Division of the tributaries emptying into the anterior surface of the superior mesenteric vein allows continued cephalad dissection. Under direct vision, the plane anterior to the superior mesenteric vein is developed, carefully avoiding branches and tumor involvement. Care is taken so as not to injure the splenic vein as it joins the superior mesenteric vein posterior to the neck of the pancreas. After the plane anterior to the superior mesenteric and portal veins is complete, a Penrose drain is looped under the neck of the pancreas.

The pancreatic neck is next divided. A free plane anterior to the portal and superior mesenteric veins and posterior to the neck of the pancreas should first be confirmed. Stay sutures are placed superiorly and inferiorly on the pancreatic remnant to reduce bleeding from the segmental pancreatic arteries running there. The Penrose drain previously placed behind the neck of the pancreas is used to elevate the pancreatic tissue to be divided and protect the underlying major veins. The neck is divided using the coagulation current of the electrocautery.

Notice is taken of the site of the main pancreatic duct so that it can be incorporated into the subsequent reconstruction.

Dissection of the head and uncinate process of the pancreas from the portal vein, superior mesenteric vein, and superior mesenteric artery is then performed. This is performed by serially clamping, dividing, and tying the smaller venous branches off the portal and superior mesenteric vessels. Dissection should be performed flush to these structures to remove all pancreatic and nodal tissue in these areas. Great care is taken not to injure the superior mesenteric artery and vein at this level, but to remove completely the pancreatic tissue and lymph nodes off the vascular structures. With these steps completed the specimen is removed and the pancreatic neck margin, uncinate margin, and common hepatic duct margins are marked for the pathologists. To speed up analysis of these frozen section margins, the common hepatic duct margin and the pancreatic neck margin may be sampled intraoperatively and sent to pathology while the pancreaticoduodenectomy specimen is still being resected.

There are multiple methods for reconstruction after pancreaticoduodenectomy. Most commonly the reconstruction first involves the pancreas, followed by the common hepatic duct and the duodenum. The issues and controversies surrounding the pancreatic and biliary reconstruction are covered in detail in separate chapters in this text. In brief, the pancreatic anastomosis can be performed to the jejunum or to the stomach.²⁰ If the jejunum is used for reconstruction, some groups favor a separate Roux-en-Y reconstruction for the pancreas or even a double Roux-en-Y reconstruction for the pancreas and the bile duct. Controversy continues regarding the best type of pancreaticojejunostomy, the importance of duct-to-mucosa sutures, and the use of pancreatic duct stents. At the Johns Hopkins Hospital, the pancreatic reconstruction is typically performed via an end-to-end or end-to-side pancreaticojejunostomy to the proximal jejunum brought through a defect in the mesocolon to the right of the middle colic artery. The pancreatic remnant should be circumferentially cleared and mobilized for 2 to 3 cm, to allow for an optimal anastomosis. The pancreaticojejunostomy is typically performed in two layers (Figure 10.6). The outer layer consists of interrupted silk sutures that incorporate the capsule of the pancreas and the seromuscular lay-

ers of the jejunum. The inner layer consists of a running absorbable suture (or interrupted absorbable sutures) that incorporates the capsule and a portion of the cut edge of the pancreas and the full thickness of the jejunum. If possible, the inner layer incorporates the pancreatic duct, to splay it open. When completed, this anastomosis nicely invaginates the cut surface of the pancreatic neck into the jejunal lumen.

The biliary anastomosis is typically performed in an end-to-side fashion as an hepaticojejunostomy approximately 10 to 15 cm down the jejunal limb from the pancreaticojejunostomy (Figure 10.7). This anastomosis is typically performed with a single layer of interrupted absorbable sutures. T-tubes are not used for this anastomosis. If the patient has a percutaneous biliary stent, then this is left in place, traversing the anastomosis.

The third anastomosis performed is the duodenojejunostomy in cases of pylorus preservation, or the gastrojejunostomy in patients who have undergone classic pancreaticoduodenectomy with distal gastrectomy. This anastomosis is typically performed 10 to 15 cm downstream from the hepaticojejunostomy, proximal to the jejunum traversing the defect in the mesocolon (Figure 10.8).

After the reconstruction is completed, closed suction drains are left in place to drain the biliary and pancreatic anastomoses. It has been our practice to not perform vagotomy, and to not place gastrostomy tubes or jejunostomy tubes.

Postoperative Care

Following a Whipple procedure, patients are generally kept in a monitored recovery setting overnight, to allow for frequent assessment of their vital signs, oxygen saturation, and fluid status. They are usually able to transfer to a regular floor bed the following day. Deep vein thrombosis prophylaxis is accomplished by a combination of subcutaneous heparin, elastic stockings, and sequential compression devices. Arterial lines (if in place) are removed on postoperative day 1 and central venous lines are removed on postoperative day 2 unless the patient has difficult intravenous access. The nasogastric tube is generally removed on postoperative day 1. The Foley catheter is removed on postoperative day 2 except in male patients with a history of obstructive uropathy. If the patient has a percutaneous biliary

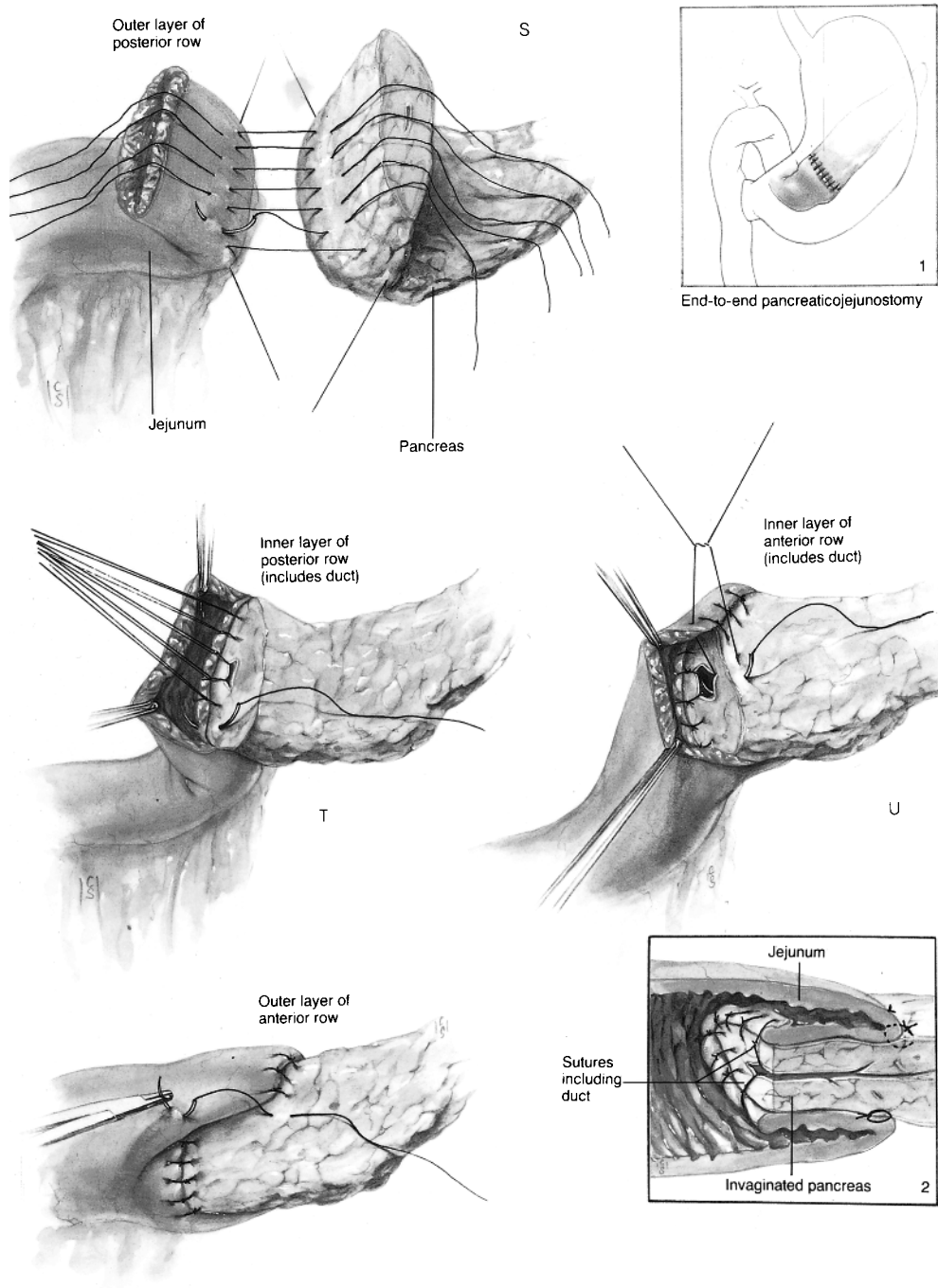


FIGURE 10.6. Two-layer end-to-end pancreaticojejunostomy. (From Cameron,²¹ by permission of B C Decker Inc.)

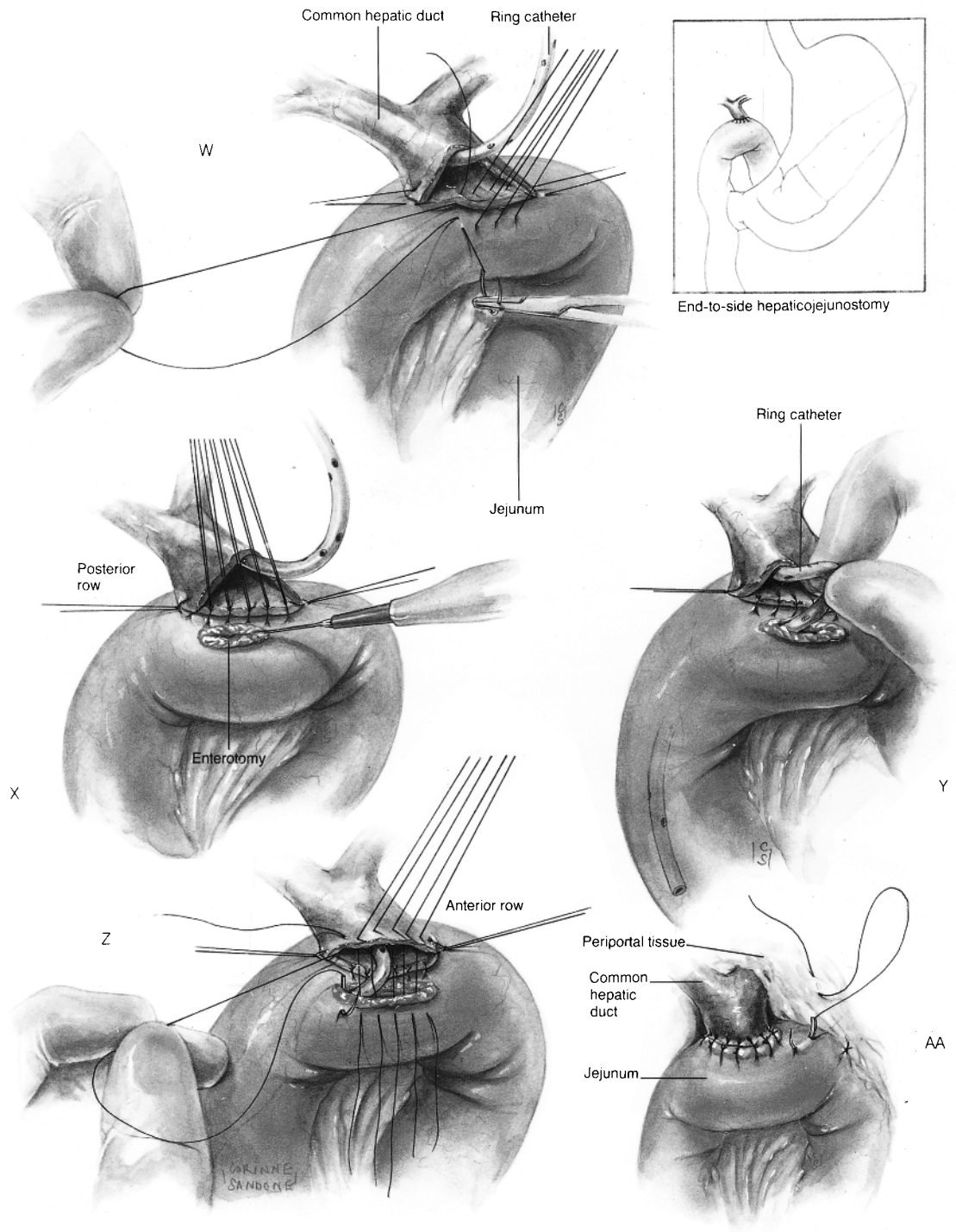


FIGURE 10.7. Single layer end-to-side hepaticojejunostomy. (From Cameron,²¹ by permission of B C Decker Inc.) While this illustration shows the anastomosis being performed over a Ring catheter, our practice now uses such tubes in a minority of patients.

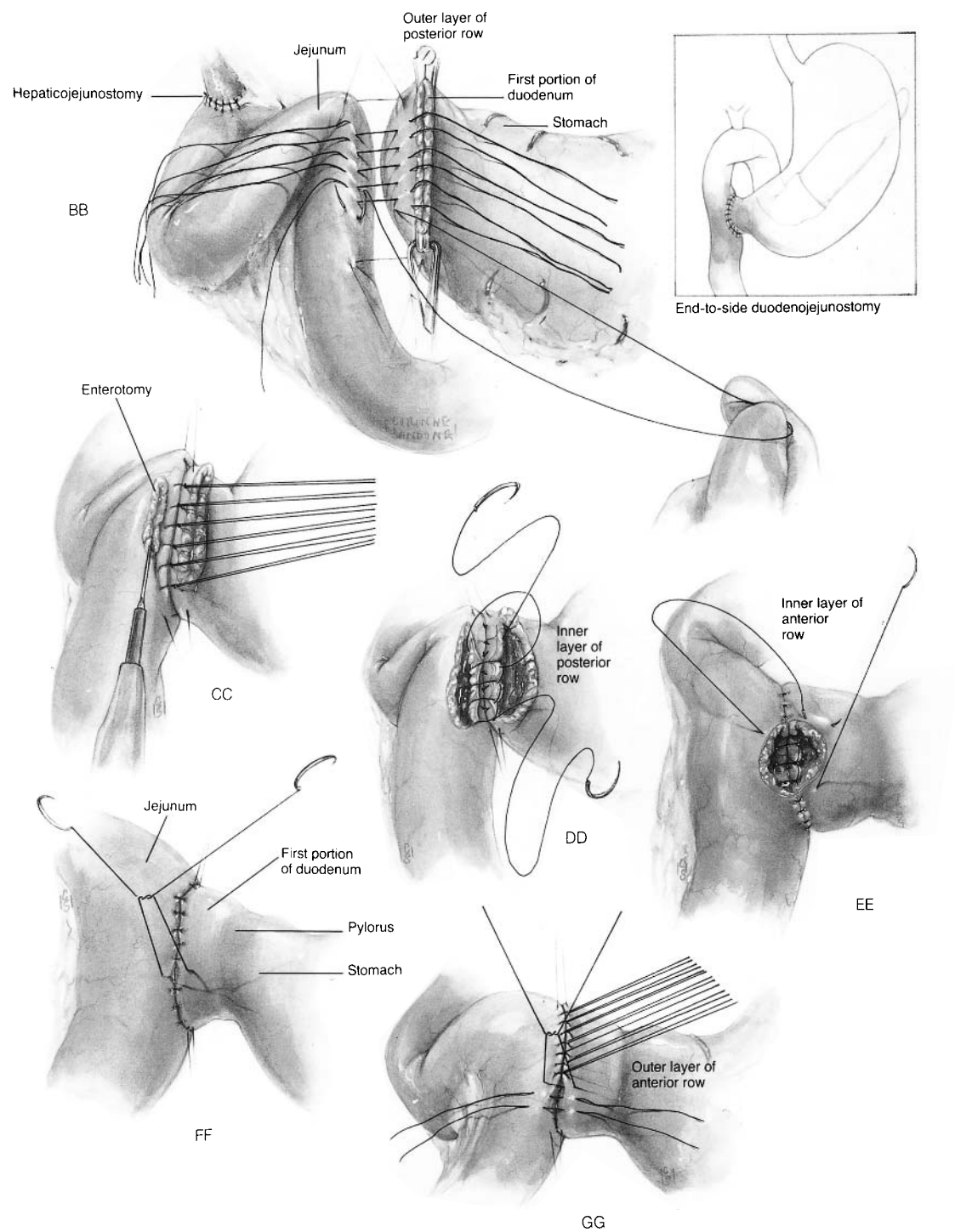


FIGURE 10.8. Two-layer end-to-side duodenojejunostomy. (From Cameron,²¹ by permission of B C Decker Inc.)

stent, this is left externalized immediately postoperatively and then internalized on postoperative day 4. Bile stent output is replaced with lactated Ringer's solution. Stents are flushed twice daily with sterile normal saline while in place.

The closed suction drains placed near the biliary and pancreatic anastomoses are observed postoperatively for any signs of biliary, pancreatic, or enteric leakage. On postoperative day 4, if any of these are draining more than 30 cc of fluid per day, they are sent for amylase determinations. If on postoperative day 4 the hepaticojejunostomy drainage output is less than 50 cc, does not have evidence of bile, and has a low amylase concentration, then it is removed. The left-sided pancreaticojejunostomy drains are removed over the next several days provided the above criteria are again met.

Antibiotics for wound infection prophylaxis are discontinued after the second postoperative dose. Patients are kept on gastric acid suppressive medication (histamine H₂ receptor antagonists or proton pump inhibitors) for at least the first postoperative month. Intravenous erythromycin is used to enhance gastric emptying and is started on the second postoperative day and continued until the patient has tolerated a regular diet for 48 hours. Pancreatic enzyme supplementation is initiated when the patient is placed on a regular diet and is adjusted to the patient's stool pattern.

Patients are encouraged to be out of bed and placed into a chair on postoperative day 1. On postoperative day 2 they are expected to ambulate with assistance, with progress to independent ambulation over the next several days. Depending on the patients' clinical status, sips of fluid are started on postoperative day 2. The next day, the patients are started on a clear liquid diet and advanced to a regular diet soon thereafter. Patients are monitored for signs of diabetes and are started on oral hypoglycemics or insulin as necessary. Routine serum chemistries and blood counts are monitored every other day and as needed during the postoperative period. Patients are referred for consultation from the medical and radiation oncology teams while hospitalized, after their pathology reports are finalized. Our current clinical pathway targets patient discharge on postoperative day 8. The majority of patients are discharged on postoperative day 8, 9, or 10. Occasional patients are ready for discharge on postoperative day 6 or 7. The occurrence of

complications such as delayed gastric emptying, pancreatic fistula, or infection may add to the postoperative length of stay.

Outcome for Pancreatic Cancer

Two recent reports from The Johns Hopkins Hospital have analyzed the outcomes of patients with pancreatic adenocarcinoma undergoing successful pancreaticoduodenectomy. In the first report, 201 patients undergoing resection between 1970 and 1994 were analyzed.¹⁶ This group of patients had an actuarial 5-year survival of 21% and a median survival of 15.5 months (Figure 10.9). Patients with negative surgical margins had a 5-year survival of 26%, whereas those with positive surgical margins had a 5-year survival of 8%. Survival was noted to improve significantly over the study period, with a 3-year actuarial survival of 14% in the 1970s, 21% in the 1980s, and 36% in the 1990s. Multivariate analysis in this study indicated the strongest predictors of long-term survival were tumor diameter less than 3 cm, negative resected lymph node status, negative resection margin status, diploid tumor DNA content, and decade of resection.

A more recent analysis evaluated 174 patients undergoing pancreaticoduodenectomy for pancreatic adenocarcinoma from October 1991 through September 1995.²² Patients had the option of participating in standard therapy, intensive therapy, or no postoperative chemoradiation therapy. Multi-

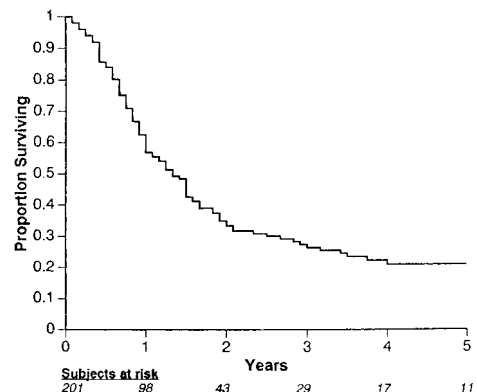


FIGURE 10.9. The actuarial survival curve (Kaplan-Meier) for 201 patients undergoing pancreaticoduodenectomy for pancreatic adenocarcinoma. (From Yeo et al,¹⁶ by permission of *Ann Surg*.)

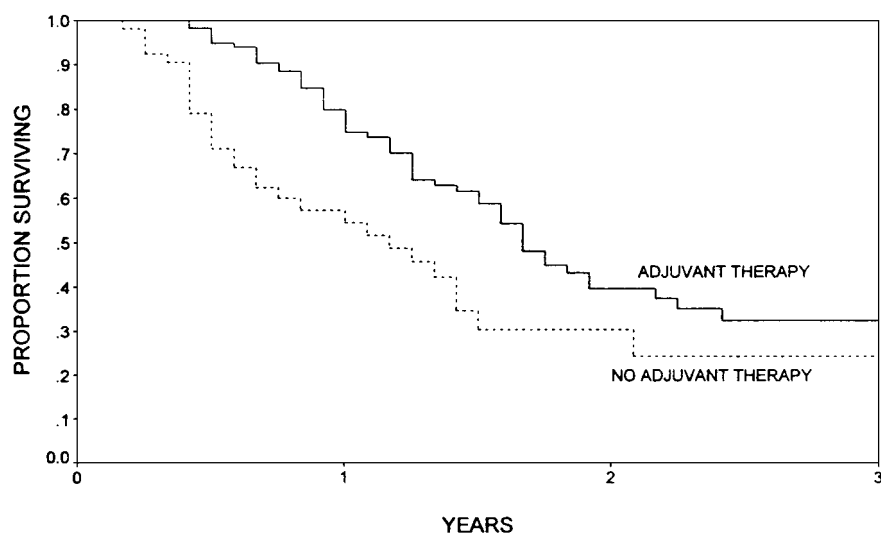


FIGURE 10.10. The actuarial survival curves for patients undergoing pancreaticoduodenectomy comparing patients receiving adjuvant therapy ($n = 120$) to those declining adjuvant therapy ($n = 53$, $P = 0.003$). (From Yeo et al,²² by permission of *Ann Surg*.)

variate analysis of this group of patients revealed the most powerful predictors of outcome to be tumor diameter, status of resection margins, intraoperative blood loss, and use of postoperative chemoradiation therapy. Postoperative chemoradiation therapy was associated with a median survival of 19.5 months, compared to 13.5 months without therapy (Figure 10.10).

Special Issues

Should Pancreaticoduodenectomy Be Performed in Octogenarians?

Advancing age is one of the risk factors for the development of pancreatic cancer. As the population in the United States ages, an increasing number of elderly patients may be considered for pancreaticoduodenal resection. A report from The Johns Hopkins Hospital studied the outcomes of patients 80 years of age and older ($n = 46$) compared to younger patients ($n = 681$) undergoing pancreaticoduodenectomy.²³ The older patients underwent pancreaticoduodenectomy for pancreatic adenocarcinoma (54%), ampullary adenocarcinoma (20%), distal bile duct adenocarcinoma (11%), duodenal adenocarcinoma (4%), cystadenocarcinoma (4%), chronic pancreatitis (4%), and cystadenoma (2%).

The elderly patients undergoing pancreaticoduodenectomy were statistically similar compared to the patients less than 80 years of age with respect to sex, race, intraoperative blood loss, and type of pancreaticoduodenectomy performed.

Patients 80 years of age or older had a shorter median operative time (6.4 hours vs 7.0 hours, $P = 0.02$), but a longer postoperative length of stay (median = 15 days vs 13 days, $P = 0.01$), and a higher complication rate (57% vs 41%, $P = 0.05$) when compared to the younger patients. The perioperative mortality of the two groups of patients was not statistically different (4.3% in the older group vs 1.6% in the younger group). In the subset of patients undergoing pancreaticoduodenectomy for periampullary adenocarcinoma, the older patients ($n = 41$) had a median survival of 32 months and a 5-year survival rate of 19%, compared to 20 months and 27% respectively, in patients younger than 80 years ($n = 454$). These differences were not statistically significant.

Age alone should not be a contraindication to pancreaticoduodenectomy. Long-term survival after pancreaticoduodenectomy is possible in carefully selected octogenarians. The operation can be performed safely in the elderly patient population, with a mortality rate comparable to that observed in younger patients.

Complications

In contrast to the low mortality rate now observed in many centers, the incidence of postoperative morbidity after pancreaticoduodenectomy can approach 50%. The leading causes of morbidity are early delayed gastric emptying, pancreatic fistula, intra-abdominal abscess, hemorrhage, wound infection, and metabolic disorders such as diabetes and pancreatic exocrine insufficiency.²⁴

Early delayed gastric emptying can occur in up to one third of patients after pancreaticoduodenectomy. The incidence appears to be equal after classic or pylorus-preserving pancreaticoduodenectomy.²⁵ The pathogenesis of early delayed gastric emptying may involve several factors, including gastric atony, ischemic injury to the antropyloric muscle mechanism, and gastric dysrhythmias secondary to anastomotic leak or the absence of duodenal motilin. Mechanical obstruction at the duodeno- or gastrojejunostomy should be excluded by upper GI series or endoscopy before making the diagnosis of early delayed gastric emptying. Standard therapy is largely supportive, including nasogastric tube decompression, nutritional support, and pharmacologic manipulation. A prospective, randomized, placebo-controlled study at The Johns Hopkins Hospital demonstrated improved gastric emptying after pancreaticoduodenectomy in patients prophylactically given erythromycin, a motilin agonist.²⁶

Pancreatic fistula is a leading cause of morbidity after pancreaticoduodenectomy. Closed suction drains are typically placed near the pancreatic-enteric anastomosis following reconstruction. If these drains are functioning properly and a patient with a fistula remains clinically stable without signs of intra-abdominal sepsis or bleeding, then the patient can

be managed conservatively (prohibition of oral intake, total parenteral nutrition, and maintenance of drain position). An abdominal computed tomography (CT) scan should be performed to exclude the presence of undrained fluid collections. Healing of the pancreatic fistula may be hastened by stepwise withdrawal of the drain(s), particularly if there is direct contact with the pancreatic-enteric anastomosis. The use of octreotide to speed the closure of such pancreatic fistulas has often been disappointing.²⁷

Fluid collections demonstrated by abdominal CT scan are common after pancreaticoduodenectomy, and in many circumstances they have no significance. In patients with an associated septic clinical picture, these collections require appropriate intervention. The presence of an intra-abdominal abscess usually indicates a leak, typically from the pancreatic-enteric anastomosis. Once recognized, focal intra-abdominal abscesses are preferably managed by percutaneous drainage in combination with appropriate intravenous antibiotics. Patients with systemic sepsis from intra-abdominal abscesses who do not promptly improve with these maneuvers should be considered for reexploration.

Quality of Life

A recent study from Johns Hopkins was undertaken to assess the quality of life and functional outcome of post-pancreaticoduodenectomy patients.²⁸ In the past, there has been a general impression that pancreaticoduodenectomy can severely impair quality of life. The study assessed quality-of-life scores for responding post-pancreaticoduodenectomy patients (n = 192) in three domains: physical, psychological, and social. The quality-of-life scores were comparable to those of patients who had undergone

TABLE 10.2. Overall quality of life assessment.

	Pancreatico- duodenectomy patients	Laparoscopic cholecystectomy patients	Healthy controls
Domains	(n = 192)	(n = 37)	(n = 31)
Physical (15 items)	78%	83%	86%
Psychological (10 items)	79%	82%	83%
Social (5 items)	81%	84%	83%

Quality of life was assessed by a visual analog scale, with the highest score being 100% in each domain. There are no significant differences when comparing between any of the groups.
From Huang et al,²⁸ by permission of *Ann Surg*.

laparoscopic cholecystectomy (n = 37) and to healthy controls (n = 31) (Table 10.2). When the pancreaticoduodenectomy patients were subgrouped into pancreatic adenocarcinoma (n = 54), other periampullary cancers (n = 55), chronic pancreatitis (n = 34), and other benign disease (n = 49), patients resected for chronic pancreatitis and pancreatic adenocarcinoma did have significantly lower quality-of-life scores in the physical and psychological domains. Overall these data indicate that pancreaticoduodenectomy survivors compare favorably to controls in the broad area of global quality of life.

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11

Regional Lymph Node Dissection for Pancreatic Adenocarcinoma

Peter W. T. Pisters and Murray F. Brennan

Introduction

Over the past two decades, a series of reports on retrospective, nonrandomized studies have focused attention on regional lymph node dissection as part of the overall surgical approach to pancreatic adenocarcinoma. Recently, two prospective, randomized clinical trials have evaluated the role of extended lymph node dissection in the management of pancreatic and periampullary malignancies. As will be outlined below, despite these two phase III trials, there is still significant debate about the possible therapeutic benefit of extended node dissection. This ongoing controversy is the stimulus for additional phase III trials.

This chapter will review the data from the retrospective, nonrandomized studies and the two recently published prospective phase III trials. The latter trials will be reviewed in detail, with discussion of the overall findings and their implications for the design of future phase III trials.

Frequency and Anatomic Distribution of Involved Lymph Nodes

Reliable data outlining the frequency and anatomic distribution of lymph node metastases from adenocarcinoma of the head of the pancreas are quite limited. In addition, there is very little published information outlining the stage-specific distribution of metastases. The majority of studies have been done in patients with locally advanced and/or

metastatic adenocarcinoma; this makes it difficult to discern the frequency of lymph node involvement in different anatomic nodal stations for patients who have localized, potentially resectable pancreatic adenocarcinoma.

One of the most widely cited reports on lymph node involvement is a study of 22 pancreatectomy specimens by Cubilla et al.¹ These investigators classified the peripancreatic lymph nodes into five anatomic groups: superior, inferior, anterior, posterior, and splenic. Not surprisingly, these investigators also demonstrated that specimens from patients undergoing regional pancreatectomy (defined as standard pancreaticoduodenectomy plus en bloc resection of the superior mesenteric artery and vein with adjacent soft tissues) contained significantly more lymph nodes than did standard pancreaticoduodenectomy specimens (70 vs 33 lymph nodes). On the basis of the pathologic findings in this small series of 22 specimens, the authors concluded that one third of patients undergoing pancreaticoduodenectomy for adenocarcinoma of the pancreatic head may have metastases to lymph nodes that are outside the scope of the traditional lymphadenectomy performed as part of standard pancreaticoduodenectomy. The authors' pathologic findings were a significant component of the proposed oncologic rationale for regional pancreatectomy but were limited in that they did not evaluate or comment on the numbers of patients that had regional nodal disease without visceral metastatic disease. This is important in that the latter group is the only subset of patients that might reasonably be expected to benefit from more aggressive local treatment strategies, including extended regional lymph node dissection.

More recently, Kayahara and colleagues have reported on the anatomic distribution of lymph node metastases in a series of 49 patients with adenocarcinoma of the pancreatic head.² Thirty-seven patients (76%) in this series had involvement of at least one regional lymph node. The most commonly involved lymph node groups were the posterior pancreaticoduodenal lymph nodes (51%), the superior mesenteric nodes (37%), the anterior pancreaticoduodenal lymph nodes (33%), and the para-aortic nodes (19%). It is difficult to assess whether the patient population included patients with locally advanced disease, but this seems likely given the surprisingly high frequency of nodal involvement in para-aortic lymph nodes. Such involvement is a very uncommon finding among patients with localized pancreatic cancer considered resectable by objective radiographic criteria.

As anticipated, autopsy series of patients with early-stage pancreatic adenocarcinoma are quite rare. Nagai and colleagues reported on 8 such autopsy specimens with extensive analysis of regional lymph node involvement.³ The patient population was diverse and included patients with locally advanced disease and patients with adenocarcinoma arising in the body and tail of the pancreas. Only 4 of the patients had T1 primary tumors that were confined to the pancreas. Two of the four patients with localized disease had no evidence of microscopic lymph node metastasis. The remaining 2 patients with localized disease had involved lymph nodes in lymph node stations that would have been included in a standard pancreaticoduodenectomy. However, the small numbers of patients evaluated and the heterogeneity of anatomic site and tumor stage preclude meaningful conclusions about site- and stage-specific nodal involvement.

The pathologic analyses reported in these relatively small series, with their heterogeneity of tumor site and stage, do not provide reliable information on the frequency and distribution of lymph node involvement in localized pancreatic cancer considered resectable by traditional objective radiographic criteria. Thus, the data derived from the meticulous pathology analysis of extended lymphadenectomy specimens performed as part of the Johns Hopkins randomized trial,⁴ described below, should be regarded as the best currently available data on the frequency and anatomic distribution (first vs second echelon

nodal groups) of nodal metastases from pancreatic adenocarcinoma.

Retrospective Nonrandomized Studies

Several retrospective, single-institution, nonrandomized studies have compared standard pancreaticoduodenectomy to a spectrum of "radical" operations. Often termed "extended" or "regional" pancreatectomy, these aggressive surgical procedures involve resection of the entire duodenum and head of the pancreas together with a spectrum of extended soft tissue and/or vascular resections. Among the most well-described and widely cited of these procedures is regional pancreatectomy, as described by Fortner at the Memorial Sloan-Kettering Cancer Center (MSKCC).⁵ The rationale for regional pancreatectomy was in part the limited pathologic study reported by Cubilla et al,¹ which suggested that as many as one third of patients with pancreatic adenocarcinoma (albeit many of whom had locally advanced disease) have metastatic adenocarcinoma in lymph nodes that are not generally included in the scope of the lymphadenectomy performed as part of standard pancreaticoduodenectomy. Although there was some oncologic rationale for considering this, Fortner's 1984 report of 35 patients undergoing a regional pancreatectomy demonstrated a significant operative mortality rate (23%) and no 5-year survivors.⁶ The absence of long-term survivors despite the most aggressive surgical strategy possible suggests that isolated nodal metastases (without visceral metastatic disease) are uncommon in pancreatic adenocarcinoma and does not provide circumstantial evidence to support a therapeutic benefit to extended node dissection.

By the late 1980s and early 1990s, additional data from the United States became available, including the National Cancer Institute experience and updated comparative data from MSKCC. In 1989, Sindelar reported the National Cancer Institute experience with regional pancreatectomy.⁷ In that report of 20 patients, a 20% operative mortality rate and a disappointing 3-year survival rate of 10% were noted.⁷ Four years later, an updated retrospective review of the MSKCC experience with pancreaticoduodenectomy for pancreatic adenocarcinoma re-

vealed no differences in median survival between the 35 patients treated with regional pancreatectomy (median survival 22 months) and 79 patients treated with standard pancreaticoduodenectomy (median survival 18 months).⁸ On the basis of this aggregate experience, regional pancreatectomy fell out of favor in the United States, with few, if any, centers pursuing radical surgery as part of their therapeutic approach to pancreatic cancer.

In Japan, however, many investigators have pursued a spectrum of radical forms of pancreatic resection for decades. This enthusiasm has been based in part on Fortner's preliminary findings and single-institution retrospective reports from Japan.⁹⁻¹¹ In the late 1980s, Ishikawa et al⁹ and Manabe et al¹⁰ reported independent experiences with radical pancreatectomy. In the report by Ishikawa and colleagues, the outcome of 37 patients who underwent standard pancreaticoduodenectomy between 1971 and 1981 was compared to a subsequent cohort of 22 patients who underwent radical pancreaticoduodenectomy between 1981 and 1983.⁹ The operative mortality was 3 times higher in the standard pancreaticoduodenectomy group, likely reflecting historical differences in preoperative imaging, patient selection, perioperative care, and acquired surgical experience. The 5-year survival rates were 28% in the radical pancreaticoduodenectomy group versus 9% in the standard pancreaticoduodenectomy group; these differences were not statistically significant. One year later, Manabe and colleagues reported the outcomes of 32 patients treated by radical pancreatectomy and 42 patients treated by standard pancreaticoduodenectomy.¹⁰ Although there was a significantly improved survival rate in the patients who underwent radical pancreatectomy, the two treatment groups were not balanced for known prognostic factors. Indeed, 79% of the patients in the standard pancreaticoduodenectomy group had positive lymph nodes compared with 37% in the radical pancreatectomy group. Thus, given the significant imbalance of node-positive patients between treatment groups and the inherent limitations of retrospective nonrandomized data, this experience should be interpreted with caution.

The overall results reported by Ishikawa et al and Manabe and colleagues stimulated further interest in radical pancreatectomy in Japan. Satake and colleagues pooled the operative experience from 59

institutions in Japan and looked at the outcome of patients with early-stage (T1) pancreatic adenocarcinoma treated by standard or radical pancreaticoduodenectomy.¹¹ This analysis of 185 patients demonstrated an overall 5-year survival rate of 27% in both the radical and standard pancreaticoduodenectomy groups. Thus, even in a sizable cohort of patients with early-stage pancreatic cancer (ie, the cohort most likely to benefit from improved methods of regional lymph node clearance), the aggregate retrospective experience from Japan does not support the earlier observations of Manabe et al¹⁰ and Ishikawa et al.⁹

Henne-Bruns et al, from the Universities of Kiel and Hamburg, have recently reported a retrospective series of 72 patients with pancreatic adenocarcinoma.¹² Results of 26 patients who underwent partial pancreatectomy with standard lymphadenectomy were compared with those of 46 patients who underwent extended lymphadenectomy that included retroperitoneal lymphatic and soft tissue clearance. The extended node dissection included removal of all lymph nodes along the hepatoduodenal ligament, the celiac axis including the first 3 cm of the splenic artery, the right side of the superior mesenteric artery, and the anterior surface of the inferior vena cava and renal veins. There were no survival differences noted between patients treated by routine versus extended lymphadenectomy. Of note, 76% of patients treated with extended lymphadenectomy had severe postoperative diarrhea, likely owing to a significant degree of circumferential or near-circumferential dissection around the superior mesenteric artery with secondary denervation of the midgut. This complication of circumferential superior mesenteric artery dissection has also been reported by other investigators.¹³⁻¹⁶

One relative strength of the report by Henne-Bruns and colleagues was the pathologic analysis of the extended lymphadenectomy specimen.¹² The retroperitoneal lymphadenectomy specimen was harvested separately from the primary tumor and the adjacent lymph nodes retrieved from the primary tumor specimen. This allowed for accurate assessment of the yield of positive nodes in the extended lymphadenectomy specimen and secondary assessment of the contribution of extended lymphadenectomy to improved staging. Among the 46 patients who underwent extended lymphadenectomy, only 4 patients (9%) had positive para-aortic nodes; all 4 patients also had positive nodes

in the standard lymphadenectomy specimen. Thus, no patients with “skip metastases” were identified, and staging was not improved in these 4 patients by extended lymphadenectomy. As discussed below and reviewed elsewhere,¹⁷ the frequency of involved second echelon lymph nodes is an exceedingly important variable in the design of appropriately powered phase III trials to examine the potential therapeutic benefit of extended lymphadenectomy.

A detailed comparison of the weight of nonrandomized evidence supporting extended lymph node dissection for gastric versus pancreatic adenocarcinoma is beyond the scope of this chapter. However, even a superficial review of the retrospective^{18,19} and prospective nonrandomized data²⁰ to support extended lymph node dissection for gastric cancer yields a far more compelling justification for extended lymphadenectomy for gastric cancer than does the cumulative retrospective experience with radical surgery for pancreatic cancer summarized above.

Given the overall experience with radical pancreaticoduodenectomy and the limitations of the published retrospective, nonrandomized studies, there is no clear evidence to suggest that radical pancreaticoduodenectomy improves long-term outcome. Indeed, review of the Eastern and Western experience with radical pancreaticoduodenectomy does not provide an overly compelling case for prospective randomized trials of this treatment.

Prospective, Randomized Trials of Extended Lymph Node Dissection

The data from retrospective nonrandomized studies from Japan and Europe have formed the basis for two randomized, phase III trials evaluating the potential therapeutic benefit of extended lymphadenectomy in the management of adenocarcinoma of the pancreas. These studies are the Italian multicenter trial²¹ and the ongoing trial at Johns Hopkins University.⁴

Italian Multicenter Trial

The Italian Multicenter Lymphadenectomy Group has recently reported results of a multi-institutional

trial evaluating standard versus extended lymphadenectomy for adenocarcinoma of the pancreatic head.²¹ The protocol was designed to replicate the study design of Ishikawa et al⁹ in that patients were stratified on the basis of tumor size (using 4 cm as a cutoff) and then were randomly assigned to undergo standard or extended lymphadenectomy. The published report does not describe how the trial was statistically powered, the planned sample size, or the anticipated therapeutic gain of lymphadenectomy. Standard lymphadenectomy included removal of the anterior and posterior pancreaticoduodenal, pyloric, biliary duct, superior and inferior pancreatic head, and pancreatic body lymph node stations. Extended lymphadenectomy included removal of all lymph nodes encompassed in this standard lymphadenectomy plus removal of lymph nodes from the hepatic hilum and along the aorta from the diaphragmatic hiatus to the inferior mesenteric artery (para-aortic) and laterally to both renal hila. This extended lymph node dissection included circumferential removal of soft tissue at the origin of the celiac trunk and proximal superior mesenteric artery. Adjuvant therapy was not utilized in either patient group.

Eighty-one patients undergoing pancreaticoduodenectomy were randomized to undergo standard ($n = 40$) or extended ($n = 41$) lymphadenectomy. Demographic and clinicopathologic characteristics were comparable between the groups. Transfusion requirements, postoperative morbidity and mortality rates, and overall survival rates did not differ between the two groups. However, using an unconventional *a posteriori* analysis that was not planned at the time of the study design, there was a statistically significantly improved survival rate ($P < 0.05$) in node-positive patients following pancreaticoduodenectomy with extended lymphadenectomy. Multivariate analysis of all patients demonstrated that long-term survival was affected by tumor differentiation (well versus moderately versus poorly differentiated, $P > 0.001$), diameter (≤ 2 cm vs > 2 cm, $P < 0.01$), lymph node metastasis (absent vs present, $P < 0.01$), and the need for 4 or more units of transfused blood (< 4 vs ≥ 4 , $P < 0.01$). Importantly, however, the extent of lymphadenectomy did not emerge as an independent favorable prognostic factor in the multivariate analysis, suggesting that the findings of the *a posteriori* analysis should be interpreted with caution.

At least four specific issues in this trial warrant further comment:

- 1. *Definition of extended lymphadenectomy:* The extended lymphadenectomy as defined in this study was a much more extensive procedure than generally performed elsewhere in the West. Notably, this procedure involved complete skeletonization of the aorta from the diaphragmatic hiatus to the origin of the inferior mesenteric artery, with circumferential soft tissue clearance at the level of the celiac axis and proximal superior mesenteric artery. This dissection clearly adds complexity and time to the procedure (although the time differences as reported were strikingly similar given the complexity of the additional dissection) and has been reported to result in disabling diarrhea, largely mediated by denervation of the small bowel associated with the extended circumferential perivascular soft tissue clearance.^{13–16}
- 2. *Rate of positive margin resection:* Twenty (25%) of the 81 patients in the trial had positive resection margins defined at the end of the procedure based on the surgeon’s perception of whether complete “oncologic clearance” had been achieved. Since this assessment was made at the conclusion of the operation (before final pathologic margin status was available), it can be inferred (although this was not clearly stated) that patients who had “incomplete oncologic clearance” in fact had residual gross disease (R2 resection). It is noteworthy that in the subset of patients with node-positive disease (ie, the subset of patients who might benefit from extended lymph node dissection), fully 50% (16 of 32 patients) underwent an R2 resection. One can spec-

ulate that the frequency of grossly and microscopically positive resection margins in this group was well in excess of 50%. This is of significance since patients who undergo pancreaticoduodenectomy with a grossly or microscopically positive retroperitoneal margin have a median survival of 8 to 10 months (without chemoradiotherapy)^{22–29} (Table 11.1) and, by definition, have undergone a palliative rather than potentially curative procedure. In this respect, any potential survival advantage of extended lymph dissection is obscured by the fact that more than 50% of the patients in the node-positive group underwent resection with grossly or microscopically positive margins.

- 3. *Histopathologic analysis:* As demonstrated in the randomized phase III trials of extended lymph node dissection in gastric cancer,^{30–32} careful pathologic analysis is essential in any trial evaluating the potential therapeutic benefit of extended lymphadenectomy. The Italian investigators did not include any standardized form of pathologic analysis or anatomically based subdissection of specimen lymph nodes to determine the frequency of positive lymph nodes in the additional lymph node groups removed with the extended lymphadenectomy procedure. Thus, it is not possible to identify the subset of patients who may have benefited from extended lymphadenectomy. This issue is of critical importance in that only patients who have involvement of the lymph nodes in the extended lymphadenectomy specimen and who undergo complete surgical resection *in the absence of visceral metastatic disease* can derive a survival benefit from the extended procedure.

TABLE 11.1. Median survival following R1 or R2 pancreaticoduodenectomy.

Reference (year)	No. R1 or R2 resections	Margin status	Median survival, mo
Tepper (1976) ²²	17	R1/2	8
Trede (1990) ²³	54	R1/2	10
Whittington (1991) ²⁴	19	R2	*
Willet (1993) ²⁵	37	R1/2	11
Nitecki (1995) ²⁶	28	R2	9
Yeo (1995) ²⁷	58	R1/2	10
Lillemoe (1996) ²⁹	64	R1/2	12
Breslin (2000) ²⁸	16	R1	25*

R1, microscopically positive margin; R2, grossly positive margin.

*2 patients alive at 18 months follow-up.

**Following preoperative chemoradiotherapy.

4. *Statistics*: The Italian investigators did not publish the details of the biostatistical plan that was formulated prior to the initiation of the trial. In particular, they did not report the anticipated improvement in overall survival and calculated sample size. For the reasons outlined below, the trial is probably substantially underpowered. In addition, the unplanned subset *a posteriori* analysis performed after completion of the trial has been questioned by many biostatisticians.

In summary, the equivocal findings in this trial combined with the significant issues of biostatistics, trial design, R2 resection rate, and pathology quality control outlined above make it difficult to draw any conclusions about the potential contribution of extended lymphadenectomy from the Italian trial.

Johns Hopkins University Trial

Preliminary results of a second phase III trial of extended lymphadenectomy have been reported by investigators at Johns Hopkins University.⁴ At the time of that report, with approximately 50% of planned accrual completed, 114 patients with peripapillary adenocarcinoma (including 72 with pancreatic adenocarcinoma) had been randomized to the standard or extended lymphadenectomy treatment groups. The standard lymphadenectomy group underwent a pylorus-preserving pancreaticoduodenectomy with removal of adjacent lymph node groups including the anterior pancreaticoduodenal, posterior pancreaticoduodenal, and lower hepatoduodenal lymph nodes and the lymph nodes along the right lateral aspect of the superior mesenteric vessels. The extended lymph node dissection group underwent a standard pancreaticoduodenectomy plus an additional 30% to 40% distal gastrectomy including lymphoareolar tissue within the lesser omentum and greater omentum along the right gastric and right gastroepiploic vessels. In addition, patients in the extended lymphadenectomy group underwent a retroperitoneal lymph node dissection with removal of all lymphoareolar tissue extending from the right renal hilus to the left lateral border of the aorta in the horizontal axis and from the portal vein to below the third portion of the duodenum at the level of the origin of the inferior mesenteric artery (Figure 11.1). This trial was designed to detect an improvement in overall 5-year survival from

20% to 35% with 80% power. A total projected study population of 242 patients was planned at the beginning of the trial, but the target total accrual has recently been increased (C. J. Yeo, written communication, September 2000).

At the time of the most recent trial update, 56 patients had been randomized to the standard pancreaticoduodenectomy group and 58 patients to the group treated by standard pancreaticoduodenectomy plus extended lymphadenectomy and distal gastrectomy. The groups were balanced for known prognostic factors. There were no identifiable differences between the groups in intraoperative blood loss, transfusion requirements, operative morbidity, or mortality rates. However, operative time was statistically significantly longer in the extended lymphadenectomy group. Survival durations in the standard and extended lymphadenectomy groups were comparable.

When comparing the Johns Hopkins trial to the Italian multicenter trial, the following issues are noteworthy:

1. *Inclusion/exclusion criteria*: The Italian trial was restricted to patients with pancreatic adenocarcinoma, whereas the Johns Hopkins trial included patients with pancreatic, distal bile duct, ampullary, and duodenal adenocarcinoma. These four tumor types have different natural histories that may complicate the interpretation of the trial results. In addition, the Johns Hopkins trial was restricted to patients undergoing potentially curative (R0/1) pancreaticoduodenectomy whereas the Italian investigators included patients who underwent resection with gross residual disease (R2). This difference is important in that it is unlikely that extended lymph node dissection could offer a therapeutic benefit if the primary tumor is incompletely removed (see discussion below on implications for the design of clinical trials).
2. *Scope of extended lymphadenectomy*: The extended lymphadenectomy performed as part of the Johns Hopkins trial is substantially different from that performed as part of the Italian study.²¹ In particular, the Johns Hopkins extended lymph node dissection did not include circumferential dissection of the soft tissues around the proximal superior mesenteric artery, celiac axis, or supraceliac aorta. Lymph node sampling from the celiac axis (chiefly the hepatic artery lymph

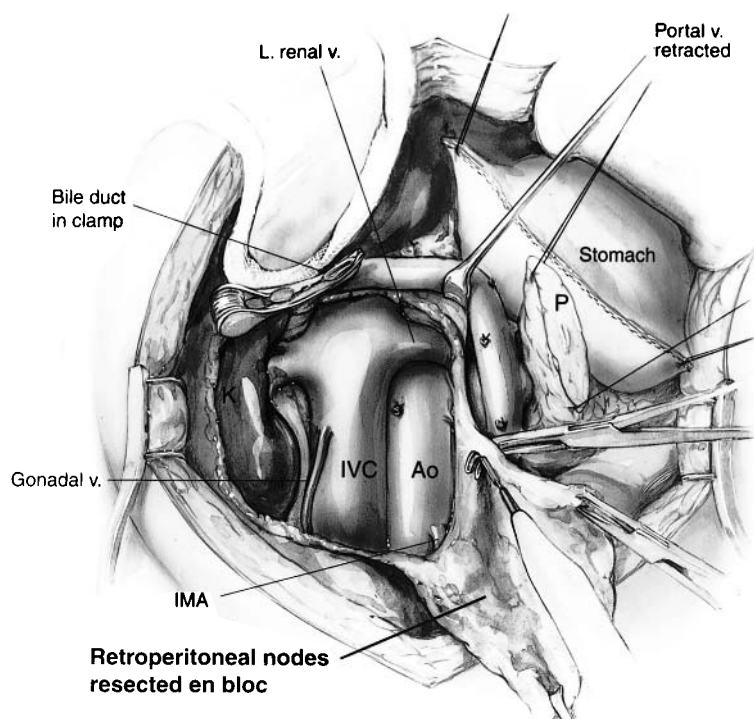


FIGURE 11.1. The retroperitoneal lymph node dissection performed as part of the Johns Hopkins University phase III trial of extended lymph node dissection for periampullary adenocarcinoma.⁴ Lymphoareolar tissue is cleared from the hilum of the right kidney (K) to the left lateral border of the aorta (Ao) in the mediolateral axis. In the cranio-caudad axis, dissection extends from the portal vein to the origin of the inferior mesenteric artery (IMA). A median of 7.4 lymph nodes were retrieved and analyzed from this additional dissection. The frequency of pathologic involvement of these nodes was 10%.⁴ Abbreviations: P, pancreatic remnant; IVC, inferior vena cava; v, vein. Source: Yeo et al.⁴ Reprinted by permission from Lippincott Williams and Wilkins, Inc.

nodes) was performed in the Johns Hopkins trial, but no formal celiac lymphadenectomy was done.

3. *R1/R2 resection rate:* Patients undergoing R2 pancreaticoduodenectomy (with gross residual disease) were specifically excluded from participation in the Johns Hopkins trial. This is in contrast to the Italian study, in which 20 (25%) of the 81 patients in the trial underwent presumed R2 resection.²¹ In the Johns Hopkins trial, 10 patients (9%) underwent an R1 resection, with a microscopically positive uncinate process margin of resection in 7 patients, duodenal (precise anatomic site unspecified) margin in 2 patients, and pancreatic parenchymal margin in 1 patient. This low positive-margin rate likely reflects (1) the single-institution nature of this trial performed by experienced pancreatic surgeons, (2)

inclusion of 42 patients (37%) with nonpancreatic periampullary adenocarcinoma, and (3) appropriate trial design that specifically excluded patients undergoing R2 resection from participation in the trial. In all probability, the 9% R1 resection rate reported in this single-institution experience from a highly experienced pancreatic surgery group represents the lowest possible R1 resection rate achievable. The difference in the positive-margin resection rate between the Johns Hopkins and Italian multicenter trials is striking and likely reflects differences in trial design (inclusion/exclusion criteria) and the unavoidable differences in pretreatment imaging and patient selection that occur when clinical trials are performed in a multicenter fashion.

4. *Histopathologic analysis:* The Johns Hopkins trial involves more careful pathologic analysis

than was performed in the Italian multicenter study. In particular, pathologic information on lymph node retrieval from lymph node stations included in the extended lymphadenectomy portion of the procedure is available. As anticipated, more lymph nodes were retrieved from patients undergoing extended lymphadenectomy (mean, 27 nodes) than standard lymphadenectomy (mean, 16 nodes). The mean number of additional lymph nodes retrieved as part of the extended lymphadenectomy including distal gastrectomy was 11 nodes. Pathologic involvement of the lymph nodes retrieved as part of the extended retroperitoneal lymph node dissection (second echelon nodes) was noted in only 6 patients (10%). All of the patients with pathologically involved second echelon lymph nodes had pathologically involved first echelon nodes that were resected as part of the standard pancreaticoduodenectomy. Thus, no patient's tumor was up-staged by the additional pathologic information provided by extended lymph node dissection.

5. *Statistics:* In retrospect, the Johns Hopkins trial may be significantly underpowered because data on the frequency of positive lymph nodes in the second echelon lymph nodes were not available at the time of trial design and thus could not be used in the sample size estimate. In addition, as summarized below, estimates of long-term outcome following resection of node-positive tumors should be incorporated in the sample size calculations in an effort to define the fraction of patients who have *isolated* lymph node involvement without extranodal metastatic disease. This subset of node-positive patients represents the only patients who can reasonably be expected to derive a potential survival benefit from improved locoregional nodal clearance. Such estimates are essential for accurate sample size calculations.

The Johns Hopkins trial continues to accrue patients, with accrual now at 268 patients (C. J. Yeo, written communication, September 2000). In the meantime, given the aggregate results of the Italian and Johns Hopkins trials and the high probability that these trials are underpowered for their primary endpoints, additional phase III trials are planned to address this question.

Implications of Presently Available Data for the Design of Future Trials

On the basis of the retrospective, nonrandomized study data and the equivocal results of the two randomized trials summarized above, considerable thought has been given to the design of additional phase III trials that, it is hoped, will be designed and powered to more definitively address the potential therapeutic benefit of extended lymphadenectomy. However, the design, implementation, and interpretation of lymphadenectomy trials in cancer surgery is complex. This is certainly the case with lymphadenectomy for pancreatic cancer, which (for anatomic reasons) is much more difficult to study than lymphadenectomy for melanoma, breast cancer, or even gastric cancer. Several issues specific to pancreatic cancer are relevant in the design of future phase III trials.

Biostatistical Issues

The pathologic data from the retrospective study of Henne-Bruns et al and from the prospective Johns Hopkins trial provide important information for the design of a definitive randomized trial of extended lymph node dissection. Specifically, attention should be focused on the frequency of involvement of second echelon (N2 disease) lymph nodes and the complete (R0) resection rate. Henne-Bruns and colleagues performed a separate para-aortic lymphadenectomy and found metastatic disease in 4 (9%) of 46 patients.¹² More important, all of these 4 patients had involved first echelon lymph nodes. This percentage of patients with involved N2 nodes is virtually identical to that reported by Yeo and colleagues in the Johns Hopkins trial.⁴ Since the removal of pathologically negative lymph nodes is assumed to confer no therapeutic benefit, only about 10% of patients who undergo extended lymphadenectomy could potentially benefit from the procedure.

The estimated R0 resection rate is another variable critical to clinical trial design. Henne-Bruns and colleagues reported an R0 resection rate of 83% (60 of 72 resected patients).¹² This R0 resection rate is higher than that reported in the Italian multicenter randomized trial of extended lymph node

dissection, in which 50% of patients with node-positive disease underwent R2 resection.²¹ Therefore, the R0 resection rate for patients with pancreatic adenocarcinoma (ie, distinct from duodenal, ampullary, or distal bile duct adenocarcinoma where R0 resection is easier to attain) is unlikely to be greater than 80%. Further, one can assume that only patients who undergo an R0 resection can benefit from extended lymph node dissection; ie, taking out additional lymph nodes is unlikely to confer a survival advantage if there is residual cancer in the operative field.

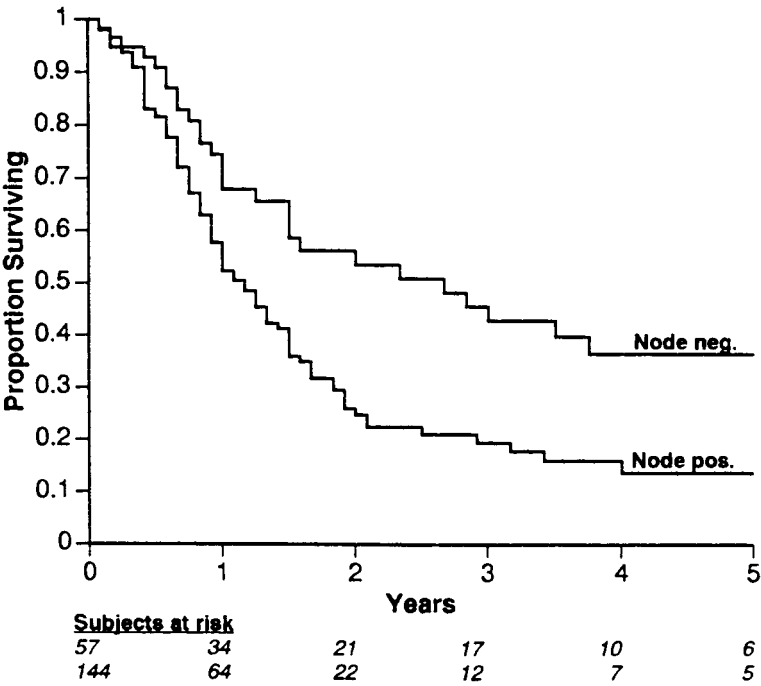
It is also important to recognize that lymphadenectomy will confer a survival benefit only if patients truly have no visceral metastatic disease (M0) at the time of surgery. Unfortunately, the number of patients with node-positive, M0 pancreatic adenocarcinoma is very low. The percentage of such patients can be estimated by evaluating the long-term outcome of patients with node-positive pancreatic adenocarcinoma if one assumes that only patients with truly M0 disease at the time of pancreaticoduodenectomy are capable of achieving long-term survival. The experience at Johns Hopkins University demonstrated 5 long-term survivors (3%) among 144 patients with node-positive pancreatic adenocarcinoma²⁷ (Figure 11.2). At MSKCC, 132

patients with node-positive pancreatic adenocarcinoma underwent pancreaticoduodenectomy between 1983 and 1994 and had a minimum 5 years of follow-up. Of these, 7 patients (5%) were alive 5 years after surgery; 4 of these 7 patients subsequently died of metastatic pancreatic adenocarcinoma (Figure 11.3). Thus, 3 (2%) of 132 patients may have had node-positive but M0 disease (Table 11.2).

In estimating the required sample size and feasibility of a randomized prospective trial of extended lymphadenectomy, the pathologic findings of Henne-Bruns et al¹² and Yeo et al⁴ can be included in a statistical model using the following assumptions:

- Complete gross and microscopic primary tumor removal is required for extended lymphadenectomy to confer a possible survival benefit. The R0 resection rate achievable in a multi-institutional trial is no higher than 80% (0.80).
- Only patients who have pathologically involved second echelon lymph nodes (N2 disease) can benefit from extended lymphadenectomy; ie, the removal of pathologically negative lymph nodes is unlikely to be therapeutic. The frequency of involved second

FIGURE 11.2. Actuarial plot of overall survival for patients with node-negative and node-positive pancreatic adenocarcinoma treated by pancreaticoduodenectomy at Johns Hopkins University. Survival was significantly improved in patients with node-negative disease ($P = 0.0018$). Of note, among 144 patients with node-positive disease, 5 (3%) were alive at 5 years. Source: Yeo et al.²⁷ Reprinted by permission from Lippincott Williams and Wilkins, Inc.



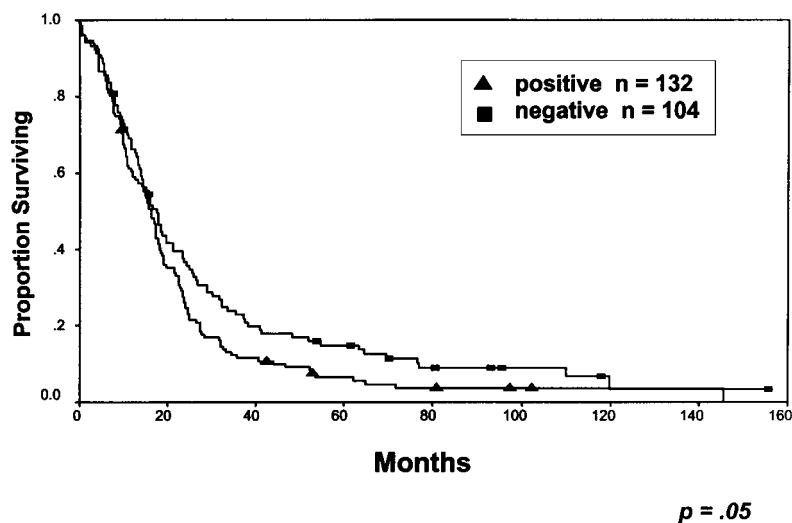


FIGURE 11.3. Long-term survival of a cohort of 236 patients with pancreatic adenocarcinoma who underwent pancreaticoduodenectomy (between 10/15/83 and 10/14/94) at MSKCC. The minimum follow-up time of this cohort was 60 months. Of the 132 patients with node-positive disease, 7 (5%) patients were alive ≥ 5 years after surgery. Of these 7 patients, 4 subsequently died of metastatic pancreatic adenocarcinoma. Thus, 3 (2%) of 132 patients with node-positive pancreatic adenocarcinoma are believed to have survived long-term.

echelon lymph nodes in patients with radiographically resectable pancreatic cancer is estimated to be 10% (0.10).^{4,12}

- Only patients who have involved lymph nodes *without* visceral metastatic disease are likely to derive a survival benefit from the removal of additional pathologically involved lymph nodes. The fraction of patients with node-positive but truly M0 pancreatic adenocarcinoma is 5% (0.05) or less.²⁷

The implications of these literature-based assumptions on sample size are outlined in Table 11.3. These assumptions imply that only 0.4% ($0.80 \times 0.10 \times 0.05 \times 100\%$) of the patients who undergo an extended lymphadenectomy may achieve a long-term survival benefit. Therefore, to definitively demonstrate the benefits of extended lymphadenectomy would require a prohibitively large sample size. As an illustration, if we use the

historical 12-month median survival with grossly complete (R0/R1) surgical resection (and conventional lymphadenectomy),^{33,34} then even if extended lymphadenectomy leads to long-term survival for every patient with N2M0 disease, the anticipated improvement in the survival rate for the entire population would be no more than 0.4%. To detect such a difference in a randomized trial with 80% power would require 238,000 patients randomized to each of the two arms. Based on a reasonable accrual rate of 150 patients per year to a phase III trial, this would translate into a study with 3,173 years of accrual. Even if one assumes a “best case scenario” with a 100% R0 resection rate, twice as many patients with involved second echelon lymph nodes (as a consequence of more rigorous or sensitive pathology techniques for identification of positive nodes), and a 15% rate of patients with involved second echelon lymph nodes but truly M0 disease, the time to complete a definitive trial with

TABLE 11.2. Survival of patients with adenocarcinoma of the pancreatic head treated by pancreaticoduodenectomy (1983–1994) at MSKCC with minimum follow-up of 5 years.

Lymph node status	No. patients	Alive ≥ 3 years	Alive ≥ 5 years	DOD > 5 years	Long-term survivors*
Positive nodes	132	15 (11%)	7 (5%)	4 (3%)	3 (2%)
Negative nodes	104	23 (22%)	14 (13%)	7 (7%)	7 (7%)
Total	236	38 (16%)	21 (9%)	11 (5%)	10 (4%)

DOD, dead of disease (pancreatic cancer).
*Patients who remain free of disease more than 5 years after pancreaticoduodenectomy for adenocarcinoma of the pancreas.

TABLE 11.3. Sample size estimates for phase III trials of extended lymph node dissection for pancreatic adenocarcinoma using evidence-based or liberal clinicopathologic assumptions.

Parameter	Clinicopathologic assumptions	
	Evidence-based	More liberal
Median survival with PD alone, mo	15	15
Frequency of:		
N2	0.10	0.20
N2 but M0	0.05	0.15
R0	0.80	1.00
Power, %	80	80
Calculated sample size	476,000	8,412
Years to complete trial*	3,173	56

PD, pancreaticoduodenectomy; N2, pathologically involved second echelon nodes (nodes removed during the extended lymphadenectomy); N2 but M0, involved second echelon lymph nodes but no visceral metastatic disease (as estimated from long-term survival data for node-positive patients); R0, potentially curative resection with histologically negative resection margins.

*Assumes accrual of 150 patients/year.

similar parameters would still be 56 years. A trial of extended lymphadenectomy with survival as an endpoint is clearly impractical. Any study looking at extended lymph node dissection can only effectively focus on morbidity, mortality, postoperative gastrointestinal function, or secondary biologic endpoints.

Surgical/Pathology Quality Control

As demonstrated in the randomized trials evaluating extended lymph node dissection for gastric cancer, issues of quality control for both surgery and pathologic analysis are critical to the success and interpretation of the trial.^{30,35} In the Dutch gastric cancer trial, intensive efforts were made to ensure satisfactory surgery and pathology quality control.³⁵ This included involvement of a Japanese proctoring surgeon who spent months operating with the Dutch investigators to develop a small group of 8 skill-verified proctoring surgeons who were trained and certified in the performance of extended lymph node dissection according to the Japanese rules for lymph node dissection.³⁶ This subset of proctoring surgeons attended every one of the extended lymphadenectomy procedures. Proctoring surgeons themselves microdissected the

specimens to facilitate accurate lymph node retrieval and pathology analysis of the anatomic lymph node group stations that were supposed to be harvested as part of the protocol. This arduous effort to ensure surgical and pathologic quality control was supplemented with videotapes and regular investigators meetings. The efforts made to ensure quality control in these critical areas were beyond those ever observed in *any* prior surgical trial. Unfortunately, notwithstanding these exhaustive efforts to maintain quality control, protocol violations in the form of contamination (dissection of lymph nodes from more than 2 lymph node stations that were not supposed to be harvested) in the limited node dissection (D1) group and noncompliance (absence of lymph nodes from more than 2 lymph node stations that were supposed to be harvested) in the extended node dissection (D2) group occurred in 57% of patients (Table 11.4).^{37,38} To the extent that contamination occurred in the D1 group (6%) and noncompliance occurred in the D2 group (51%), there was partial homogenization of treatment and control groups that may have obscured any therapeutic advantage to extended lymph node dissection.

The lessons learned from the Dutch trial are exceedingly important in the design of subsequent phase III trials of extended lymph node dissection in gastrointestinal cancers. In the case of protocol design for node dissection trials in pancreatic cancer, extensive thought will need to be given to how to avoid the problems in the Dutch gastric cancer trial.

The biostatistical, clinicopathologic, and quality control issues described here clearly help to frame

TABLE 11.4. Impact of noncompliance and contamination on Dutch phase III trial of extended node dissection for gastric cancer.

Type of protocol violation	No. patients affected (%) by dissection type	
	D1 (n = 380)	D2 (n = 331)
Contamination*	22 (6)	23 (7)
Noncompliance**	137 (36)	169 (51)

*Proof of lymph nodes from more than 2 lymph node stations that were not supposed to be harvested

**Absence of lymph nodes from more than 2 lymph node stations that were supposed to be harvested

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the practical, logistical, and biostatistical challenges associated with clinical trial development in this area. Considering these difficulties and the inherent aggressive biology of pancreatic cancer, it may be impossible to ever definitively answer the question of whether extended lymphadenectomy improves survival in patients with pancreatic cancer.

Summary

Retrospective, nonrandomized studies (primarily from Japan) suggest a survival benefit from extended lymph node dissection for localized pancreatic cancer. Unfortunately, a pooled retrospective experience from 59 Japanese centers and the 2 underpowered phase III trials that have addressed extended lymphadenectomy for pancreatic cancer do not demonstrate a clear survival benefit to extended lymphadenectomy. Moreover, careful analysis of the published clinicopathologic data and outcome analysis of patients undergoing resection of node-positive pancreatic adenocarcinoma suggest that this issue cannot be settled by a logistically feasible randomized clinical trial. As a result, it appears reasonable to conclude that there is currently no definable therapeutic advantage to extended lymph node dissection as part of the standard management of pancreatic cancer.

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12

Pylorus Preservation versus Standard Pancreaticoduodenectomy: Oncologic Controversies

Karen E. Todd and Howard A. Reber

Introduction

The standard pancreaticoduodenectomy or Whipple procedure was first performed by Alan O. Whipple for carcinoma of the ampulla of Vater, and a description of the procedure was published in 1935. A similar operation had actually been performed earlier by the German surgeon Kausch, and the results published in 1912. In 1945, Whipple performed a one-stage pancreaticoduodenectomy, and although a number of modifications have been made to that procedure over the ensuing years, it evolved into what became the most widely done operation for neoplastic disease of the periampullary region.

Historically, the operation was associated with a significant mortality rate (20% to 25%) and, in those who survived the perioperative period, nutritional problems such as weight loss, diarrhea, and dumping symptoms were quite common. Multiple factors must have been responsible for this. For example, the operation performed for many years included a subtotal (two thirds to three quarters) gastrectomy with a Billroth II reconstruction and a truncal vagotomy. After a truncal vagotomy and partial gastrectomy, it is known that both liquids and solids often empty from the gastric remnant more rapidly than normal, and food particles that are too large to be digested completely may enter the intestine. The small gastric remnant limited the size of a meal that could be comfortably ingested, so many patients ate less. The large experience with surgery for duodenal ulcer disease showed that about 25% of all patients who underwent a truncal vagotomy (even without a gastrectomy) suffered

from diarrhea, presumably secondary to altered small bowel motility and rapid intestinal transit. The usual gastrointestinal reconstruction after the Whipple resection consists first of a pancreaticojejunostomy, then a hepaticojejunostomy, and the gastrojejunostomy, which is last. Thus, the pancreatic juice and bile enter the intestine some distance proximal to the point where the food does. This creates a situation where even if the remaining pancreas is normal, and the amount of digestive enzymes is adequate for normal absorption, malabsorption may result from inadequate mixing of the gastric chyme with bile and pancreatic juice.

Several of the modifications of the original operation have lessened that gastrointestinal morbidity. These included elimination of the truncal vagotomy, and resection only of the gastric antrum (30% to 40% gastrectomy), instead of up to three quarters of the stomach. These changes may have been the result of the gradual introduction of more effective acid antisecretory measures to decrease the frequency of marginal ulceration, as well as a growing appreciation of the role of the gastric antrum and the hormone gastrin that originates there. In addition, new and more effective pancreatic enzyme supplements have made the management of pancreatic enzyme insufficiency more effective today than it was in the 1960s and 1970s. Indeed, by 1 year after a standard Whipple operation today, most patients have stabilized at a new weight that is only about 5% less than their preoperative one. Diarrhea is virtually never a clinically significant problem. In some cases, this requires dietary modifications and/or drug therapy; in others, in spite of the alterations described, no adjustments are required.

These older accounts of uncontrolled diarrhea and profound weight loss that followed the standard Whipple operation left a lasting impression on many surgeons.¹ In an effort to reduce some of the morbidity, the British surgeon Watson first performed a pylorus-preserving pancreaticoduodenectomy (PPW) in a patient with carcinoma of the ampulla of Vater, and reported the case in 1944.² This modified operation received little attention until 1977, when Traverso and Longmire at UCLA published their experience with the procedure in 2 patients with chronic pancreatitis.³ They hoped that the incidence of marginal ulcer, which had been as high as 20% following the standard Whipple (SW), would be less if the entire stomach and pylorus were preserved. Although they originally intended that the operation be used only in patients with benign disease, it was eventually applied to patients with pancreatic and other periampullary cancers as well.^{4,5} It has generally been accepted that this did not compromise the chance for cure of the cancer.

Technical Considerations

The operation is done in the same way as the SW resection, except that a gastrectomy is not performed. Instead, we transect the duodenum with an automatic stapling device 2–3 cm distal to the pylorus after cleaning its surface circumferentially. The transection is usually at about the level of the gastroduodenal artery as it passes behind the duodenum. Although the gastroepiploic arcade is preserved as far distally along the greater curve of the stomach as possible, we make no particular effort to either identify or preserve the right gastric artery or nerves that enter along the lesser curve near the pylorus. The reconstruction is the same for the biliary and pancreatic anastomoses as in an SW operation. We perform a retrocolic duodenojejunostomy in a two-layer end-to-side fashion using 3-0 absorbable suture for an inner running layer and 3-0 silk for an interrupted outer layer. A nasogastric tube is left in place overnight and removed on the first postoperative day.

It is generally accepted that operative mortality rates are no different between PPW and SW. With either operation, in the hands of experienced surgeons around the world, the operative mortality from pancreatoduodenectomy is less than 5%. In

the last 145 patients at UCLA, the senior author has had 1 death (0.7% operative mortality rate). Controversy exists about differences in postoperative morbidity, however.

Comparisons of the PPW versus the SW

Since the reintroduction of the PPW, its proponents argue that this modification results in less dumping, better weight gain, improved quality of life, and shorter operative times. Proponents of the SW argue that the standard approach is a better cancer operation and results in less delayed gastric emptying. In fact, for most of these claims, very little supportive data exist.

Length of Operation

The duodenojejunostomy may be marginally easier to perform than the gastrojejunostomy. A Polya reconstruction involves a much longer anastomosis to the jejunum; a Hofmeister reconstruction requires separate closure of the lesser curve side of the resected stomach. However, Lin and Lin examined operative times, estimated blood loss, and transfusion requirements in 16 patients undergoing PPW and 15 patients undergoing SW. There were no differences between the two groups.⁶ Unpublished data from a prospective, randomized trial at UCLA support these findings. In 12 patients undergoing PPW and 10 undergoing SW by a single surgeon, length of operation (6.8 hours vs 7.4 hours) and estimated blood loss (371 cc vs 430 cc) were not significantly different between the 2 groups. None of the patients received a blood transfusion.

Delayed Gastric Emptying

Most surgeons believe that delayed gastric emptying is increased in patients undergoing the PPW compared to the SW, although the published results are less than conclusive. Sumida et al examined the influence of preservation of the superior pyloric branches of the vagus on the physiologic function of the stomach, duodenum, and jejunum in 27 patients undergoing PPW. Thirteen patients had the right gastric artery and superior pyloric branches of

the vagus divided, while 14 patients had these structures preserved. Gastric juice output, gastric emptying, and antral and duodenal motility as well as plasma gastrin levels were measured. There was no difference in any of these parameters between the two groups. Sumida et al concluded that preservation of the pyloric vagal branches did not influence gastric exocrine or endocrine secretion or emptying.⁷

Kobayashi et al measured gastric emptying in 14 patients after PPW at a mean of 38 days after surgery. They found that solid phase emptying was significantly delayed in the postoperative period in the PPW group. Liquid phase emptying was also delayed, but less so. Liquid phase emptying returned to normal in 2 to 5 weeks; solid phase emptying took 3 months to recover.⁸ This study involved a very small number of patients; moreover, the authors did not correlate the delayed solid phase emptying with changes in the patients' postoperative weight or nutritional status.

Thor et al studied myoelectric activity and gastric emptying in 18 patients undergoing SW and 10 patients undergoing PPW. Myoelectric activity was measured by electrogastrography using cutaneous electrodes. Bradygastria and tachygastria were measured to determine gastric dysrhythmia after solid and liquid meals. A strong relationship was noted between solid gastric emptying and dysrhythmia in patients undergoing the PPW.⁹ Again, there were a small number of patients in this study and they were not randomized to SW versus PPW. In the previously mentioned prospective randomized comparison of PPW and SW by Lin and Lin, the authors found that delayed gastric emptying was more frequent in 16 PPW patients (38%) than 15 SW patients (7%).⁶ Braasch et al reported that 50% of patients experienced delayed gastric emptying after PPW.¹⁰ However, no comparison was made with patients undergoing the SW in this retrospective review. In a retrospective study using historical controls, Morel et al found that 20 patients undergoing PPW required gastric suction longer than 18 patients undergoing SW (7 days vs 4.5 days).¹¹ All of the SW patients had chronic pancreatitis, while some of the patients in the PPW group had chronic pancreatitis and some had cancer.

Warshaw's group from the Massachusetts General Hospital published their retrospective analysis of SW versus PPW for patients with chronic pan-

creatitis. They found an incidence of delayed gastric emptying (defined as lack of significant postoperative oral intake for more than 14 days, and requiring institution of parenteral nutrition) of 33% in PPW patients compared to 12% in those who had SW.¹² In an accompanying editorial, Cameron referred to the Johns Hopkins experience and indicated that their incidence of delayed emptying after PPW for either benign or malignant disease had fallen to about 15%.¹³ He attributed this to increasing experience with the operation, as well as the routine use of erythromycin given as a motilin-like promotility agent, which they use routinely in these patients.

The importance of a more objective assessment of this issue is pointed out by a retrospective review at UCLA, where delayed gastric emptying occurred in 41% of patients after SW and 61% of patients after PPW.¹⁴ The review encompassed a period of 6 years, and the operations were performed by 7 different surgeons. Delayed emptying was defined in this study as failure to remove the nasogastric tube by postoperative day 7. In a subsequent prospective randomized study at the same institution in which all operations were performed by the same surgeon, full oral diets were resumed at a similar time in both groups (7.8 days in the 12 PPW patients and 8.4 days in the 10 SW patients). These 2 studies with disparate results from the same center emphasize the need for prospective controlled studies.

In a recent, as yet unpublished prospective study from Buchler et al in Switzerland, delayed gastric emptying was no different in 77 patients undergoing PPW ($n = 37$) versus SW ($n = 40$). This prospective, randomized study with resection performed by 3 different surgeons appears to support our own institution's prospective study. However, the issue remains unresolved.¹⁵

Marginal Ulceration

Marginal ulceration is thought to occur more commonly after the SW in which an antrectomy is performed. Grace et al studied 331 patients who had undergone PPW and found that marginal ulceration occurred in 3.6%, compared to 10% of patients undergoing SW. The data for SW were pooled from a number of other published studies, however.¹⁶ Fink et al found no difference in marginal ulcera-

tion as diagnosed by upper endoscopy in 12 patients undergoing PPW (6) versus SW (6) (0 ulcerations in either group).¹⁷

We have not seen marginal ulceration in our patients who routinely are managed during the postoperative period and for at least the first 3 months after discharge on acid antisecretory medication. However, in patients with pancreatic cancer who have undergone PPW, their limited life expectancy must limit the development of marginal ulceration to some degree. Other patients must be lost to surgical follow-up, so the true incidence of marginal ulceration is unknown. It should also be mentioned that patients with chronic pancreatitis who undergo PPW may have a higher incidence of marginal ulceration than those with an otherwise normal pancreas. This is because of the impaired bicarbonate secretion in the pancreatitis patients, which may not neutralize the gastric acid as completely. Thus, studies in which patients with chronic pancreatitis are used as controls for those with pancreatic cancer are inappropriate.

Nutritional Status

Weight gain has been reported to be better in patients undergoing PPW compared to those who have an SW. Takada et al found that 24 PPW patients gained 103% of their preoperative weight versus 90% in 14 SW patients.¹⁸ However, 11 of these patients underwent pancreatic resection for low-grade malignancies such as cystadenocarcinoma, and it is unclear from this retrospective study whether these 11 patients underwent SW or PPW. This is important because some of the weight loss experienced by patients who have had resections for cancer must be due to the underlying malignancy rather than the effects of the operation itself.

In another study by Braasch et al, all 63 patients undergoing PPW achieved 101% of preoperative weight and 95% of preillness weight.¹⁰ Seventy-eight percent of these patients were able to eat 100% of their former meal capacity. However, no comparisons were made with patients who had undergone an SW in this retrospective analysis. Kozuschek et al compared 43 patients undergoing PPW and 15 patients undergoing SW. Eighty-six percent of the PPW group achieved their preoperative weight compared to 43% of the SW group by 12 months after surgery. The PPW group also had

increased capacity for food intake.¹⁹ In this retrospective study, the 2 groups were poorly matched. Thus, there were patients with carcinoid and islet cell tumors in the PPW group, but only patients with pancreatic ductal carcinoma in the SW group. Patti et al studied 10 patients who had undergone the PPW between 1 and 45 months after surgery. Ninety-four percent of patients achieved their preoperative weight, and 89% reached preillness weight.²⁰ There were no direct comparisons made with the SW. Morel et al noted a 7.4 kg weight gain in 19 patients undergoing PPW versus a 4.2 kg weight gain in 9 patients undergoing SW ($P < 0.001$).¹¹ In this study, the SW group consisted of historical controls.

Fink et al measured levels of vitamin A, vitamin B12, carotene, folate, serum iron, and total iron-binding capacity in 6 patients undergoing PPW and 6 others undergoing SW. There were no differences in these nutritional parameters between the two groups.¹⁷ Van Berge Henegouwen et al examined postoperative weight gain at intervals after SW in 56 patients and PPW in 69 patients. They found no difference in overall weight gain between the PPW group and SW group at any time point. Interestingly, there was a difference in postoperative weight gain in both groups with positive resection margins or recurrence compared with those patients with negative margins or tumor-free status.²¹ Our data at UCLA also revealed that change from preoperative weight in the PPW group was similar to that of the SW group at 9 to 12 months postoperatively (10.4 lb vs. 11.1 lb).

In summary, we are unaware of any well-designed studies to answer the ongoing question about the nutritional superiority of PPW compared to the SW operation. The issue remains unresolved.

Adequacy as a Cancer Operation

Since less tissue is removed in a PPW, there has been concern that it may be a less adequate cancer operation than the SW. Although they are rarely involved with metastases in patients with pancreatic cancer, lymph nodes along the greater and lesser curves of the distal stomach as well as the anterior and posterior pyloric nodes are not resected routinely as part of the PPW. Occasionally an extensive cancer in the head of the pancreas does involve the duodenum, so there is the potential that a PPW

may result in tumor being left behind at the duodenal resection margin. Examples of this have been reported. Sharp et al described 3 patients with intramural spread of pancreatic cancer within the duodenum, and warned that spread of pancreatic cancer along nerves, vessels, and lymphatics and directly across tissue planes may be underappreciated by surgeons.²² The only study that suggested that PPW might be a less adequate cancer operation was performed by Roder et al. They analyzed 53 patients with pancreatic and 57 patients with other periampullary cancers. Thirty-one patients in each group underwent SW; the remainder underwent PPW. There was no difference in survival between PPW and SW for patients with periampullary cancer. However, for patients with pancreatic cancer, SW conferred a significantly better survival than PPW at 10 months (80% vs 40%). This was especially true for stage III tumors.²³ These data have not been duplicated by others.

Several retrospective studies have examined long-term survival following PPW in patients with periampullary malignant disease, and found no differences compared to patients who underwent SW. Takao et al compared long-term survival and tumor recurrence between PPW and SW in 113 patients. There was no difference in actuarial survival curves or 5-year survival between the 2 groups. There were also no differences in tumor recurrence rates or types of recurrence.²⁴ Takada et al assessed 24 patients undergoing PPW and 14 patients undergoing SW. There was no difference in the survival rate between these 2 groups.¹⁸

Support for the idea that the PPW is an adequate cancer operation comes from a study by Cooperman. He examined 140 Whipple specimens and found no evidence for tumor extension or lymph node involvement proximal to the pylorus and no lymph node involvement along either curvature of the stomach.²⁵

Grace et al reviewed 96 patients who underwent pancreatoduodenectomy at UCLA between 1975 and 1984. Forty-one patients had malignant disease; 30 underwent SW and 11 had a PPW. Overall actuarial survival was 13% for pancreatic cancer, 62% for ampullary cancer, 60% for duodenal cancer, and 52% for bile duct cancer. There was no difference in survival between patients undergoing the PPW versus the SW for periampullary malignancy. However, most of these lesions were early,

and only 3 patients had stage II disease. Six of the PPW patients and 10 of the SW patients had negative lymph nodes.²⁶

Braasch et al reviewed 87 patients who had undergone PPW for a variety of periampullary cancers or chronic pancreatitis. Five-year survival for the patients with cancers of the head of the pancreas was 17%, which was similar to the authors' previous experience after SW.¹⁰

This question is likely to remain unresolved. Thus, because of the low survival rate associated with the standard Whipple operation, an enormous number of patients would need to be randomized between the 2 operations to reveal a significant survival difference between them.

Quality of Life

Quality of life is stated to be better after PPW compared to SW, but little objective data supports this notion. Takada et al assessed 24 patients undergoing PPW and 14 patients undergoing SW. Quality of life was measured by a questionnaire that examined social activity postoperatively. In patients undergoing PPW, 23 of 24 reported complete recovery or recovery with voluntary retirement compared to 6 of 14 patients undergoing the SW who fared that well ($P < 0.05$).¹⁸ The patients undergoing SW had more extensive disease including positive lymph nodes and invasion of the duodenum or pylorus. Thus, the groups were not well matched, which may explain why patients in the SW had a worse quality of life postoperatively. Patel et al also studied quality-of-life issues in 19 patients undergoing SW and 4 patients undergoing PPW. They concluded that there was no difference in quality of life based on these patient interviews.¹⁴ Both of these studies were retrospective, however.

Quality of life was also explored in our prospective, randomized trial of 12 patients undergoing PPW and 10 patients undergoing SW. Self-administered questionnaires were mailed to patients at 3-month intervals after surgery. Quality-of-life scores assessed at 9 to 12 months postoperatively showed no differences between the PPW and SW groups (6 vs 7.2; best score = 10). Similarly, symptom scores and activity scores were not significantly different between groups (K.E.T. and H.A.R., unpublished data, 1998).

Conclusions and Recommendations

In our opinion, the theoretical nutritional benefits of the PPW and the improvement in the quality of life that might have been expected to accompany them have yet to be proven. Although certain physiologic differences undoubtedly result from removal of the antrum and destruction of the antropyloric mechanism in the SW operation, it is not at all clear that these translate to recognizable clinical symptoms in most patients. We are convinced that the technical aspects of the two operations are equivalent, and that the adequacy of the PPW as a cancer operation, while not proven rigorously, is not likely to be significantly different than that of the SW. Unfortunately, each has major limitations in this regard. There probably is an increased incidence of delayed gastric emptying after the PPW, but the problem occurs as well after the SW, and it is easily managed in most patients.

In our own practice at UCLA, the PPW has become the standard operation for most pancreatic and periampullary malignancies. This is done because it seems illogical to remove more tissue than necessary, unless clear benefit would be derived from doing so. SW is reserved for larger pancreatic cancers that appear to have encroached upon the area of the pylorus or gastric antrum. In every case where a PPW is done, an intraoperative frozen section of all of the resection margins, including the duodenum, is performed. If the duodenal margin is involved, PPW is converted to SW (this has never been necessary).

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13

Vascular Resection and Reconstruction for Localized Pancreatic Cancer

Charles R. Scoggins, Ingrid M. Meszoely, Steven D. Leach, and A. Scott Pearson

Introduction

Pancreatic cancer is a common disease that is rarely cured. There are 29,000 new cases of pancreatic cancer diagnosed each year, and this malignancy is the fourth leading cause of cancer-related death within the United States.¹ Fewer than 50% of patients in whom exploration is performed undergo successful tumor resection, as a result of unsuspected vascular involvement or extrapancreatic disease as determined by the surgeon during laparotomy.² Many pancreatic surgeons consider superior mesenteric venous involvement by tumor a contraindication to resection. When tumor resection is performed in this setting without resection of the involved vascular segment, a positive retroperitoneal margin results. Patients who undergo resection with positive margins have been shown to have short survival rates similar to patients with localized disease treated nonsurgically with chemoradiotherapy.^{3–8} Methods to increase the number of patients in whom a negative margin can be obtained are therefore attractive, providing that these methods do not increase morbidity and mortality. In order to expand the subset of patients who are candidates for resection, several centers have evaluated the role of en bloc resection of mesenteric vascular structures involved by pancreatic cancer.

Does vascular involvement by pancreatic cancer result from enhanced biological aggressiveness, or is this a function of tumor location? A study from the M. D. Anderson Cancer Center has evaluated this issue.⁹ Pathological specimens from patients in whom standard pancreaticoduodenectomy was performed were compared to specimens from patients

in whom pancreaticoduodenectomy with en bloc resection of the superior mesenteric portal vein (SMPV) confluence was required because of vascular involvement by tumor. There were no differences in median tumor size, tumor DNA aneuploidy, or rate of nodal metastases between the 2 groups.⁹ In addition, analysis of the rate of major morbidity and mortality between these 2 groups demonstrated no difference in the length of hospital stay, perioperative morbidity, or perioperative mortality.⁹ Based on these observations, involvement of the SMPV confluence by pancreatic adenocarcinoma in the head of the pancreas appears to be related to tumor location, and not a marker of biological aggressiveness.^{9,10} Survival data demonstrate that patients in whom venous resection is performed have survival times equal to those of patients that do not require venous resection.¹⁰

Indications

The decision to perform pancreaticoduodenectomy with concomitant mesenteric vascular resection for pancreatic adenocarcinoma must be based on a firm understanding of the regional anatomy, both normal and variant. The relatively high number of variations in mesenteric vascular anatomy dictates a thorough preoperative delineation of each patient's anatomy prior to resection. In addition, the total extent of disease, both local and distant, must be known before laparotomy. Preoperative data regarding invasion of the vessels bordering the pancreatic head allow for proper patient selection. These anatomical issues are best delineated by dy-

dynamic contrast-enhanced computed tomography (CT). The ability of this imaging modality to identify tumor extension to the mesenteric vasculature and correctly predict the need for mesenteric venous resection in conjunction with pancreaticoduodenectomy has been evaluated. Of patients in whom preoperative CT demonstrated venous involvement by tumor, 84% required venous resection at the time of definitive operation.¹¹ Assuming that en bloc resection of the SMPV confluence will be undertaken if necessary, the criteria for resectability based on thin-cut dynamic CT include: (1) absence of extrapancreatic disease; (2) no evidence of tumor extension to the superior mesenteric artery (SMA) or celiac axis as defined by the presence of a normal fat plane between the tumor and these arterial structures; and (3) a patent SMPV confluence¹² (Figure 13.1). In our experience, 20% to 30% of patients meeting these criteria will have local tumor involvement of the SMPV confluence, requiring en bloc vascular resection.

Currently, evidence of celiac axis and/or SMA involvement is considered a contraindication for resection.² Unlike the SMPV confluence and more distal aspects of the hepatic artery, the celiac axis and proximal SMA are ensheathed by a dense plexus of autonomic nerves. When these vessels are involved by tumor, extensive perineural invasion typically precludes a successful margin-negative resection. Preoperative determination of absence of SMA involvement is especially critical, as direct

assessment of this tumor-vessel relationship during exploration is typically possible only during the final step in tumor resection.¹⁰ In addition, when the SMPV confluence is inseparable from the tumor as determined intraoperatively, the SMA cannot be directly evaluated without performing either venous resection and medial retraction of the interposition graft or splenic vein division and lateral retraction of the specimen and attached SMPV confluence.^{13,14}

Resection of the SMPV confluence requires a thorough understanding of normal mesenteric venous anatomy and frequent variants. The portal vein is formed by the junction of the superior mesenteric vein (SMV) and the splenic vein posterior to the pancreas. The inferior mesenteric vein may empty into the splenic vein (most commonly), into the SMV, or into a common confluence with the SMV and splenic veins. Early tributaries of the SMV include the right gastroepiploic vein and the middle colic vein, both of which are sacrificed during resection of the SMPV confluence. In a cadaveric study, these two veins shared a common trunk before joining the SMV in 27% of specimens (I. M. M., unpublished data, 1999). The first jejunal vein branches join the SMV just a few centimeters distal to the middle colic vein, and may be jeopardized with lengthy venous involvement by tumor.

In addition to CT, endoscopic ultrasound (EUS) has been evaluated as a method to determine vascular involvement by tumor. Reports of 95% sensitivity and 92% specificity for the diagnosis of tumor invasion into the portal vein have been published with histological correlation of ultrasonographic and pathologic data.^{15,16} In this study, EUS was shown to be comparable to CT for delineation of SMPV confluence involvement. There appear to be limitations to the application of EUS data regarding SMA status, however, as this technique has less than 20% sensitivity in detecting SMA involvement by pancreatic cancer.¹⁷ EUS-based data have led to the development of four distinct criteria that support the diagnosis of venous invasion by tumor. The presence of venous irregularity, indistinct borders between the tumor and vein, proximity of the tumor and vein, and large tumor size all suggest venous invasion by tumor. Of these criteria, venous wall irregularity appears to be the most specific.¹⁶ While EUS may indeed be

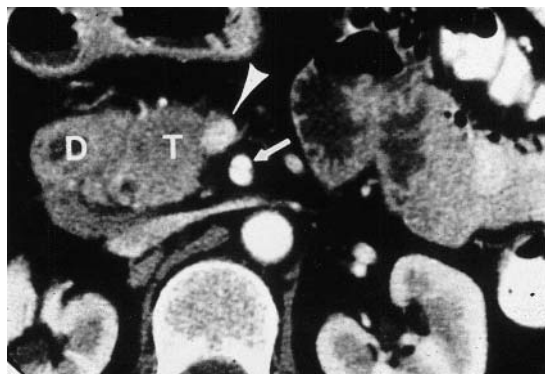


FIGURE 13.1. Pancreatic cancer involving superior mesenteric vein (SMV) as demonstrated by loss of fat plane between tumor (T) and patent SMV (arrowhead). Intact fat plane between tumor and superior mesenteric artery (arrow).

useful in determining the presence or absence of venous involvement, this modality remains limited in terms of providing extensive anatomic detail. In this regard, dynamic CT clearly remains superior.

Some centers consider mesenteric angiography a vital component of the preoperative evaluation when contemplating curative resection for patients with pancreatic cancer. Angiography allows only for determination of changes in the vascular lumen, as evidenced by alterations in contour and patency. In addition, critical anatomical variations, most commonly variants of hepatic arterial origin, may be identified prior to resection of the pancreatic head. In cases of reoperative pancreaticoduodenectomy, angiography plays a crucial role in the preoperative evaluation. Prior attempts at resection and surgical biliary drainage procedures induce periportal scarring, which puts the portal vascular structures at an increased risk of iatrogenic injury. Preoperative delineation of aberrant hepatic arterial anatomy in this setting is therefore useful.^{18,19} Angiography also allows for determination of clinically occult celiac stenosis, which may require revascularization secondary to loss of collateral hepatic blood flow when the gastroduodenal artery is ligated during pancreaticoduodenectomy.^{20,21} Of note, the blood supply to the biliary-enteric anastomosis is derived from the celiac axis, and may be at risk in cases of celiac stenosis.²¹ Preoperative angiography may also be of use in selected cases involving very large tumors in which intraoperative delineation of vascular anatomy may prove difficult.

An angiographic system for grading SMPV confluence involvement by adenocarcinoma of the pancreas has been reported.²² The authors classified the angiographic contour of the SMPV confluence during the venous phase of the SMA angiogram. Five types of involvement were described, with type I demonstrating a normal SMPV contour, suggesting no involvement by tumor. Type II demonstrated a smooth shift in the SMPV without venous narrowing, while type III was characterized by unilateral narrowing of the venous confluence. Type IV involvement was described as bilateral SMPV narrowing, implying near-or-total circumferential involvement by tumor. Type V involvement was demonstrated by bilateral narrowing with the presence of collateral veins. Correlation of the preoperative angiographic data with

pathological analysis of the venous specimens revealed an accuracy rate of 54%. Angiography tended to underestimate the true extent of disease, with 40% of cases being underdiagnosed preoperatively.²²

The combination of detailed preoperative radiographic evaluation and intraoperative evaluation by an experienced surgeon detects most of the cases in which tumor adherence to the SMPV confluence mandates vascular resection to achieve negative retroperitoneal margins. There are, however, cases where there is no objective evidence of venous involvement by the malignant process. In this setting, the role of touch-prep cytologic evaluation of the SMPV confluence in cases where this venous structure is apparently spared involvement by a nearby tumor has been evaluated. In one report, touch-preparations performed on SMPV confluences in patients undergoing pancreaticoduodenectomy demonstrated tumor cells on the venous wall in 30% of patients, resulting in the decision to perform venous resection. Histological confirmation of tumor cells within the wall of the resected vein correlated well with the cytological results.²³ The authors concluded that this technique may increase the number of patients in whom a margin-negative resection may be performed using venous resection techniques.

Technique

Pancreaticoduodenectomy with en Bloc Vascular Resection

Pancreaticoduodenectomy for carcinoma of the pancreatic head may be divided into 6 defined steps. This systematic approach to resection allows for standardization and optimal exposure, facilitating vascular resection and reconstruction.¹¹ The general concepts of preparation for operation are similar between standard pancreaticoduodenectomy and pancreaticoduodenectomy with venous resection with the addition of exposing the left neck and both groins for potential vein harvest sites.

1. A Cattell-Braasch maneuver is performed by mobilizing the right colon and incising the visceral peritoneum to the ligament of Treitz. This allows for cephalad retraction of the viscera, exposing the entire duodenum. The lesser sac is entered by sep-

arating the greater omentum from the transverse colon. The middle colic and right gastroepiploic veins are identified and ligated adjacent to their junction with the SMV. This allows for greater exposure of the SMV as well as prevention of traction injury during further dissection. The infrapancreatic SMV is identified and isolated prior to Kocherization of the duodenum.

2. The Kocher maneuver is performed, beginning near the ureter-right gonadal vein junction. The fibrofatty retroperitoneal tissues overlying the inferior vena cava are elevated with the duodenum and pancreatic head. Preoperative demonstration of an intact fat plane between the SMA and the tumor as demonstrated by CT obviates the use of palpation to determine the relationship between the tumor and the SMA; palpation may be difficult to accurately perform secondary to peripancreatic fibrosis and the proximity of the uncinate process to the SMA.

3. Dissection of the portal structures begins with exposure of the common hepatic artery. The gastroduodenal artery is subsequently divided. In selected cases, encasement of a short segment of the hepatic artery may require segmental resection and reconstruction with reversed saphenous vein interposition graft. The gallbladder is mobilized from the liver bed and the common bile duct is transected cephalad to the cystic duct–common duct junction. The anterior wall of the portal vein may then be exposed. There is no attempt to develop a plane between the SMPV and the posterior pancreatic head, as this maneuver does nothing to evaluate invasion of the lateral or posterior wall of the SMPV confluence. Invasion of the anterior wall of the SMPV confluence by tumors in the pancreatic head rarely occurs, and invasion of the posterolateral aspect of the SMPV confluence can only be determined after gastric and pancreatic transection.

4. Gastric transection is performed at the level of the third or fourth transverse vein on the lesser curvature and the level of the confluence of the gastroepiploic veins on the greater curvature.

5. Jejunal transection is performed approximately 15 cm distal to the ligament of Treitz. The duodenal mesentery is divided to the level of the aorta, and the duodenum and jejunum are reflected beneath the mesenteric vessels.

6. After pancreatic transection, the SMPV may be resected if, in the opinion of the surgeon, the tu-

mor is inseparable from the lateral wall of the SMV or portal vein. SMPV resection is performed in cases where resection of the venous structures is required for attainment of negative retroperitoneal margins. Inflow occlusion (SMA) is performed to prevent bowel edema due to outflow obstruction, and systemic heparinization is used at the discretion of the operating surgeon. The technique of venous resection and reconstruction is dictated by the extent of tumor involvement.

Venous Resection

The majority of pancreatic cancers arise within the pancreatic head and uncinate process. Local extension of these tumors may subsequently encroach nearby vascular structures. The most frequently involved of the major retroperitoneal vessels is the SMV, with or without concomitant involvement of the SMV confluence with the portal vein. Options for venous resection may be based on the extent of tumor adherence to the vein wall. Tumors that involves less than one third of the total circumference of the vein may be adequately resected tangentially, with reconstruction performed with a saphenous vein patch^{9–11,13} (Figure 13.2). In instances of more extensive venous involvement by tumor, segmental resection of the SMPV confluence may be undertaken, with reconstruction by autologous internal jugular vein interposition graft¹¹ (Figure 13.3). This allows for prompt restoration of

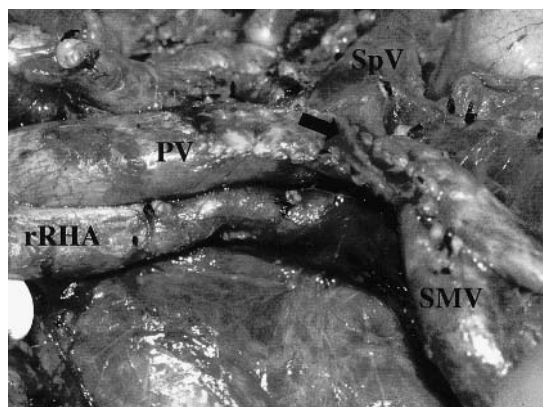


FIGURE 13.2. Intraoperative photograph of tangential SMPV confluence resection reconstructed with saphenous vein patch (arrow). PV = portal vein; rRHA = replaced right hepatic artery; SpV = splenic vein.

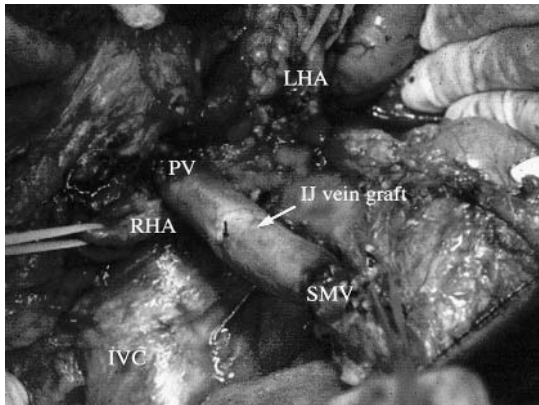


FIGURE 13.3. Total pancreatectomy with resection of SMV reconstructed with internal jugular vein interposition graft (arrow). IJ = autologous internal jugular vein interposition graft; PV = portal vein; IVC = inferior vena cava; RHA = right hepatic artery; LHA = left hepatic artery.

mesenteric venous drainage while providing a mobile length of reconstructed mesenteric vein. Medial retraction of the reconstructed venous segment allows for access to the retroperitoneum for dissection of the tumor from the lateral wall of the SMA¹⁰ (Figure 13.4).

When necessary, partial resection of the inferior vena cava has also been performed in selected patients with good results. These patients may be reconstructed with either saphenous vein patch in the case of tangential caval resection, or polytetrafluoroethylene (PTFE) interposition grafting in cases where segmental caval resection has been performed.

Preoperative CT data seem to be less accurate in predicting inferior vena caval involvement when compared to involvement of the SMPV confluence.¹¹

Vascular Reconstruction

Following major vascular resection, the surgeon is faced with the task of reconstructing the vessel in a way that will provide adequate blood flow and patency without compromising exposure during the completion of the retroperitoneal dissection. There are two major options for restoration of vessel continuity following segmental resection: primary anastomosis and interposition grafting. Several Japanese authors have advocated primary anastomosis after resection of a segment of the SMV.^{22,24} This approach allows for adequate restoration of portal circulation and avoids a second incision at a vein harvest site (Figure 13.5). However, it has the

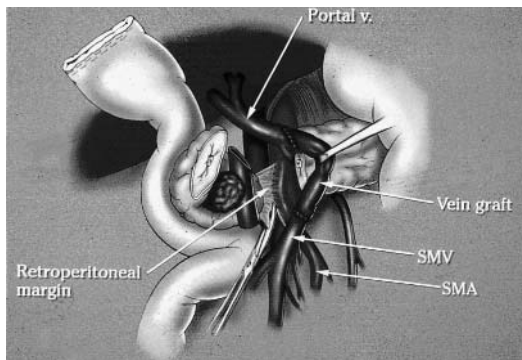


FIGURE 13.4. Internal jugular vein interposition graft reconstruction following segmental resection of SMPV confluence. Medial retraction of graft allows access to retroperitoneum for dissection of tumor from lateral wall of SMA. (From Leach et al¹⁰ by permission of *Br J Surg*.)

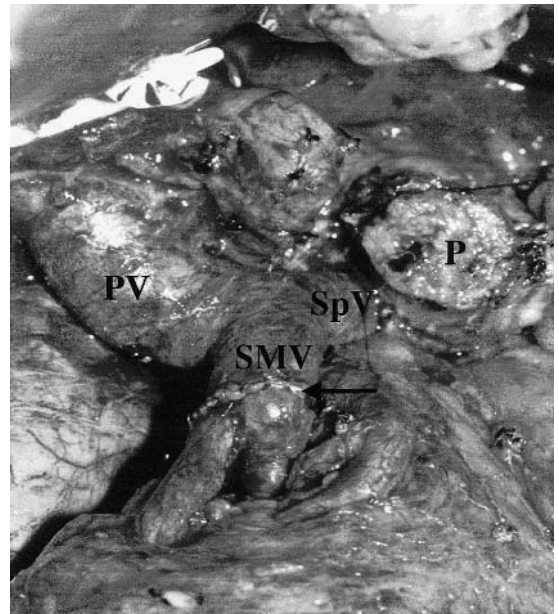


FIGURE 13.5. Pancreaticoduodenectomy with en bloc resection of SMPV confluence with primary anastomosis reconstruction (arrow). PV = portal vein; SpV = splenic vein; P = pancreas. Splenic vein division is often required in order to provide adequate vascular mobilization to perform tension-free vascular anastomosis. Short segment of resected SMV allowed splenic vein preservation in this case.

potential for producing tension on the anastomosis, leading to the frequent need for disconnection of the splenic vein in order to gain adequate vascular mobilization. In addition, primary SMPV reconstruction may restrict access to the lateral wall of the SMA root during final dissection of the uncinate process. Reconstruction of the SMPV with interposition graft has the dual benefits of producing a tension-free reconstruction that is mobile, thus providing adequate exposure during the completion of the retroperitoneal/SMA dissection.¹⁰ There are several choices of conduit for interposition grafting, including PTFE and autologous vein. There are reports of patients in whom PTFE has been used to reconstruct segmental resections of SMPV,¹¹ although a high rate of graft thrombosis is reported. In contrast, autologous vein interposition grafting is associated with high rates of long-term patency, as well as minimizing infectious risks associated with prosthetic material. Reversed saphenous vein is commonly used to reconstruct both segmental arterial and tangential venous defects. In our experience, the internal jugular vein is frequently utilized for segmental reconstruction of the SMPV, as it is ideally sized for this vessel.

Arterial Resection

In addition to the possible involvement of the SMPV by pancreatic cancer, neighboring arterial structures, most notably the hepatic arteries, may require en bloc resection during extended pancreaticoduodenectomy. Approximately 15% to 20% of the population has variations of hepatic arterial anatomy. A replaced right hepatic artery arising from the SMA may course posterior to, or through, the pancreatic head and is thus at high risk for tumor encasement. Short segments of hepatic artery that are encased by tumor have been successfully resected and reconstructed by either primary anastomosis or reversed saphenous vein interposition graft.¹¹ This is especially important in that the proximal bile duct receives nearly all of its blood supply from the right hepatic artery following division of the gastroduodenal artery. The common hepatic artery may also become encased by tumor, most commonly near the origin of the gastroduodenal artery. In cases where this encasement occurs without involvement of the SMA or celiac axis, isolated segmental resection of the common hepatic artery may be performed and reconstructed with either

primary anastomosis or reversed saphenous vein interposition. In reoperative cases or cases where there has been extensive fibrosis from preoperative radiotherapy, reconstruction of a resected common hepatic artery may be accomplished with a reversed saphenous vein graft anastomosed with the aorta.¹¹ Unlike the more proximal celiac axis or SMA, these hepatic branches may be involved by tumors without extensive involvement of the retroperitoneum, allowing for margin-negative resection to be performed.

Results

Survival Following Vascular Resection

Analyses of survival data from several studies of pancreaticoduodenectomy with en bloc mesenteric vascular resection demonstrate that, in patients in whom venous resection is performed, the survival time is no different than in patients that do not require venous resection.^{10,24,25} Thus, selected patients with traditionally unresectable tumors are provided a survival benefit similar to those patients with traditionally resectable tumors. At the M. D. Anderson Cancer Center, a prospective study comparing standard pancreaticoduodenectomy and pancreaticoduodenectomy with en bloc SMPV confluence resection demonstrated an overall survival time of 21 months, with survival of the venous resection group of 22 months and survival of the nonvenous resection group of 20 months over a median follow-up time of 17 months.¹⁰ The median tumor size in the venous resection group was 3.5 cm, compared to 3.0 cm in the nonvenous resection group. These data are consistent with the hypothesis that SMPV confluence involvement by pancreatic head cancer is a function of tumor location and possibly size, and not an independent predictor of patient survival.¹⁰ When necessary for complete tumor extirpation, venous resection converts a patient's predicted survival time from that of an unresectable patient population to that of a patient population in whom complete tumor removal can be performed without venous resection.¹⁸ Other studies have demonstrated median survival times of 13 to 14 months for patients in whom venous resection was performed, compared to 17 to 25 months for patients in whom no vascular resection was performed.^{24,25} Some long-term survivors have been reported after en bloc vas-

cular resection, and histological data demonstrated negative retroperitoneal margins in these patients.²⁴ Another report did not demonstrate a survival advantage in patients treated with SMPV confluence resection.²⁶ This study, however, failed to document the rate with which a margin-negative resection was achieved, thus raising uncertainty as to the final pathological staging of these patients. When resection requires inclusion of arterial structures in addition to the SMPV confluence, survival is not favorable, probably owing to extensive retroperitoneal infiltration by tumor cells.²⁴

Correlation of angiographic staging data and survival has been performed by Ishikawa and colleagues.²² They separated patients in whom pancreaticoduodenectomy with en bloc SMPV confluence resection was performed into 2 groups: those with survival times less than 3 years, and those with survival times greater than 3 years. The major differences between patients that survived less than 3 years and those with survival greater than 3 years were angiographic evidence of bilateral venous narrowing and longer segments of venous involvement. Both of these are markers of high-grade venous involvement, and are associated with shorter survival times in their study.²² A normal or low-grade venous phase angiogram and less than 1.2 cm of involvement of vein are associated with a 3-year survival of over 50%. The survival falls to 0% when the angiogram suggests a high-grade lesion or involvement of greater than 1.2 cm of vein. Higher angiographic grading, based on bilateral narrowing or encasement of the SMPV confluence, may represent cases in which the SMA is involved by tumor, thus conferring a poor survival time. Thin-cut dynamic CT accurately delineates the intimate relationship of the tumor, SMV, and SMA, which lies just posterior-medial to the SMV. Based on these data, it is likely that this classification system provides data similar to that provided by helical CT, with bilateral SMPV narrowing (type IV) an indicator for involvement of the adjacent SMA and a resulting inability to achieve a margin-negative resection.

Complications of Vascular Resection

Pancreaticoduodenectomy with en bloc resection of the SMPV confluence has been shown to carry acceptable mortality rates that are similar to those of standard pancreaticoduodenectomy. Studies from

multiple institutions have shown that inclusion of venous resection and reconstruction does not increase the mortality rate when performed by experienced surgeons.^{9,19,25,26} In contrast, when the resected specimen is extended to include major arterial structures and/or a total pancreatectomy, the mortality rate increases.²⁴ In addition, the morbidity rates after venous resection are equivalent to those of standard pancreaticoduodenectomy. Complication rates of 22% to 30% after venous resection have been reported, compared to complication rates of 28% to 34% for standard pancreaticoduodenectomy.^{9,11,26} There are some distinct differences in hemorrhagic complications between standard pancreaticoduodenectomy and pancreaticoduodenectomy with en bloc SMPV confluence resection. Pancreaticoduodenectomy with venous resection and reconstruction is associated with a significant increase in operative blood loss and associated transfusion requirement.^{9,10,25} The reoperation rate after venous resection is 12%, about the same for pancreaticoduodenectomy without venous resection. In addition, the indications for reoperation are similar, suggesting that inclusion of SMPV confluence resection is not associated with novel complications not observed following standard resection.²⁵

In the postoperative period, thrombosis of the reconstructed SMPV confluence is a major concern. Acute SMV thrombosis is associated with significant morbidity and mortality, notably from ascites, nutritional wasting, and portal hypertensive hemorrhage.¹⁰ In a study from M. D. Anderson, the 3-year venous patency rate after SMPV resection and reconstruction was 75%.¹⁰ Some authors have recommended long-term low-dose aspirin therapy for patients in whom resection of the SMPV confluence has been performed.^{10,11}

Status of the Retroperitoneal Margin

Resection of the SMPV confluence is performed when necessary for complete extirpation of the tumor, thereby allowing a margin-negative resection of an otherwise unresectable tumor. Patient survival is directly influenced by the status of the retroperitoneal margin. In patients with pancreatic adenocarcinoma, positive retroperitoneal margins after pancreaticoduodenectomy, either with or without concomitant venous resection, are associated with a median survival of less than 1 year.^{2,14} Data regarding the retroperitoneal status from mul-

multiple series of pancreaticoduodenectomy with concomitant venous resection have been quite variable. Reported percentages of patients having positive retroperitoneal margins after venous resection range from 13% to 68%.^{10,24-27} While the ability to predict margin-negative resection based on preoperative imaging is not absolute, it is generally true that patients undergoing vascular resection with a positive retroperitoneal margin have not been well selected for surgery.

Conclusions

Pancreaticoduodenectomy with en bloc SMPV resection may be safely performed, with rates of morbidity and mortality that are similar to those of standard pancreaticoduodenectomy. Thin-cut dynamic contrast-enhanced CT is the staging modality of choice, with selective supplementation by visceral angiography. In carefully selected patients, resection of the SMPV confluence may provide for a negative retroperitoneal margin, thus allowing patients traditionally deemed unresectable on the basis of venous involvement a median survival equivalent to those in whom standard pancreaticoduodenectomy is performed. Vascular resection for localized pancreatic cancer represents an aggressive approach to local tumor control, and is ideally combined with other aggressive local-regional therapies, including preoperative or postoperative chemoradiation and (where available) intraoperative radiotherapy. Pancreaticoduodenectomy with vascular resection also requires a high degree of institutional expertise, and is optimally undertaken in high-volume centers combining accurate preoperative imaging, high-volume surgical experience, and specialized postoperative care.

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14

Techniques for Biliary and Pancreatic Reconstruction After Pancreaticoduodenectomy

Jeffrey A. Drebin and Steven M. Strasberg

Introduction

Pancreaticoduodenectomy is one of the most complex abdominal surgical procedures.¹ This procedure is commonly known as the Whipple procedure after the American surgeon who popularized resectional approaches to the management of periampullary malignancies.² Pancreaticoduodenectomy involves not only resection of a periampullary tumor, but also the removal of the pancreatic head, gallbladder and bile duct, duodenum and, in some cases, a portion of the stomach, from the underlying mesenteric blood vessels. Following removal of the tumor and adjacent structures, biliary, pancreatic, and gastric anastomoses are created in order to restore continuity with the upper gastrointestinal tract. The procedure commonly takes 4 to 6 hours, and may be associated with significant blood loss.

The outcomes of patients undergoing pancreaticoduodenectomy have undergone a dramatic improvement over the past 20 to 30 years.¹ Prior to this time, the morbidity and mortality of pancreaticoduodenectomy was such that some prominent surgeons questioned whether the procedure should be performed at all.³ With perioperative mortality rates of 20% to 30%, major morbidity in virtually all patients, and fewer than 5% of patients surviving for 5 years after undergoing pancreaticoduodenectomy for malignant disease, support for palliative bypass procedures, rather than tumor resection, was generated in some institutions.^{1,3}

Results from the past two decades, however, demonstrate a striking reduction in perioperative mortality among patients undergoing pancreatico-

duodenectomy.¹ Perioperative mortality rates at high-volume surgical centers are well under 5%.¹ There has also been a corresponding improvement in the long-term survival of patients undergoing resection for malignant disease.¹ These results have led to increased acceptance of pancreaticoduodenectomy as the preferred surgical approach to patients with periampullary malignancies, and have also led to the use of pancreaticoduodenectomy in the management of certain patients with benign disorders, such as chronic pancreatitis.¹

Despite improvements in perioperative mortality, patients undergoing pancreaticoduodenectomy still have significant perioperative morbidity, with approximately half of patients in large series experiencing at least one significant postoperative complication.^{1,4,5} Some of this morbidity is due to events that can occur after any major surgical procedure, such as wound infection, myocardial infarction, and pneumonia. However, approximately half of the post-pancreaticoduodenectomy morbidity noted is the result of postoperative leakage at sites of biliary and pancreatic reconstruction.^{1,4,5} This chapter will review biliary and pancreatic anastomotic techniques with an emphasis on recent improvements that appear to minimize the development of postoperative complications.

Biliary Reconstruction

Whipple's original technique, published in 1935, utilized the gallbladder, anastomosed to the stomach, to permit biliary drainage into the gastrointestinal tract following the resection of a peri-

ampullary tumor.² This was modified within a few years to include the now standard resection of the gallbladder and common bile duct, followed by biliary-enteric anastomosis. Leakage at this anastomosis remains fairly common, however, with 5% to 10% of patients developing biliary leaks or fistulas in a number of large series.¹ Moreover, this leak rate has not changed significantly in recent decades. While rarely fatal, these anastomotic problems often require additional operative or radiological procedures to control ongoing bile leakage, and may predispose patients to infectious complications.

The Importance of Biliary Blood Supply

The greatest advance in our understanding of factors leading to the failure of biliary-enteric anastomoses was the result of studies by Terblanche on the biliary vasculature.⁶ It had long been known that the common bile duct contains two arteries running longitudinally at the medial and lateral aspects of the duct—the so-called three o'clock and nine o'clock arteries. Terblanche made the critical observation that the blood supply to these bile duct arteries arises distally from branches originating in the celiac and superior mesenteric arteries and proximally from branches originating in the right or common hepatic artery. About 60% of the arterial supply originates distally with 38% arising proximally—only 2% of the arterial supply originates from collateral vessels in the middle third of the bile duct. Thus the middle third of the bile duct is a vascular watershed area, receiving relatively poor supply from above and below.

Since the distal blood supply is disrupted in the course of resecting the distal bile duct during pancreaticoduodenectomy, the remaining duct is dependent on blood supply from above. If the duct is transected in the middle third, where blood supply is relatively poor, the cut edge of the residual bile duct may become ischemic, and if used for biliary-enteric anastomosis may result in poor healing with resultant biliary leakage or the late development of a biliary stricture. In order to avoid such complications, it is common practice to divide the bile duct higher, in the upper region of the common hepatic duct. In addition, the residual bile duct should be mobilized no more than necessary in order to avoid disruption of collateral circulation or possible compromise of the longitudinal arteries.

The Role of Stenting in Biliary-Enteric Anastomoses

The use of biliary stents or T-tubes in post-pancreaticoduodenectomy biliary reconstruction is controversial. Although preoperative biliary decompression has not been shown to improve outcomes in patients undergoing pancreaticoduodenectomy,^{1,7,8} some patients with periampullary malignancies have preoperative transhepatic biliary drainage catheters, such as Ring catheters, placed in order to palliate symptoms of jaundice or cholangitis prior to definitive surgery. During pancreaticoduodenectomy, the catheter can be preserved at the time of bile duct transection and subsequently used to stent the hepaticojejunostomy. It can be left open to drainage for the first few days after surgery and then capped if there is no evidence of bile leakage into the operatively placed drain. While such tubes can be used for routine postoperative cholangiography, we have found that routine cholangiography adds little in patients with normal liver function tests and no evidence of bile in the operative drains.

A related but distinct question is whether or not to place a T-tube or other stent in the bile duct during reconstruction in patients who have not had preoperative transhepatic catheters placed. The technical difficulties and cost associated with transhepatic stent placement make their use relatively uncommon at most institutions; the majority of patients undergoing preoperative biliary decompression have stents placed endoscopically prior to surgical referral. In such cases, the preoperative stent is generally removed or left in the operative specimen at the time of bile duct transection during pancreaticoduodenectomy.

If the bile duct is more than a few millimeters in diameter, it is not necessary to stent the biliary-enteric anastomosis. The results of carefully constructed nonstented anastomoses are such that the placement of a T-tube is rarely of benefit and may be a source of complications. However, if the hepatic duct is very small, a stent, such as a 5 French pediatric feeding tube, may be placed retrograde into the jejunum and positioned across the anastomosis. The stent is then brought out through a Witzel tunnel in the jejunal limb. The placement of such a stent is useful both to ensure patency of the duct during suture placement and to decompress the biliary tree above a small anastomosis in the immediate postoperative period.

Other Considerations in Performing a Biliary-Enteric Anastomosis

Additional technical factors that may improve the results of hepaticojejunostomy after pancreaticoduodenectomy include the careful construction of a duct-to-mucosa anastomosis using absorbable monofilament sutures. This can be most easily accomplished as a single-layer technique. The use of double-armed sutures and $2.5\times$ loupe magnification is helpful, particularly when anastomosing a small duct. Care should be taken when suturing the bile duct to minimize torquing of the needle with resulting trauma to the duct; needle holes are potential sites of biliary leakage. Similarly, when tying down sutures the knots must be snug but not so tight as to cut through the bile duct and produce a leak. As in all surgical procedures, the Halstedian principles of gentle tissue handling and minimizing operative tissue trauma will result in superior results.

Reconstruction after pancreaticoduodenectomy requires 3 distinct anastomoses involving the bile duct, pancreas, and stomach. In order to minimize postoperative complications, a number of surgeons have proposed different orders of anastomosing these structures to a single jejunal loop, as well as construction of a Roux-en-Y loop with separation of 1 anastomosis from the other 2.⁹ What is probably most critical with regard to post-pancreaticoduodenectomy bile duct reconstruction, as with all biliary-enteric anastomoses, is to avoid the reflux of food and gastric acid into the liver. While this may be accomplished by placing the biliary and gastric anastomoses on different limbs of a Roux-en-Y loop, it is more simply done by placing the hepaticojejunostomy at least 45 cm upstream of the gastrojejunostomy on the same jejunal loop.

Hepaticojejunostomy: A Specific Technique

While there is some variation among surgeons in the specific technical approaches to constructing a hepaticojejunostomy, this aspect of reconstruction after pancreaticoduodenectomy is, in fact, much less variable than performance of the pancreaticoenteric anastomosis. Our practice is to initially place double-armed sutures of 5-0 or 6-0 PDS (or Maxon) through the anterior wall of the bile duct and hold them on tags, in order to splay open the

bile duct orifice. A jejunotomy is made on the antimesenteric border of the jejunum, and the posterior row of sutures is then placed between the full-thickness jejunum and the bile duct, with care taken to incorporate jejunal mucosa in each suture bite. The sutures are held on tags until the entire posterior row is completed and then the sutures are sequentially tied down. The posterior sutures may be placed such that knots will be tied outside or inside the biliary-jejunal lumen. Our practice is to place the posterior suture knots on the inside in order to provide good apposition of the mucosa and to minimize trauma to the bile duct when tying down sutures.

Following the completion of the posterior row, the sutures previously placed through the anterior wall of the bile duct are passed through the jejunum. Again sutures may be placed so that knots are tied inside or outside the anastomotic lumen; our practice is to place them to allow the anterior row to be tied outside the lumen. After all anterior sutures are placed, they are sequentially tied down, completing the anastomosis. A closed suction drain is left posterior to the anastomosis at abdominal closure. Using such technique we have noted a biliary anastomotic leakage rate of less than 1% (J.A.D. and S.M.S., unpublished data, 2000).

Management of Biliary Anastomotic Leaks

While careful attention to technique can avoid the development of leakage at sites of biliary-enteric anastomoses in the vast majority of patients, a small percentage of patients will develop a postoperative bile leak, as evidenced by the presence of bile in the surgical drain. The management of biliary leaks is relatively straightforward and often requires minimal intervention. Bile leaks noted in the first few postoperative days will usually resolve spontaneously and, assuming there is adequate drainage and no evidence of biliary obstruction or sepsis, can simply be observed. Surgical reexploration of patients for evidence of biliary leakage in the first few days after pancreaticoduodenectomy is rarely necessary.

Persistent drainage of bile beyond the seventh postoperative day, or bile leakage in the setting of laboratory tests suggesting biliary obstruction, should be approached more aggressively. Trans-

hepatic cholangiography is critical to delineate the anatomic nature of the biliary leak. Furthermore, for small anastomotic leaks between sutures or through suture holes, the placement of a transhepatic catheter across the leak is generally all that is required to achieve healing. More significant leaks, as occur with complete disruption of the anastomosis, or early biliary obstruction due to technical problems in constructing the hepaticojejunostomy, may require surgical intervention; in experienced hands such biliary catastrophes should be exceedingly rare. In over 200 pancreaticoduodenectomies performed over the past 4 years by our section, there have been no patients requiring surgical re-exploration for management of leakage or early obstruction at a biliary-enteric anastomosis, and only 2 patients requiring placement of a transhepatic catheter for small biliary leaks.

Pancreatic Reconstruction

The fragile nature of the pancreatic parenchyma and the ability of activated pancreatic enzymes to injure tissue ("autodigestion") make surgical manipulation of the pancreas a technical challenge. Pancreaticocenteric anastomotic leakage, generally defined as the drainage of 50 cc or more of amylase-rich drainage postoperatively for 3 consecutive days, including or after the tenth postoperative day, may be a significant source of morbidity and mortality.^{10,11} Pancreatic anastomotic leakage rates on the order of 10% to 20% are still reported in large series from major centers.^{1,10,11} Furthermore, such patients almost invariably require a more prolonged hospital stay and other interventions such as the initiation of parenteral nutrition.⁹⁻¹¹ Pancreatic anastomotic failure may also lead to the development of intra-abdominal abscesses requiring percutaneous or surgical drainage,¹ and though less common than in previous decades, remains a significant cause of postoperative mortality.^{1,11}

Ablative Approaches to Avoiding Pancreatic Anastomotic Failure

The technical challenge of managing the residual pancreas following pancreaticoduodenectomy has been approached in a variety of ways. One approach is to resect the entire pancreas (total pan-

createctomy), rather than simply performing a pancreatic head resection. This procedure has been advocated in the past as a superior cancer operation,^{12,13} with the added advantage that there is no need to construct a pancreaticocenteric anastomosis and thus no risk of developing a pancreatic leak. However, patients undergoing total pancreatectomy all develop diabetes, which can be quite difficult to manage in some cases. Furthermore, the purported advantages of total pancreatectomy from an oncologic perspective have not resulted in improved patient survival compared with the survival of patients undergoing more limited pancreatic resections.¹²⁻¹⁴ The development of improved techniques for performing pancreaticocenteric anastomoses, and for managing leaks if they do occur, no longer justifies performance of total pancreatectomy as a means to avoid pancreatic anastomotic leakage. The indications for total pancreatectomy are currently limited to the relatively rare instances when an otherwise resectable pancreatic malignancy extends from the pancreatic head into the body and tail of the gland, and for patients with mucinous ectasia of the entire pancreatic duct.

In Whipple's original description of pancreaticoduodenectomy, the pancreatic duct was suture ligated rather than reanastomosed to the gastrointestinal tract.² This approach may avoid leakage at a pancreaticocenteric anastomosis, and has been advocated by some surgeons.⁹ Duct ligation is associated with a high rate of pancreatic leakage at the suture site, with the resulting formation of a pancreaticocutaneous fistula along surgical drain tracts. In addition to the formation of pancreatic fistulae, patients undergoing duct ligation universally develop pancreatic exocrine insufficiency. Proponents of this approach point out that pancreatic juice, in the absence of contact with biliary or enteric secretions, has a far lower fraction of activated enzymes and is thus less caustic and damaging to surrounding tissues.⁹ Furthermore, it has been suggested that the rate of postoperative pancreatic fistula development and exocrine insufficiency is no higher with duct ligation than with construction of pancreaticocenteric anastomoses.⁹ A variant of duct ligation, particularly popular in some European centers, is occlusion of the pancreatic duct by the injection of rubber.⁹ This approach may have a lower pancreatic fistula rate than is seen following suture ligation of the pancreatic duct.⁹

While there may be advantages to pancreatic duct occlusion compared with construction of pancreatocenteric anastomoses that have a high likelihood of leakage, this is clearly a suboptimal approach. The resulting pancreatic exocrine insufficiency results in significant lifelong morbidity, and the long-term use of pancreatic enzyme supplements is both inconvenient and costly. Furthermore, as will be discussed later in this chapter, newer approaches to construction of pancreatocenteric anastomoses have reduced the incidence of anastomotic leakage dramatically. It is therefore our view that duct occlusion techniques have little role in the management of the pancreatic stump after pancreaticoduodenectomy.

Restoration of Pancreatocenteric Continuity

The surgical anastomosis of the residual pancreas to the gastrointestinal tract, with drainage of pancreatic juice back into the gut, represents the most “physiological” approach to managing the pancreatic remnant following pancreaticoduodenectomy. The pancreatocenteric anastomosis is an unusual gastrointestinal anastomosis in that a hollow organ is being sewn to the side of the pancreas, a solid organ with a small and often eccentrically placed duct. This anastomosis is further complicated by the small size of the pancreatic duct, which often measures only 1 to 3 mm in diameter. The two principal approaches to restoring pancreatocenteric continuity are anastomosis of the pancreas to the jejunum (pancreaticojejunostomy) or to the stomach (pancreaticogastrostomy). While both pancreaticojejunostomy and pancreaticogastrostomy have their proponents, pancreaticojejunostomy has a longer history, has been studied far more extensively, and will be discussed first.

Pancreaticojejunostomy: Invagination Techniques

The cut edge of the pancreas measures several centimeters in diameter and, on average, is about the same diameter as the lumen of the jejunum. In contrast the pancreatic duct is rarely larger than 5 mm in diameter and, as noted above, is often much smaller. This has led to the development of approaches to pancreaticojejunal anastomosis in

which the entire pancreas is invaginated into the jejunal lumen (Figure 14.1A). This approach has been referred to as a “dunking” anastomosis, since the cut edge of the pancreas is dunked into the lumen of the bowel. In this approach there is little or no effort to approximate the pancreatic duct to the jejunal mucosa. Instead the pancreas is invaginated 2 to 4 cm into the cut end or side of the jejunum, and the capsule of the pancreas is sutured to the jejunum. This is clearly a very different approach to constructing an anastomosis than that used in biliary reconstruction or most other gastrointestinal anastomoses. The closest analogy is to the Kasai procedure, in which the bowel is sewn to the liver capsule in infants with biliary atresia.

Advantages of constructing an invaginated anastomosis relate to its (relative) technical simplicity and avoiding the need to deal with a small and sometimes friable pancreatic duct. The end of the

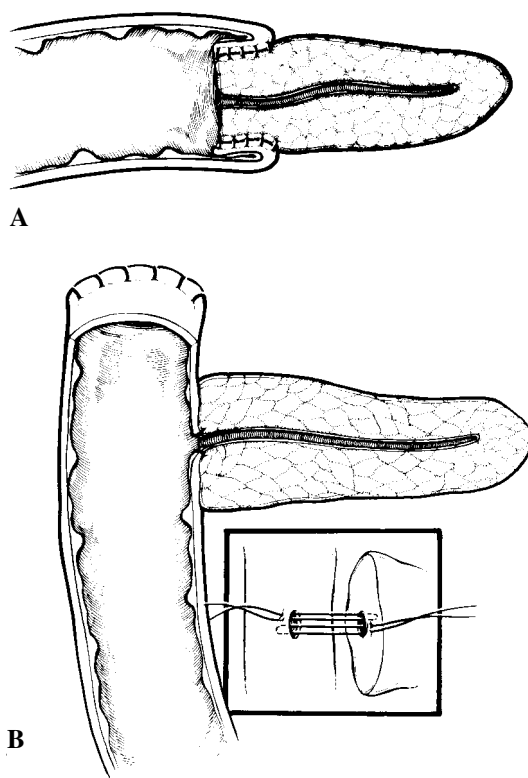


FIGURE 14.1. Methods for the construction of pancreaticojejunal anastomoses: (A) Invagination/dunking technique. (B) Duct-to-mucosa technique. (From Strasberg et al,¹ by permission of *Gastroenterology*.)

pancreas commonly fits easily into the cut end of the duodenum; when the pancreas is relatively large and the jejunum relatively small, the administration of glucagon may relax the small bowel muscularis and allow an end-to-end invaginating anastomosis. When the size discrepancy is too great for an end-to-end anastomosis, the pancreas can be intussuscepted into the side of the jejunum with little compromise of the efficacy of the anastomosis. Furthermore, the placement of sutures between the pancreatic capsule and the jejunum, while still delicate, is simpler than placement of sutures into the pancreatic duct and parenchyma.

The advantages of an invaginated pancreaticojejunal anastomosis are counterbalanced by certain specific problems. These include the need to extensively mobilize the pancreatic remnant and a relatively high rate of anastomotic failure. The need for pancreatic mobilization is clear when one considers that the entire circumference of the residual pancreas is intussuscepted into the jejunum. If the pancreas is to be advanced 2 to 4 cm into the jejunal lumen, it must be mobilized circumferentially over this distance from all surrounding tissues. While mobilization of the pancreas is relatively straightforward along its superior and inferior borders, the posterior border of the pancreas may be intimately associated with, and give off small branches draining into, the splenic vein. Mobilization of the pancreas off the splenic vein and division of small feeding branches is tedious and can on occasion be associated with bleeding that is difficult to control. In addition, the available data suggest that invaginated pancreaticojejunal anastomoses are associated with a relatively high rate of leakage. Several series, including the largest series in the published literature,^{1,5,9} suggest that 15% to 20% of patients reconstructed with an invaginated anastomosis develop significant pancreatic leaks or fistulae. The use of a two-layer technique does not seem to obviate this problem. Thus reconstruction using a dunking technique, though effective in the majority of patients, is associated with relatively high leak rates.

Pancreaticojejunostomy: Duct-to-Mucosa Techniques

An alternative approach to restoration of pancreaticoenteric continuity is to construct a duct-to-mucosa anastomosis by directly suturing these structures together (Figure 14.1B). This is commonly

done using fine monofilament absorbable sutures. Although running and interrupted suturing techniques have been advocated, the precise placement of sutures allowed by an interrupted technique has led to the more widespread adoption of this technique, particularly with relatively small pancreatic ducts. Gentle tissue handling and precise needle placement yield the most satisfactory results when sewing a delicate organ like the pancreas. Following completion of the duct-to-mucosa anastomosis, a second layer of sutures is generally placed between the seromuscular layer of the jejunum and the capsule of the pancreas. This serves to reinforce and take tension off the pancreatic ductal anastomosis.

Advantages of the duct-to-mucosa anastomosis include avoiding the need to extensively mobilize the pancreatic remnant, and a generally lower rate of anastomotic failure than that reported after intussuscepting anastomoses, though the latter point remains controversial.^{1,9} However, the construction of a duct-to-mucosa anastomosis can be both technically more demanding and more time consuming than the construction of an intussuscepted anastomosis. The placement of sutures can be challenging, particularly in patients with small pancreatic ducts, and the tying of sutures in the setting of a friable pancreas can require considerable skill. Approaches we have adopted to optimize this type of anastomosis will be described in greater detail below.

Pancreaticogastrostomy

An alternative to pancreaticojejunostomy is the construction of an anastomosis between the pancreas and the stomach—pancreaticogastrostomy. The pancreatic remnant sits in close apposition to the posterior wall of the stomach, permitting a tension-free anastomosis. The stomach holds sutures well and has a rich blood supply. Furthermore, gastric fluid can be readily obtained and assayed for amylase to evaluate pancreatic exocrine function. Pancreaticogastrostomy can be done as either an invaginating or a duct-to-mucosa type anastomosis, though the relatively greater thickness of the gastric wall has led to the more widespread acceptance of the dunked type of anastomosis. Advocates of pancreaticogastrostomy suggest it is less likely than pancreaticojejunostomy to lead to pancreatic anastomotic failure, and several case series appear to support this view.⁹ However, a large prospective randomized trial comparing pancreaticogastros-

tomy with pancreaticojejunostomy failed to find a difference in outcome between the 2 procedures.¹⁰ It is our belief that the specific anastomotic method used to restore pancreaticoenteric continuity is less important than careful handling of tissues and preservation of blood supply at the cut edge of the pancreas in performing the anastomosis, as will be discussed below.

The Role of Pancreatic Blood Supply in Anastomotic Healing

The blood supply of the pancreas has until recently received relatively little attention from pancreatic surgeons.¹⁵ Like the bile duct, the pancreas carries longitudinal arteries supplied from proximal and distal vascular arcades. In addition, the body and neck of the pancreas frequently are supplied by the dorsal pancreatic artery, which is variable in

anatomic location (Figure 14.2). Because the blood supply from the pancreatic head, and sometimes from the dorsal pancreatic artery, is sacrificed during a pancreaticoduodenectomy, the pancreatic neck may become a relatively poorly perfused vascular watershed. An ischemic pancreatic neck, if used as the site of pancreatic anastomosis, is more likely to lead to anastomotic failure, regardless of the specific method of restoring pancreaticoenteric continuity. This is analogous to the situation of the middle third of the bile duct, as described above.

The recognition of the importance of pancreatic anastomotic blood supply has led to alterations in technique that have the potential to dramatically reduce the rate of leakage at pancreaticojejunal anastomoses.¹⁵ The specific alterations are: (1) the use of a scalpel to transect the pancreas, with suture ligation rather than cautery used to control bleeding vessels at the cut surface of the pancreas that

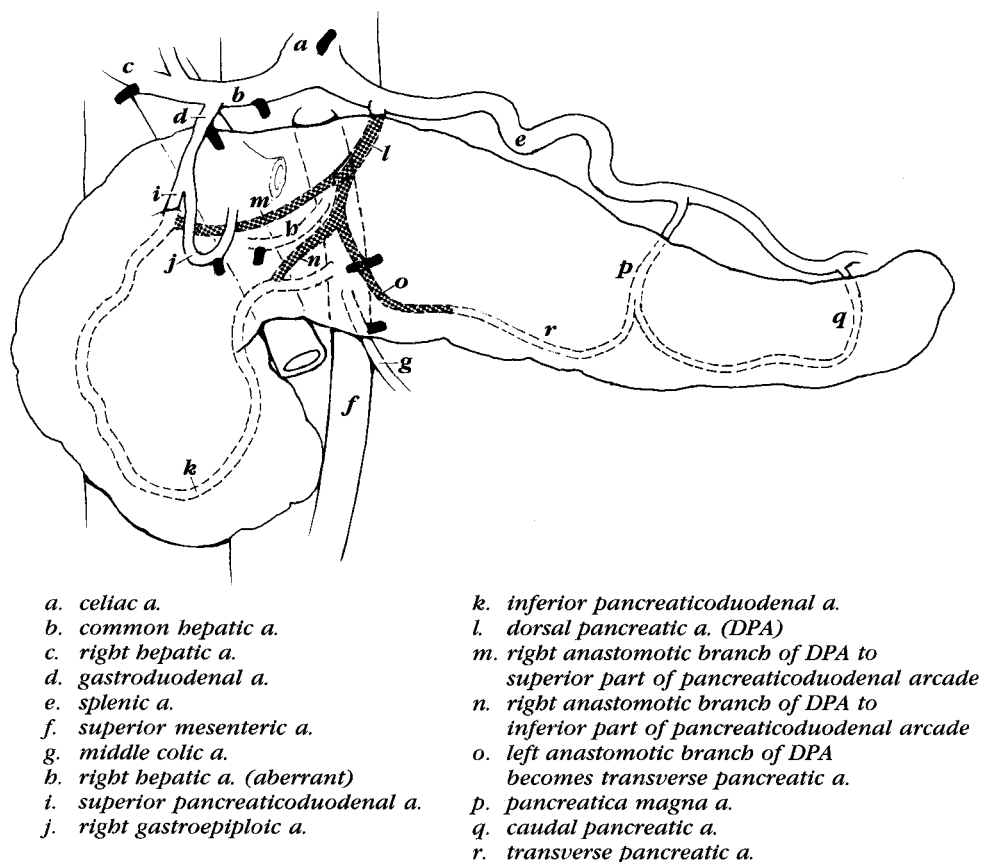


FIGURE 14.2. Vascular arcades in the pancreas. Note the variable sites of origin of the dorsal pancreatic artery, as indicated by short dark segments. (From Strasberg and McNevin,¹⁵ by permission of *Journal of the American College of Surgeons*.)

are in close proximity to the pancreatic duct; and (2) examination of the degree of bleeding at the transected pancreatic neck and "cutting back" the pancreas an additional 1 to 2 cm if reasonably brisk bleeding is not noted at the cut edge. The application of these principles, along with the use of loupe magnification in the construction of two-layer duct-to-mucosa anastomoses, has resulted in a pancreatic anastomotic leakage rate of under 2% in over 100 patients undergoing pancreaticoduodenectomy over the past 2 years at our institution (J. A. D. and S. M. S., unpublished data, 2000).

Stenting of Pancreatic Duct-to-Mucosa Anastomoses

The pancreatic duct is often only 1 or 2 mm in diameter and may be eccentrically placed on the cut edge of the pancreas. The desire to maintain the patency of the delicate anastomosis of the pancreatic duct to the jejunal mucosa has led surgeons to utilize several distinct approaches to stenting this anastomosis. The simplest means of stenting is the placement of a nonfixed stent, such as a 16 French angiocath tip, across the anastomosis during suture placement. This stent will eventually migrate from the anastomosis into the intestine and pass in the stool. A more complex fixed stent can be made by placing a 5 French pediatric feeding tube across the pancreaticojejunostomy, bringing it out of the jejunum through a pursestring and Witzel tunnel, and then externalizing it through an abdominal wall stab incision.

Advantages of stenting include the assurance that sutures do not occlude the duct lumen, the preservation of duct-to-mucosa patency/continuity during anastomotic healing, and, if the stent is brought out of the body, a means of controlling pancreatic juice drainage in the event of a leak. Disadvantages of stenting include the potential of a poorly functioning stent to occlude the stented lumen, the possibility that the stent tract will itself become a source of pancreatic or enteric leakage, and potential complications such as the stent eroding into adjacent structures, migrating, or fracturing during attempted withdrawal.

In our experience, duct-to-mucosa anastomoses to pancreatic ducts 3 mm in diameter and larger can be performed without the need for stenting. However, for very small ducts, 2 mm in diameter or less, we feel that the placement of a retrograde

feeding tube stent, as described above, is warranted to provide additional security that the duct is not compromised in suture placement and tying, and to allow duct decompression during the first few postoperative days.

The Use of Octreotide to Prevent Pancreatic Leaks

A controversial issue in pancreatic surgery is the utility of the somatostatin analogue octreotide in the prevention of postoperative fistulae. Several prospective randomized European studies suggest that, taken as a group, patients undergoing a variety of pancreatic surgical procedures have a lower fistula rate if treated with octreotide in the postoperative period.^{16,17} However, these studies have been criticized on a number of grounds. Some of the cases in these studies involved patients undergoing duct ablative procedures with ligation or rubber injection, which should lead to a higher risk of pancreatic fistula than is seen in patients undergoing resection and reconstruction with a pancreatocenteric anastomosis. Furthermore, these studies represent a variety of resectional and drainage procedures; in the one study that looked specifically at the subgroup of patients undergoing pancreaticoduodenectomy, there was no advantage of postoperative octreotide.¹⁶

There has now been a prospective randomized study, performed at a major American pancreatic surgery center, evaluating the utility of octreotide in the prevention of pancreatic anastomotic failure, specifically in the setting of patients undergoing pancreaticoduodenectomy.¹⁸ This study has demonstrated convincingly that there is no benefit of octreotide in preventing postoperative pancreatic fistula development after pancreaticoduodenectomy. Furthermore, octreotide is relatively expensive, and, if administered subcutaneously, it is painful for patients as well. It is therefore not our practice to routinely administer octreotide to pancreaticoduodenectomy patients in the postoperative setting.

Pancreaticojejunostomy: A Specific Technique

There is greater diversity among experienced surgeons in the technical approaches to pancreaticojejunostomy than to hepaticojejunostomy. We have

utilized a duct-to-mucosa technique, with careful attention to blood supply at the cut edge of the pancreas and selective stenting, that has reduced the incidence of pancreatic fistulae to “near zero.”¹⁵ The evaluation of blood supply at the cut edge of the pancreas is made at the time of pancreatic transection. As noted above, using a scalpel rather than cautery to transect the pancreatic neck allows direct evaluation of bleeding from the cut edge. If this bleeding is abundant and includes pulsatile arterial bleeding, it is adequate to achieve anastomotic healing. If the bleeding is sparse or questionable, it is our practice to mobilize an additional 1 to 2 cm of pancreas and to resect well to the left of the portal vein. This almost invariably is associated with improved bleeding from the cut pancreatic stump. Bleeding is then controlled with a combination of cautery and suture ligation; cautery is not used in close proximity to the pancreatic duct.

The pancreatic duct to jejunal mucosa anastomosis is performed prior to the hepaticojejunostomy, using an inner layer of 5-0 or 6-0 monofilament absorbable double-armed sutures, such as Maxon or PDS, and loupe magnification. The anterior row of sutures is placed in the pancreatic duct and held on tags, which serves to splay open the duct for posterior suture placement. A small jejunotomy is then made along the antimesenteric border of the jejunum using cautery. It is our practice to fix and evert the mucosa at this site using full-thickness interrupted 6-0 sutures placed 90 degrees apart, to ensure that anastomotic sutures incorporate all layers of the bowel wall. If a stent will be used, it is brought through a flank stab incision, placed through a pursestring and Witzel tunnel in the downstream jejunum, and advanced through the jejunotomy prior to proceeding with the anastomosis. The posterior row of sutures is then placed between the pancreatic duct and the jejunum, with double-armed sutures passed so that knots are on the outside. These sutures are sequentially tied down, and the stent, if present, is then advanced into the pancreatic duct. The anterior sutures, previously placed in the pancreatic duct and tagged, are then passed through the jejunotomy; following the placement of all sutures, they are sequentially tied down, again with knots on the outside.

Upon completion of the inner layer of the anastomosis, a second layer of interrupted 3-0 silk sutures is placed from the edge of the pancreatic cap-

sule to the seromuscular layer of the jejunum. These sutures are initially placed along the anterior surface of the pancreas and are tied sequentially prior to placing the next suture. The superior and inferior edges of the pancreatic capsule are then similarly approximated to the jejunum. Finally, the jejunum is rotated anteriorly to permit sutures to be placed along the posterior edge of the pancreatic capsule. Placing the anterior and side sutures first avoids undue traction on the inner suture line when rotating the jejunum for placement of the outer posterior row of sutures.

Admittedly, this is an atypical way to construct a two-layered gastrointestinal anastomosis. The more common approach to this situation, for example in a bowel anastomosis, is to place the outer posterior row of sutures as the first step, before proceeding with the inner layer. However, the geometry of the cut edge of the pancreas and of the pancreatic duct is such that placing the outer posterior sutures first may compromise precise suture placement at the more critical duct-to-mucosa inner layer. We have found that placing the anterior outer row first, and avoiding excessive traction when rotating the jejunum for the posterior outer row sutures, results in a very satisfactory two-layer anastomosis. Although leaks are rare, we continue to place a closed suction drain posterior to the pancreaticojejunostomy and to check drain amylase levels on postoperative day 5. Utilizing this approach we have observed only 2 leaks in over 100 pancreaticoduodenectomies performed over the past 2 years. A very similar approach, resulting in the complete avoidance of leaks (in a smaller series of patients), has been described by Howard.¹⁹

Management of Pancreatic Anastomotic Leaks

With experience and careful attention to surgical technique, the incidence of pancreatic anastomotic leakage (pancreatic fistula) should be well under 5%. When such leaks occur they are usually of little consequence if adequately drained. A computed tomography scan may be of particular use in establishing the absence of undrained collections, or in identifying such collections for percutaneous drain placement. While octreotide does not completely resolve such pancreatic fistulae, it does decrease pancreatic secretion and may facilitate fis-

tula healing. For these reasons we continue to use octreotide on those rare occasions when significant leaks occur. Because alimentation may stimulate pancreatic exocrine secretion, it is our practice to limit patients to clear fluids for comfort and to use hyperalimentation for nutrition during fistula healing. Antibiotics are indicated only if a specific infected collection is identified in association with clinical evidence of sepsis. Pancreatic fistulae, and the maneuvers used to facilitate their healing, result in significant patient morbidity, prolonged hospitalization, and increased medical expenses, but rarely are a source of patient mortality at present.^{1,10,11}

Conclusions

Pancreaticoduodenectomy has become the established surgical approach to the management of most pancreatic and other periampullary malignancies, and is also increasingly utilized for the treatment of benign disorders such as chronic pancreatitis. Problems resulting from leakage at sites of biliary or pancreatic anastomoses remain frequent causes of morbidity, and occasional causes of mortality, after pancreaticoduodenectomy. Attention to specific technical aspects when performing these anastomoses, particularly optimization of blood supply at the cut edge of the bile duct and pancreas, in conjunction with careful suture placement and tissue handling, can significantly lower the incidence of biliary and pancreatic anastomotic leakage, with resulting improvement in patient outcomes.

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15

Transduodenal Local Resection for Periapillary Neoplasms

Bryan Clary, Theodore N. Pappas, and Douglas Tyler

Introduction

Ampullary neoplasms are uncommon and represent less than 10% of pancreatic and periampullary tumors. Although the majority of these are adenocarcinomas, the spectrum of neoplasms occurring in this location is broad and includes benign as well as malignant diseases (Table 15.1). The definitive treatment of these tumors has been primarily surgical since Halsted reported the first local excision of an ampullary carcinoma in 1899.¹ Improvements in surgical morbidity and mortality following pancreaticoduodenectomy have led to significant controversy over the role of local resection in patients with known malignant neoplasms and conditions that are potentially malignant, including adenomas and neuroendocrine tumors. This chapter reviews the nature and management of ampullary neoplasms with specific attention to the role and technique of transduodenal ampullary resection. Distinction from periampullary neoplasms is important given the different prognoses and technical considerations involved in the operative approaches. This chapter deals specifically with tumors involving the ampullary structure.

Anatomical Considerations

Although illustrated first by Bottfried Bidloo in 1685, credit for the discovery of the ampulla is generally given to Abraham Vater, who in 1720 described the structure in detail. Located in the posteromedial wall of the second portion of the duodenum, it is formed by the confluence of the

intraduodenal bile duct (CBD), terminal pancreatic duct (PD), and duodenal mucosa. It is surrounded by the muscular sphincter of Oddi, and empties into the duodenum through a prominence in the duodenal wall, the papilla of Vater. The common channel formed by the confluence of the CBD and PD is present in approximately half of individuals. In a significant proportion of patients, the CBD and PD enter as separate channels onto the duodenal papilla or duodenal wall without a common channel.

As a consequence of these anatomical relationships, tumors of the ampullary region may arise from any of the three epithelia (CBD, PD, duodenum) as well as the wall of the duodenum (connective tissue, neuroendocrine cells). The differentiation between ampullary tumors and tumors arising in the adjacent pancreas and distal common bile duct appears to be a clinically relevant distinction, as survival rates for patients with ampullary malignancies are reportedly higher than those for patients with pancreatic and distal CBD tumors. In addition to the prognostic significance, the anatomical relationships are such that definitive treatment of these tumors must take into account the maintenance or reestablishment of biliary, pancreatic, and gastrointestinal continuity following tumor removal.

Histology

Adenoma and Adenocarcinoma

Adenomas occurring at the ampulla may be sessile or pedunculated and may contain tubular, villous, or tubulovillous features. Adenomas of the duode-

TABLE 15.1. Ampullary neoplasms.

Benign Neoplasms
Adenoma
Hyperplastic polyp
Hemangioma
Leiomyofibroma
Lipoma
Neuroma
Neuroendocrine tumor
Malignant
Adenocarcinoma
Adenosquamous carcinoma
Gastrointestinal stromal tumor (leiomyosarcoma)
Fibrosarcoma
Neuroendocrine carcinomas (small cell and non-small cell)
Local invasion from other primary tumors (duodenal, pancreatic, bile duct, colon, gallbladder)
Metastases (melanoma, renal cell carcinoma, lymphoma)

num, of which the ampulla is the most common site, clearly possess malignant potential and have been postulated to be premalignant lesions within an adenoma-carcinoma sequence similar to that present in colon cancer. In support of this, investigators have documented the presence of adenomatous tissue at the periphery of ampullary carcinomas in up to 40% of cases.²⁻⁴ In addition, patients with ampullary adenomas tend to be younger than patients with ampullary carcinoma.⁵ Periapillary and duodenal adenomas are reported to be present in up to 80% of patients with familial adenomatous polyposis and confer a relative risk of developing ampullary adenocarcinoma that is 120 times greater than in the general population.⁶⁻⁹ Adenomas with villous features appear to have a greater risk of malignant transformation, as do those that are larger in size.³ The sum of these observations lends considerable support to the contention that these lesions are premalignant and if given enough time may transform into frankly malignant lesions.

TABLE 15.2. TNM staging of ampullary carcinoma.

Tumor			
T1 = tumor limited to ampulla of Vater			
T2 = tumor invading the duodenal wall			
T3 = tumor invading ≤2 cm into pancreas			
T4 = tumor invading >2 cm into pancreas and/or into adjacent organs			
Nodes			
N0 = no regional lymph node metastases			
N1 = regional lymph node metastases			
Metastases			
M0 = no distant metastases			
M1 = distant metastases			
Stages	T	N	M
Stage I	T1	N0	M0
Stage II	T2-3	N0	M0
Stage III	T1-3	N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

Given the poor prognosis of ampullary adenocarcinomas and the frequent finding of small foci of adenocarcinoma within ampullary adenomas, these lesions should be strongly considered to have a malignant component until proven otherwise.

Adenocarcinoma is by far the most common malignant ampullary neoplasm. Although it accounts for only 5% to 10% of all periampullary malignancies,¹⁰ the resectability rate is greater than that of pancreatic and distal common bile duct tumors, such that it constitutes a larger proportion (20% to 25%) of resectable periampullary malignancies.¹¹ The current TNM staging of ampullary carcinoma is presented in Table 15.2. Only 20% to 30% of resectable ampullary adenocarcinomas are limited to the ampulla or sphincter of Oddi (T1).^{10,12-14} Approximately 40% to 55% of resected ampullary adenocarcinomas are associated with lymph node metastases (Table 15.3).^{10,12-15} Nodal status appears to be related to primary tumor stage, but even

TABLE 15.3. Regional nodal involvement in resected ampullary carcinoma.

Authors	All patients		T1		T2-3	
	n	Node positive	n*	Node positive	n*	Node positive
Roder et al 1995 ¹³	66	42%	20	20%	44	50%
Beger et al 1999 ¹²	126	55%	18	22%	73	37%
Yamaguchi and Enjoji 1987 ¹⁴	107	52%	12	0	55	44%
Howe et al 1996 ¹⁰	101	45%	22	NS	73	NS
Talamini et al 1997 ¹⁵	106	39%	NS	NS	NS	NS

*NS indicates not stated.

T1 lesions are associated with a 20% incidence of nodal metastases. Factors that have been found to be influential in the prognosis of patients with ampullary adenocarcinomas include nodal status, resectability, and tumor grade.^{10,15}

Neuroendocrine Tumors

Neuroendocrine tumors arising within the ampulla have been demonstrated to have a broad spectrum of malignancy, ranging from low-grade carcinoid tumors to intermediate non–small cell carcinoma to high-grade small cell carcinoma.¹⁶ Carcinoid tumors are the most common primary neuroendocrine tumor of the ampulla and can be recognized histologically by the monotonous sheets of small round cells with uniform nuclei and cytoplasm. Mineralized concretions termed psammoma bodies are frequently present in somatostatin-rich duodenal carcinoid tumors. Approximately 25% to 30% of ampullary carcinoids occur in patients with von Recklinghausen's neurofibromatosis.^{17,18} Ampullary carcinoids occurring in patients with neurofibromatosis are most often somatostatin-rich and have been called somatostatins. Carcinoids cannot be distinguished as either benign or malignant based on the histology of the primary lesion. This can be unequivocally determined only in the presence of metastases to the regional nodes or distant sites. The incidence of metastases is approximately 20% to 33%^{17–19} and in contrast to other duodenal carcinoids, there does not appear to be a direct correlation between size and the risk of malignancy. Carcinoids of the ampulla less than 1 cm in size and without involvement of the muscularis propria have been shown to metastasize. Regional lymph node metastases are the most common site of initial metastases followed by the liver. Poorly differentiated neuroendocrine carcinomas of the ampulla are very rare and have a distinctly poorer prognosis than the more benign-appearing carcinoid tumors.¹⁶ As of 1999, less than 80 cases of ampullary carcinoids had been reported in the literature. Given this paucity of data, the natural history and consequently the appropriate management of these lesions are unclear. A significant proportion of patients exhibit a benign course without synchronous or subsequent metastases. Although the size doesn't appear to be predictive of behavior, this observation is based on small numbers of pa-

tients and is contrary to experience with other foregut carcinoids. Ampullary resection appears to be reasonable in patients with localized, small (<1 cm) carcinoids and for those with liver metastases. A more radical approach to those with lymph node metastases in the absence of liver metastases is also reasonable, as some of these patients have been shown to die of disease progression without liver metastases. The likelihood of deriving a therapeutic benefit from a more radical approach appears to be small given the biology and usual clinical course of this disease. Patients with large tumors that pose a technical challenge in reestablishing biliary and pancreatic drainage should also be managed with pancreaticoduodenectomy.

Other Tumors

A number of other benign tumors of the ampulla have been reported. These include inflammatory and hyperplastic polyps, lipomas, hemangiomas, and leiomyofibromas. Local excision in patients with symptomatic lesions when technically feasible is an appropriate form of management. Endoscopic polypectomy/resection may also be possible in some of these lesions. Gastrointestinal stromal tumors (formerly leiomyoma/leiomyosarcoma) have also been reported to occur at the ampulla of Vater. The distinction of malignancy in these tumors is problematic. All should be considered potentially malignant. The patterns of failure include local recurrence, peritoneal spread, and liver metastases. Metastases from other malignancy are equally uncommon. Melanoma and renal cell carcinoma are the most frequently cited examples.

Presentation

The most common presenting symptom in patients with ampullary neoplasms is jaundice resulting from obstruction of the distal bile duct. Although jaundice occurs in approximately 70% of patients with adenocarcinoma, it is curiously less frequent (20% to 30%) in patients with adenomas and other benign lesions.^{5,15,20} Pruritus accompanying the jaundice is also frequently reported. Weight loss, abdominal pain, nausea, and fever are also reported in these patients. Pancreatitis has been described and in our experience appears to be more frequent

in patients with adenomas compared to patients with adenocarcinoma.⁵ Anemia secondary to bleeding from the tumor into the gastrointestinal tract can occur given the friable nature of these tumors. The classic triad of fluctuating painless jaundice, a palpable gallbladder, and anemia is uncommonly present.²⁰

Diagnostic Evaluation

Nearly all patients have abnormalities of serum liver function tests. Alkaline phosphatase and γ -glutamyl transferase, which are sensitive markers of cholestasis, are very frequently abnormal. Other findings on laboratory testing include hyperbilirubinemia, a microcytic anemia, and, more infrequently, hyperamylasemia. In patients with longstanding jaundice, the prothrombin time may be elevated secondary to malabsorption of vitamin K, a fat-soluble vitamin. Physical findings may include jaundice, scleral icterus, a palpable gallbladder, evidence of recent weight loss, evidence of pruritus, and hemoccult-positive stool.

The goal of imaging studies in patients with ampullary tumors is to obtain information on diagnosis, staging, and the determination of resectability. In patients presenting with abdominal pain and jaundice, abdominal ultrasound is often the initial test and helps to establish the presence of obstructive jaundice. This simple, noninvasive test is useful in demonstrating gallstones, evaluating for choledocholithiasis, and determining the level of ductal obstruction. Ampullary masses are only rarely seen on transabdominal ultrasound. In patients suspected of having a neoplastic process involving the ampulla or periampullary region, computed tomography (CT) scanning of the abdomen with intravenous and oral contrast is then recommended. In addition to being able to identify the

presence of a periampullary mass, this modality also is useful in establishing the absence or presence of hepatic metastases. CT scanning is superior to transabdominal ultrasound in its ability to assess the local extent of periampullary malignancies and has a positive predictive value of approximately 80% in determining resectability. Conversely, it has a positive predictive value of approximately 90% for determining unresectability.²¹ The failures of CT scanning are represented by small volume peritoneal and liver metastases, and occasionally more extensive local disease than suspected on the basis of the preoperative imaging. Although prevalent in the past, angiography is very seldom helpful in patients with ampullary and periampullary tumors in the presence of a CT scan suggesting resectability.

Endoscopic retrograde cholangiopancreatography (ERCP) has been extremely valuable in evaluating patients documented to have obstructive jaundice in the absence of a mass lesion. Through this approach, ampullary lesions are visualized and may be biopsied. Ductograms of the common bile and pancreatic ducts obtained with ERCP provide information on the extent of neoplastic changes along these structures. In lesions thought to be amenable to local resection based on histology, endoscopic views detail the size and extent of circumferential involvement. Many investigators (Table 15.4) have questioned the accuracy of preoperative biopsies in predicting the final histology of ampullary lesions.^{5,12,22–24} The ability to predict that an ampullary mass is benign (negative predictive value for malignancy) ranges from 19% to 93%, as we have reported.⁵ Although it is not our experience, it can be concluded from these studies that preoperative endoscopic biopsies are often unreliable in precluding the diagnosis of a malignant process. The value of frozen-section analysis during surgical resection of ampullary lesions is not

TABLE 15.4. Endoscopic biopsy in the diagnosis of ampullary tumors.

Authors	N (patients)	% Carcinoma (final histology)	(+) Predictive value (malignant)	(–) Predictive value (malignant)
Sauvanet et al 1998 ²³	28	69	100%	46%
Clary et al 2000 ⁵	34	50	100%	78%
Komorowski et al 1991 ²²	44	41	100%	93%
Yamaguchi et al 1999 ²⁴	78	94	100%	19%
Beger et al 1999 ¹²	90	70	94%	43%

well discussed in the literature. We recently reported a negative predictive value for malignancy of 94% in 20 patients undergoing resection of ampullary neoplasms thought to be benign on the basis of preoperative endoscopic appearance and biopsy. In our experience, frozen-section analysis complemented preoperative biopsy and allowed for the application of local resection to these patients with benign adenomas.

Endoscopic ultrasound (EUS) has been reported to provide a superior assessment of lymph node involvement, portal vein invasion, and tumor size.^{25,26} EUS is significantly more sensitive in detecting ampullary masses than CT or transabdominal ultrasound and is capable of detecting mass lesions of the ampulla that are less than 10 mm.²⁷ Whether this modality is more sensitive than direct visualization at identifying small ampullary tumors is uncertain. Although EUS may provide a better assessment of portal and mesenteric venous invasion, this modality does not accurately assess the hepatic parenchyma and is less accurate in evaluating the celiac axis. EUS therefore should not be seen as a replacement for CT scanning to address these issues of resectability.

In recent reports, EUS has been shown to accurately predict the primary tumor stage (Table 15.5).^{23,27–30} The positive predictive value in these studies in documenting early-stage (T1) lesions is reported to be between 67% and 100%. Within these EUS series the proportion of patients with T1 lesions is approximately 80%, a value that is significantly higher than the 20% reported in the larger series of resectable ampullary tumors.^{10,12–15} As the proportion of patients who are truly T1 is relatively high, this likely has artificially inflated the positive predictive value in these EUS series. The accuracy in predicting the status of the regional

nodes by EUS in these same series is approximately 65% to 80%.^{23,28,29} The false-negative rate for lymph node metastases is as high as 20% to 40%. In summary, EUS has difficulty in accurately staging the primary and nodal status of patients with ampullary carcinomas. EUS may be helpful in determining resectability in patients suspected of having portal or mesenteric vein invasion. Its use, though, in selecting patients with adenocarcinoma for local resection is hazardous given the significant percentage of patients understaged by this modality. Whether the use of frozen-section analysis can identify these understaged patients who should be converted to pancreaticoduodenectomy has not been addressed.

Therapeutic Options and Indications

Options for excision of ampullary neoplasms include endoscopic resection, laparotomy with local excision, and pancreaticoduodenectomy. Endoscopic removal of ampullary adenomas has been described in the literature. Recurrence rates are relatively high (25%)^{31,32} and complications occur, including bleeding and pancreatitis. Endoscopic polypectomy may be acceptable for pedunculated lesions that can be completely excised and are without foci of adenocarcinoma. Given the high rates of recurrence, difficulty achieving negative margins, and uncertainty with preoperative biopsies, endoscopic approaches should in general be limited to those individuals with significant medical comorbidities and small benign lesions amenable to snare polypectomy.

Although there are many case reports and a few series on the treatment of ampullary neoplasms by local ampullary resection, the indications for this

TABLE 15.5. Endoscopic ultrasonography in the staging of ampullary tumors.

Authors	T1 by EUS (n)	Final pathology (n)		Positive predictive value (Stage I)
		T1	T2–4	
Sauvenet et al 1997 ²³	12	9	3	67%
Cannon et al 1999 ²⁸	20	16	4	80%
Kubo et al 1999 ²⁹	8	6	2	75%
Menzel et al 1999 ³⁰	4	4	0	100%
Mukai et al 1992 ²⁷	8	7	1	88%
Total	52	42	10	81%

procedure remain controversial. For small lesions thought to be benign on the basis of preoperative endoscopic appearance and biopsy, ampullary resection is well accepted. We have recently reviewed our experience with ampullectomy for benign adenomas.⁵ In this series, intraoperative frozen-section analysis with careful step-sectioning correctly predicted the final histology of adenoma in 16 patients undergoing ampullectomy. Furthermore, when all patients ($n = 39$) with ampullary neoplasms treated by the senior author (T.N.P.) were included in the analysis, the negative predictive values of preoperative endoscopic biopsy and frozen-section analysis were 78% and 94%, respectively. The morbidity of ampullectomy was significantly less than that experienced following pancreaticoduodenectomy, consistent with the findings of other groups.^{12,33} Ampullary resection is also reasonable for small, benign-appearing carcinoids and low-grade neuroendocrine tumors. Larger lesions and lesions with regional nodal metastases within the field of resection should in general undergo pancreaticoduodenectomy, although the data to support this are limited.

The majority of the controversy surrounding local ampullary resection is in its application to adenocarcinoma of the ampulla. Reports of local resection in the management of adenocarcinoma exist, but suffer from the small numbers of patients in these studies. Long-term survival following pancreaticoduodenectomy for ampullary cancer has been demonstrated in a number of recent studies, with 5-year survival reaching 35% to 40% in resected patients.^{10,13,15} The declining mortality associated with pancreaticoduodenectomy^{10,34} coupled with the survival statistics from these large studies has led many to conclude that pancreaticoduodenectomy is the procedure of choice for patients with adenocarcinoma involving the ampulla who are medically fit. In addition, relatively high rates of local recurrence and difficulty in achieving a margin-negative resection have been reported in series of local ampullary resection.

Technique of Transduodenal Local Resection

The abdomen is entered through a standard right subcostal incision. After complete examination of the peritoneal cavity to rule out metastatic disease, an

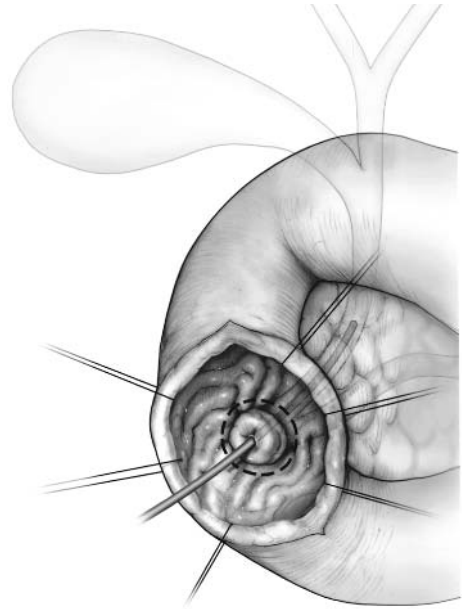


FIGURE 15.1. Exposure of the ampulla via a longitudinal duodenotomy with retrograde cannulation of the common bile duct.

extended Kocher maneuver of the duodenum is performed. With the duodenum mobilized, stay sutures are placed and a generous longitudinal duodenotomy is performed over the junction of the second and

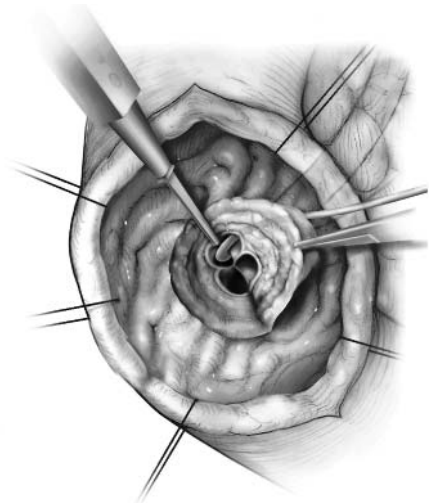
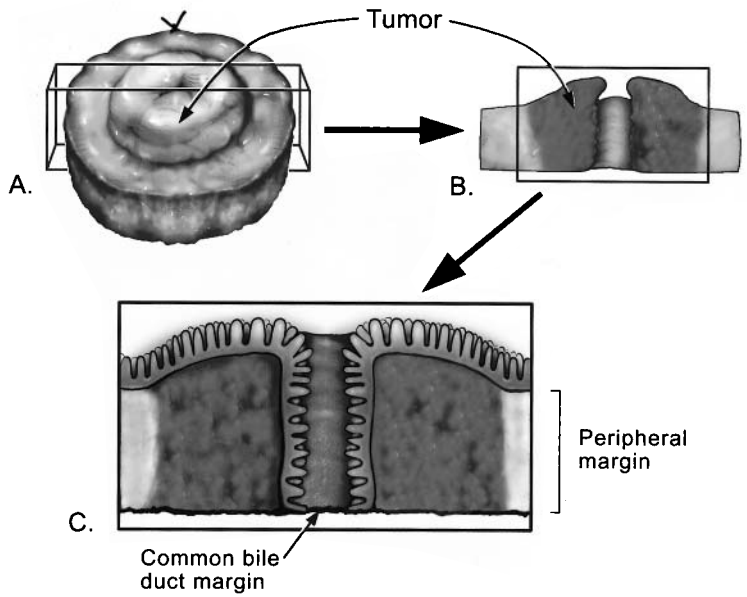


FIGURE 15.2. Excision of the ampulla in the submucosal plane with a 0.5–1.0 cm cuff of normal duodenal mucosa.

FIGURE 15.3. Frozen-section analysis of lesions thought to be benign to assess for malignancy and margin status. Step-sectioning is performed through the specimen with identification of the inked peripheral and deep (CBD) margins.



third portion of the duodenum. Palpation of the lesion is usually possible through the unopened wall of the duodenum and serves as a guide. Exposure of the duodenum is shown in Figure 15.1. The ampulla can be cannulated through the center of the mass as demonstrated in the figure or retrograde through the cystic duct after a cholecystectomy is performed.

A needle tip cautery is used to excise the ampullary mass and a 0.5 to 1.0 cm margin of normal mucosa tissue. It is helpful to initially cauterize an outline of the resection margin on the mucosal surface. The excision should start laterally with the depth of dissection being in the submucosal plane. The resection proceeds in a lateral to medial direction so that the common bile duct is transected before the pancreatic duct as shown in Figure 15.2.

Once the specimen is completely excised it is oriented for the pathologist so that serial sectioning can be performed to determine if the margins are negative and to see if there is an invasive component.⁵ Figure 15.3 demonstrates how the specimen is oriented and sectioned by the pathologists at our institution. If negative margins cannot be achieved or if an invasive cancer is identified, then pancreaticoduodenectomy is performed. When negative margins have been achieved for a benign lesion, then the reconstruction can begin. Since the pancreatic duct is usually of a small caliber, we recommend creating a common channel between the

common bile duct and the pancreatic duct using scissors as shown in Figure 15.4.

The duodenal mucosa is then reapproximated to the common channel using 5-0 Vicryl on a TF needle with simple sutures as shown in Figure 15.5. Figure 15.6 depicts the completed anastomosis. Once the ductal anastomosis is completed, the duodenum is then closed in a transverse direction (Figure

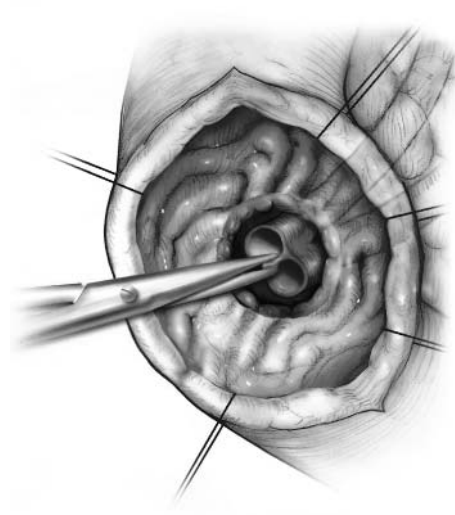


FIGURE 15.4. Creation of a common channel through incision of the intervening septum.

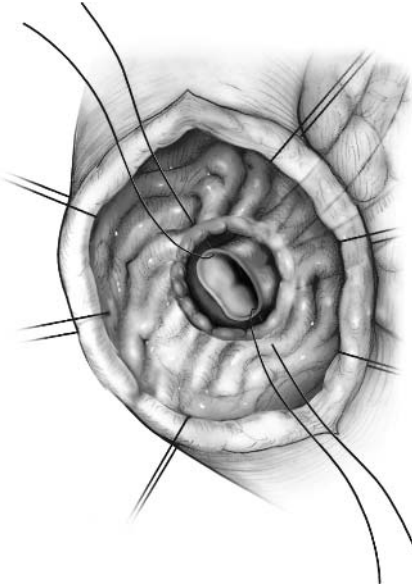


FIGURE 15.5. Reimplantation of the common orifice to the duodenal mucosa.

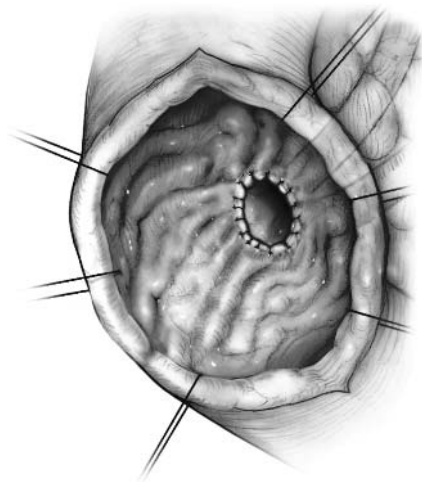


FIGURE 15.6. Completed anastomosis.

15.7). We routinely place a closed suction drain in the right upper quadrant prior to closing the abdomen.

Results of Surgical Therapies

Local resection of the ampulla is an uncommonly performed procedure that in small series has been demonstrated to be associated with less morbidity and mortality than pancreaticoduodenectomy (Table 15.6).^{5,33,35} In our recently reported series, there were no operative deaths and 4 complications in 18 patients undergoing local resection. Complications encountered included delayed gastric emptying, pancreatitis, CBD stricture, and cholangitis. The mean operative time and blood loss were 169 minutes and 192 ml, respectively. The average length of stay was 10 days. Perioperative deaths are uncommon following local resection, but still occur. In patients with adenocarcinoma, this procedure has historically been applied to individuals with significant comorbidity such that a direct comparison with pancreaticoduodenectomy is not possible. In recent years advances in preoperative and postoperative care, anesthesia, and surgical technique have resulted in a declining morbidity rate following pancreaticoduodenectomy.^{10,12,34} De-

spite this, operative mortality in large, recently reported series continues to be 3% to 6%, and overall morbidity 30% to 65%.^{10,12,13,15,36,37}

For lesions that are benign and technically feasible, local resection has been shown to be efficacious and associated with low rates of recurrence.^{5,12,33} For larger lesions precluding a safe ductal reimplantation or in patients with additional

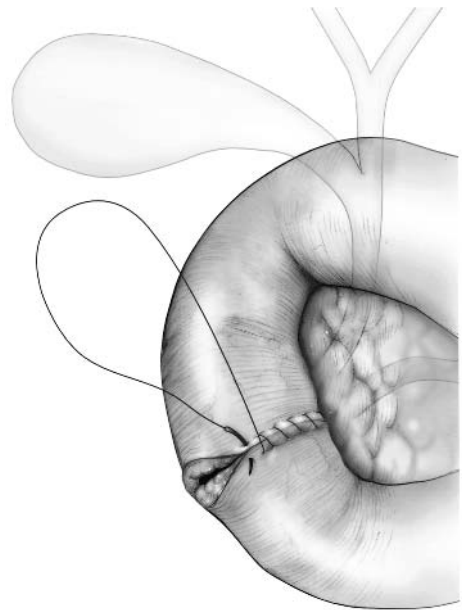


FIGURE 15.7. Transverse closure of the duodenum following completion of the anastomosis.

TABLE 15.6. Local resection and pancreaticoduodenectomy in the treatment of adenocarcinoma of the ampulla.

Authors	N	pT1** (%)	Positive margin* (%)	5-Year survival* (%)	Operative morbidity (%)*	Operative mortality (%)
Local Resection						
Beger et al 1999 ¹²	10	100	60	NR	NR	0
Klein et al 1996 ³⁸	9	100	11	44	22	0
Knox and Kingston 1986 ³⁹	25	NR	0	51	NR	0
Newman and Pittam 1982 ⁴⁰	9	NR	33	43	22	11
Robertson and Imrie 1986 ⁴¹	8	NR	NR	44	NR	25
Tarazi et al 1986 ⁴²	11	NR	28	41	NR	9
Pancreaticoduodenectomy						
Beger et al 1999 ¹²	88	9	NR	52	NR	3.2
Talamini et al 1997 ¹⁵	105	NR	2	38	47	3.8
Roder et al 1995 ¹³	66	30	8	35	29	4.5
Howe et al 1998 ¹⁰	101	23	4	46		5.0
Monson et al 1991 ^{37†}	104	NR	NR	34	NR	5.7
Allema et al 1995 ³⁶	62	NR	25	50	65	6.0

*NR indicates not reported.

**pT1 indicates pathologic T1.

†Includes 17 patients undergoing total pancreatectomy.

duodenal polyps (familial polyposis), pancreaticoduodenectomy is often necessary. In our experience, benign lesions greater than 3 cm generally require a pancreaticoduodenectomy for technical considerations alone, not to mention the increased risk of an unsuspected carcinoma seen with larger lesions. As demonstrated in Table 15.6, reports of local resection for adenocarcinoma are limited to small numbers of patients. Most patients in these reports have disease limited to the ampulla (T1) or have significant comorbidities. Despite the trend toward earlier lesions, a positive margin following local resection occurs in between 0% and 60% of patients. Survival following local resection for adenocarcinoma is difficult to interpret given the small number of patients, but has been reported to be 40% to 50% at 5 years,^{38–42} a figure comparable to the 35% to 50% seen in the much larger pancreaticoduodenectomy series.^{10,12,13,15,37} It is important to note, however, that these series likely represent completely different patient populations. When patients with high-risk lesions are excluded (T3 or T4, involved nodes, poorly differentiated) the results following pancreaticoduodenectomy appear significantly better, with survivals of 60% to 80%.^{10,13,37,43} The inability to accurately define the T-stage and nodal stage preoperatively combined with the excellent results of the large pancreaticoduodenectomy series lead us to conclude that local resection should not be routinely applied in patients

with apparently early ampullary adenocarcinoma. In patients with significant comorbidities that greatly increase the risk of surgery, local resection is an option for small tumors and may be associated with long-term survival.

Conclusion

Local resection of the ampulla is an appropriate form of therapy in the treatment of small benign ampullary neoplasms. This procedure appears to be less morbid than more radical procedures including pancreaticoduodenectomy, although the latter is clearly indicated in fit patients with advanced malignant processes or large benign tumors where reimplantation of the ducts is not technically feasible. Patients with invasive carcinoma who are not medically fit for pancreaticoduodenectomy may be considered for local resection. Patients with small, well-differentiated neuroendocrine tumors may also be candidates for local resection in the absence of known or suspected metastatic disease, although the data to support this are limited.

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16

Distal Pancreatectomy for Pancreatic Cancer

Gulam Abbas and Gary R. Gecelter

Introduction

Adenocarcinoma of the body and tail of the pancreas represents approximately one third of all cases of carcinoma of the pancreas, while that of the head and uncinate process accounts for two thirds. The presentation of left-sided primaries is delayed compared to that of periampullary carcinomas, as the lead-time advantage usually gained as a result of the early onset of clinical jaundice is lost. The nonspecific symptoms associated with these tumors further add to the delay in diagnosis. Consequently, carcinoma of the body or tail of the pancreas is resectable at the time of diagnosis, representing only 8% to 10% of patients taken to surgery.¹ In contrast to tumors of the head of the pancreas, there has been no significant increase in the rate of resection for left-sided tumors. Rather, advances in modern imaging and minimally invasive staging have led to a significant decrease in the number of patients undergoing unnecessary laparotomy. In this chapter we focus on new technical considerations such as preoperative assessment of the pancreas and the emerging role of staging laparoscopy. Spleen conservation as well as conventional distal pancreatectomy are discussed.

Diagnosis

Carcinoma of the body or tail of the pancreas is diagnosed more frequently by abdominal computed tomography (CT) than by any other modality. The role of transabdominal ultrasound is reserved for evaluation of the gallbladder and bile duct diame-

ter rather than for direct evaluation of the mass, which is commonly obscured by overlying stomach gas. Diagnostic confirmation is made by procurement of tissue using one of the following modalities: CT-guided percutaneous aspiration, endoscopic retrograde cholangiopancreatography (ERCP), exfoliative brush cytology, endoscopic ultrasound-guided transduodenal fine-needle aspiration, or staging laparoscopy/laparotomy.

CA 19-9 represents the best serum marker of suspected pancreas carcinoma, especially if the level is above 500 μ /l. Further, a return of CA 19-9 to normal levels postoperatively is indicative of adequate resection, making it a valuable surveillance tool.

Preoperative Staging

The sensitivity of new noninvasive and invasive imaging and diagnostic modalities has significantly reduced the rate of laparotomy for unresectable pancreatic tumors from 49% to 21%.² The modern staging armamentarium includes thin-slice “pancreas protocol” CT scanning, endoscopic ultrasound (EUS), and magnetic resonance imaging (MRI). Thin-slice spiral CT is the cornerstone of the preoperative evaluation, capable of providing necessary detail regarding tumor encasement of the portal vein and celiac and common hepatic arteries. In addition, it is able to document liver involvement by metastatic lesions as small as 1 cm if contrast timing is optimized. In cases of obvious unresectability as evidenced by liver metastasis and ascites, CT-guided needle biopsy or paracentesis can confirm the diagnosis and obviate further investigation.

EUS provides excellent detail of the relationship of the tumor to the portal and splenic veins although it is less useful for detecting superior mesenteric vein involvement.³ In our experience, the advantage of EUS is the ability of the side-fire devices to facilitate transduodenal fine-needle aspiration for cytologic diagnosis.

MRI has not added significantly to the diagnostic or staging accuracy of distal pancreatic carcinomas, although magnetic resonance angiography represents a noninvasive alternative to conventional angiography in evaluating the relevant peripancreatic vasculature. Further, magnetic resonance cholangiopancreatography provides a noninvasive morphologic assessment of the pancreatic and bile ducts that can function as an alternative to conventional diagnostic ERCP. The role of ERCP lies in its ability to provide diagnostic brush cytology rather than in its use as a staging modality.

The role of positron emission tomography (PET) in the diagnosis and staging of pancreatic adenocarcinoma is currently under evaluation. Although false-positive PET scans are reported in patients with pancreatitis, series are beginning to emerge that attest to the added value of this modality in decision-making when applied to known pancreatic cancers. One recent series reports the PET findings altered the management of 28 of 65 patients (43%).⁴

Laparoscopic Staging

The role of laparoscopic staging of upper gastrointestinal malignancies has expanded over the past 5 years with the ubiquitous assumption of general surgical laparoscopy. Laparoscopy contributes to staging by providing the ability to acquire detailed visual information regarding the surface of the peritoneum, including identifying and facilitating biopsy of planar, 2-dimensional metastases that are not visualizable by CT, MRI, or EUS. Approximately 30% of all patients found to be resectable on the basis of CT will show evidence of metastatic disease at laparoscopy.⁵ As patients with left-sided pancreatic carcinomas will less frequently require palliative surgery than those with carcinoma of the head, laparoscopy will obviate unnecessary laparotomy in the majority of cases. Peritoneal cytology, acquired during laparoscopy, has been shown to correlate strongly with peritoneal spread and consequently poor prognosis.⁶ The addition of laparoscopic ul-

TABLE 16.1. Comparison of prediction of resectability by pre-operative CT & EUS versus addition of laparoscopy.

Site	Total cases referred	Resectable on basis of Helical CT and EUS	Resectable after addition of laparoscopy
Head	55	39	31
Body and tail	11	4	1

trasound allows sensitive inspection of the liver and also provides information regarding the vascular relationships of the tumor similar to the detail obtainable using EUS. Memorial Sloan-Kettering Cancer Center recently reported that this modality altered staging and subsequent management in 14% of 90 patients.⁷ At our institution, laparoscopy obviated laparotomy in 11 of 66 patients with carcinoma of the pancreas where preoperative evaluation, including “pancreas protocol” CT scans, suggested resectability (Table 16.1). The addition of laparoscopic ultrasound has been applied mainly to liver evaluation where we have found it to upstage an additional 5 patients previously deemed resectable (Table 16.2). At our institution, during the past 4 years we have preceded every pancreatic exploration with staging laparoscopy, adding laparoscopic ultrasound in the last 2 years. We have routinely used flexible laparoscopy (Figure 16.1) (LTFV, Olympus Endosurgery), which has greatly facilitated our ability to visualize the surface of the liver and spleen as well as to gain access to the lesser sac through a small window in the gastrocolic ligament.

Preoperative Preparation

Pneumoccal, as well as hemophillus and meningococcal vaccine, is routinely administered to all patients once exploration of a tumor of the body or tail of the pancreas is anticipated, ideally 2 weeks prior to surgery. Bowel preparation is reserved for patients

TABLE 16.2. Upstaging by addition of laparoscopic ultrasound.

Site	N	Flexible laparoscopy	Addition of laparoscopic ultrasound
Head	36	21	16
Body	4	0	0
Tail	4	2	1

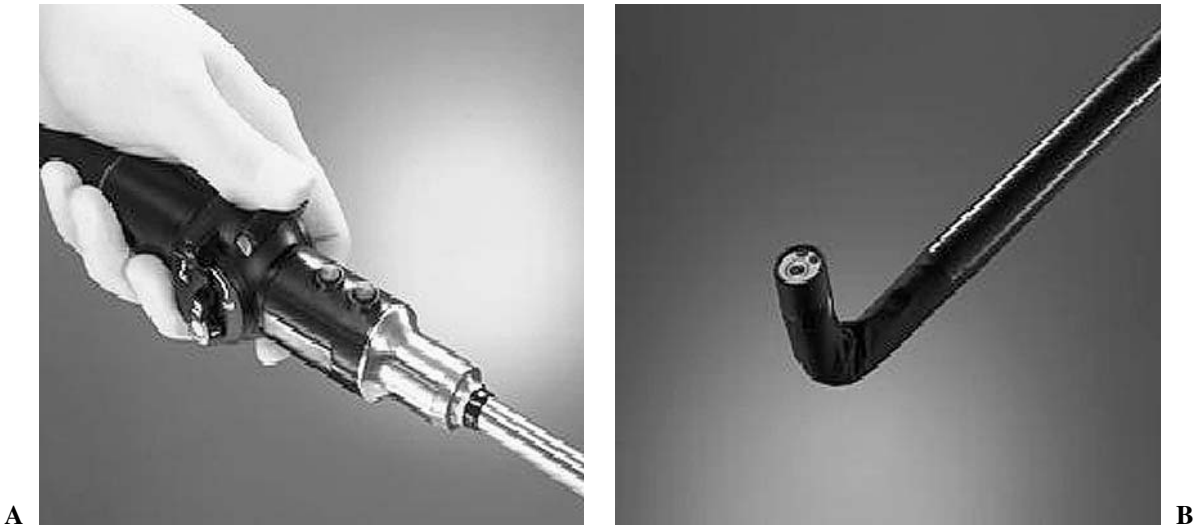


FIGURE 16.1. Deflecting tip Laparoscope

in whom a colonic resection is expected and, in our institution, for all patients who are undergoing reoperation. Regarding the use of somatostatin analog, a randomized double-blind multicenter trial from Germany and Austria has shown a decrease in post-operative pancreatic fistula formation (3.8% vs 11%) in patients receiving perioperative octreotide for 7 days.⁸ Scrutiny of the data from this study documented fistulas in normal, soft glands and in patients with downstream main pancreatic duct strictures. It is probably feasible to reserve the use of octreotide for these high-risk situations. A single dose of a second-generation cephalosporin should be administered preoperatively.

Surgical Technique

Distal Pancreatectomy and Splenectomy

Following induction of general anesthesia, the patient is prepped and draped for a chevron or mid-line laparotomy incision. With the use of a transparent camera housing trocar (Optiview, Ethicon Endosurgery), laparoscopic access into the abdominal cavity is gained through an incision in the left rectus muscle at the level of the umbilicus. We have found that this technique is rapid and the incision can be closed without fascial sutures, as the track of the trocar traverses the anterior and posterior rectus sheaths at different levels. After the pneumoperitoneum is created, the deflecting tip video

laparoscope (LTFV, Olympus Endosurgery) is introduced into the abdomen. This is immediately followed by the placement of 2 additional trocars in the right subcostal region. Port site selection is based upon body habitus and tumor location as well as the site of necessary scrutiny, as determined by initial survey. Initial survey includes surface examination of the supracolic compartment of the abdomen with the patient in steep reverse Trendelenburg position and rotated left or right for examination of the liver and spleen, respectively (Figure 16.2). Examination of the liver is facilitated

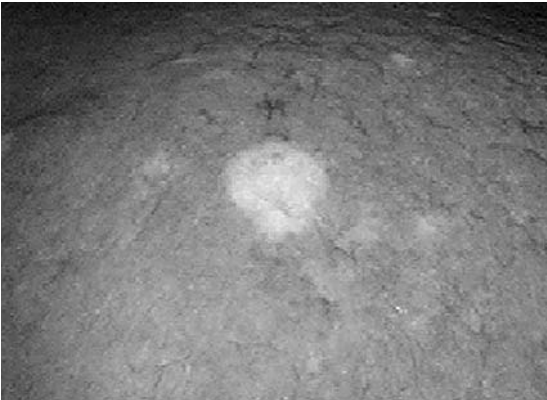


FIGURE 16.2. Surface hepatic metastases not identified by helical computerized tomography. This is a 57-year-old morbidly obese female with a 3 cm left-sided pancreatic mass confirmed to be adenocarcinoma by percutaneous needle aspiration cytology (note hepatic steatosis).

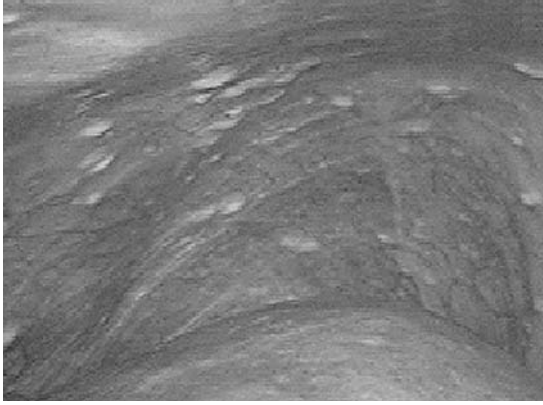


FIGURE 16.3. Coalescing 'stud' metastases in the gastrohepatic ligament. This is a 76-year-old male with a pancreatic tail carcinoma invading the splenic hilum



FIGURE 16.5. Technique of elevation of the transverse colon with patient in steep trendelenburg position

by the placement of a 5-mm liver retractor to elevate the visceral surface of the liver anteriorly. Any lesions identified on the surface of the liver or peritoneum are immediately biopsied for frozen section prior to commencement of further laparoscopic survey or ultrasound (Figures 16.3 and 16.4). During this phase, the flexible laparoscope is particularly useful to achieve an *en face* view of the dome of the liver and both left and right triangular ligaments.

Next the patient is turned into a steep Trendelenburg position for examination of the true pelvis

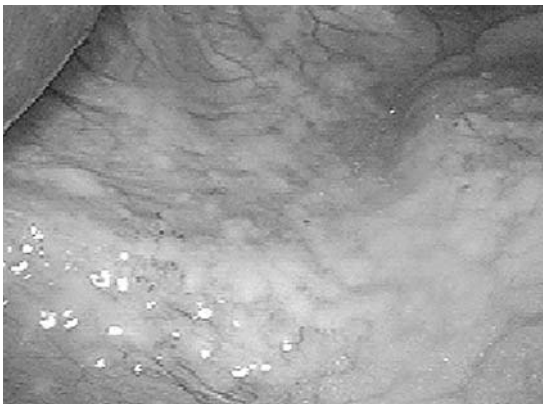


FIGURE 16.4. Metastatic seed metastases tracking the hepatic branch of the vagus nerve in the gastrohepatic ligament in a 73-year-old male with 3 cm carcinoma of the body of the pancreas

and infracolic structures. During this phase it is important to rotate the transverse colon over the stomach and liver so as to visualize the ligament of Treitz and the posterior leaf of the transverse mesocolon, which is critical (Figure 16.5). Typically, invasion of this structure presents as an umbilication with firmness to the touch, particularly along the inferior surface of the pancreatic body. Any suspicious lesions are biopsied for frozen section.

Laparoscopic ultrasound follows a strict routine. With the use of a flexible 7.5-MHz laparoscopic linear array duplex probe (Aloka USA), introduced through a 10-mm trocar, the liver is scanned commencing with delineation of the hepatic veins followed by sequential evaluation of segments and concluding with a view of the gallbladder and hilar structures. Key to the success of this procedure is the assessment of the liver parenchyma as the transducer is passed over the surface of the liver following portal venous branches out to their periphery. This smooth, sustained motion facilitates identification of abnormal lesions as an interruption of the flow of the real-time image. All suspicious lesions are scanned in 2 dimensions at right angles to ensure that they are spherical and not tubular structures.

Attention is then turned to the pancreas, which is evaluated by commencing at the aorta in both longitudinal and cross-section at the level of the celiac axis. In this way one is immediately able to identify the relationship of the tumor with the axial vessels, moving leftward to evaluate the splenic

artery and vein and rightward to evaluate the confluence of the splenic and superior mesenteric veins, the portal vein, and the common hepatic artery. Transduction is performed over the anterior surface of the stomach with the left lobe of the liver retracted anteriorly. Evaluation of lymph nodes at the “fountain head” of the celiac axis is the easiest, whereas superior and inferior pancreaticoduodenal nodes are more subtle. A valuable routine is to scan the pancreas from body to head and then body to tail in cross-section, commencing at the aorta while focusing on keeping the center of the image on the pancreatic duct. We find it difficult to image the pancreatic duct in longitudinal section for any length of time because of the fixity of the fulcrum of the trocar. Switching camera and transducer trocars helps to increase the degrees of freedom. Vascular evaluation is aided by the use of duplex scanning, which is able to identify turbulent flow in the portal or splenic veins; such flow may suggest unresectability.

At this point, if the lesion appears to be resectable, the pancreas is examined directly. This is performed by creating a window in the gastrocolic ligament through a translucent window, usually to the left of the midline. The greatest advantage of the flexible laparoscope is that it can be advanced through a small avascular window in the gastrocolic ligament and then rotated like an endoscope within the lesser sac to scan the surface of the pancreas to the splenic hilum (Figures 16.6, 16.7). The only limitation to this maneuver is the presence of

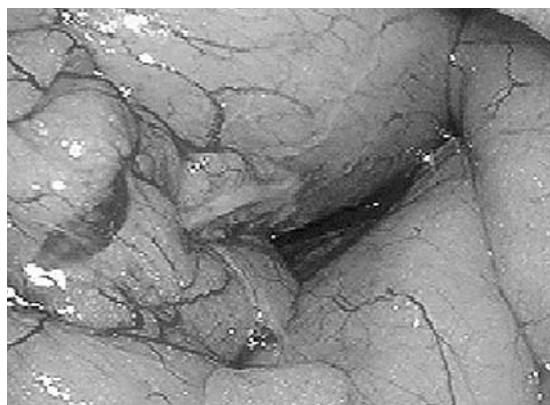


FIGURE 16.6. Dilated lymphatics on the posterior lesser curve of the stomach. This image was obtained via a window in the gastrocolic ligament

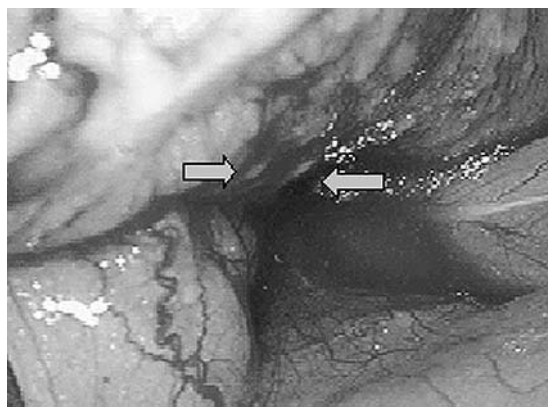


FIGURE 16.7. Visible metastases (largest 3 mm) in the gastrotrohepatic ligament seen in the same patient as Fig. 16.6

congenital and postpancreatic retrogastric adhesions.

If staging laparoscopy suggests resectability, we proceed to skeletonize the greater curvature of the stomach, moving leftward toward the spleen. Here dissection is facilitated by ultrasound coaptive coagulation (Harmonic Scalpel, Ethicon Endosurgery). With full mobilization of the stomach off the spleen we proceed to laparotomy and completion of the distal pancreatectomy. We have not completed a laparoscopic distal pancreatectomy for adenocarcinoma but have performed this procedure in the case of islet cell tumors and cystic neoplasms. We do not routinely perform peritoneal lavage for cytology, as it does not influence our decision to proceed with resection if cytology is positive. Further, there is an unavoidable delay in obtaining cell-block evaluation that precludes intraoperative decision-making.

After the laparoscopic evaluation has been completed in the described manner, conversion to open resection ensues once resectability has been suggested. Our preference is for a left subcostal or chevron incision with table-mounted subcostal retraction. The laparoscopic findings are confirmed by palpation and direct inspection, after which resection is commenced. Traditionally, splenectomy has been a part of distal pancreatectomy. During the last decade, however, authorities have increasingly recommended preservation of the spleen for benign disease.⁶⁻⁹ Splenic preservation in malignant disease (conservative splenectomy, conservative distal splenectomy) is debatable. Warsaw et al¹⁰ recom-

mend conservative pancreatectomy if it is technically feasible. In a recent review of splenectomy during pancreatectomy, Schwartz et al¹¹ reported a significant reduction in survival among patients undergoing a splenectomy compared to those in whom splenectomy was avoided (17.8 months vs 12.2 months; $P < 0.005$). In this series, splenectomy was performed in 28 of 42 (66%) distal pancreatectomies for adenocarcinoma, and the pursuant recommendation was to avoid splenectomy wherever possible. Splenectomy¹² should always be performed if the tumor extends to the hilum of the spleen or dissection of the tail of the pancreas from the hilum compromises the adequacy of the resection.

Spleen-Preserving Distal Pancreatectomy

If not already completed laparoscopically, the gastrocolic ligament is opened further along the greater curvature of the stomach, off the epiploic arcade, and halted just before vasa brevia. The retroperitoneum is opened along the inferior margin of the pancreas and the avascular plane behind the pancreas is entered. The retroperitoneum is also opened along the superior border of the pancreas. The tail of the pancreas is separated from the splenic hilum. The splenic vessels are clamped and usually ligated together if they branch close to the hilum. Alternately, individual ligation of the branches is performed if the vessels form a leash prior to entering the spleen. Blunt and sharp dissection is used to elevate the pancreas off its bed, advancing toward the portal vein. Once the tumor is elevated from the retroperitoneum, the splenic vein can be ligated at a variable distance from its junction with the portal vein. If necessary, the inferior mesenteric vein can be ligated without any additional morbidity. By fastidious dissection, the neck of the pancreas is separated from the portal vein. The neck is then divided, leaving a 1–2-cm gross margin from the tumor, using either a linear stapler device or electrocautery and closing the stump with a row of mattress sutures. If frozen section analysis of margins is suspicious of cancer, further resection should be undertaken.

An alternative technique to the aforementioned method is a retrograde dissection.¹³ The neck of the pancreas is dissected off the portal vein and is

transected using a linear stapling device or electrocautery. The splenic vein is invariably embedded in the posterosuperior aspect of the pancreas while the artery usually runs along the superior border of the pancreas. Dissection is carried out toward the tail of the pancreas, carefully securing the pancreatic branches of the splenic artery and vein as encountered. The tail of the pancreas is separated from the hilum of the spleen and is then carefully evaluated for any unintentional injuries.

Standard Distal Pancreatectomy

In conventional distal pancreatectomy, a splenectomy is routinely performed. The splenic artery is isolated and ligated early in the procedure. Dissection is further carried out along the greater curvature. Short gastric vessels are carefully clamped and ligated. The inferior pole of the spleen is separated from the splenic flexure of the colon and the lienocolic ligament is taken down. With the surgeon's left hand retracting the spleen medially toward the midline, the posterior splenic attachments are taken down, bringing the spleen out of the retroperitoneum. Retracting the spleen and tail of the pancreas medially further mobilizes the tail and body of the pancreas out of the retroperitoneum. The loose areolar tissue plane of the retroperitoneum, just anterior to the left kidney, renal vein, and left adrenal gland, is entered, with care taken not to injure these structures. At particular risk is the adrenal gland, which lies on the ventromedial surface of the upper pole of the kidney. The splenic vein lies embedded in the pancreatic tissue in its posterosuperior surface. The junction of the splenic vein and inferior mesenteric vein is identified and the splenic vein is mobilized just distal to this junction. The splenic vein tributaries draining the pancreatic parenchyma are ligated. Next the splenic vein itself is ligated and cut upstream from the inferior mesenteric vein. The neck of the pancreas is separated from the superior mesenteric and portal veins posteriorly. The pancreatic neck is then transected. This can be facilitated by either preplacing a linear stapling device or by freehand division and subsequent oversewing of the stump with mattress sutures. Our preference is to place 2 figure-of-eight sutures at the superior and inferior borders of the pancreatic neck, which can be tied immediately fol-

lowing transection. This helps minimize bleeding that occurs predominately from the superior and inferior pancreaticoduodenal vessels that run along the superior and inferior borders of the pancreatic neck.

The pancreatic stump is drained with a closed suction silastic drain brought out through the left upper quadrant. The abdominal cavity is copiously irrigated with warm saline solution, and hemostasis is secured using electrocautery and ligatures. The abdomen is closed in mass closure using #1 PDS loop suture and the skin is closed using skin staples.

In tumors deemed unresectable, celiac ganglion neurolysis is performed for pain control. Our preference is to perform ultrasound-guided installation of 25 mL of 50% ethanol on either side of the celiac axis.

Complications

Postoperative complications following distal pancreatectomy for adenocarcinoma have been acceptable. It should be possible to perform the operation with a mortality rate of less than 5%, with some series reporting zero mortality.¹³ The most common complication occurring in a large series from the French Association of Surgery was hemorrhage, which occurred in 6% of 128 resections; pancreatic fistula occurred in 3%.¹⁴ Late diabetes and steatorrhea were reported in 11% and 5%, respectively.

Outcome

Resection of adenocarcinoma of the body and tail of the pancreas occurs more infrequently than that of a similar cancer arising in the pancreatic head. Resectability ranged between 8% and 12% in 3 large series (Nordback, Johnson, Brennan) and less so in our small series (Table 16.2). The median survival in patients who are unresectable is less than 6 months, and few seldom live more than a year after surgery. Following resection, survival can be expected to double, and occasionally 5-year survivors are encountered (approximately 10%). As with tumors of the head, tumor size, lymph node status, and the adequacy of surgical margins all contribute to the predictability of long-term survival.¹

Conclusions

Carcinoma of the body and tail of the pancreas is a dreaded disease that is usually diagnosed at an inoperable stage. In approximately 10% of all patients, resection is possible, resulting in a survival expectation that mirrors that of carcinoma of the head of the pancreas. Although no significant therapeutic advances have taken place in the past 2 decades, preoperative staging, including laparoscopy, has led to a decrease in the number of patients subjected to unnecessary laparotomy. If feasible, when performing distal pancreatectomy, splenic preservation is favored and is associated with a lower perioperative and long-term mortality risk. Early detection of pancreatic cancer remains elusive, as the heralding symptoms are nonspecific and common among the aging population most likely to be affected.

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Risks of Perioperative Mortality with Pancreaticoduodenectomy

Laura A. Lambert and John D. Birkmeyer

Introduction

Outcomes of pancreaticoduodenectomy have improved substantially since Whipple first described his 2-staged procedure for en bloc resection of the pancreatic head and duodenum in 1935.¹ As recently as 20 years ago, operative mortality rates commonly exceeded 20%, substantially higher than 5-year survival rates after this procedure. Moreover, a number of studies showed that patients with resectable pancreatic cancer undergoing palliative bypass did as well as, if not better than, patients undergoing pancreaticoduodenectomy.^{2,3} For these reasons, many questioned the value of pancreaticoduodenectomy in the treatment of even early-stage pancreatic cancer.⁴

In the 1980s, however, many centers began reporting much better results.^{5–23} (Table 17.1). In addition to reports of improving 5-year survival after pancreaticoduodenectomy (20% to 30% in patients with pancreatic cancer, higher for bile duct and ampullary malignancies), operative mortality rates below 5% became commonplace. One case series of 650 consecutive patients undergoing pancreaticoduodenectomy described a 1.7% mortality rate.²² Other studies with more than 100 patients reported no perioperative deaths.^{9,20} Average lengths of stay after pancreaticoduodenectomy, which generally exceeded 30 days 2 decades ago, fell to 10 to 15 days.^{10,22} Reasons for the dramatic reductions in morbidity and mortality rates associated with pancreaticoduodenectomy are likely multifactorial. In addition to improvements in operative techniques, better intensive care, anesthesia techniques, infection prophylaxis, and nutritional support may have also played a role.

However, despite increasing enthusiasm for pancreaticoduodenectomy in patients with cancer of the head of the pancreas (some authorities are even asserting the value of this procedure for palliation in patients formerly considered “unresectable”²⁶), caution is advised in applying recent data about surgical morbidity and mortality to individual patients. Because the therapeutic margin of pancreaticoduodenectomy (difference between its risks and benefits) remains small relative to other procedures, surgical risks must be considered carefully and account for the characteristics of individual patients (eg, age and comorbidity). Provider characteristics may be even more important. Strong evidence has accumulated from the volume-outcome literature that results being reported in case series from highly selected, high-volume referral centers do not reflect outcomes in the “real world.”^{12,27–29} Thus, estimates of risks associated with pancreaticoduodenectomy must account for *where* patients are being treated. Examining data from surgical case series as well as more recent information from population-based studies, this chapter considers the importance of various patient and provider factors in mortality risks with pancreaticoduodenectomy.

Patient Characteristics Related to Operative Mortality

Age

Although advanced age was considered a contraindication to pancreatic resection as recently as the early 1980s,^{13,30–32} more recent studies have

TABLE 17.1. Mortality from pancreaticoduodenectomy reported in case series with over 100 patients.

Authors	Years	Patients (n)	Mortality (%)
Monge et al ²⁴	1940–1962	239	19
Warren et al ²⁵	1942–1972	348	16
Smith ¹⁷	1943–1969	224	7
Herter et al ¹³	1945–1973	102	15
Su et al ¹⁸	1965–1995	132	14
Yeo et al ²³	1974–1994	201	5
Andersen et al ⁶	1976–1990	117	8
Tsao et al ²¹	1980–1992	101	2
Nitecki et al ¹⁶	1981–1991	186	3
Allema et al ⁵	1983–1992	176	5
Bakkevold and Kambestad ⁷	1984–1987	108	11
Gordon et al ^{12*}	1985–1990	271	2
Bottger and Junginger ⁸	1985–1997	221	3
Swope et al ¹⁹	1987–1991	299	8
Cameron et al ^{9*}	1988–1991	145	0
Yeo et al ^{22*}	1990–1996	650	1
Fernandez-del Castillo et al ¹⁰	1991–1994	231	0

*Overlapping series from same institution; thus some patients reported likely more than once.

challenged this view (Table 17.2). At least 11 studies have compared mortality rates with pancreaticoduodenectomy in patients over 70 years with those under 70.^{8,14,33–41} Combining data from these studies, patients over 70 had a 6% mortality rate

(24/430), only modestly higher than the 4% (54/1388) observed in younger patients.

Risks of pancreaticoduodenectomy in the very elderly have also been examined. For example, Sohn et al examined the outcomes of 46 octogenarians undergoing pancreaticoduodenectomy at a single institution between 1986 and 1996. Compared to patients under 80 (n = 681), octogenarians had higher complication rates and longer hospital stays. However, older patients had only modestly increased risks of in-hospital and 30-day mortality—4.3% vs 1.6% in non-octogenarians—a difference not found to be statistically significant.⁴²

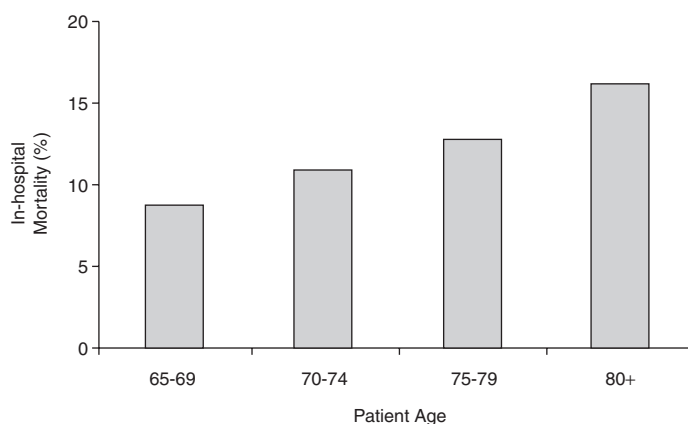
Population-based data suggest, however, that age is a considerably more important risk factor for mortality with pancreaticoduodenectomy than is apparent in case series from highly selected referral centers. Using the national claims database, we reviewed in-hospital mortality in all 7,229 Medicare patients (age 65+) undergoing this procedure in US hospitals between 1992 and 1995. The overall in-hospital mortality rate (10.8%) was substantially higher than rates appearing in the surgical literature. After accounting for other patient characteristics, increasing age had a near linear effect on mortality risks with pancreaticoduodenectomy (Figure 17.1). Mortality rates in octogenarians (n = 733) were approximately twice as high as those in patients age 65 to 69 (16% vs 8%, *P* < 0.0001).

TABLE 17.2. Influence of age on perioperative mortality with pancreaticoduodenectomy.

Authors	Perioperative mortality (%)				
	Patients < 70		Patients 70+		P
	n	deaths (%)	n	deaths (%)	
al-Sharaf et al ³³	47	4 (4)	27	2 (7)	NS
Bottger and Junginger ⁸	185	6 (3.2)	43	1 (2.3)	NS
DiCarlo et al ³⁴	85	3 (3.5)	33	2 (6)	NS
Magistrelli et al ⁴⁰	73	5 (6.8)	29	0 (0)	NS
Richter et al ⁴¹	283	4 (1.4)	45	2 (4.3)	NS
Chijiwa et al ³⁵	51	3 (5.9)	18	0 (0)	NS
Fong et al ³⁶	350	14 (4)	138	8 (6)	NS
Karl et al ³⁸	13	0 (0)	14	0 (0)	NS
Kayahara et al ¹⁴	102	7 (7)	28	5 (18)	NS
Hannoun et al ³⁷	179	8 (4.5)	44	4 (10)	NS
Kojima et al ³⁹	20	0 (0)	11	0 (0)	NS
Total	1388	54 (4)	430	24 (6)	

Table restricted to data from studies published after 1990 and those explicitly comparing mortality rates in patients under and over age 70.

FIGURE 17.1. Relationship between patient age and in-hospital mortality rates in 7,229 US Medicare patients undergoing pancreaticoduodenectomy between 1992 and 1995.



Functional Health Status

A relationship between functional health status and mortality risks with pancreaticoduodenectomy is clinically intuitive but not fully established. In a single prospective, multicenter study of 108 patients, Bakkevoeld and Kambestad used multiple regression analysis to examine the relative importance of numerous potential risk factors. Among all variables evaluated, only a low Karnofsky index (a functional status measure developed for cancer patients) was predictive of perioperative mortality.⁷ In contrast, 2 different studies have failed to detect a relationship between mortality with pancreaticoduodenectomy and American Society of Anesthesiologists Physical Status score (ASA score).^{8,35} However, these studies were based on relatively small numbers of patients and thus lacked the statistical power needed to detect statistically and clinically important effects of patient ASA score.

Hypoalbuminemia

Serum albumin, which reflects nutritional status and perhaps illness chronicity, is a well-known predictor of postoperative mortality for patients undergoing elective surgery.^{18,43-48} Hypoalbuminemia is also among the most important mortality predictors for pancreaticoduodenectomy. In subgroup analysis of data from one prospective trial of surgical resection for periampullary cancer by Chou et al, patients with preoperative albumin levels below 30 g/L had markedly higher mortality rates (6/37, 16%) than patients with higher albumin levels (1/56, 2%) ($P = 0.01$).⁴⁹ Another retro-

spective study made similar observations about the importance of hypoalbuminemia. Mortality rates in patients with albumin levels below 30 g/L were 30% (8/26), compared to 11.3% (12/106) in patients with higher albumin levels ($P < 0.05$).¹⁸

Surgical Indication

Mortality risks with pancreaticoduodenectomy are largely independent of the disease process for which it is performed. Several case series^{8,10,22,50} and at least one population-based study²⁷ indicate that patients undergoing this procedure for benign and malignant conditions have similar mortality rates. Also, patients with pancreatic cancer have similar risks with pancreaticoduodenectomy as those with ampullary, bile duct, or duodenal cancer.

Hyperbilirubinemia

Whether preoperative hyperbilirubinemia increases mortality risks with pancreaticoduodenectomy remains controversial. Prolonged biliary obstruction can result in portal endotoxemia and its sequelae, including gastrointestinal bleeding, renal insufficiency, hepatic insufficiency, respiratory insufficiency, and disseminated intravascular coagulopathy.^{51,52} Numerous studies of various surgical procedures have shown that preoperative jaundice is associated with increased mortality risks.⁵³⁻⁵⁸

However, the literature describing the importance of hyperbilirubinemia in pancreaticoduodenectomy is mixed. Several studies have not found this variable to be an important predictor of mor-

tality.^{7,49,59} These studies were based on relatively small sample sizes, however, so the possibility that their null findings represent Type II errors cannot be excluded. Conversely, other studies have identified hyperbilirubinemia as a risk factor for mortality after pancreaticoduodenectomy. In their retrospective analysis of 279 patients, Braasch et al noted that patients with serum bilirubin levels exceeding 200 mg/100 ml had significantly higher mortality rates (6/28, 22%) than patients with lower bilirubin levels (29/251, 11.5%).⁵⁴ A smaller case series and a more recent prospective study have also suggested increased mortality risks in patients with hyperbilirubinemia.^{8,30}

Treatment-Related Factors

Preoperative Biliary Drainage

Although patients with hyperbilirubinemia may have increased mortality risks with pancreaticoduodenectomy, it is not clear whether risks can be reduced by preoperative biliary drainage. One case series by Lygidakis et al noted that preoperative decompression of biliary obstruction (by endoscopy) was associated with fewer patients developing postoperative complications (16% vs 74%, $P < 0.05$).⁶⁰ However, mortality was not reduced by preoperative biliary drainage, echoing the findings of at least six prospective trials as well as numerous retrospective analyses.^{6,7,49,59–69}

Preoperative biliary drainage may even be harmful. In one retrospective study of 240 patients at a major referral center, Povoski et al noted an increased rate of perioperative mortality in patients receiving preoperative biliary drainage procedures (8%), compared to patients not receiving such procedures (3%) ($P = 0.037$).⁶⁷ Because biliary instrumentation alone (ie, diagnostic endoscopic retrograde cholangiopancreatography) had no association with surgical risks with subsequent pancreaticoduodenectomy, the authors hypothesized that increased mortality risks with drainage procedures were related to establishing free communication between the biliary and gastrointestinal tract or exterior. Although a causal relationship between preoperative biliary drainage and mortality after pancreaticoduodenectomy may be plausible, inferences from the analysis by Povoski et al are limited by the strong potential for referral bias and confounding in this study.

Although its effects on mortality risks are not fully established, the preponderance of evidence does not suggest significant benefits of preoperative biliary drainage procedures. For this reason, these procedures should be reserved for patients with biliary sepsis or intolerably symptomatic jaundice and for patients in whom the feasibility of resection is in doubt.

Neoadjuvant Chemoradiation

Because of high disease recurrence rates and low survival rates after pancreaticoduodenectomy for pancreatic cancer,^{70–74} interest in adjuvant chemoradiation is growing. There is also increasing awareness of the advantages of preoperative (neoadjuvant) chemoradiation over postoperative therapy. Chemoradiation may be more efficacious before surgical devascularization of the tumor bed. There are also practical advantages to preoperative therapy: Prolonged recovery times after pancreaticoduodenectomy are common and preclude many patients from receiving postoperative chemoradiation.

There is no evidence to date that neoadjuvant chemoradiation increases morbidity or mortality after pancreaticoduodenectomy. Although there are no randomized clinical trials assessing this issue, several case series have described outcomes of patients undergoing pancreaticoduodenectomy after neoadjuvant or intraoperative therapy.^{71,73,75–79} Among a total of 160 patients in these series, there were only 2 deaths (1.3% mortality). Moreover, 2 studies suggest that preoperative chemoradiation may reduce the incidence of pancreatic anastomotic leak, an important source of morbidity and mortality after pancreaticoduodenectomy.^{80,81}

Perioperative Octreotide

Pancreatic fistulas—usually resulting from anastomotic leaks from the pancreaticojejunostomy—are an important cause of both morbidity and mortality after pancreaticoduodenectomy.⁸² Many advocate prophylactic administration of octreotide (a somatostatin analogue) perioperatively to reduce the incidence of postoperative pancreatic fistulas by inhibiting pancreatic exocrine function.

To date, the effectiveness of perioperative octreotide after pancreatic resection has been evaluated in 4 prospective, randomized, double-blind,

TABLE 17.3. Summary of 4 prospective, randomized, double-blind, placebo-control trials of perioperative octreotide in patients undergoing pancreatic resection for neoplastic disease and/or chronic pancreatitis.

Authors	Overall n (% PD)†	Octreotide		Placebo	
		n	Mortality n (%)	n	Mortality n (%)
Buchler et al ⁸³	246 (62)	125	4 (3.2)	121	7 (5.8)
Friess et al ⁸⁴	274 (25)	122	2 (1.6)	125	1 (0.8)
Pederzoli et al ⁸⁶	252 (40)	122	2 (1.5)	130	5 (3.8)
Montorsi et al ⁸⁵	218 (40)	111	9 (8.1)	107	6 (5.6)

Statistical comparisons between octreotide and placebo groups. **P* < 0.05, ***P* < 0.01. All mortality differences nonsignificant.

†PD indicates pancreaticoduodenectomy.

placebo-control, multicenter trials^{83–85} (Table 17.3). All 4 trials demonstrated a significant reduction in the postoperative complication rate in patients with octreotide treatment. Overall, 21.5% of patients in the octreotide group (103/480) experienced complications, as compared with 37.5% (181/483) receiving placebos. However, none of the trials found a significant difference in mortality rates. The overall mortality rate was 3.5% (17/480) for patients receiving octreotide, versus 3.9% (19/483) in the placebo groups of these trials.

These trials enrolled patients with both neoplastic disease and chronic pancreatitis, who received a variety of pancreatic resections. Only 1 prospective, randomized trial—based at a single referral center—has evaluated the use of perioperative octreotide specifically in patients undergoing pancreaticoduodenectomy for malignant disease. As in previous trials, mortality rates were similar for patients receiving octreotide (2%, 1/57) and those receiving placebo (0%, 0/53). In contrast to the other trials, however, this study did not find a significant reduction in morbidity rates in patients receiving octreotide.⁸¹

Surgical Technique and
Extent of Resection

Whether operative mortality with pancreaticoduodenectomy is influenced by specific modifications in standard surgical technique for this procedure or the extent of resection is uncertain. Most studies addressing this question consist of case series from highly selected referral centers or randomized trials too small to adequately study mortality risks.

Extended Lymphadenectomy

Two recent prospective randomized trials have examined outcomes with standard pancreaticoduodenectomy for malignancy with and without extended lymphadenectomy. In one trial by Pedrazzoli et al patients undergoing extended lymphadenectomy had nearly identical mortality rates (5%, 2/41) as those receiving standard pancreaticoduodenectomy (5%, 2/40).⁸⁷ In a second trial by Yeo et al, 114 patients with periampullary adenocarcinoma underwent either standard pancreaticoduodenectomy (n = 56) or radical pancreaticoduodenectomy (including distal gastrectomy and retroperitoneal lymphadenectomy, n = 58). Mortality rates for the two groups (5.4% for standard versus 3.4% for radical) were not significantly different.⁸⁸

Portal Vein Resection

Although tumor involvement of the portal vein had previously been considered a contraindication to pancreatic resection,^{88,90} many centers are accruing experience with pancreaticoduodenectomy with portal vein resection and graft reconstruction. Several case series suggest that this procedure can be performed with relatively low operative mortality.^{91–95} In one series described by Leach et al⁹⁵ patients underwent pancreaticoduodenectomy with en bloc resection of the superior mesenteric vein/portal vein confluence and reconstruction, with no perioperative deaths.⁹⁵ Other investigators have reported mortality rates of 5% to 15% for patients undergoing portal vein resection with pancreaticoduodenectomy, as compared with 3% to 7% in patients undergoing pancreaticoduodenectomy alone.^{91–93}

Other Modifications of Standard Pancreaticoduodenectomy

Pylorus-preserving pancreaticoduodenectomy has been proposed to preserve antral and pyloric function, with hopes of improving long-term nutrition. Evaluated by several randomized controlled trials, this procedure seems to be associated with modestly shorter operation times but a higher incidence of delayed gastric emptying in the early postoperative period. Both perioperative and late mortality are comparable to mortality rates after standard pancreaticoduodenectomy.⁹⁵

Various technical modifications of the standard pancreaticoduodenectomy have been proposed for reducing risks of postoperative pancreatic fistulas, including suture ligation of the pancreatic stump, end-to-side versus end-to-end pancreaticojejunal anastomosis, pancreaticogastrostomy versus pancreaticojejunostomy, and total pancreatectomy versus resection of the pancreatic alone. Although these procedures vary in terms of their effects on pancreatic fistula rates and other clinical sequelae, they have no demonstrable effect on risks of perioperative mortality.⁹⁶

Hospital Characteristics and Procedural Volume

Case series from individual hospitals report considerably lower mortality rates with pancreaticoduodenectomy than population-based studies describing outcomes for all patients undergoing this

procedure.^{10,22,27} This discrepancy may reflect in part publication bias: Centers with low mortality rates are more likely to report their experience than hospitals with poorer performance.

However, the difference between mortality rates at national referral centers and hospitals surrounding them is so large that other factors must be at work. For patients undergoing pancreaticoduodenectomy in New York State between 1984 and 1991, the combined mortality rate at the state's 2 nationally recognized referral centers was 5.5% compared to 14% at all other hospitals.²⁹ Between 1988 and 1993, Maryland patients undergoing surgery at the Johns Hopkins Hospital (54% of all patients) had substantially lower mortality rates (2.2%) than patients at the remaining 38 hospitals in the state (13.5%) ($P < 0.001$).⁹⁷ These analyses underscored the importance of hospital procedural volume (and surgeon volume²⁹) as a predictor of perioperative mortality with pancreaticoduodenectomy.

The so-called "volume-outcome effect" is not restricted to nationally known referral centers. Birkmeyer et al performed a national study of all 7,229 US Medicare patients undergoing pancreaticoduodenectomy between 1992 and 1995. An approximately linear volume-outcome relationship was observed (Figure 17.2). More than 50% of patients received care at hospitals averaging fewer than 2 pancreaticoduodenectomies per year in Medicare patients. In-hospital mortality rates at these very-low-volume (0 to 1 procedures per year) and low-volume (1 to 2 procedures per year) hospitals were 3- to 4-fold higher than rates at centers performing

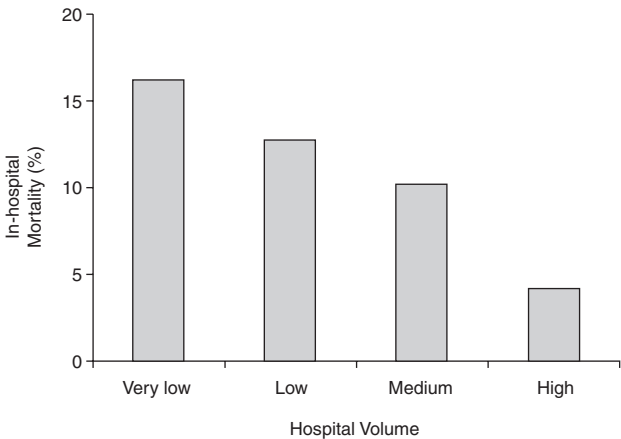
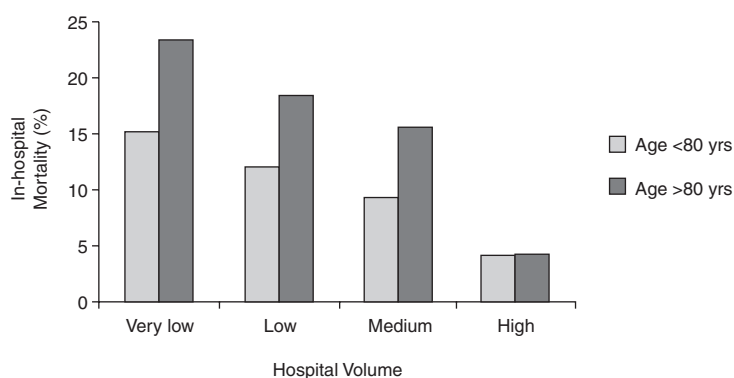


FIGURE 17.2. Association between hospital volume (average number of pancreaticoduodenectomies per year in Medicare patients) and in-hospital mortality, adjusted for age, sex, comorbidity score, and surgical indication. (Very low = <1/yr; low = 1–1.9/yr; medium = 2–4.9/yr; high = 5+/yr). (From Birkmeyer et al,²⁷ by permission of *Surgery*.)

FIGURE 17.3. Association between hospital volume and in-hospital mortality in 7,229 US Medicare patients undergoing pancreaticoduodenectomy between 1992 and 1995, stratified by patient age. Octogenarians had substantially higher mortality risks than younger patients at very low (<1/yr) and low (1–1.9/yr) volume centers, but not at high (5+/yr) volume centers. (Medium = 2–4.9/yr.)



more than 5 pancreaticoduodenectomies per year (16% and 12%, respectively, versus 4%) ($P < 0.001$). The 10 US hospitals averaging more than 10 such procedures annually (in Medicare patients) had lower mortality rates than those performing 5 to 10 per year (2.1% versus 6.2%, $P < 0.01$). In this study, differences in mortality rates by hospital volume strata could not be attributed to measurable differences in patient characteristics or referral bias.²⁷

Hospital volume may “attenuate” the effect of patient characteristics on mortality risks with pancreaticoduodenectomy. For example, although population-based data suggest that advanced patient age is an important surgical risk factor, increased risks in the very elderly are most pronounced in low-volume hospitals (Figure 17.3). In the US Medicare population, octogenarians undergoing surgery at very-low-volume institutions (0 to 1 procedures per year in Medicare patients) had higher mortality rates (20.9%) than younger patients at these facilities (13.7%) ($P < 0.001$). In contrast, at high-volume institutions (5+ procedures per year), mortality rates for octogenarians (5.1%) were not significantly different from those for patients less than 80 years old (4.1%).

Summary

Over the past decade, pancreaticoduodenectomy has become a much safer operation, with mortality risks at many centers considerably below 5%. Clinical decision making should account for patient characteristics that increase these risks. Although information about patient-level risk factors is currently imperfect

given the lack of large population-based studies based on clinical data, factors that may increase mortality risks include advanced age, diminished functional status, hypoalbuminemia, and hyperbilirubinemia. Currently, there is little evidence that surgical mortality is influenced by treatment-related variables, including preoperative biliary drainage, neoadjuvant chemoradiation, perioperative administration of octreotide, or the extent of surgical resection. However, mortality risks with pancreaticoduodenectomy are heavily influenced by *where* surgery is performed. Given considerably lower mortality rates observed at high-volume centers for pancreatic surgery, patients being evaluated in other settings should be given the option of referral.

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18

Endoscopic Palliation for Locally Advanced and Metastatic Disease: Biliary and Duodenal Stents

Kiranpreet S. Parmar and Irving Waxman

Introduction

Pancreatic cancer is one of the leading causes of cancer mortality in the United States. It is difficult to diagnose in the early stage when resection is the only curative modality, in part because currently no screening tests are available for early detection. By the time most patients are symptomatic, 70% to 80% have advanced disease and a curative resection is not an option.¹ In patients with advanced disease, clinicians must rely on less invasive palliative treatments for their patients' symptoms, the predominant ones being pain, jaundice, pruritus, anorexia, and gastric outlet obstruction. Jaundice occurs in 90% of patients at the time of diagnosis, significant pain in 33% and gastric outlet obstruction in about 13%.² Palliative treatment for these symptoms has been the realm of surgery until the introduction of endoscopic and radiologic techniques (biliary and, more recently, duodenal stents) (see Figure 18.1).

Soehendra and Reynders-Frederix first introduced endoscopic biliary decompression and stent placement in 1979 for malignant bile duct obstruction,³ a treatment that has now become the initial treatment of choice for patients with obstructive jaundice due to pancreatic cancer. Endoscopic stent placement is less invasive and almost as effective as surgical interventions.⁴ It not only reduces the bilirubin but also decreases pruritus, nausea, and abdominal discomfort due to hyperbilirubinemia.

A choice has to be made between placing a metal or a plastic stent. The decision is based on the patient's clinical condition and prognosis. Patients

likely to survive more than 6 months will be better off with metal stents, as the occlusion rate is lower and hence there is less chance of obstruction.

The second use of biliary stents is to deliver intraluminal brachytherapy for head of pancreas carcinoma. Iridium-192 wires of homogeneous linear activity are used and placed manually across the stenotic portion of the duct under fluoroscopic guidance.⁵ The dose used is 20 to 50 Gy.⁶

Only recently have patients with pancreatic cancer with duodenal obstruction had a choice of palliative treatment. Duodenal and gastric outlet obstructions were previously managed by gastrojejunostomy or placement of J tubes, but advancements in endoscopic techniques have led to the development of a less invasive method. This consists of dilating the stenosis with a balloon and placing a self-expanding metal stent.

Biliary Stents

The overall median survival for pancreatic cancer has not changed in recent years. Palliative procedures with low morbidity and mortality are the treatments of choice for patients with this condition. Options available today are surgical decompression and percutaneous or endoscopic stent placement (see Figure 18.1). The treatment depends on the patient's clinical condition and expected survival duration.

Surgical procedures used in the past were biliary enteric anastomoses such as cholecystojejunostomy, choledochojejunostomy, or Whipple resection and required an exploratory laparotomy.⁷

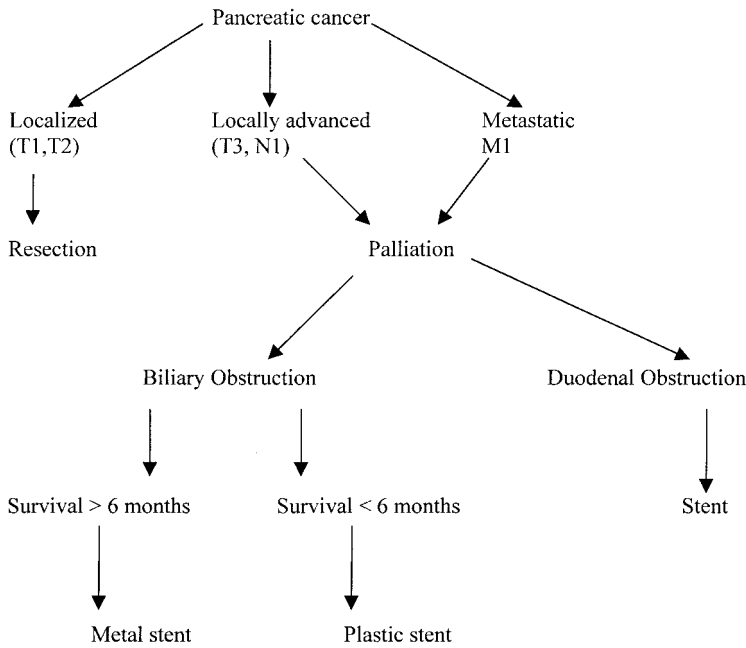


FIGURE 18.1. Algorithm for placement of stents in pancreatic cancer.

Choledochojejunostomy usually involves the formation of a Roux-en-Y loop and involves two anastomoses. The only important, though uncommon, late complication is anastomotic stricture formation, which requires either endoscopic or more likely percutaneous transhepatic therapy.⁸ The complication is due to difficulty in identifying the opening of the bile duct into the small bowel, secondary to distortion of the normal anatomy. Cholecystojejunostomy is performed when the gallbladder is dilated and is also associated with early and late complications such as cystic duct and stomal stenosis. These procedures can still be used in patients who have early noncurable disease, are good surgical candidates, and in whom it is impossible to place a biliary stent. The main concern with these procedures is the increased postsurgical hospital time required and the high mortality rate of up to 20%⁹ related to the surgery.

Biliary Stent Techniques

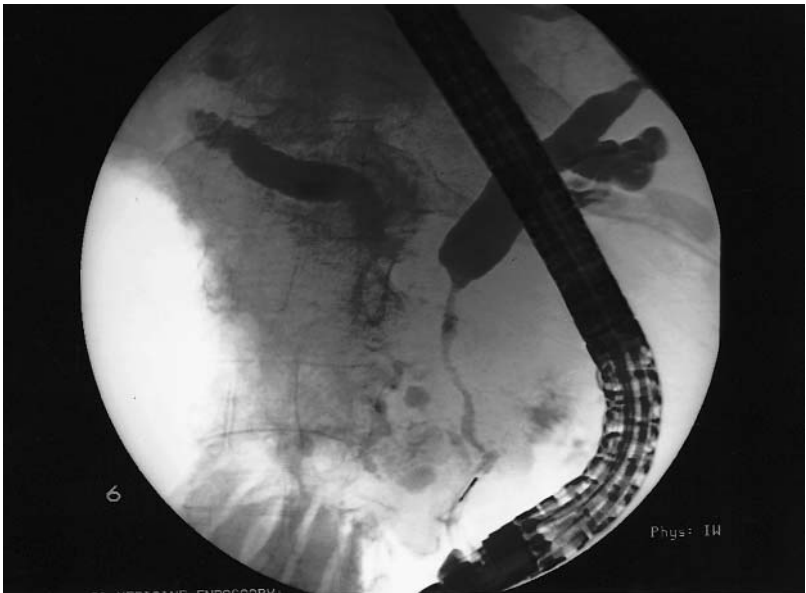
Stent placement has become the method of choice for palliative treatment of bile duct stenosis due to pancreatic cancer (Figure 18.2). This method is less invasive and equally effective when compared to surgical inventions.¹⁰ It can be performed by radiologic percutaneous, endoscopic, or combined

percutaneous-endoscopic methods (“rendezvous”). In all these methods either a metal or plastic stent can be placed. With the percutaneous method the drainage can be either internal into the duodenum or external, and with the endoscopic method the stents are completely internalized.

The percutaneous method requires performing a percutaneous fine-needle transhepatic cholangiogram to identify the biliary tree and select an optimal bile segment for puncture, after the use of local and intravenous anesthetic agents. A guide-wire is placed through the puncture sheath. Biliary stents can then be placed either externally, internally, or both. In the external technique the bile drains percutaneously into a bag, while with internally placed stents the bile drains into the duodenum. Both internal and external stents can be placed if a patient requires stent exchanges or flushing; either metal or plastic stents can be used. Complications of this procedure include bile leakage, pneumothorax, pleural effusion, cholangitis, peritonitis, sepsis, subphrenic abscess, and hemorrhage.

Endoscopic stent placement is performed with the aid of a side-viewing therapeutic duodenoscope with an instrument channel of 3.8 to 4.2 mm, which is required for stent placement. The biliary stents are placed internally (Figure 18-3). Intravenous prophylactic antibiotics (such as ampicillin, gen-

FIGURE 18.2. Endoscopic retrograde cholangiopancreatography illustrating a classic double duct associated with pancreatic cancer.



tamycin, cefuroxime, or ciprofloxacin) are administered before the procedure. In the hands of an experienced endoscopist this procedure has a success rate of 90% to 95% in patients with low bile duct stricture and 70% to 80% in those with hilar strictures.^{11,12}

The rendezvous procedure is performed by using both of the above techniques. It is believed that by decreasing the size of the percutaneous puncture some of the complications of the percutaneous route can be avoided. The procedure is performed by placing a guidewire through the percutaneous

route into the duodenum. The guidewire is then captured by the endoscopist and pulled through the therapeutic channel. Over this wire various catheters and stents can be passed as in a regular endoscopic procedure.

Indications for Biliary Stents

Obstructive jaundice occurs in 90% of pancreatic head cancers and 70% of all pancreatic cancers.¹³ It occurs secondary to direct invasion of the bile duct by cancer or due to extrinsic compression by

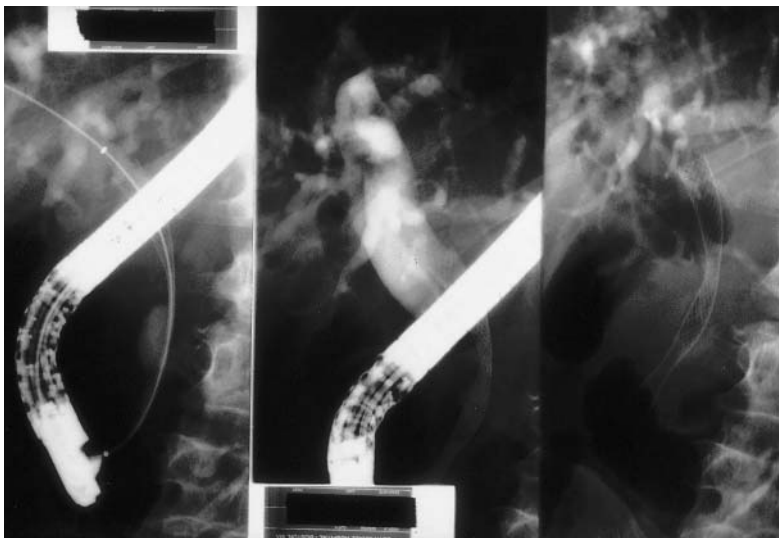


FIGURE 18.3. Fluoroscopic images illustrating biliary stent deployment.

mass or lymphadenopathy. It is the most important and the most common indication for placement of biliary stents, because hyperbilirubinemia can lead to pruritis, abdominal discomfort, nausea, malabsorption, and renal and hepatic dysfunction. The placement of a biliary stent not only relieves hyperbilirubinemia and pruritis in patients with obstructive jaundice due to pancreatic cancer but also relieves other constitutional symptoms. Ballinger et al¹⁴ reported on 19 patients with head of the pancreas cancer who underwent biliary stent placement. All patients had anorexia before stent insertion, with 13 patients having moderate to severe anorexia. This was significantly improved in all patients 1 week after stent placement and was maintained at 12 weeks. Sixteen patients complained of indigestion before the procedure, moderate to severe in 11. This was significantly better in 1 week and completely relieved in 6 to 8 weeks after stenting. Fifteen patients felt that their mood was good to very good before stent placement and that remained unchanged at the 12-week assessment. In this series biliary stenting not only relieved jaundices and pruritis but also had a significant impact on other disease-related symptoms.

Patients who develop cholangitis either spontaneously or secondary to diagnostic endoscopic retrograde cholangiopancreatography (ERCP) will also require stent placement. Stents are placed to provide drainage of obstructed bile, which is necessary in all patients who have undergone diagnostic cholangiography.

Placement of iridium-192 wires for intraluminal brachytherapy is another potential role for biliary stent placement. Biliary stents are placed at the stricture site and the iridium-192 wires are placed along the stricture. The correct position is evaluated by fluoroscopy. Montemaggi et al reported on 7 patients with pancreatic cancer who received intraluminal brachytherapy. The iridium-192 wires were placed in the duct of Wirsung or in the common bile duct. The minimum follow up was 18 months, median survival was 11.5 months, and 1 of 7 patients was alive at 29 months. Two patients had reduction in tumor size as determined by computed tomography scan and ultrasound. All patients had adequate control of jaundice. No statistically significant survival advantage was seen.⁶ Another study done by Levitt et al for treatment of malignant bile duct obstruction showed that placement

of iridium-192 wires was a safe and technically feasible technique.¹⁵ In both studies the ability of the intraluminal brachytherapy to improve palliation or lengthen survival remains uncertain. Multicenter prospective controlled trials are essential.

Prophylactic placement of biliary stents for pancreatic cancer has not been studied so far, but due to the risk of cholangitis, the need for periodic replacement of stents, and the additional procedural risks, it does not seem to be a warranted indication.

Biliary Stent Types

Metal

Metal stents available in the market today include Wallstent and Diamond by Microvasive, Endocoil 2 by Intra Therapeutic, and Z-Stent by Wilson-Cook (see Table 18.1). These stents are designed to expand after they have been deployed, resulting in up to a 30-French diameter. They arrive with delivery systems preloaded with the metal stents. Membrane-covered metal stents have been developed but are not currently available in the United States.

The Wallstent is a cylindrical wire mesh made of a stainless steel alloy. It is pliable and can conform to the shape of the biliary tract in the long axis. The stent has its own delivery system and must be primed with sterile saline before use. The diameter of the delivery system is 7.5 French and the deployed length is 40 to 80 mm, with a maximum diameter of 30 French. Care must be taken while deploying this stent because the fully deployed length of the Wallstent is reduced by 25% from the original.

The Diamond stent is made up of nitinol wire, which is again arranged in a tubular mesh. There are 5 platinum-iridium markers at each end of the

TABLE 18.1. Metal stents.

Types	Delivery system (Fr)	Deployed length (mm)	Deployed diameter (mm)
Diamond	9.25	40, 60, 80	10
Endocoil	10	40, 50, 60	7.3
Wallstent	7.5	40, 60, 80	8
		42, 68, 80	10
Z-Stent	12	40, 60, 80	10

Source: Reproduced in part from *Gastrointest Endosc.* 1999;50: 939, with permission.

stent to help with deployment. The delivery system of this stent is 9.5 French and the deployed length and diameter are the same as those of the Wallstent. This stent, however, has less retraction in length than the Wallstent.

The Endocoil is composed of nickel titanium wire arranged in a coil. Its delivery system is 10 French and the deployed length is 40 to 60 mm, with a maximum diameter of 7.3 mm. This stent shortens by 50% on deployment, but it is thought that its compact coils may prevent tumor ingrowth.

The Z-Stent is made up of stainless steel wire arranged cylindrically. The delivery system has the largest diameter, 12 French, and the deployed length and diameter are the same as those of the Wallstent.

The costs of these stents are quite high compared to plastic stents and range from \$1000 to \$1500.

Plastic

Plastic biliary stents available in the market are produced by a number of companies (see Table 18.2). They are made from either polyethyelene or Teflon. The length of the polyethylene stents ranges from 4 to 15 cm and the size extends from 5 to 15 French. The shape of these stents is double pigtail and straight, or curved with sideflaps. The Teflon stent has a length of 5 to 15 cm and the size varies from 8.5 to 11.5 French. These stents are straight without sideholes. The stents larger than 10 French require a 4.2-mm instrument channel duodenoscope. The price of these stents is much lower than the metal stents: from about \$40 to \$60, with the delivery system costing about another \$100.

A comparison between Teflon and standard polyethylene stents was recently conducted. Fifty-

seven patients with malignant biliary stenosis were included in the study (38 with pancreatic cancer, 17 with cholangiocarcinoma, 2 with ampullary cancer). Patients were prospectively randomized to 10-French, 7-cm-long Teflon stents (29 patients) or standard polyethylene (28 patients). The patients were evaluated clinically and if necessary by biochemical tests each month until death or requirement for surgery. Stents were replaced, if they became occluded, with the same type of stent. The two groups were comparable in age, sex, and diagnosis. No statistically significant difference was found when comparing the mean duration of function of the first, second, and third stents. The median duration of stent function was 96 days in the Teflon group and 75.5 days in the polyethylene group. No significant difference was found in either survival time or stent patency. This study showed no significant advantage of Teflon over standard polyethylene with regard¹⁶ to survival and stent patency.¹⁷

The diameter of the stent does seem to make a difference in complication rates as well as the duration of stent patency. One study comparing 8-French stents with 10-French stents showed a higher rate of cholangitis (34% versus 5%, respectively). The stent patency in this trial for 10 French was a median of 32 weeks versus 12 weeks for 8 French.¹⁷ Another study compared 10 French with 11.5 French and found both stents to have the same efficacy in palliative management of pancreatic cancer.¹⁸ We currently recommend using a minimum of a 10-French stent when treating patients with pancreatic cancer palliation for biliary obstruction.

Recommendations on prophylactic stent exchanges still remain controversial. The main complication of stent placement is occlusion. This can

TABLE 18-2. Plastic stents.

Material	Size (Fr)*	Shape	Length (cm)
Polyethylene	7, 8.5, 10, 11.5	Straight/curved with sideflaps	5, 7, 9, 12, 15
Solopass (polyethylene)	5, 6, 7, 10	Double pigtail	4, 7, 10
	7, 10	Straight with sideflaps	5, 7, 10, 12, 15
Teflon	8.5, 10, 11.5	Straight without sideholes	5–15

*Stents > 10 French require a 4.2-mm channel duodenoscope
Source: Reproduced in part from *Gastrointest Endosc.* 1999;50:939, with permission.

be avoided by exchanging the stent every 3 to 6 months¹⁹ or using the alternative approach, which is to exchange the stent only when there are signs and symptoms of occlusion.^{20,21} Our practice has been to leave the stent in place till there are signs of stent occlusion and then replace it, as many patients will die from their disease before replacement is required.

Metal versus Plastic

The decision to use plastic or metal biliary stents is based on not only the life expectancy of the patient but also on his or her ability and willingness to undergo repeat procedures. Even with the above criteria it is difficult to decide which stent should be placed.

Plastic stents had a median patency of 4.5 months in a study done on 204 patients, 101 randomized to surgery and 100 to stent placement,⁴ with the development of stent occlusion in 20% to 30% of patients within the first 3 months of stent placement.²² The occlusion of plastic stents is mainly due to sludge buildup in the stent. Median period of stent patency was found to be 8.2 months in metal stents.²³ The mechanism of stent stenosis is tumor ingrowth rather than the sludge. In another prospective randomized trial of 105 patients, 49 patients received metal stents and 56 a straight polyethylene stent. Median patency of the first polyethylene stent group was 126 days as compared to metal stents, which had a patency of 273 days. There was no survival advantage in either group, but cost analysis showed that initial placement of metal stents resulted in a 28% decrease in the number of endoscopic procedures.²¹

A recent study by Prat et al shed more light on the dilemma of whether to use metal or plastic biliary stents.¹⁹ A total of 101 patients with malignant strictures of the common bile duct were included in the study. Patients were randomized to receive either an 11.5-French polyethylene stent to be exchanged in case of dysfunction (33 patients), an 11.5-French stent to be exchanged every 3 months (34 patients), or a self-expanding metallic Wall-stent (34 patients). The procedure was successful in 97% of the patients. Procedure-related mortality was 2.9% and morbidity was 11.9%. Overall survival was not different in the three groups but the complication rate was higher in the first group. Cost

analysis showed that metal stents were preferred in patients surviving for more than 6 months and plastic stents were preferred for patients surviving for 6 months or less. Metal stents or plastic stents exchanged every 3 months were valuable for increasing complication-free survival in patients with malignant common bile duct strictures.

Our recommendations on which stent to use are based on life expectancy. If it is greater than 6 months, we prefer to use metal stents; however, patient choice and convenience are important factors, as some of our patients have to travel great distances to receive medical care. Also important is the patient's ability to recognize stent complications, as well as patient compliance.

Complications of Biliary Stents

The complications of stent placement in pancreatic cancer are due to ERCP and sphincterotomy, if necessary, and to stent placement. The complications due to stent placement can be divided into early and late.

Complications resulting from ERCP are pancreatitis, which occurs in the range of 1% to 10%,²⁴ cholangitis, which occurs in the range of 0.6% to 0.8%,²⁵ and complications related to any endoscopic procedure, such as perforation, bleeding, aspiration, and reaction to medication or dye, which occur in less than 1% of patients.

The complications of endoscopic sphincterotomy include increased incidence of hemorrhage, pancreatitis, and perforation. In a study by Margulies et al²⁶ the overall incidence of complications was higher in patients undergoing sphincterotomy (8.3% vs 1.2%). This study was done with 10-French plastic stents. Interestingly, a higher incidence of stent migration was noted in patients without sphincterotomy. Currently the majority of gastroenterologists do not perform sphincterotomy before stent placement.

Early complications of biliary stenting are hemorrhage, pancreatitis, duodenal perforation, bile duct perforation, cholangitis, and cholecystitis. Late complications include stent migration and obstruction leading to jaundice, pancreatitis, and cholangitis. In a study of 221 patients with pancreatic cancer who were stented for palliation of obstructive jaundice, a success rate of 90% was achieved for stenting. Procedure-related mortality was 2%, and the 30-day mortality was 10%. Early

cholangitis was 8% and late stent occlusion was 21% at a mean of 5 months.²²

Stent obstruction in plastic stents is due to sludge formation. The initial event is formation of biofilm by bacteria on the stent surface. This leads to deposition protein, amorphous substance, and deconjugated bilirubin, which is the composition of sludge causing the blockage.²⁷ Attempts have been made to prolong stent patency. A randomized prospective trial comparing Tannenbaum Teflon and the standard polyethylene stent as described above did not show any benefit. No substantial difference was found in plastic stents in a randomized trial when omitting side holes in biliary stents as compared to stents with side holes.¹⁶ Placement of stents above or across the sphincter of Oddi to prevent migration of bacteria also showed no significant difference in the overall stent performance.²⁸ Another study did not show any benefit of treatment with antibiotic and ursodeoxycholic acid in prolonging stent patency or improving survival.²⁹ A study by Speer et al did show a difference in stent patency when using a 10-French plastic stent as compared to an 8-French stent,¹⁷ suggesting that the internal diameter of the stent is a critical factor in stent patency. The cause of stent obstruction in metallic stents—tumor in growth—is slower to occur.³⁰ The median patency was 8.2 months with a range of 1 to 32.5 months. Patients who do become obstructed can be treated by balloon dilatation or placement of a plastic stent.²³

Biliary Stents Versus Surgery

In the past surgery was the only option for palliative treatment of pancreatic cancer, and biliary enteric anastomosis was the procedure of choice, as all patients with obstructive jaundice underwent exploratory laprotomy. Now more choices exist for the palliative treatment of pancreatic cancer. Multiple randomized studies have been done comparing surgery with endoscopic treatment.^{4,9} Both methods have been shown to relieve jaundice, but the operative mortality is higher and there is increased length of hospital stay in the surgical group. In a randomized prospective trial 204 patients were randomized to surgical or endoscopic biliary decompression. Technical success was achieved in 98% of surgical and 95% of endoscopic patients. The procedure-related mortality was 3% in stented

patients compared to 14% in surgical patients. Length of hospital stay was a median of 8 days for the stent group compared to 13 days for the surgical group. Late gastric outlet obstruction occurred in 17% of the stented group and 7% of the surgical group. There was no difference in the median survival for both groups.⁴

Duodenal Stents

Gastric outlet and duodenal obstruction is a devastating complication of pancreatic cancer, which presents with symptoms of nausea, vomiting, weight loss, and abdominal pain. It may be the presenting symptom of pancreatic cancer or it may occur in up to 10% of patients in the course of their disease. Until recently the only palliative options were open gastrojejunostomy or percutaneous jejunostomy tube placement with a gastrostomy. Because of the poor clinical condition of these patients the surgical procedures carried significant risk for morbidity and mortality. Other modalities have been tried in the past, such as dilation or ablation of stenosis, with varying degrees of success. The use of self-expanding metallic stents as an alternative to the above treatments has met with some success. The method is minimally invasive and has a low morbidity rate.

Duodenal Stent Techniques

Two methods are available for the placement of duodenal stents. One is percutaneous and the other is endoscopic.

Percutaneous placement requires the placement of a percutaneous endoscopic gastrotomy tube. After this has been placed a guidewire is maneuvered through the stenosis and then the Wallstent assembly is introduced over the guidewire. The Wallstent is released under fluoroscopic guidance in the stricture.

The endoscopic placement of stents requires dilatation of the stricture if the stent cannot be passed. Endoscopic balloon dilatation is carried out with an increasing diameter of “through the scope” balloons until the scope can be passed through the obstruction. The length of the stricture is determined and a stent 2 cm longer than the stricture is deployed. The deployment is done under endoscopic and fluoro-

scopic guidance. More than one stent can be used in the same procedure in an overlapping fashion.

Indications for Duodenal Stents

Duodenal stents are indicated for duodenal or gastric outlet obstruction caused by unresectable locally invasive or metastatic pancreatic cancer for palliation.

Duodenal Stent Types

The enteral Wallstent is the most common type of stent used. It is a self-expanding metallic stent that is 16 to 22 mm in diameter and comes in 60-, 83-, and 90-mm lengths. It is made up of stainless steel alloy and comes with its own delivery system, which is 10 French in size. The stent is pliable and flexible. Other stents available are the Z-Stent, Ultraflex, and Endocoil.

A prospective study by Carre-Locke³¹ was conducted to determine the feasibility of enteral stents. Twelve patients with gastric outlet obstruction (3 due to pancreatic cancer) were studied. After stenting 6 patients were able to eat a regular diet, 3 could have a pureed diet, and 3 were unsuccessful due to the presence of multiple obstructions. Two pancreatic cancer patients were able to eat a regular diet and 1 a pureed diet. Three procedures were done as out-

patient and 3 patients were discharged within 24 hours of the procedure. This study suggests that stent placement is the optimal procedure when compared to surgery in terms of decreased morbidity, mortality, and cost. The overall quality of life was better and life expectancy was similar for both procedures.

In another retrospective study, 10 patients with malignant duodenal strictures secondary to pancreatic cancer were analyzed.³² Gastric outlet obstruction was relieved in all patients after placement of duodenal stents, and 3 patients required biliary stents for palliation of jaundice (Figure 18.4). No recurrence of gastric outlet obstruction was noted in a follow-up period of 1 to 5 months.³²

A third retrospective report on 8 patients found relief of symptoms in 7 patients, 5 of whom had undergone previous surgeries.³³ Different types of stents were used in this study (Wallstent, Z-Stent, Ultraflex, and Endocoil). Thus the enteral stent provides a safe and effective method of palliation in gastric outlet obstruction secondary to pancreatic cancer.

Conclusion

Until recently surgery was the only option available to patients with biliary and duodenal obstruction caused by pancreatic cancer. This meant pa-



FIGURE 18.4. Fluoroscopic still image demonstrating a double biliary (thin arrow) and duodenal (thick arrow) outlet stent placement.

tients had high morbidity, high mortality, and a decreased quality of life due to the bypass surgical procedures, with long recovery periods. With the advent of palliation by stent placement, patient care has been revolutionized. The need for hospital stay and the cost of taking care of these patients have decreased, but most important of all, their quality of life has improved without affecting survival.

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19

Celiac Plexus Block Versus Systemic Opioid Medication in the Management of Pancreatic Cancer Pain

Suresh K. Reddy and Larry L. Zhou

Introduction

Pain is one of the most common presenting symptoms encountered in a cancer patient. Pain occurs in 30% to 50% of patients in active therapy and as many as 60% to 90% of patients with advanced disease.^{1–5} The etiology of pain (eg, from tumor, cancer therapy, or unrelated to either of the two causes) will greatly influence the choice and the efficacy of pain treatment options.

Patients with pancreatic cancer present with myriad symptoms, of which pain at some point during the course of the disease may be severe and difficult to treat.⁶ Pain was the most common symptom (82%) among patients referred to a palliative care service in one study.⁷ Pain in pancreatic cancer tends to be central abdominal or epigastric, perceived as deep in the abdomen and usually chronic in nature rather than episodic. In some patients pain radiates to the upper back and rarely to the shoulder. Metastasis to the liver, to the peritoneum with ascites, to retroperitoneal nodes, to vascular structures, and to the epidural space may result in intractable pain.⁸ Pain may be caused by stretching of the visceral nerve fibers caused by bulky tumor masses in the retroperitoneum. Pain also may be caused by direct invasion of the autonomic nerves by the tumor, and possibly by pancreatic ductal obstruction leading to ductal dilatation. Finally, invasion of the somatic nerves in advanced disease may result in somatic pain.⁹ Drapiewski found direct tumor invasion in pancreatic nerves in 84% of patients in an autopsy series.¹⁰

Treatment of Pancreatic Cancer Pain

The treatment of any cancer pain involves a thorough assessment of pain and other associated symptoms. Pancreatic cancer is often associated with symptoms such as anorexia, nausea, vomiting, asthenia, cachexia, and psychological distress,⁶ in particular depression.^{11–13} Hence a thorough multidisciplinary assessment is mandatory. Failure to treat pain in some pancreatic cancer patients may be due to our inability to identify factors influencing pain. Some of the tools utilized in assessing the patient's pain and other symptoms at M. D. Anderson Cancer Center include the Anderson Symptom Assessment Scale (ASAS) (Figure 19.1) to quantify various symptoms, the Mini-Mental Examination Score,¹⁴ and CAGE,¹⁵ which is sometimes employed as a baseline to monitor cognitive function as well as to exclude alcohol problems. CAGE is a mnemonic for attempts to Cut back on drinking, being Annoyed at criticisms about drinking, feeling Guilty about drinking, and using alcohol as an Eye-opener. A thorough physical exam combined with correlation of imaging studies will lead to the appropriate diagnosis of the pain syndrome.

The treatment of the pancreatic cancer pain includes surgical neurectomy,¹⁶ pharmacotherapy,¹⁷ chemotherapy,¹⁸ radiation therapy,^{19–21} and anesthetic interventions, including celiac/splanchnic neurolytic blocks^{22,23} and neuraxial infusion of medications, both epidural and intrathecal.^{24–27}

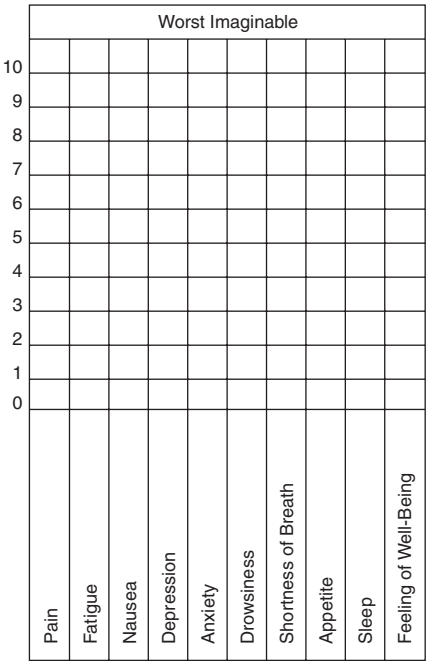


FIGURE 19.1. Anderson Symptom Assessment Scale. Courtesy M. D. Anderson Cancer Center. Driver LC Bruera E, eds. *M. D. Anderson Palliative Care Handbook*. Houston, TX: M. D. Anderson; 2000.

Celiac Plexus Block or Pharmacotherapy

Historical Background

After the introduction of the celiac plexus block by Kappis²⁸ in 1914 in Germany, it was widely used in Germany as a regional anesthetic technique for upper abdominal visceral surgery,²⁹ done predominantly by the surgeons. During the 1940s celiac plexus block became popular among anesthesiologists, both for surgical anesthesia and pain relief. Celiac plexus block was used to treat many types of chest and abdominal pain as well as vascular disease,³⁰ and splanchnic blocks were used to treat pain and hypertension.³¹ From 1914 until the 1950s these blocks were performed guided only by surface anatomy. Bonica first mentioned radiographic guidance for celiac plexus block in 1953.³² Since then the technique has become popular among radiologists, who subsequently started publishing research on it. Several methods of performing the blocks were described in the literature.³³

Celiac plexus block has been performed by us-

ing both a percutaneous posterior and an anterior approach.³⁴ The posterior approach can be either retrocrural^{28,35} (splanchnic nerve block), or anterocrural (Figure 19.2). The most commonly used approach is the percutaneous posterior method. Recently, a new technique of endosonography-guided celiac plexus neurolysis (EUS CPN) has been described,³⁶ but this new technique needs well-defined prospective studies to confirm the results claimed by the authors.

The celiac plexus block was claimed to be among the most effective and most commonly utilized blocks to provide pain relief in pancreatic cancer pain. Success was generally thought to be in the range of 80% to 90%. It was also claimed that celiac plexus block should be employed early in the course of the disease to decrease the dose of opioids and to improve pain control and performance status. The technique itself became popular because of the profound pain relief observed and the simple percutaneous approach with relatively fewer side effects than other techniques, favoring the risk-benefit ratio. This popularity continued at the time when the myths and misconceptions surrounding opioid use were profound. Concerns included fear of addiction, poor knowledge and training in the use of opioids, cultural and social stigmas, and the fear of side effects. However, these concerns have been questioned, citing reporting irregularities and lack of good scientific evidence.^{1,2}

Cancer pain treatment in recent years has undergone tremendous change due to better understanding of the pathophysiology of cancer pain and associated symptoms and a better understanding of pharmacokinetics and pharmacodynamics of opioid medications. The traditional view about the cause of nociception has been questioned, leading to the emergence of new concepts in the causation of pain and other symptoms in the cancer patient (Figures 19.3, 19.4). This has led to a revolutionary multidisciplinary approach to treating pain and other symptoms in cancer patients both during active treatment and in the terminal phases of malignancy.

Celiac plexus block was considered as a standard first-line therapy for pancreatic cancer pain for a number of years. However, its efficacy and methodology have recently been questioned, resulting in a fierce debate about the success rate of the celiac plexus block previously claimed for pancreatic cancer pain.^{37,38} This debate in the future

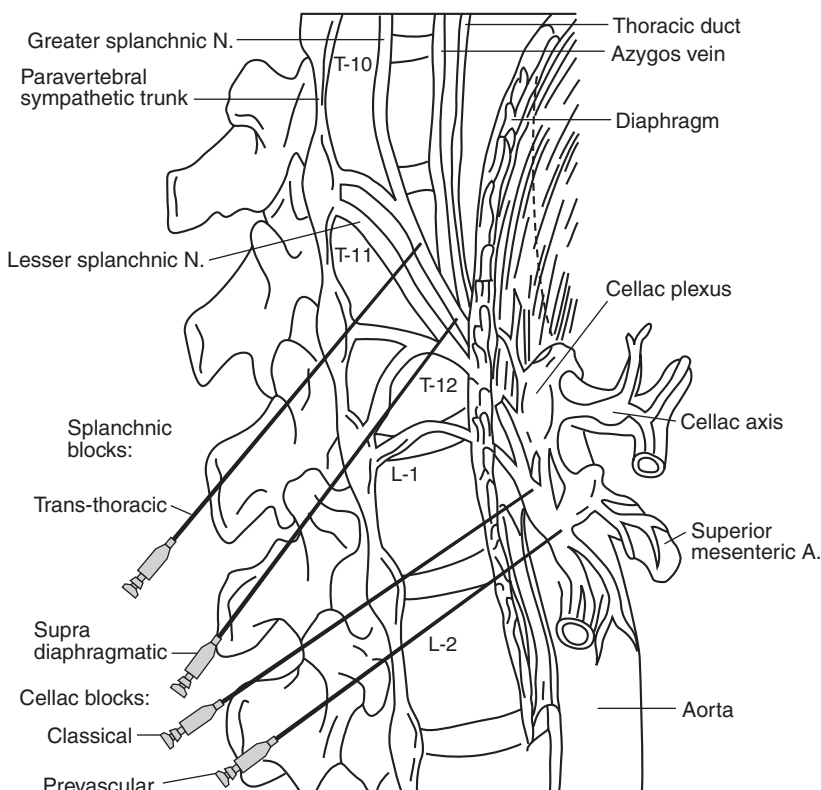


FIGURE 19.2. Anatomy and two approaches to celiac and splanchnic nerve block. Lateral projection showing the splanchnic nerves and celiac plexus in relation to the diaphragm vertebrae and aortic vessels. Placement of needles for two approaches to the celiac plexus and two approaches to the splanchnic nerves. See text for anatomic details. Reproduced with permission. Abrams SE, Boas RA. Sympathetic and visceral nerve blocks. In: Benumof JL, ed. *Clinical Procedures in Anesthesia and Intensive Care*. Philadelphia, Pa: JP Lippincott; 1992:88:787–806.

may help us to conduct outcome-based research as opposed to the anecdotal approach in interventional therapy for cancer pain management.

Literature Debating Celiac Plexus Block

Lebovits and Lefkowitz³⁷ reviewed the literature on celiac plexus block from 1964 to 1988 (Table 19.1) and concluded that there were several shortcomings. No study used the traditional experimental design; there was a lack of comparison groups. Moreover, criteria for objective quantification were rarely specified, most studies were not homogenous with regard to diseases studied, and studies have been done for other indications. Lebovits and Lefkowitz also noted that retrospective chart review studies have major limitations: charts are often incomplete, missing, or illegible. Short survival hampers evaluation of the incidence and duration

of pain relief. Finally, in these studies patient demographics were rarely reported.

Sharfman and Walsh³⁸ reviewed the literature on celiac plexus block since 1964 and reviewed 15 published series in a total of 480 patients. At least satisfactory response to neurolytic celiac plexus block (NCPB) was reported in 418/480 (87%) of the patients. Deficiencies found included the failure of any study to describe a detailed history of analgesic intake preblock; limited postblock data, no information given about postblock analgesic requirements; omission of the duration of analgesia in most series; and the failure to provide data supporting claims of decreased nausea, decreased constipation, and increased appetite. The authors concluded that data available on NCPB for pancreatic cancer pain are insufficient to judge meaningful efficacy, long-term morbidity, or cost-effectiveness. Rigorous evaluation of the technique is required.

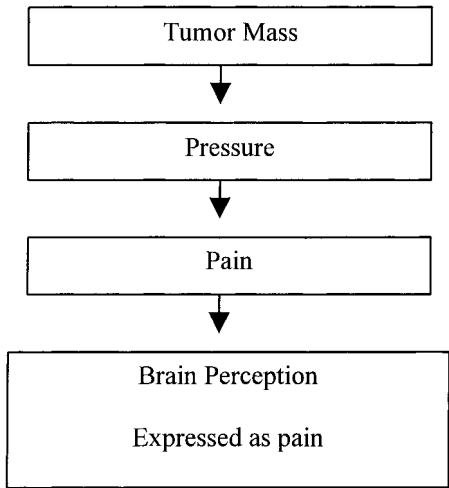


FIGURE 19.3. Traditional view of production of pain. Compiled by the Department of Symptom Control and Palliative Care at M. D. Anderson Cancer Center.

They suggested that NCPB is an option in the treatment of pancreatic cancer pain, but that it cannot be recommended as the treatment of choice.

Literature Supporting Celiac Plexus Block

Mercadante³⁹ did a study comparing celiac plexus block versus analgesics in pancreatic cancer pain in 20 patients. After 1 week of treatment with a World

Health Organization recommended method,⁴ which included a nonsteroidal anti-inflammatory drug–opioid sequence, 10 patients were continued on this sequence (Group A), while the other 10 patients underwent celiac plexus block (Group B). Both groups received opioids as needed. Parameters noted to calculate the effective analgesic dose included a visual analogue scale and opioid consumption at weekly intervals until death. Mean survival rate was 51 days.

The results in Group A showed a gradual increase in the pain score, but pain was controlled with opioids. There was progressive increase in opioid use, with side effects noted. Group B showed a decrease in the pain score. There was a reduction in opioid use, with fewer side effects (drowsiness and tiredness). However, no reduction in the incidence of nausea and constipation between the two groups was observed.

This study attempted to improve on the methodology of the previous studies. However, the number of patients enrolled remained small, the difference in pain scores between the two groups was not statistically significant, and the side-effect profile favored block, but did not imply a significant difference. The 1-week stabilization on analgesics is a short period for escalating medications. This study also raises the ethical issue of doing a neurolytic procedure when pain control in both groups is similar. Other factors contributing to pain such as anxiety, depression, and other psychosocial is-

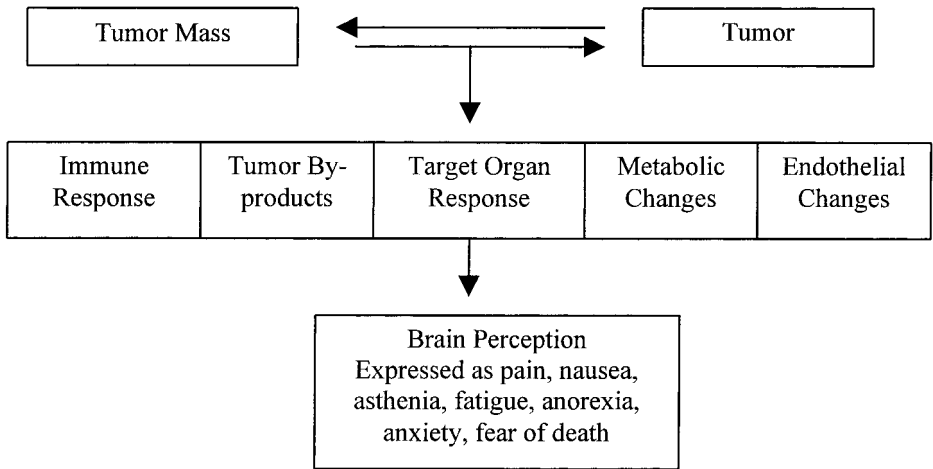


FIGURE 19.4. Emerging view of production and expression of pain and other symptoms. Compiled by the Department of Symptom Control and Palliative Care at M. D. Anderson Cancer Center.

TABLE 19.1. Studies using the celiac plexus block with patients who have pancreatic carcinoma.

Sample of patients with pancreatic cancer		Pain relief		Neurolytic agent, bilateral volume and concentration	Most prevalent complication	Recommended increased usage	
Total sample		Effectiveness	Duration				
(a) Case studies							
Gorbitz and Levens (1971)	10	5	1 pt "fair" to "good." 2 pts. partial. 2 pts. lost to follow-up	Range 1–5 mo	50 ml of 50% alcohol	Postural hypotension: pain	Not addressed
Galizia and Lahiri (1974)	1	1	Pain-free	1 mo (death)	Attempted 10 ml of 6% aqueous phenol	Paraplegia	Not addressed
Kune et al. (1975)	25	12	83% "good"	Unspecified	20 ml of absolute alcohol	Postural hypotension	Yes
Jones and Gough (1977)	100	14	Unspecified	Unspecified	16 ml of absolute alcohol	Hypotension	Yes
Thompson et al (1977)	100	676	Unspecified	Unspecified	50 ml of 50% alcohol	Postural hypotension	Yes
Moore et al (1981)	20	20	95% "marked" to "complete"	Through 72 h, not followed after	50 ml of 50% alcohol	None	Not addressed
Leung et al (1983)	36	13	85% "complete"	64% median duration, 3 mo (death), maximum 11 mo	50 ml of 50–75% alcohol	Postural hypotension	Yes
Owitz and Koppolu (1983)	138	80	85% "satisfactory"	Range 3 to 18 mo	20 ml of absolute alcohol unilateral	"Fairly severe" postural hypotension	Yes
Ischia et al. (1983)	28	7	71% "complete"	Unspecified	30 ml of 75% alcohol (transaortic)	Diarrhea lasting 38–48 h	Yes
Coombs and Savage (1985)	1	1	Pain-free	1 mo (death)	50 ml of 70% alcohol	None	Yes
(b) Retrospective chart review studies							
Bridenbaugh et al (1969)	41	23	96–100% "free" of pain	Unspecified	40–50 ml of 50% alcohol	Pain: hypotension	Yes
Black and Dwyer (1973)	100	18	70% "success"	At least 1 mo or until death	40 ml of absolute alcohol	Weakness/numbness in thigh/groin	Yes
Brown et al (1987)	136	136	85% "good"	75% until death, 13% more than 50% of duration of life	50 ml of 50% alcohol	Postural hypotension	Yes

Modified with permission. Lebovits AH, Lefkowitz M, Pain management of pancreatic cancer. *J Pain Symptom Manage.* 1989;36:267–271.

sues were not considered. The article draws some broad conclusions and favors celiac plexus block in pancreatic cancer patients, but it does not provide us with the guidance for appropriate patient selection and timing for this therapy.

Lillemoe et al⁴⁰ did a double-blind, prospective study comparing intraoperative chemical splanchnicectomy with 50% alcohol versus a placebo in patients with histologically proven unresectable pancreatic cancer. No difference in hospital mortality, complications, oral intake, or length of stay was observed. Patients were stratified based on the presence or absence of pain preoperatively. In the group with no preoperative pain, in the alcohol group the intensity of pain lessened significantly compared with the placebo group. In the pain group, the alcohol group had increased survival in addition to decreased pain. The study concluded that intraoperative chemical splanchnicectomy with alcohol significantly reduced the intensity of pain or prevented it.

This was a well-designed prospective double-blind randomized study, offering some validity to the use of interventional therapy in pancreatic cancer pain. The authors observed a significant reduction both in the intensity of pain and in opioid consumption in patients treated with alcohol injection of the celiac plexus intraoperatively. They also demonstrated an improvement in the survival duration of patients with preoperative pain who received alcohol injection. But this study is yet to be reproduced, partly due to a decrease in the number of laparotomies performed in patients with unresectable pancreatic cancer (as a result of thin-sectioned contrast-enhanced computer tomography to accurately predict resectability of pancreatic neoplasms).⁴¹ The results in the study have also not been reproduced to date by percutaneous celiac plexus block. The improved survival data in the preprocedure pain group should be interpreted with caution because of the small numbers available for interpretation. Even though a reduction in opioid dosing is mentioned in the celiac plexus block group, no detailed quantitative data on the morphine equivalent daily dose are provided.

Eisenberg et al⁴² performed a meta-analysis of the efficacy and safety of the neurolytic celiac plexus block (NCPB) for cancer pain. Their literature search yielded 59 studies (Tables 19.2,

TABLE 19.2. Cancer diagnosis for which neurolytic celiac plexus block (NCPB) was performed.

Type of cancer	Patients	
	n	%
Pancreas	707	63
Nonpancreatic:	410	37
Gastric/esophagus	113	10
Colon/rectum	65	6
Liver metastases	58	5
Lung	28	3
Gallbladder, liver, bile duct	22	2
Renal/adrenal	21	2
Gynecologic breast	14	1
Other (lymphoma, sarcoma, teratoma, gastrocolic and pancreatic metastasis, or unknown)	89	8
Total	1117	100

Pancreatic cancer is the most common diagnosis and is the basis for NCPB in approximately two-thirds of the patients. Tabulated data reflect 21/24 (85%) of studies that involved 1106 of 1126 (98%) of patients whose outcomes were analyzed.

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19.3). Data on 2 or more patients were available in only 24 papers. Twenty-one studies were retrospective, one was prospective, and only 2 out of 59 studies were randomized and controlled. They noted that pain relief was 89% during the first 2 weeks after NCPB. Long-term follow-up beyond 3 months revealed persistent benefit. Persistent pain relief also was reported until the time of death. Common side effects were transient and included local pain (96%), diarrhea (44%), and

TABLE 19.3. Pain assessment in the literature reviewed.

Pain assessment	Studies (n = 24)		Patients (n = 1145)	
	N	%	N	%
Duration	4	17	181	16
Location	6	25	189	16
Quality	3	12	165	14
Intensity	5	21	125	11
Intractability	6	25	291	17

Only six papers reported intractable pain as an indication for neurolytic celiac plexus block. Pain intensity prior to block was reported in 11% of the patients. No paper assessed all 5 pain characteristics.

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hypotension (38%). Complications occurred in 2% of patients.

The authors concluded that meta-analysis applied to this literature is not suitable. Most published reports focus on the block technique rather than on the assessment of patient pain. Crucial information such as pain intensity, duration, location, and quality exists for only a minority of patients (25% or fewer). Evidence to support the celiac plexus block as superior to oral analgesic therapy was not sufficient. However, the celiac plexus block was effective in controlling pancreatic cancer pain, with a favorable risk-benefit ratio. As the authors themselves noted, the lack of uniformity in precise criteria for patient enrollment, type of outcome reported, and duration of follow-up make meta-analysis a misnomer. The conclusions that pain relief persisted beyond 3 months and until the time of death are misleading.

Summary

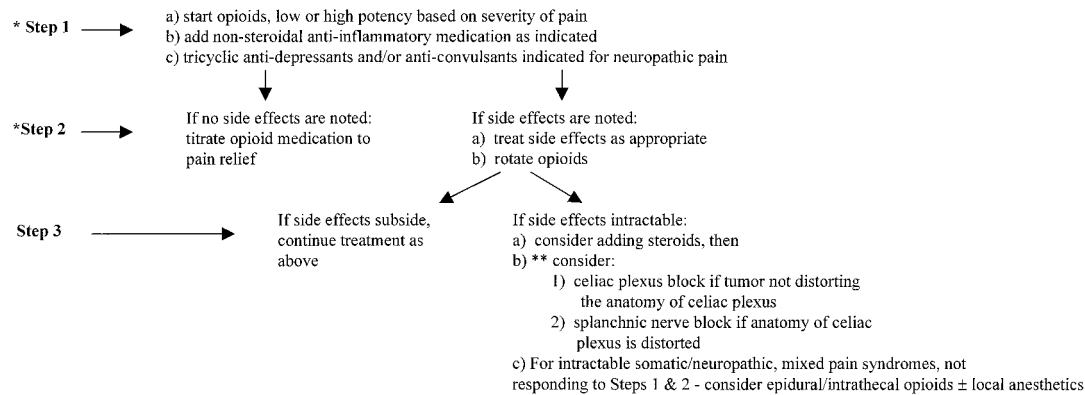
The debate on the superiority of celiac plexus block versus pharmacotherapy provides some interesting insights into the methodology used by interventional therapists to assess the success of the block. Studies comparing the two different modalities will continue to pose a daunting challenge to proponents of both. Reasons include the inconsistency of the technique used; inability to assess the extent and progression of the disease; problems encountered in the delineation of pain other than visceral, psychosomatic, and psychosocial issues complicating the optimal assessment of nociception; and pain assessment complicated by ongoing treatment of cancer (chemotherapy, radiation therapy, and sometimes surgery, alone or in combination). The superiority of one method over the other may never be established. But the traditional model of noci-

A. Multidisciplinary Assessment Is the Key

B. Diagnose the Type of Pain in Pancreatic Cancer

Visceral Pain (Most Common) Example: pancreatic mass liver/adrenal/renal metastasis small bowel obstruction	Somatic Pain Example: bone metastasis peritoneal implants (Carcinomatosis)	Neuropathic Pain Example: radioclopathy spinal core compression	Mixed Pain Syndrome Example: ascites pancreatic mass with bone metastasis

C. Treatment of Pancreatic Cancer Pain:



D. Diagnosis and Treatment of Other Symptoms as Appropriate

Example: nausea, anorexia, loss of weight, depression, anxiety, fear of death

* Steps 1 & 2 may take up to 2-3 weeks to titrate.

** Indicated for visceral pain and mixed pain syndrome with predominant visceral pain component. Also consider risk vs benefit ratio.

FIGURE 19.5. M. D. Anderson Cancer Center algorithm for treating pancreatic cancer pain.

ception in cancer pain (Figure 19.3) is slowly being replaced by a newer model, wherein the symptoms of any cancer are believed to be caused by a number of tumor products giving rise to a multitude of symptoms (pain being one of them) (Figure 19.4). In this setting the interventional treatment of cancer pain should be offered as an adjuvant to other treatment modalities: pharmacotherapy, psychosocial support, and concomitant treatment of other symptoms, including but not limited to nausea, fatigue, loss of appetite, cachexia, dyspnea, anxiety, fear of death, and depression. In our experience, when pain and other symptoms are believed to be caused by tumor-related nociception, celiac/splanchnic block offers complete resolution of symptoms in an occasional patient, but pain usually returns to a varying degree requiring pharmacotherapy and other support. Even if pain control is optimal, the majority of patients need continued support and treatment of terminal symptoms.

We propose the stepwise approach for the treatment of pancreatic cancer pain that is followed at the M. D. Anderson Cancer Center Pain Section (Figure 19.5).

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20

Postoperative Adjuvant Therapy: Past, Present, and Future Trial Development

William F. Regine

Introduction

Despite the obvious evolution in our 3 major cancer treatment modalities—surgery, radiation, and chemotherapy—the overall survival rate of patients with pancreatic cancer remains dismal. In the United States, the pancreatic cancer death rate has been steadily increasing and the 2000 estimated mortality rate (28,200 per year) is expected to nearly equal its estimated incidence (28,300 per year).¹ While this may be in part attributed to the limitations and less than optimal integration of our current treatment modalities, it is in large part due to the biologically aggressive nature of this cancer problem. Only 10% to 15% of patients with pancreatic cancer are able to undergo “potentially” curative resection, despite which the 5-year survival in these patients is <20%.^{2–7} Even among the most favorable subset of patients, those with tumor diameters of <3 cm and/or negative nodal status, as well as microscopically negative margins of resection, the 5-year survival is no more than 36%.^{3,5–9} Studies by the Gastrointestinal Tumor Study Group (GITSG) have evaluated external beam radiation therapy with or without 5-fluorouracil (5-FU) in patients with locally unresectable disease. These studies have shown a definite survival advantage to the use of 5-FU in combinations with radiation therapy.¹⁰ In addition, series in which the patterns of disease recurrence following pancreatic resection have been mapped suggest that both local and distant recurrences are frequent and thus the addition of chemoradiation may be beneficial.^{11,12} The recent, current, and future status of postoperative adjuvant therapy for pancreatic cancer is presented in

this chapter. The results of randomized trials will be discussed, evolving institutional and cooperative group experiences reviewed, and the status of ongoing trials updated.

Postoperative Adjuvant Therapy: Past Randomized Trials

Among patients who have undergone a potentially curative resection, phase 3 evaluation of postoperative adjuvant therapy has been limited, until recently, to a single trial evaluating chemoradiation and a single trial evaluating chemotherapy alone. The GITSG trial, although terminated early due to poor patient accrual, showed a statistically significant doubling in median survival and modest improvement in 5-year survival for patients receiving adjuvant split-course chemoradiation as compared to observation. This trial was limited to 43 patients with pancreatic adenocarcinoma who were felt to have undergone potentially curative resections with negative margins. Therapy involved 40 Gy in 6 weeks, with a 2-week break, and 3-day infusions of 5-FU at the start of weeks 1 and 5, followed by weekly infusions of maintenance 5-FU. Twenty-one patients randomized to adjuvant split-course chemoradiation had a median survival of 21 months, 2-year survival of 43%, and 5-year survival of 19% compared to 11 months, 18%, and 5%, respectively, for the observation group ($P = 0.03$). There were no life-threatening complications or deaths attributable to therapy.^{13,14} These results were duplicated in an additional cohort of 30 patients treated on a nonrandomized basis achieving

median and 2-year survivals of 18 months and 46%, respectively, and a 5-year survival of 17%, further substantiating the benefit in patients receiving this adjunctive therapy.^{14,15} Only 2 of the 51 treated patients (4%) in the GITSG study developed possible late treatment-related complications.

A less often referred to Norwegian trial showed a statistically significant doubling in median survival, with no associated significant improvement in long-term survival, for patients receiving adjuvant adriamycin-mitomycin-5-FU (AMF) chemotherapy as compared to observation. Within this trial of 61 patients with pancreatic or ampullary carcinoma, a total of 43 patients with pancreatic adenocarcinoma were randomized to observation or postoperative adjuvant chemotherapy. Therapy involved AMF chemotherapy once every 3 weeks for 6 cycles.¹⁶ Overall, 30 patients randomized to adjuvant chemotherapy had a median survival of 23 months as compared with 11 months in the control group ($P = 0.02$). This did not translate into a significant difference in long-term prognosis, with 2-, 3-, and 5-year survival in the treatment group being 43%, 27%, and 4% compared to 32%, 30%, and 8% in the observation group ($P = 0.10$). In addition, the toxicity of combination chemotherapy was substantial and included hematologic toxicity, cardiotoxicity, nephrotoxicity, and at least 73% of treated patients requiring hospitalization.

Postoperative Adjuvant Therapy: Recent Randomized Trials

The European Organization for Research and Treatment of Cancer recently completed a phase 3 trial (EORTC protocol #40891) evaluating the same split-course chemoradiation regimen as in the GITSG trial but without maintenance therapy, versus observation in patients after curative resection for cancer of the pancreatic head or periampullary region. Patients were stratified by tumor location. A total of 218 patients were randomized (110 to treatment and 108 to observation) of which 114 (52%) had pancreatic head lesions—60 to treatment arm and 54 to observation. Not surprisingly, therapy was well tolerated, with maximal World Health Organization toxicity being grade 3 and observed in only 7 patients; however, 21 patients in the treatment arm (20%) did not receive

any treatment because of postoperative morbidity or patient refusal. In the treatment arm, overall, the median survival was 24.5 months, and 2-year survival was 51% compared to 19 months and 41% for the observation group ($P = 0.208$). When analyzed by tumor location the median, 2-year, and 5-year survival for patients with periampullary cancers randomized to treatment was 39.5 months, 70%, and 38%, respectively, as compared to 40.1 months, 64%, and 36%, respectively, for those randomized to observation ($P = 0.737$). In the pancreatic head cancer group the median, 2-year, and 5-year survival for those randomized to treatment was 17.1 months, 37%, and 20%, respectively, as compared to 12.6 months, 23%, and 10% respectively, for those randomized to observation ($P = 0.099$).¹⁷ The results of this trial, while of interest and discussed in more detail in Chapter 22, cannot be considered definitive given the lack of use of maintenance therapy in the adjuvant regimen, the inclusions of patients with positive resection margins without stratification, the lack of radiation therapy quality assurance, and lack of statistical power. For example, to have had an 80% chance of detecting an increase in 2-year survival from 40% to 55% would have required randomization of more than 300 patients, roughly 3 times the number of patients with pancreatic cancer actually entered.¹⁸ Similar concerns can also be applied to the GITSG trial.

The Japanese Study Group of Surgical Adjuvant Therapy (JSGSAT) for Carcinomas of the Pancreas and Biliary Tract have recently reported their results, in abstract, of a randomized trial of postoperative adjuvant chemotherapy for resected pancreatic/biliary carcinomas. Between 1988 and 1992 this trial randomized 508 patients with resected pancreatic/biliary tract carcinomas to mitomycin-5-FU (MF) combination chemotherapy ($n = 232$) vs observation ($n = 204$). Therapy involved mitomycin given the day of surgery and 5-FU given during weeks 1 and 3 after surgery and then continuing with 5-FU alone for 1 additional year beginning 5 weeks after surgery. Primary tumor sites included bile duct, gallbladder, and ampullary; 158 patients (31%) had resected pancreatic carcinoma. Combination chemotherapy was well tolerated, with greater than 80% of patients receiving their planned doses. However, a significant improvement in 5-year survival with postoperative MF ad-

juvant therapy was limited only to the subgroup of patients with gallbladder carcinoma (26% vs 14%, $P = 0.03$). Among the patients with pancreatic carcinoma the 5-year survival for treated patients was 11.5% as compared to 18% for the observation group ($P = \text{NS}$).¹⁹

By the end of 1999 the European Study Group of Pancreatic Cancer (ESPAC) is expected to close a postoperative adjuvant trial, ESPAC-1, which will have accrued nearly 560 patients with pancreatic or ampullary adenocarcinoma to a 4-arm design that will include observation. The remaining 3 arms evaluate radiotherapy and/or chemotherapy similar to that used in the GITSG/EORTC trials. Patients are stratified according to the presence or absence of positive resection margins, and the study combines 2 methods of quality-of-life assessment, one by the patient and one by the physician.²⁰ Preliminary results of the ESPAC-1 trial suggest no benefit to postoperative adjuvant split-course radiotherapy or chemotherapy in patients with adenocarcinoma of the pancreas. When all patients are analyzed there is suggestion of a benefit in favor of postoperative 5-FU chemotherapy. The status and additional details of this ESPAC-1 trial, and a soon-to-be activated follow-up trial ESPAC-3 that will evaluate 5-FU vs gemcitabine vs observation in the postoperative setting, is provided in Chapter 22.

The Evolution Toward More Dose-Intensive Postoperative Adjuvant Chemoradiation

Postoperative adjuvant therapy for pancreatic carcinoma and other gastrointestinal sites has evolved into use of higher-dose, nonsplit course, and potentially more toxic chemoradiation regimens as compared to that utilized in the GITSG and EORTC/ESPAC trials.^{21–24} Phase 3 evaluation of such an approach in rectal carcinoma, with use of radiation doses of 50.4 to 54 Gy in 6 weeks combined with continuous infusion (CI) 5-FU, has been associated with a significant improvement in survival when compared to a less dose-intensive postoperative adjuvant chemoradiation regimen.²⁵ Whether similar improvements can be achieved without significant upper abdominal toxicity in pa-

tients with pancreatic carcinoma, where the death rate due to disease recurrence is more exponential, needs evaluation; however, phase 2 experiences have been promising.

The Mayo Clinic experience among 29 patients treated with postoperative chemoradiation following potentially curative resection of adenocarcinoma of the pancreas is reflective of an evolution toward dose-intensive postoperative adjuvant therapy. Nine patients were treated with split-course therapy, while the remainder were treated with continuous-course therapy. The median dose of radiation used was 54 Gy with a range of 35 to 60 Gy. Twenty-seven of 29 patients also received concurrent bolus 5-FU chemotherapy. The median, 2-year, 3-year, and 5-year survival for the group was 23 months, 48%, 24%, and 12%, respectively. Seventeen percent developed late treatment-related complications, while the rate of small bowel obstruction requiring operation among those receiving >45 Gy was 4.2%.²¹

The Johns Hopkins Hospital evolving experience with increasingly intensive chemoradiation following resection for adenocarcinoma of the pancreas^{9,23} has been updated in 173 patients undergoing 3 options of postoperative adjuvant therapy: (1) 99 patients receiving “standard” split-course or continuous-course radiation to doses of 40 to 45 Gy in conjunction with concurrent bolus 5-FU; (2) 21 patients receiving “intensive” therapy involving continuous-course radiation of 50.4 to 57.6 Gy to the tumor bed and prophylactic hepatic irradiation of 23.4–27 Gy with CI 5-FU plus leucovorin concurrently and as maintenance for 4 months, and (3) 53 patients having no therapy. The “intensive” therapy group experienced increased toxicity and had no survival benefit when compared to the “standard” therapy group. However, patients receiving adjuvant therapy had a median and 2-year survival of 19.5 months and 39%, respectively, as compared to 13.5 months and 30%, respectively, for the patients who received no therapy ($P = 0.03$).²⁴

The M. D. Anderson Hospital experience with postoperative adjuvant therapy among a cohort of 19 patients made use of infusional chemoradiation (50.4 Gy in 28 fractions over 5½ weeks with CI infusion 5-FU at 300 mg/m² per day) and intraoperative electron-beam radiation therapy (10 to 15 Gy). The median, 2-year, and 3-year survival for these patients is 22 months, 55%, and 39%, respectively.²⁶

The Eastern Cooperative Oncology Group reported the results of a phase I trial²⁷ evaluating the maximum tolerated dose (MTD) of CI 5-FU with concurrent radiation in 25 patients with unresectable, residual, or recurrent carcinoma of the pancreas or bile duct. Beginning at 200 mg/m² per day, CI 5-FU was given concurrently with radiation therapy (59.4 Gy in 33 fractions over 6 to 7 weeks). Chemotherapy began and continued through the entire course of treatment. After each cohort of 5 patients had been treated and observed, the daily dose was escalated in 25 mg/m² increments until dose-limiting toxicity was encountered. The dose-limiting toxicity was oral mucositis and the MTD of CI 5-FU was found to be 250 mg/m² per day.

Table 20.1 summarizes the reviewed results of postoperative adjuvant therapy. While more dose-intensive chemoradiation regimens, similar to that used in the above experiences, have become more of the community norm, an associated improvement in patient outcome has, at most, been modest. In addition, the survival results achieved with utilization of 5-FU-based chemoradiation/combination chemotherapy regimens have plateaued at

median, 2-year, 3-year, and 5-year survivals in the order of 22 months, 45%, 30%, and ≤20%, respectively. The problem of distant metastases continues to be a major factor for poor prognosis in these patients. The need for more effective chemotherapy to reduce the incidence of distant metastases, with the potential for further improvements in patient survival, is clear.

Gemcitabine

In 1996 the US Food and Drug Administration (FDA) approved gemcitabine for use in patients with pancreatic cancer. This is the first chemotherapeutic agent since 5-FU to be approved for use in patients with pancreatic cancer in 35 years. Gemcitabine is also unique in that the FDA approved the compound despite an objective response rate of 5.4%.²⁸ The FDA approved gemcitabine based on 2 registration trials in patients with symptomatic stage IV pancreas cancer.^{28,29} These trials introduced the concept of clinical benefit response. Pancreatic cancer patients almost always have devas-

TABLE 20.1. Pancreatic carcinoma—results of postoperative adjuvant therapy.

<i>Split-Course Chemoradiation</i>	Survival			
	Median (mo)	2-Year (%)	3-Year (%)	5-Year (%)
GITSG				
Randomized to Rx (n = 21)	21	43	24	19
GITSG				
Registered to Rx (n = 30)	18	46	—	17
EORTC				
Randomized to Rx (n = 60)	17.1	37	31	20
<i>Chemotherapy Alone</i>				
Norwegian				
Randomized to Rx (n = 23)	23	43	27	4
JSGSAT				
Randomized to Rx (n = ~79)	—	—	—	11.5
<i>Dose-Intensive, Nonrandomized</i>				
Mayo (n = 29)	23	48	24	12
Hopkins (n = 120)	19.5	39	33	—
M. D. Anderson (n = 19)	22	55	39	—

—indicates no data.

tating symptoms including pain and weight loss. These patients also experience declining performance status. Objective responses are also frequently difficult to assess noninvasively. Investigators combined changes in pain control, weight loss, and performance status to create clinical benefit response. The FDA accepted this patient-centered endpoint for testing of gemcitabine in pancreas cancer. In the first trial investigators randomized 126 stable symptomatic patients to gemcitabine 1000 mg/m² weekly for 7 weeks followed by a week's rest, then 4 cycles consisting of 3 weekly doses of gemcitabine at 1000 mg/m² and 1 week rest.²⁸ The other patients received bolus 5-FU. The clinical benefit response was 24% for gemcitabine and 5% for 5-FU ($P = 0.0025$). In addition, patients achieving symptomatic relief did so within 6 weeks of initiating therapy. Symptomatic benefit lasted an average of 12 weeks. The second registration trial was a single-arm phase 2 trial in 63 patients who had failed 5-FU.²⁹ Treatment was with gemcitabine at the same dose and schedule. The clinical benefit response was 27% and the 1-year survival 4%. The median survival was 3.9 months. Based on these 2 trials the FDA approved gemcitabine to treat advanced pancreas cancer both in front-line and salvage therapy. The toxicity of gemcitabine was mild to moderate. Only 10% of patients discontinued therapy due to toxicity and the most common toxicity was myelosuppression. Other toxicities include hepatitis, nausea, vomiting, hair loss, skin rash, and fever. Gemcitabine is given as a 30-minute infusion intravenously. Gemcitabine is now used in the primary therapy for metastatic pancreas cancer. In the meantime, a phase 3 evaluation of adjuvant treatment of resected pancreatic adenocarcinoma is needed to prove or disprove increased efficacy as well as establish the best "standard" adjuvant regimen for use in the community and future trials.

Postoperative Adjuvant Therapy: Current and Future Randomized Trials

The Radiation Therapy Oncology Group (RTOG) study #97-04 is a phase 3 Intergroup trial of pre- and postchemoradiation 5-FU versus pre- and

postchemoradiation gemcitabine for postoperative adjuvant treatment of resected pancreatic adenocarcinoma. It is the first US phase 3 cooperative group study in nearly a quarter century (GITSG trial activated February 1974) evaluating adjuvant chemoradiation in patients with localized and resected adenocarcinoma of the pancreas. Participating Intergroup members include the Eastern Cooperative Oncology Group and the Southwest Oncology Group. The study schema is detailed in Figure 20.1. All patients will receive the same 5-FU-based chemoradiation while the study evaluates gemcitabine in the postoperative adjuvant setting and its effect on overall survival, local-regional, and distant disease control, and/or disease-free survival as compared to 5-FU. This study incorporates modern therapeutic principles of chemoradiation and current developments in chemotherapy in pancreatic cancer. The study will also be the first of its kind to require prospective radiation therapy quality assurance. All patients will have their radiation treatment fields, along with preoperative diagnostic information, reviewed for compliance to protocol requirements prior to the start of chemoradiation (ie, during prechemoradiation chemotherapy). A minimum 3- or 4-field approach is required, with the intent of minimizing potential treatment toxicity. Schematics used as guidelines for recommended/required fields, according to preresection primary tumor location, are shown in Figure 20.2. The study will also be the first to prospectively evaluate the ability of postresectional CA19-9 to predict outcome among adjuvantly treated patients who have undergone a potentially curative resection for adenocarcinoma of the pancreas. The study was activated in April 1998 with a projected accrual rate of 5 patients per month and a target total accrual goal of 330 patients. Through 1999 the study accrued 128 patients, with recent 6-month accrual averaging >12 patients per month. This outstanding accrual is no doubt in part reflective of the long absence of a US phase 3 randomized trial in this setting. To date, this trial will be the largest and most statistically powered in evaluating adjuvant therapy exclusively in patients with pancreatic adenocarcinoma. Results of this trial will provide data to compare to currently available phase 3 results with less intensive chemoradiation regimens (GITSG, EORTC/ESPAC-1) and future phase 3 results from ESPAC-3.

U.S. INTERGROUP/RTOG #97-04

A PHASE III STUDY OF PRE AND POST CHEMORADIATION 5-FU VS. PRE AND POST CHEMORADIATION GEMCITABINE FOR POSTOPERATIVE ADJUVANT TREATMENT OF RESECTED PANCREATIC ADENOCARCINOMA

SCHEMA

S	<u>Nodal Status</u>	R	
	1. Uninvolved		<u>Arm 1:</u> Pre-CRT + Chemoradiation (CRT) + Post-CRT
T	2. Involved	A	5-FU 5-FU
R	<u>Tumor Diameter</u>	N	
	1. < 3cm		
A	2. ≥ 3cm	D	
T	<u>Surgical Margins</u>	O	<u>Arm 2:</u> Pre-CRT + Chemoradiation (CRT) + Post-CRT
	1. Negative		Gemcitabine Gemcitabine
I	2. Positive	M	
	3. Unknown		
F		I	
Y		Z	
		E	

PRE-CRT CHEMOTHERAPY: Starting 3-8 weeks after definitive tumor-related surgery;

- Arm 1:** 3 weeks of continuous infusion (CI) 5-FU at 250 mg/m²/d.
Arm 2: 3 weeks of Gemcitabine at 1000 mg/m²/d, once weekly.

CHEMORADIATION (CRT): Starting 1-2 weeks after completion of pre-CRT chemotherapy and no later than 13 weeks after definitive tumor-related surgery;

Arms 1&2: 50.4 Gy/5 1/2 wks @ 1.8 Gy/fx [field reduction at 45 Gy] and CI 5-FU, 250 mg/m²/d, during radiation.

POST-CRT CHEMOTHERAPY: Starting 3-5 weeks after completion of CRT;

- Arm 1:** 2 cycles of CI 5-FU (one cycle = 4 wks of CI 5-FU at 250 mg/m²/d followed by 2 wks rest) for a duration of 3 months.
Arm 2: 3 cycles of gemcitabine (one cycle = 3 wks of gemcitabine at 1000 mg/m²/d, once weekly followed by 1 wk rest) for duration of 3 months.

FIGURE 20.1. Schema for US Intergroup/RTOG trial #97-04, a phase 3 study evaluating postoperative adjuvant therapy in patients with pancreatic adenocarcinoma.

For the future there are many questions still remaining to be answered in the adjuvant management of patients with pancreatic adenocarcinoma. Optimized intergration of conventional treatment modalities of radiation, chemotherapy, and surgery still need to be established. Radiation dose, fractionation, and volume are critical issues that remain to be resolved, especially in conjunction with new cytotoxic agents that pose their own unique set of questions, including mode of drug delivery, combination of drugs, and dose intensification. The use of specialized programs such as intraoperative radiation has also not been fully explored. Evolving knowledge in the biological behavior of these tumors based on genetic fingerprints could provide useful guides to designing customized treatment

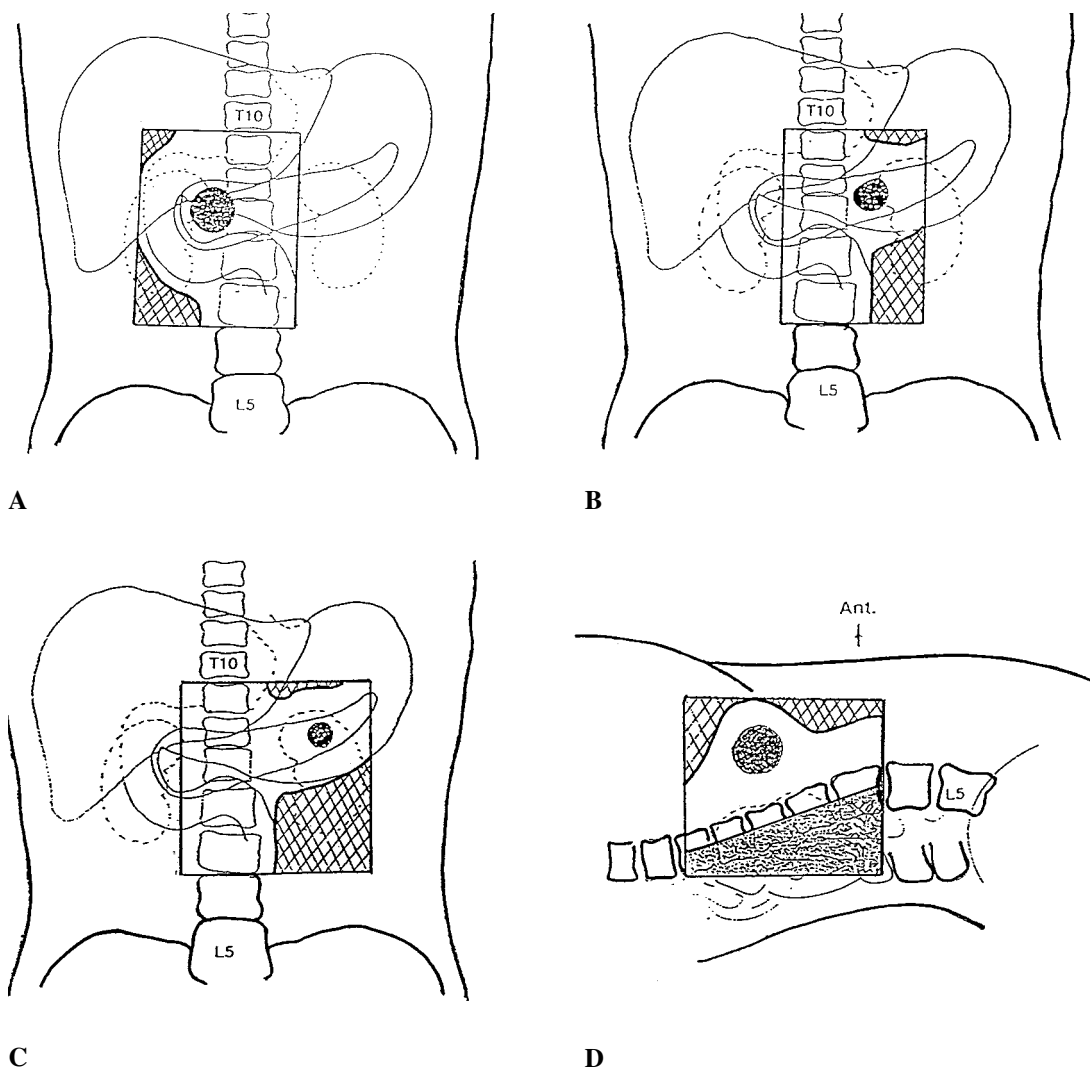


FIGURE 20.2. Schematics of recommended/required radiation therapy fields used in US Intergroup/RTOG trial #97-04. (A) For pancreatic head tumors. (B) For pancreatic body tumors. (C) For pancreatic tail tumors. (D) Lateral fields.

strategies. The US Intergroup study will have a companion biomolecular basic science study. Genes and/or mutations involving *p53*, *ras*, and *HER2/neu* have been implicated in conferring enhanced resistance to conventional adjuvant treatment strategies. Understanding the genetic profile of pancreatic cancers will be of immense value in shaping future treatment strategies, inclusive of the potential for use of gene therapy. In the meantime future trials, for the first time in over a quarter century, will have the opportunity to build upon modern, broad-based, quality-controlled, randomized trials that integrate and evaluate the potential of ba-

sic science, serum tumor markers, and quality-of-life issues.

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Preoperative (Neoadjuvant) Therapy for Resectable Adenocarcinoma of the Pancreas

Wayne A.I. Frederick, Robert A. Wolff, Christopher H. Crane, Jeffrey E. Lee, Peter W.T. Pisters, and Douglas B. Evans

Introduction and Background

Patients who undergo pancreaticoduodenectomy alone for adenocarcinoma of the pancreatic head or uncinate process have a median survival duration of 12 months and a high (50%–80%) incidence of local tumor recurrence resulting from positive retroperitoneal margins of resection, a common finding upon pathologic evaluation of pancreaticoduodenectomy specimens.¹ Recent prospective and retrospective data suggest that the combination of pancreaticoduodenectomy with postoperative adjuvant fluorouracil (5-FU) and external-beam radiation therapy (EBRT) improves survival duration and local-regional tumor control.^{2–5} However, the morbidity and often prolonged recovery associated with pancreaticoduodenectomy prevent the timely delivery of postoperative chemoradiation (chemotherapy and EBRT) in at least 25% to 30% of eligible patients.^{4,6} For example, in the Gastrointestinal Tumor Study Group (GITSG) trial, 5 (24%) of the 21 patients in the adjuvant chemoradiation arm could not begin chemoradiation until more than 10 weeks after pancreaticoduodenectomy. Thus, despite the obvious selection bias in patient accrual, the patients likely to be considered for protocol entry were those who recovered rapidly from surgery and had a good performance status. Similar findings were recently reported by the European Organization for Research and Treatment of Cancer (EORTC).⁵ Between 1987 and 1995, 218 patients who had undergone pancreaticoduodenectomy for adenocarcinoma of the pancreas or periampullary region were randomized to receive either chemoradiation (40 Gy in a split course and 5-FU given as

a continuous infusion at a dose of 25 mg/kg/day during EBRT) or no further treatment. Eleven patients were deemed ineligible for analysis due to incomplete resection in the setting of extensive local disease. Of the remaining 207 patients, 114 (55%) had pancreatic cancer. The overall median survival duration was 24.5 months for the group who received adjuvant therapy and 19 months for the group who received surgery alone ($P = 0.2$); for patients with pancreatic cancer, the median survival time was 17.1 months for those who received adjuvant therapy and 12.6 months for those who received surgery alone ($P = 0.099$). Although these differences were not significant, the wide confidence interval (CI) for the subset of patients with pancreatic cancer (relative risk, 0.7; 95% CI, 0.5–1.1) preserves the possibility that the chemoradiation arm had a clinically meaningful improvement in survival that was obscured by the small sample size. As in the GITSG trial, patients in the EORTC trial were considered for enrollment after recovery from pancreaticoduodenectomy. Despite this selection bias, 21 (20%) of 104 evaluable patients assigned to receive chemoradiation did not receive the intended therapy because of patient refusal, medical comorbidities, or rapid tumor progression.

The risk of delaying postoperative adjuvant chemoradiation prompted investigators to initiate studies in which patients with potentially resectable adenocarcinoma of the pancreas received chemoradiation before pancreaticoduodenectomy.⁷ The preoperative use of chemoradiation is supported by several considerations.⁶ First, positive gross or microscopic margins of resection along the right lateral border of the superior mesenteric artery (SMA)

are common following pancreaticoduodenectomy, suggesting that surgery alone may be an inadequate strategy for local tumor control.⁸ Second, because chemoradiation is given first, delayed postoperative recovery does not affect the delivery of multimodality therapy. Third, patients with disseminated disease evident on restaging studies after chemoradiation are not subjected to laparotomy. Fourth, recent data suggest that preoperative chemoradiation may decrease the incidence of pancreaticojejunal anastomotic fistula, the most common complication following pancreaticoduodenectomy.⁹

Critical to clinical trials evaluating preoperative or postoperative adjuvant therapy is the incorporation of standardized approaches to patient selection (pretreatment staging), operative technique, and pathologic evaluation of surgical specimens. This chapter briefly outlines our system for standardizing these important variables as defined in the following paragraphs.

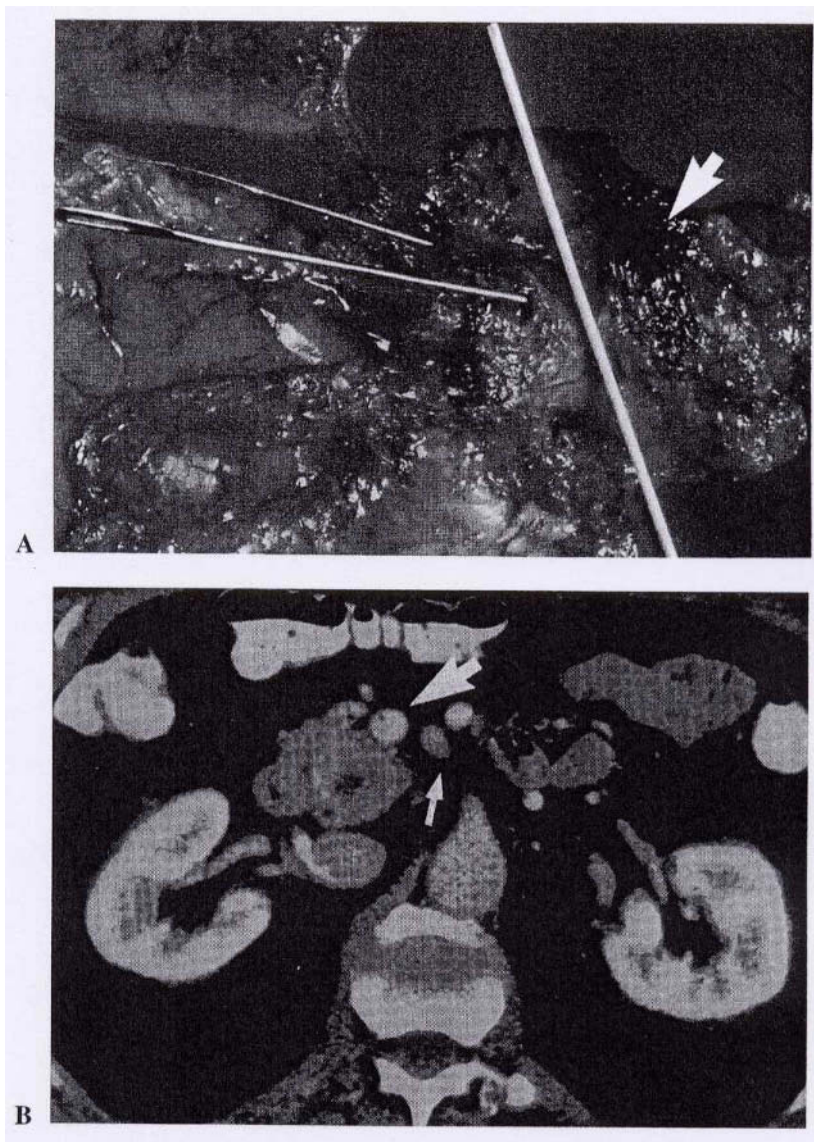
Radiographic Staging. High-quality contrast-enhanced helical computed tomography (CT) is used to define the relationship of the tumor to the celiac axis and the superior mesenteric vessels. The following CT criteria are used at our institution to define potentially resectable disease:¹⁰ (1) the absence of extrapancreatic disease; (2) the absence of direct tumor extension to the SMA and celiac axis, as defined by the presence of a fat plane between the low-density tumor and these arterial structures; and (3) a patent superior mesenteric-portal vein (SMPV) confluence. The third criterion is based on the assumption that resection and reconstruction of the superior mesenteric vein (SMV) or SMPV confluence are possible. In the absence of extrapancreatic disease, the main goal of preoperative imaging studies is to determine the relationship of the low-density tumor mass to the SMA and celiac axis. This information enables accurate prediction of the likelihood of obtaining a negative retroperitoneal margin of resection. The retroperitoneal margin, also termed mesenteric margin, corresponds to the tissue along the proximal 3–4 cm of the SMA (Figure 21.1).

Surgical Technique. The operative technique of pancreaticoduodenectomy currently performed at our institution comprises six clearly defined steps, as previously described.^{11,12} The most oncologi-

cally important and difficult part of the operation is step six, during which the pancreas is divided and the specimen is removed from the SMPV confluence and the right lateral border of the SMA. Only after full medial mobilization of the SMV is identification of the SMA (lateral to the SMV) possible. The pancreatic head and all soft tissue to the right of the SMA are then removed by direct ligation of the inferior pancreaticoduodenal artery or arteries. Failure to mobilize the SMPV confluence may result in a positive resection margin due to the incomplete removal of the uncinate process and the mesenteric soft tissue adjacent to the SMA.

Pathologic Evaluation of the Pancreaticoduodenectomy Specimen. Accurate pathologic assessment of surgical specimens is critical for both the evaluation of innovative preoperative treatment strategies and the development of reproducible prognostic predictors of patient survival and treatment failure. Retrospective pathologic analysis of archival material does not allow accurate assessment of the margins of resection or of the number of lymph nodes retrieved. The first step in the standard pathologic evaluation of the pancreaticoduodenectomy specimen developed at our institution¹³ is to perform a frozen-section evaluation of the common bile duct transection margin and the pancreatic transection margin. Either transection margin, if positive, is treated with re-resection. The retroperitoneal or mesenteric margin is defined as the soft-tissue margin directly adjacent to the proximal 3–4 cm of the SMA. This margin is evaluated by permanent-section microscopic examination and is identified and inked by the surgeon and pathologist (Figure 21.1). Re-resection to treat a microscopically positive margin is not possible in the retroperitoneum, where the aorta and SMA origin limit the extent of surgical resection; therefore, frozen-section evaluation of this margin is not performed. Importantly, this margin cannot be retrospectively evaluated after the gross evaluation of the specimen has been completed. Samples of multiple areas of each tumor, including the interface between the tumor and adjacent uninvolved tissue, are submitted for paraffin-embedded histologic examination (5–10 blocks). The final pathologic evaluation of permanent sections includes a description of tumor histology and differentiation; gross and microscopic evaluation of the tissue of origin (pan-

FIGURE 21.1. Illustration of the retroperitoneal margin as identified on pathologic evaluation of the resected pancreaticoduodenectomy specimen (A) and the corresponding orientation of this margin with respect to the superior mesenteric artery and vein as seen on contrast-enhanced computed tomography (CT) (B). A, Photograph of a pancreaticoduodenectomy specimen; the retroperitoneal margin (tissue adjacent to the superior mesenteric artery) is inked for evaluation of margin status on permanent section histologic evaluation. A small probe is in the bile duct and just inferior to that, a slightly larger probe is seen within the pancreatic duct. A white Q-tip applicator stick lies over the indentation from the superior mesenteric-portal vein confluence. B, Contrast-enhanced helical CT scan demonstrating a resectable adenocarcinoma of the pancreatic head. Note the normal fat plane between the low-density tumor and the superior mesenteric artery (small arrow); this is the retro-peritoneal margin. The superior mesenteric vein is identified by the large arrow.



creas, bile duct, ampulla of Vater, or duodenum); an assessment of maximal transverse tumor diameter; and a report of lymph node status. Metastatic disease in regional lymph nodes, poorly differentiated histology, and increased size of the primary tumor have been associated with decreased survival duration.^{14–16} In patients who receive preoperative chemoradiation, the grade of treatment effect is assessed on permanent sections (Table 21.1).¹⁷

To determine which patient subsets may benefit from the most aggressive treatment strategies, accurate pathologic staging and histologic assessment of response to preoperative therapy are mandatory.

Preoperative Chemoradiation Strategies for Localized Pancreatic Cancer

Potentially Resectable Pancreatic Cancer

The first reports of preoperative chemoradiation and pancreaticoduodenectomy from our institution used a standard-fractionation treatment schema.^{14,18} Radiation therapy was delivered over 5.5 weeks with 18-MeV photons using a four-field technique to administer a total dose of 50.4 Gy prescribed to the 95% isodose at 1.8 Gy/fraction (28 fractions)

TABLE 21.1. Grading system for chemoradiation treatment effect.

Grade	Histologic appearance
I	Characteristic cytologic changes of malignancy are present, but little (<10%) or no tumor cell destruction is evident
II	In addition to characteristic cytologic changes of malignancy, 10%–90% of tumor cells are destroyed
IIA	Destruction of 10%–50% of tumor cells
IIB	Destruction of 51%–90% of tumor cells
III	Few (<10%) viable-appearing tumor cells are present
IIIM	Sizable pools of mucin are present
IV	No viable tumor cells are present
IVM	Acellular pools of mucin are present

Data from Evans et al.¹⁴

for 5 days/week. 5-FU was given concurrently by continuous infusion at a dosage of 300 mg/m²/day, 5 days/week, through a central venous catheter. Thirty-eight patients were evaluable for analysis of patterns of treatment failure; one perioperative death occurred.¹⁹ Tumor recurrence was documented in 29 patients: 8 recurrences (21%) were local-regional (in the pancreatic bed and/or peritoneal cavity), and 30 (79%) were distant (in the lung, liver, and/or bone). The liver was the most frequent site of tumor recurrence, and liver metastases were a component of treatment failure in 53% of patients (69% of all patients who had recurrences). Isolated local or peritoneal recurrences were documented in only 4 patients (11%). In contrast, previous reports of pancreaticoduodenectomy alone for adenocarcinoma of the pancreas documented local recurrence in 50% to 80% of patients.^{8,19,20} The improvement in local-regional control with preoperative chemoradiation was seen, even though 14 of 38 evaluable patients had undergone laparotomy with tumor manipulation and biopsy prior to referral for chemoradiation and reoperation. Excluding these 14 patients, local or peritoneal recurrence was a component of treatment failure in only 2 patients (8%). However, this 5.5-week chemoradiation program was associated with gastrointestinal toxicity (nausea, vomiting, and dehydration) that required hospital admission of one third of the patients.¹⁴ In addition, the recently reported multicenter Eastern Cooperative Oncology Group (ECOG) trial documented the need for hospital admission of 51% of patients during or within 4 weeks after completing chemoradiation.²¹

These findings prompted a change in the delivery of preoperative chemoradiation at our institution in favor of rapid fractionation or short-course EBRT. In a prospective trial of 35 patients at our institution, rapid-fractionation chemoradiation at a total dose of 30 Gy (3 Gy/fraction [10 fractions] 5 days/week) was delivered over 2 weeks. 5-FU was given concurrently by continuous infusion at a dosage of 300 mg/m²/day, 5 days/week.²² This chemoradiation program was designed to avoid the gastrointestinal toxicity seen with standard-fractionation chemoradiation (5.5 weeks) while attempting to maintain the excellent local tumor control achieved with multimodality therapy. As with other neoadjuvant treatment schemas, restaging with chest radiography and abdominal CT was performed 4 weeks following completion of chemoradiation in preparation for pancreaticoduodenectomy. Twenty-seven of the 35 patients were taken to surgery, and 20 (74%) underwent successful pancreaticoduodenectomy.²² Local tumor control and patient survival were equal to the results with standard-fractionation (5.5 weeks) chemoradiation: local-regional recurrence developed in only 2 (10%) of the 20 patients who underwent resection, and the median survival duration for all 20 patients was 25 months (Table 21.2).

A more recent report from our institution of 132 consecutive patients who received preoperative chemoradiation and pancreaticoduodenectomy for adenocarcinoma of the pancreas also supports the use of rapid-fractionation chemoradiation.²³ Forty-four patients received standard-fractionation (45–50 Gy, 1.8 Gy/fraction/day) EBRT, and 88 patients received rapid-fractionation EBRT (30 Gy, 3.0 Gy/fraction/day). Thirty-six patients (27%) had undergone an unsuccessful attempt at tumor resection prior to referral, and 57 (43%) required vascular resection and reconstruction at the time of the pancreaticoduodenectomy. Intraoperative radiation therapy (IORT) was delivered to 74 of the 105 patients. At the discretion of the operating surgeon, IORT was not performed following long, difficult operations. The overall median survival duration from the time of tissue diagnosis was 21 months. The dose of preoperative EBRT, the chemotherapy agent used, and the delivery of IORT did not influence survival duration. Univariate and multivariate analyses revealed superior survival lengths among patients who had no evidence of lymph node metas-

TABLE 21.2. Recent preoperative adjuvant chemoradiation studies in patients with resectable pancreatic cancer.*

Reference	No. patients†	EBRT dose, Gy	Chemotherapy agent(s)	Median survival, mo
Breslin et al ²³	132	30–50.4	5-FU‡	21
Pisters et al ²²	20	30	5-FU	25
Spitz et al ⁶	41	30–50.4	5-FU	19.2
Hoffman et al ²¹	24	50.4	5-FU + Mito	15.7

*EBRT indicates external-beam radiation therapy; 5-FU, 5-fluorouracil; and Mito, mitomycin C.
†All patients underwent a pancreatectomy with curative intent.
‡A small number of patients received either paclitaxel or gemcitabine as a radiation-sensitizing agent.

tasis ($P = 0.03$). The data suggested that short-course chemoradiation (30 Gy in 2 weeks) combined with pancreaticoduodenectomy performed on accurately staged patients may be equivalent to standard-fractionation chemoradiation (45–50 Gy in 5–6 weeks).

Further, the recently reported interim results of the European Study Group of Pancreatic Cancer (ESPAC)-1 study suggest that EBRT may not be an essential treatment component.²⁴ The ESPAC-1 trial is a four-arm study with a 2×2 factorial design that compares the effects of adjuvant chemoradiation (40 Gy in a split course and 5-FU), adjuvant chemotherapy (5-FU and folinic acid), chemoradiation followed by chemotherapy, and observation alone following pancreaticoduodenectomy for pancreatic or periampullary carcinomas. Accrual for this study began in 1994, and medical centers in 11 countries have randomized 530 patients. The majority of patients have been entered into the randomized 2×2 factorial design. However, either because of lack of access to EBRT or because of specific institutional bias, 188 patients were randomized to receive only chemotherapy or no chemotherapy, and 68 patients were randomized to receive either chemoradiation or no chemoradiation. In the latter two nonfactorial groups, patients could receive nonstandardized therapy at the discretion of their treating physicians. For example, patients who were randomized in the nonfactorial design to chemotherapy or no chemotherapy could receive EBRT. Importantly, nonrandomized treatments were not standardized. Preliminary results suggest no benefit of postoperative chemoradiation.²⁴ Although we are not ready to remove EBRT from the study of protocol-based treatment for localized pancreatic cancer, the availability of more potent radiation-sensitizing agents and techniques

to ensure complete surgical resection makes the study of shorter-course, less toxic EBRT attractive in contemporary clinical trial design.

Repeat staging CT after chemoradiation reveals liver metastases in approximately 25% of patients among those who receive chemoradiation prior to planned pancreaticoduodenectomy.^{6,22} If these patients had undergone pancreaticoduodenectomy at the time of diagnosis, it is probable that the liver metastases would have been subclinical; these patients would therefore have undergone a major surgical procedure only to have liver metastases found soon after surgery. In the trials reported from our institution, patients who were found to have disease progression at the time of restaging had a median survival duration of only 7 months.⁷ The avoidance of a lengthy recovery period and the potential morbidity of pancreaticoduodenectomy in patients with such a short expected survival duration represent a distinct advantage of preoperative over postoperative chemoradiation. When delivering multimodality therapy for any disease, it is beneficial, when possible, to deliver the most toxic therapy last, thereby avoiding morbidity in patients who experience rapid disease progression not amenable to currently available therapies.

Despite surgeons' ability to perform pancreaticoduodenectomy safely, the procedure is too extensive and complex to enable the consistent postoperative delivery of standard-fractionation adjuvant chemoradiation.^{4,5} In the absence of compelling data demonstrating superior survival results with either a preoperative or postoperative treatment approach, all available data suggest that a greater proportion of patients receive potentially beneficial adjuvant therapy, with a reduced overall treatment time, when chemoradiation is administered in a neoadjuvant set-

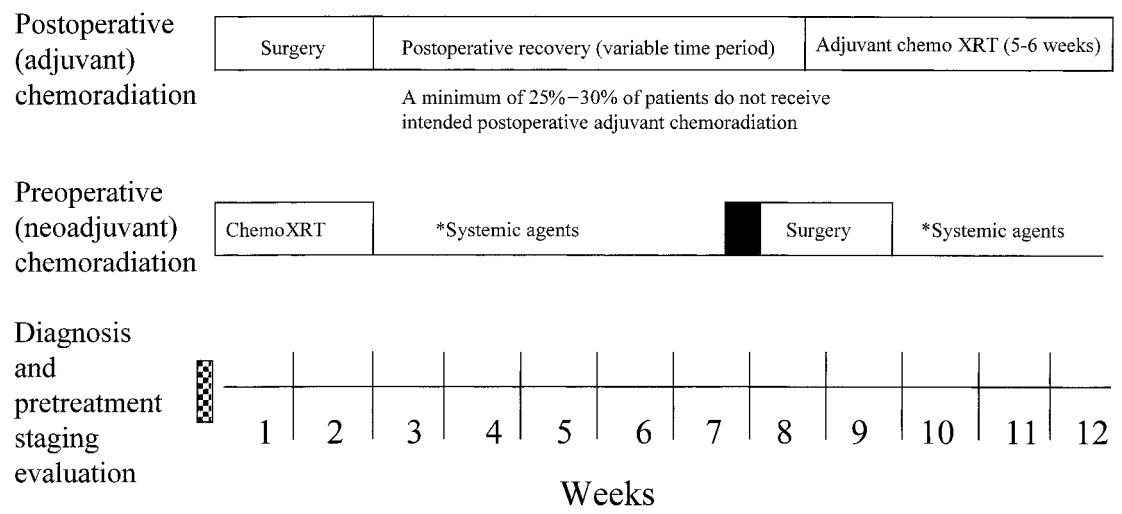


FIGURE 21.2. A comparison between postoperative and preoperative chemoradiation schemas for patients with potentially resectable adenocarcinoma of the pancreatic head. The delivery of postoperative adjuvant therapy is complicated by the prolonged recovery time of at least 25% to 30% of patients following pancreaticoduodenectomy. Current neoadjuvant treatment schemas combine short-course, rapid-fractionation chemoradiation (which avoids the gastrointestinal toxicity of standard-fractionation chemoradiation) with more potent radiation-sensitizing agents. Future treatment schemas (currently being studied in the protocol setting) emphasize the importance of improving systemic disease control with new systemic therapies. Postoperative (adjuvant) chemoXRT: 50.4 Gy; 1.8 Gy/FR; 5-FU 300 mg/m²/d, M-F; preoperative (neoadjuvant) chemoXRT: 30 Gy; 3.0 Gy/FR; with improved radiation-sensitizing agents (gemcitabine). *Systemic agents: cytotoxic therapy (gemcitabine alone or in combination) and/or novel sytemic agents (see Figure 21.3).

ting (Figure 21.2). Further, preoperative chemoradiation treatment strategies will spare many patients the morbidity and mortality associated with laparotomy, as up to one fourth of patients will show evidence of metastatic disease at the time of properative restaging following chemoradiation and thus will not benefit from surgery.

The Treatment of Biliary Obstruction During Preoperative Chemoradiation

The major concern over the delivery of chemoradiation prior to pancreaticoduodenectomy is that the presence of prosthetic biliary drains in a chronically infected biliary tree may increase the risks of chemoradiation-related morbidity and subsequent operative morbidity and mortality.^{21,25} In a recently published ECOG study, tumor- or stent-related recurrent biliary obstruction with cholangitis was believed to be the cause of 38% of hospital admissions.²¹ This finding prompted a review of the risk of stent-related morbidity at our institution.²⁶ Among a cohort of 154 patients treated with pre-

operative chemotherapy and concurrent EBRT (30 or 50.4 Gy), nonoperative biliary decompression was performed in 101 patients (66%): endobiliary stent placement in 77 and percutaneous transhepatic catheter placement in 24 patients. Stent-related complications (occlusion or migration) occurred in 15 patients. Inpatient hospitalization for antibiotics and stent exchange was necessary in 7 of 15 patients (median hospital stay, 3 days). No patient experienced uncontrolled biliary sepsis, hepatic abscess, or stent-related death. The overall risk for biliary stent occlusion (with or without cholangitis) among patients receiving chemoradiation was approximately 15%.²⁶ We concluded that preoperative chemoradiation for pancreatic cancer is associated with low rates of hepatic toxicity and biliary stent-related complications and that the need for biliary decompression should not be viewed as a contraindication to preoperative treatment. However, morbidity may be minimized by treatment in a regional referral center with a multispecialty team approach, by the placement of larger-caliber biliary stents, and by early recognition of stent occlusion.

Recent retrospective studies have also suggested that the placement of biliary drains and subsequent bacterial colonization of the biliary tree may increase the morbidity^{25,27,28} and mortality²⁵ of pancreaticoduodenectomy. Povoski et al²⁵ found that preoperative biliary drainage was associated with increased perioperative morbidity and mortality in 240 consecutive patients who underwent pancreaticoduodenectomy. The operative mortality rate in patients who underwent pancreaticoduodenectomy after biliary drainage was 7.9%, compared with 1.8% in patients who did not undergo preoperative biliary drainage ($P < 0.037$). This fourfold increase in the operative mortality rate among patients with stents caused the authors to recommend that preoperative biliary drainage be avoided whenever possible. In contrast, Sohn et al²⁸ reported an operative mortality rate of 1.7% in 408 patients who underwent biliary decompression prior to pancreaticoduodenectomy. Multivariate analysis showed that the only complication associated with preoperative biliary decompression was wound infection ($P = 0.03$). In a series of 300 consecutive patients who recently underwent pancreaticoduodenectomy at our institution, 172 patients (57%) had preoperative biliary drainage (stent group), 35 patients (12%) underwent surgical bypass prior to referral, and the remaining 93 patients (31%) received no form of preoperative biliary decompression (no-stent group).²⁹ The overall operative mortality was 1% (4 patients). Multivariate logistic regression showed no differences between groups in the incidences of all complications, major complications, infectious complications, intra-abdominal abscess, pancreaticojejunal anastomotic leak, or death. Wound infections were more common in the stent group than in the no-stent group ($P = 0.029$). Thus, our experience suggests that preoperative biliary decompression may increase wound infection rates but does not increase the risk of major postoperative complications or operative mortality.

Chemoradiation to “Downstage” Locally Advanced Pancreatic Cancer

5-FU-based Chemoradiation

Chemoradiation has been administered to patients with locally advanced, unresectable pancreatic cancer in an effort to improve survival duration and,

more recently, to downstage advanced local-regional disease to allow surgical resection. The GITSG randomized patients with locally advanced pancreatic cancer to receive 40 Gy of EBRT plus 5-FU, 60 Gy plus 5-FU, or 60 Gy without chemotherapy.³⁰ The median survival duration was 10 months in each of the chemoradiation groups and 6 months for patients who received 60 Gy without 5-FU. All patients had undergone laparotomy and therefore had been surgically staged; only patients with disease confined to the pancreas and peripancreatic organs, regional lymph nodes, and regional peritoneum were eligible for the study. Thus, although surgical staging resulted in a more uniform study population, it also introduced significant selection bias: only rapidly recovering patients were considered for treatment. Comparison of these data with the results of future studies must account for this selection bias. In contrast to the GITSG data, an ECOG study suggested no benefit of chemoradiation over 5-FU alone: the median survival was 8.3 months in the group who received chemoradiation and 8.2 months in the group who received 5-FU alone.³¹ Both the GITSG studies and the ECOG trial clearly showed that patients with locally advanced, unresectable pancreatic cancer who are asymptomatic to the point of not being fully ambulatory do not benefit from anticancer therapy.

Because surgical resection of the primary tumor remains the only potentially curative treatment for pancreatic cancer, preoperative chemoradiation has been studied for its ability to convert locally unresectable pancreatic cancer to resectable disease (Table 21.3).^{32–42} Early studies were based on the use of 5-FU and EBRT. In a report from the New England Deaconess Hospital, 16 patients who had locally advanced, unresectable pancreatic cancer were treated with 45 Gy of EBRT and infusional 5-FU to enhance resectability.⁴² Only 2 (13%) of the patients were able to undergo resection. Similarly, investigators from Duke University reported that only 2 (8%) of 25 patients with locally advanced pancreatic cancer treated with 45 Gy of EBRT and 5-FU (with or without cisplatin or mitomycin C) subsequently underwent complete resection resulting in negative margins.³⁶ The available literature suggests that it is unlikely that 5-FU-based chemoradiation schemas can make unresectable lesions resectable and thereby increase the number of patients who can be cured with multimodality therapy.

TABLE 21.3. Experience with neoadjuvant chemoradiation to allow eventual surgical resection in patients with locally advanced pancreatic cancer.*

Reference	No. patients	EBRT dose, Gy	Chemotherapy	No. with radiographic response	No. surgically resected	Median survival, months
Brunner et al ³²	24	55.8	Gem cisplatin	NA	7/13	NA
DiPetrillo and Safran ³³	40	50.4	Paclitaxel	PR, 11/38 (29%)	3	8.5
Epelbaum et al ³⁴	20	50.4	Gem	PR, 4	2	12
Wilkowski et al ³⁵	10	45	Gem + 5-FU	7	5	NA
White et al ³⁶	25	45	5-FU + Mito (12) or cisplatin (10)	6/22 had decrease >1 cm	5† (CR,‡ 1)	NA
Blackstock et al ³⁷	17	50.4	Gem	PR, 3/15	0	NA
Bajetta et al ³⁸	32	50	5-DFUR	PR, 7/32	5	9
Todd et al ³⁹	38	0	5-FU + LV + Mito + dipyridamole	PR, 14; CR, 1	4 (CR,‡ 1)	15.5
Kamthan et al ⁴⁰	35	54	5-FU + STZ + cisplatin	PR, 9; CR, 6	5 (CR,‡ 2)	15
Safran et al ⁴¹	14	50	Paclitaxel	PR, 4/13	1	NA
Jessup et al ⁴²	16	45	5-FU	NA	2	9.6§

*EBRT indicates external-beam radiation therapy; 5-FU, 5-fluorouracil; 5-DFUR, doxifluridine; Mito, mitomycin C; Gem, gemcitabine; LV, leucovorin; STZ, streptozocin; CR, complete response; PR, partial response; and NA, not available.

†Three of 5 patients had positive margins of resection.

‡Histologic CR.

§Mean.

Gemcitabine-based Chemoradiation

More effective radiation sensitization may result in a greater cytotoxic effect at the local tumor site. Gemcitabine (2',2'-difluorodeoxycytidine, Gemzar) is a deoxycytidine analogue capable of inhibiting DNA replication and repair. In a randomized trial, gemcitabine was compared to 5-FU in previously untreated patients.⁴³ Patients treated with gemcitabine had a median survival period of 5.7 months compared with 4.4 months ($P = 0.0025$) in those treated with 5-FU. Twenty-four percent of patients treated with gemcitabine were alive at 9 months compared with 6% of patients treated with 5-FU. In addition, more clinically meaningful effects on disease-related symptoms (pain control, performance status, and weight gain) were seen with gemcitabine (23.8% of patients) than with 5-FU (4.8% of patients). Gemcitabine is also a potent radiation sensitizer of human pancreatic cancer cells, and laboratory studies suggest that gemcitabine's inhibitory effect on DNA synthesis (when combined with irradiation) is prolonged in tumors compared with normal tissues.⁴⁴

Investigators recently combined EBRT (45 Gy) with continuous infusion 5-FU (350 mg/m²) and gemcitabine (300 mg/m² on Days 1, 15, and 29) in

10 patients with locally advanced pancreatic cancer (Table 21.3).³⁵ Patients also received additional systemic therapy consisting of gemcitabine and cisplatin pre- and postchemoradiation. Response in the primary tumor was seen in 7 patients and 5 of the 7 underwent surgical resection. Another recent report evaluated the combination of cisplatin and EBRT (55.8 Gy) with escalated doses of weekly gemcitabine in 24 patients with locally advanced pancreatic cancer.³² Seven of the 24 patients underwent surgical resection of the primary pancreatic tumor. These studies suggest that the combination of multiple, more effective radiosensitizers with EBRT may result in significant local tumor response. However, it is important to remember that as the definition of locally advanced pancreatic cancer is broadened, results will appear to be more promising. All studies of novel chemoradiation regimens should adhere to a strict computed tomography examination of locally advanced pancreatic cancer that includes arterial involvement (low-density tumor inseparable from the SMA or celiac axis on contrast-enhanced CT) or venous (SMV or SMPV confluence) occlusion.

The encouraging data from small pilot studies provide the basis for the ongoing phase I and phase II studies of gemcitabine in combination with

EBRT in patients with locally advanced pancreatic cancer. Gemcitabine is being given in escalating doses weekly as a single agent with EBRT,³⁴⁻⁴⁵ in combination with 5-FU and EBRT,^{35,46} in combination with cisplatin and EBRT,^{32,47} at a fixed dose with escalating doses of EBRT,^{48,49} and as a twice-weekly infusion with either standard-fractionation EBRT³⁷ or split-course EBRT.⁵⁰

Researchers from our institution have reported on a phase I study of rapid-fractionation EBRT and concomitant weekly gemcitabine in patients with locally advanced adenocarcinoma of the pancreatic head.⁴⁵ Eighteen patients with pathologically proven, locally advanced adenocarcinoma of the pancreatic head were enrolled in this study. Patients received 7 weekly doses of gemcitabine with 30 Gy of EBRT (3.0 Gy/fraction, M-F) delivered during the first 2 weeks of therapy. Six patients received gemcitabine at 350 mg/m²/week, nine at 400 mg/m²/week, and three at 500 mg/m²/week. Grade 3-4 hematologic toxicity was observed in over half the patients treated. Nonhematologic toxicities were significant and included fatigue, anorexia, nausea, vomiting, and dehydration. Forty-four percent of the patients required admission to the hospital for management of nausea/vomiting and dehydration. The risk of hospitalization appeared to be dose-related; all 3 patients treated at 500 mg/m²/week required hospital admission during treatment. It is interesting to note that patients who received the highest dose of gemcitabine (500 mg/m²/week) had a lower occurrence of grades 3 or 4 hematologic toxicity, but all had grades 3 or 4 gastrointestinal side effects. Conversely, patients treated at either 350 mg/m²/week or 400 mg/m²/week were more likely to develop grade 3 myelosuppression. We postulate that the 500-mg/m²/week dose led to severe mucosal injury that precluded continued therapy on schedule. The patients assigned to receive either 350 or 400 mg/m²/week were more likely to receive gemcitabine on a weekly basis and therefore were more prone to develop hematologic toxicity. Although gastrointestinal toxicity was also common in this group, myelosuppression was likely related to the higher cumulative dose of gemcitabine. These results suggest that when gemcitabine is given weekly with concomitant radiation therapy to a dose of 30 Gy in 10 fractions, the maximum tolerated dose (MTD) of gemcitabine is between 350

mg/m²/week and 400 mg/m²/week for 7 weeks. This is approximately one third of the recommended dose of gemcitabine when administered as a single agent for the treatment of advanced pancreatic cancer. Seventeen patients were evaluated for response and 8 patients (47%) had evidence of a local anticancer effect. Four of these 8 patients (24%) had a partial response to therapy. The median survival duration for the entire group was 6 months. The 1-year survival rate for patients with an objective response to therapy was 66%. The clinical responses observed in this group of patients suggest gemcitabine is a clinically relevant radiosensitizer in patients with pancreatic adenocarcinoma. However, the toxic effects are significant, suggesting that until dose and scheduling issues are explored further, concomitant administration of gemcitabine and radiation therapy should still be considered investigational.

Because of encouraging results in patients with locally advanced disease, gemcitabine-based chemoradiation is being studied in patients with potentially resectable pancreatic cancer (as defined by CT). Hoffman and colleagues⁵¹ have reported a phase I study of preoperative standard-fractionation EBRT (50.4 Gy) and escalating weekly doses of gemcitabine (300 mg/m², 400 mg/m², and 500 mg/m²). Pancreaticoduodenectomy was completed in 8 patients. The current phase II protocol available at our institution for patients with potentially resectable pancreatic cancer is based on the results of the phase I study reported by Wolff et al⁴⁵ discussed previously. Patients receive gemcitabine-based chemoradiation followed by a complete restaging evaluation; patients with no evidence of disease progression are then taken to surgery for pancreaticoduodenectomy. To date, over 40 patients have been entered on this treatment program, and the histologic response to induction therapy (in the resected specimen) appears superior to previous regimens; follow-up is immature, which prevents survival analysis at this time.⁵²

The current treatment of patients with potentially resectable adenocarcinoma of the pancreas at our institution (Figure 21.3) emphasizes the use of new, more potent radiation-sensitizing agents and the preoperative delivery of systemic therapy. In the future, the addition of novel systemic therapies directed at specific molecular events involved in pancreatic tumorigenesis (ie, inhibition of *ras*-

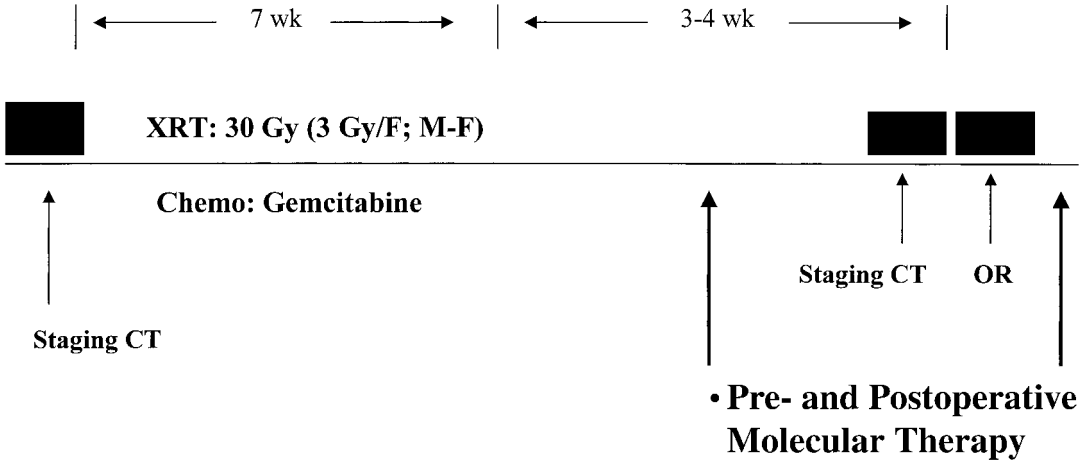


FIGURE 21.3. The future of multimodality therapy for patients with potentially resectable adenocarcinoma of the pancreatic head. Treatment schemas emphasize the importance of minimizing toxicity and treatment duration while attempting to improve therapeutic efficacy. Combining radiation therapy with more potent radiation-sensitizing agents enhances cytotoxicity. After both chemoradiation and surgery, systemic therapy is continued with systemic agents of low toxicity directed at specific molecular events involved in pancreatic tumorigenesis (ie, inhibition of angiogenesis, the use of protease inhibitors [matrix metalloproteinase inhibitors], inhibition of *ras*-dependent signal transduction, or possibly, strategies for the use of gene therapy). XRT, radiation therapy; CT, computed tomography; OR, operating room.

dependent signal transduction, the use of protease inhibitors such as matrix metalloproteinase inhibitors, or the inhibition of angiogenesis) can be used to enhance the treatment of distant microscopic metastases that exist in most patients. Such agents should be of low toxicity to permit administration during both the preoperative and the postoperative period.

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European Adjuvant Trials

Paula Ghaneh, David Smith, Jennifer Almond, Deborah Moffitt, Janet Dunn, John Slavin, Robert Sutton, Claudio Bassi, Paolo Pederzoli, Hans G. Beger, Karl H. Link, Helmut Freiss, Markus Büchler, and John P. Neoptolemos

Introduction

The incidence of pancreatic ductal adenocarcinoma has risen over recent decades and is now one of the most common causes of cancer death in Europe.¹ In the European Community, pancreatic cancer is responsible for 37,000 deaths per year.¹ The majority of patients present with advanced disease and are not suitable for potentially curative surgery, resulting in an overall long-term survival rate of 0.4%.²

At the present time, surgery offers the only chance of cure, if the tumor is resectable. The outlook for these patients undergoing resection has improved in recent years due to the development of improved and standardized surgical techniques, advances in supportive care, and increased specialization with the setting up of high-throughput centers. Results from these specialist centers have demonstrated increasing resection rates of over 10%, associated with decreasing operative morbidity and mortality rates of less than 5%.^{3,4} Unfortunately, these encouraging trends have not translated directly into better survival times for patients. The median and long-term survival periods following resection remain poor compared with other cancers. Median survival periods range from 10 to 18 months, and 5-year survival rates range from 10% to 24%.^{5–8} Most patients are never cured, despite optimum treatment for pancreatic ductal adenocarcinoma.

Some efforts to improve patient survival rates have been directed at the type of surgical resection.^{9–15} Radical surgery has been suggested, particularly by Japanese groups, as a method of elim-

inating residual local disease. There have been many nonrandomized studies of radical pancreatoduodenectomy and lymph node clearance. Although many of these studies suggest good control of local disease, there is no convincing evidence of an increase in patient survival rates.¹⁶ Radical lymph node dissection allows more accurate staging of disease and this will affect the stage-by-stage survival figures when comparing different types of resection. The tumors will tend to be upstaged following radical lymph node dissection, so it is essential to examine overall group survival within the context of randomized studies.

To perform meaningful comparisons between different series of patients, it is essential that a universal staging system be used to counteract the effects of “stage migration,” which can be seen when comparing Western and Japanese data. Satake et al¹⁷ compared Japanese Pancreatic Society (JPS) and Union Internationale Contre le Cancer (UICC) systems of staging in a large cohort of patients. Stage for stage, the JPS system revealed a better rate of survival compared with the UICC system, but the overall 5-year survival rate of 11% was obviously the same.

Extended radical resection may improve local control, but the lack of gain in survival is, in part, related to the pattern of recurrence of pancreatic cancer^{18–22} (Table 22.1). The majority of recurrences are local and occur within 1 to 2 years of surgery. The liver is a major site of pancreatic cancer recurrence and its early appearance after resection suggests the presence of hepatic micrometastases at the time of surgery.^{23,24} Microscopic peritoneal disease also occurs early after surgery.

TABLE 22.1. Patterns of recurrence following surgery for pancreatic cancer.

Series	Year	Number of patients	Overall recurrence rate		Local		Hepatic		Distant		Peritoneal	
			<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Whittington et al ¹⁸	1991	26	23	88	22	85	6	23	2	8	16	62
Westerdahl et al ¹⁹	1993	74	74	100	64	86	68	92	—	—	—	—
Kayahara et al ²⁰	1993	45	30	67	25	56	18	40	13	29	12	27
Zerbi et al ²¹	1994	39	30	77	22	56	—	—	20	51	—	—
Sperti et al ²²	1997	95	78	82	56	59	48	51	5	5	8	8

The presence of microscopic residual retroperitoneal disease and features such as perineural invasion are factors that contribute to local recurrence. Improved preoperative imaging²⁵ and laparoscopy with laparoscopic ultrasound can identify tumors that are unresectable or the presence of metastatic disease. Peritoneal cytology has been shown to be positive in 58% of patients who may have unresectable tumors or have a limited postoperative survival period.²⁶

A multicenter prospective randomized trial has compared traditional pancreatoduodenectomy with a more extensive lymph node dissection.¹⁵ Eighty-one patients were randomized to a standard (*n* = 40) or extended (*n* = 41) lymphadenectomy and retroperitoneal soft-tissue clearance. The standard lymphadenectomy included removal of the anterior and posterior pancreatoduodenal, pyloric, biliary duct and superior and inferior pancreatic head and body lymph node stations.²⁷ In addition, the extended lymphadenectomy included removal of lymph nodes from the hepatic hilum; removal of lymph nodes from along the aorta from the diaphragmatic hiatus to the inferior mesenteric artery and laterally to both renal hila; and circumferential clearance of the origin of the celiac trunk and superior mesenteric artery.²⁸ There was no significant difference in morbidity and mortality between the two groups. There was no significant difference of overall survival between the two groups, although subgroup analysis revealed that patients who had lymph node positive disease demonstrated better survival rates following an extended resection.¹⁵ In the light of these findings, further large randomized studies would be needed to confirm any survival advantage for this type of procedure.

Kayahara et al²⁰ found in a study of 30 patients who had radical surgery that the local recurrence

rate in 15 patients examined postmortem was 80%; this finding has been confirmed by others. These findings indicate that radical resection alone does not guarantee a lack of local recurrence. At the present time, the pylorus-preserving pancreatoduodenectomy has been shown to produce similar long-term survival results to the more traditional Kausch-Whipple procedure.²⁹ Significant survival factors have been identified, such as tumor stage and grade, resection margin status and the use of blood transfusion during surgery.^{30–32} Although patients with stages I and II disease and negative resection margins tend to have better survival rates, these features do not ensure cure.^{12,33,34} Since radical surgery alone cannot guarantee much improved patient survival rates, there is a need for adjuvant or neoadjuvant therapy to attempt to increase long-term survival following pancreatic resection.

Rationale for Adjuvant Therapy

Radiotherapy

The main drawback to radiotherapy is the radiosensitivity of adjacent organs, thus limiting the effective dose deliverable to the target. External beam radiotherapy (EBRT) is routinely used with concomitant 5-FU as a radiosensitizer (chemoradiotherapy). Unlike chemotherapy, there has never been a randomized trial to compare chemoradiotherapy with a control arm in advanced pancreatic cancer. In retrospective series of studies, there have been median survival times of 10 to 15 months reported,^{35,36} although these were in highly selected groups of patients. The main benefit advocated for EBRT is greater local control. In advanced pancreatic cancer, this may translate into better palli-

ation for the patient (compared with best supportive care), but there is no convincing evidence of longer survival periods.^{16,37} In the adjuvant setting, there may be a reduction in locoregional recurrence, but this does not translate into improved survival times (vide infra).

Intraoperative radiotherapy (IORT) has been used to provide a higher dose and more specific targeting of radiation to the patient by providing more precise delivery and reduction in radiation damage to neighboring organs. In advanced pancreatic cancer, nonrandomized studies of IORT used alone demonstrate median survival times of only 6 months.^{38,39} Efforts have been made to combine IORT with EBRT, but two studies have not shown any significant survival differences between EBRT (+5-FU) compared with IORT plus EBRT (+5-FU).^{40,41} Although the combination of these approaches appeared to result in good local control, this did not translate into a survival advantage. Hepatic irradiation in patients with locally advanced disease has reduced the incidence of liver metastases but without improving survival times.⁴² Further experience with this approach, in combination with chemotherapy, has resulted in unacceptable levels of toxicity and premature study termination.⁴³

Combination Chemoradiotherapy and Follow-on Chemotherapy

The combination of chemoradiotherapy and follow-on chemotherapy might provide the combined benefits of good local control as well as systemic treatment. The Gastrointestinal Tumor Study Group (GITSG) randomized three groups of patients with unresectable pancreatic cancer to either 60-Gy EBRT (with radiosensitizing 5-FU) with or without follow-on 5-FU versus 40-Gy EBRT with

radiosensitizing 5-FU and follow-on 5-FU. The median survival times per group were 40, 23, and 42 weeks, respectively.⁴⁴ A more recent study of EBRT and infusional 5-FU has reported a median survival time of 12.7 months with good local, but not extraregional, control.⁴⁵ Similarly, Garton et al⁴¹ found that the combination of EBRT and IORT and follow-on 5-FU resulted in good local control, but this was not reflected in an increase in overall survival time. The disease recurrence in the peritoneum and liver was not affected by this regimen and indicates that extrapancreatic disease was probably present at the time of treatment.

Adjuvant Chemotherapy

There have been relatively few adjuvant chemotherapy trials in pancreatic cancer^{46–48} (Table 22.2). In the early 1980s, Splinter et al⁴⁶ examined the use of 5-FU, doxorubicin, and mitomycin C in 16 patients. The 3-year survival rate was 24%, and the locoregional recurrence rates were no higher than those of patients who received EBRT. Baumel et al⁴⁸ reported on 43 patients given postoperative chemotherapy, but the median survival time was only 12 months.

There has been one randomized controlled trial in Europe (Norway). Bakkevold et al⁴⁷ randomized 61 patients who had undergone pancreatoduodenectomy for pancreatic and ampullary cancers to receive either 5-fluorouracil, doxorubicin and mitomycin C (FAM regimen) for a total of six courses or no chemotherapy. There was a significant difference in the median survival rate of 23 months for the treatment arm compared with 11 months for the control arm. This difference did not extend to long-term survival rates, which were 4% and 8% for each group, respectively. Included in each

TABLE 22.2. Survival following surgery and adjuvant chemotherapy for pancreatic cancer.*

Series	Period	Number of patients	Regimen	Median survival, mo	Actuarial survival, %		
					1 year	3 years	5 years
Splinter et al ⁴⁶	1980–1984	16	5-FU/DOX/MMC	—	—	24	—
Bakkevold et al ^{47†}	1984–1987	30	5-FU/DOX/MMC	23	70	70	4
		31	—	11	45	30	8
Baumel et al ⁴⁸	1982–1988	43	Not specified	12	—	—	—

*5-FU indicates 5-fluorouracil; DOX, doxorubicin; MMC, mitomycin C.
†Randomized controlled trial.

group were 7 patients with ampullary carcinoma. Difficulties with this regimen included substantial toxicity: of the 30 patients randomized to receive chemotherapy, only 13 managed to complete the six courses. Moreover, at the first course, 16 of 22 patients needed hospitalization for toxic side effects.

The European Study Group for Pancreatic Cancer (ESPAC) commenced the ESPAC-3 trial on June 1, 2000, to address definitively the role of adjuvant chemotherapy. Two adjuvant chemotherapy regimens are being studied against a no-treatment control: (1) 5-FU + folinic acid for 24 weeks versus (2) gemcitabine for 24 weeks versus (3) observation. Eligible patients must have undergone a potentially curative resection (R0/R1) with histologically proven ductal adenocarcinoma of the pancreas. A total of 990 patients will be recruited (330 in each arm) within 4 to 5 years, and survival analysis will be completed after 2 years of follow-up for all patients. Group 1 will receive folinic acid as follows: 20-mg/m² intravenous bolus injection, followed by a 425-mg/m² 5-FU intravenous bolus injection (with oral cryotherapy) and given on 5 consecutive days every 28 days for 6 cycles. Group 2 will receive 1000 mg/m² of gemcitabine as an IV infusion over 30 minutes, once a week for 3 weeks of every 4 weeks (one cycle) for six cycles. The primary endpoint is the 2-year survival rate. The hypothesis to be tested is an improvement by adjuvant 5-FU + folinic acid or gemcitabine over ob-

servation from a 20% 2-year survival rate to a 30% survival rate. To detect these differences with 90% power and an $\alpha = 0.05$ level of significance, 330 patients are required for each arm (total, 990 patients). With 990 patients, the study will also be able to detect a 10% difference in survival benefit after 2 years of gemcitabine treatment compared with 5-FU + folinic acid treatment at the same level of significance and with a slight loss of power (ie, 80%). These calculations are made assuming that approximately 15% to 20% of patients randomized will have positive microscopic resection margins.

Adjuvant Chemoradiotherapy

Adjuvant EBRT and IORT have been used alone and in combination (Table 22.3).^{21,34,49–52,56} The rationale for their use is based on the good local control demonstrated in advanced disease and symptom relief. Although either EBRT or IORT seem to reduce the local recurrence rate by a similar amount, the majority of studies claim either a survival advantage for EBRT compared with IORT alone, or for EBRT plus IORT compared with IORT alone. Zerbi et al²¹ showed that the local recurrence rate was reduced by 50% in 43 patients treated by adjuvant IORT when compared with the 47 patients in the nonirradiated resection group, but, typically, survival was not significantly dif-

TABLE 22.3. Survival following surgery and radiotherapy for pancreatic cancer.*

Series	Year	Number of patients	EBRT, Gy	IORT, Gy	Median survival, mo	Actuarial survival, %		
						1 year	3 years	5 years
Willett et al ³⁴	1993	16 (nm)	40–50	—	21	—	—	29
		23 (pm)	40–50	—	11	—	—	0
Johnstone et al ⁴⁹	1993	26	45–55	20	18	—	—	—
Zerbi et al ²¹	1994	43	—	12.5–20	19	71	7	—
		47	—	—	12	49	10	—
Dobelbower et al ⁵⁰	1997	14	—	—	6.5	15	0	0
		6	—	10–20	9	50	35	33
		14	50–67	—	14.5	64	28	0
		10	27–54	10–25	18	70	10	0
Farrell et al ⁵¹	1997	14	60	12–15	16	62	22	15
Hishinuma et al ⁵²	1998	34	24 EBRT	13 EBRT + IORT	13	59	—	19
Klinkenbijl et al ^{56†}	1999	54 pdc	—	—	12.6	—	—	10
		60 pdc	40	—	17.1	—	—	20

*EBRT indicates external beam radiotherapy; IORT, intraoperative radiotherapy; nm, negative resection margin; pm, positive resection margin; and pdc, pancreatic ductal adenocarcinoma.

†Randomized, controlled clinical trial.

ferent between the two groups. Two rather small studies have claimed 3- and 5-year survival rates of 53% and 29%, respectively, using a combination of IORT and radical resection.^{53,54} Despite the improved targeting of IORT, there is a significant complication rate from vascular damage, septic complications, and gastrointestinal ulcers—14% in one study.⁵⁵ At the present time, there is little to support the use of adjuvant IORT, either alone or in combination with other forms of treatment.

A large multicenter randomized phase III trial organized by the European Organisation for Research and Treatment of Cancer has recently published its results.⁵⁶ Between 1987 and 1995, 218 patients were randomized to receive either chemoradiotherapy or observation only following potentially curative surgery for pancreatic adenocarcinoma or ampullary carcinoma. Treatment consisted of two courses of radiotherapy with 5-FU chemosensitization for a total dose of 40 Gy. There was no follow-on chemotherapy. Ninety-three patients out of 110 received 40 Gy doses. In each group, 42% to 46% of patients had periampullary carcinoma. The total number of patients in each group with proven pancreatic ductal adenocarcinoma was 54 and 55, respectively. The median survival time was 19 months in the observation group and 24.5 months in the treatment group, and the 2-

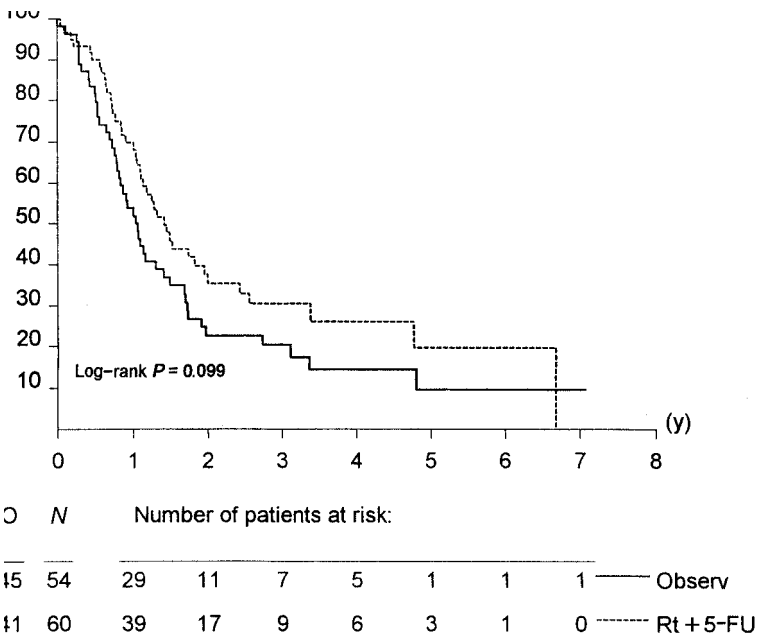
year survival rates were 41% and 51%, respectively (Figure 22.1). There was no overall significant difference between the two groups. The difference between survival of patients with pancreatic ductal adenocarcinoma in the two groups was not significant, with median survival times of 12.6 months in the observation group and 17.1 months in the treated group.

These results suggest that there is no survival advantage for adjuvant chemoradiotherapy in these patients. Unfortunately, the power of the study was rather weak and the difference in survival rates approached borderline significance ($P = 0.099$). That this is likely to be a true result, rather than a type II statistical error, is borne out by the results of the ESPAC-1 trial (vide infra). Moreover, it must be noted that, unlike the GITSG adjuvant trial,^{57,58} chemoradiotherapy was given without continuing with 5-FU on a weekly basis. Thus, the benefit that was observed in the GITSG study could have been due to the follow-up 5-FU chemotherapy.

Adjuvant Regional Therapy

The main objective of regional therapy is to deliver high doses of cytotoxic agent to the tumor site and avoid systemic side effects. The techniques in-

FIGURE 22.1. Duration of survival in patients who underwent resection for pancreatic cancer. The solid line indicates the survival curve for 54 patients with pancreatic ductal adenocarcinoma in the observation arm; the dotted line indicates the survival curve for 60 patients with pancreatic ductal adenocarcinoma undergoing adjuvant chemoradiation. Observ indicates observation; Rt indicates chemoradiotherapy; O indicates number of deaths. (From Klinkenbijl et al,⁵⁶ by permission of *Annals of Surgery*.)



volved are highly specialized and require considerable expertise. The first major adjuvant study was by Beger et al.⁵⁹ A regimen of 5-FU, folinic acid, mitoxantrone, and cisplatin was infused via the celiac artery in patients who had undergone pancreatoduodenectomy. In 20 patients (18 of whom had pancreatic ductal adenocarcinoma), the median survival time was 21 months versus 9.3 months in historical controls. The 2-year survival rate was 40%. Following use of this protocol, although disease recurrence occurred locally and in the peritoneum, the liver was hardly affected.

Although it is assumed that liver metastases gain most of their blood supply via the hepatic artery, Ishikawa et al⁶⁰ adopted a protocol of regional hepatic artery and portal vein infusion to control liver metastases. It was claimed that recurrent disease in the liver was significantly reduced and also that survival was significantly improved (3-year survival rate, 54%), but only compared with historical controls. Ozaki⁶¹ reported a 5-year survival rate of 32% in 24 patients who had resection and who were then further treated with IORT as well as regional chemotherapy.

These encouraging results provide the basis for the ESPAC-2 trial. This is a multicenter, prospective randomized controlled phase III trial to compare adjuvant postoperative intra-arterial chemotherapy and radiotherapy (Arm A) with surgery alone (Arm B) in resectable pancreatic ductal adenocarcinoma and advanced periampullary cancer. It is anticipated that 220 patients will be recruited (110 into each arm). Patients in Arm A will receive a total of six cycles of celiac artery infusion and a total of 54-Gy EBRT.⁶² It is anticipated that the study will be completed in 2007 and will provide

a definitive answer to the use of regional therapy for pancreatic cancer in the adjuvant setting.

Neoadjuvant Therapy

The majority of patients with pancreatic cancer present with advanced disease. This translates into resection rates that rarely rise above 15%. Preoperative therapy has been advocated in order to increase the resectability rates and improve survival. The use of preoperative therapy would reduce delays in starting adjuvant treatment postsurgery and might reduce the incidence of positive resection margins and early peritoneal spread.

The majority of neoadjuvant studies have originated from centers in the United States (Table 22.4). The original studies, which used radiotherapy regimens for the treatment of locally advanced disease, found that a rather small number of tumors previously irresectable could be resected following treatment. It was also claimed that there was a small improvement in survival rates. One study found that although the use of preoperative radiotherapy appeared to downstage some tumors and decrease the local recurrence rate, paradoxically, the early death rate was increased due to the rapid appearance of hepatic metastases.⁶³

More recently, there has been interest in chemoradiotherapy and multimodality neoadjuvant therapy. Hoffman et al⁶⁴ used a preoperative regimen of 50.4 Gy with 5-FU and mitomycin C to treat 53 patients with pancreatic ductal adenocarcinoma. Patients underwent resection 4 to 6 weeks after therapy. Twelve patients did not proceed to surgery due to local progression in 3 of these pa-

TABLE 22.4. Results of neoadjuvant therapy for pancreatic cancer.*

Series	Year	Number of patients	Regimen	Resection rate		Positive resection margin, n	Median survival, mo	Actuarial survival, %	
				n	%			3 years	5 years
Ishikawa et al ⁶³	1994	23	EBRT	17/23	74	—	—	—	22
Coia et al ⁶⁶	1994	27	EBRT + 5-FU + MMC	13/27	48	0/13	16	43	—
Staley et al ⁶⁷	1996	39	EBRT + 5-FU + IORT	39/39	100	7/39	19	—	19
Spitz et al ⁶⁸	1997	41	EBRT + 5-FU	41/91	51	5/41	19.2	—	—
Hoffman et al ⁶⁴	1998	53	EBRT + 5-FU + MMC	24/53	45	—	15.7	—	—

*EBRT indicates external beam radiotherapy; 5-FU, 5-fluorouracil; MMC, mitomycin C; and IORT, intraoperative radiotherapy.

tients and distant metastases in 6 patients. The median survival time was 15.7 months in the 24 patients who were able to undergo resection. The regimen was not without toxicity and there were two treatment-related deaths.

The M.D. Anderson group found that 17 (61%) of 28 patients could be resected following preoperative radiotherapy and infusional 5-FU.⁶⁵ Tumors larger than 5 cm, those obstructing the portal/superior mesenteric vein, or those encasing the superior mesenteric artery were less likely to be resected.^{66,67} A recent nonrandomized study of pre- and postoperative chemoradiation showed no significant differences between the two groups with respect to patterns of disease recurrence and median survival times: 19.2 months for the preoperative group and 22 months for the postoperative group.⁶⁸ The place of neoadjuvant therapy has yet to be established by randomized controlled trials and therefore cannot be endorsed as standard treatment.

Adjuvant Combination Therapy

The majority of combination studies have been based on the GITSG regimen, originally used in advanced pancreatic cancer⁴⁴ (Table 22.5)^{57,58,69–72} and adopted for a randomized adjuvant trial in 1973.^{57,58} Forty-three patients were randomized to receive either a 40-Gy dose combined with 5-FU or no adjuvant treatment. The median survival time in the treated group was 20 months compared with

11 months in the control group, and the 2-year survival rates were 42% and 15%, respectively.⁵⁷ Because there were so few cases, a further 30 patients were registered (not randomized) to the treatment arm, and the median survival time in this group was 18 months, with a 2-year survival rate of 46%.⁵⁸ Unfortunately, due to the small number of patients, the 95% confidence intervals of the survival curves were so large as to overlap with survival curves in patients receiving no additional treatment from other studies. Thus, no convincing conclusion could be derived from this study.

A number of nonrandomized studies have also claimed a survival benefit of adjuvant combination therapy, but all these studies are essentially biased due to patient selection. Yeo et al⁷⁰ compared three different treatment groups of patients who had undergone pancreatoduodenectomy and then received either (1) “standard therapy” with 40- to 40.5-Gy EBRT (+5-FU) to the pancreatic bed plus follow-on weekly bolus 5-FU for 4 months; (2) “intensive therapy” with 50.4- to 57.6-Gy EBRT (+5-FU) to the pancreatic bed, plus prophylactic hepatic irradiation with 23.4- to 27-Gy doses, plus protracted infusion with 5-FU and folinic acid for 5 of 7 days per week for 4 months; or (3) no further treatment. Ninety-nine patients received the “standard” regimen with a median survival time of 21 months and a 2-year survival rate of 44%. In the no-treatment group, the median survival time was 13.5 months and a 2-year survival rate of 30% (significantly worse than the standard treatment group; *P* = 0.002). Patients who received the more intensive

TABLE 22.5. Results of adjuvant combination therapy in patients who have undergone resection for pancreatic cancer.*

Series	Year	Number of patients	Radiotherapy, Gy	Chemotherapy	Median survival, mo	Actuarial survival, %			
						1 year	2 years	3 years	5 years
Kalser & Ellenberg ^{57†}	1985	21	EBRT 40	5-FU	20	67	42	24	18
		22	—	—	11	50	15	7	8
Douglass ⁵⁸	1987	30	EBRT 40	5-FU	18	—	46	—	—
Conlon et al ⁶⁹	1996	56	EBRT 45	5-FU	20	—	35	—	—
Yeo et al ⁷⁰	1997	53	—	—	13.5	—	30	—	—
		99	EBRT 40–45	5-FU	21	—	44	—	—
		21	EBRT 50–57	5-FU + FA	17.5	—	22	—	—
Neoptolemos ⁷¹	1998	35	EBRT 40	5-FU	13	56	38	29	15
Abrams et al ⁷²	1999	23	EBRT	5-FU + FA	15.9	—	—	—	—

*EBRT indicates external beam radiotherapy; 5-FU, 5-fluorouracil; FA, folinic acid.
†Randomized controlled trial.

treatment, however, had no significant survival rate (median survival time, 17.5 months and 2-year survival rate, 22%) difference from those in the no-treatment arm ($P = 0.252$). Performance status is recognized to be the single most important determinant of long-term survival,^{57,58} yet nothing was said about this in the study from Yeo et al and, significantly, was not factored into the multivariate survival analyses. It is also recognized that patients with better performance status are not only much more likely to live longer but also are more likely to elect further treatment. Indeed, Yeo and colleagues, in the Discussion to the published paper, recognize the retrospective nonrandomized nature of the study and that they are “biased” in their institution.⁷⁰

The UK Pancreatic Cancer Trials Group (UK-PACA) studied combined treatment in a total of 40 patients from nine institutions between 1987 and 1993.⁷¹ All patients underwent pancreatoduodenectomy; 34 had ductal adenocarcinoma and 6 had ampullary tumors. Radiation therapy consisted of a split course of 40 Gy separated by 2 weeks, and 5-FU was administered as a radiosensitizer, just as in the GITSG regimen.^{57,58} Two weeks following radiotherapy, 5-FU was given as a bolus once a week for a maximum of 24 weeks. No patient received the full course of chemotherapy, however, and a median of eight treatments were given. The median survival time for the 34 patients with pancreatic ductal adenocarcinoma was 13.2 months, and the 5-year survival rate was 15%. Patients with positive lymph nodes had a significantly shorter survival time compared with those without lymph node involvement (Figure 22.2A). A positive resection margin was associated with reduced survival only on multiple regression analysis, once lymph node status was taken into account (Figure 22.2B). In this study, no patient died or required hospitalization because of 5-FU toxicity. It was felt that the optimum length of follow-on treatment with 5-FU should not be beyond 6 months in the adjuvant setting.

The preliminary findings of such studies were crucial to the establishment of the ESPAC-1 trial in 1994.⁷³ This was a randomized controlled study that aimed to compare the effects of three adjuvant treatments with an untreated control group. The four groups in the study were as follows: (1) radiotherapy (with 5-FU as a chemosensitizer); (2) 5-

FU plus folinic acid (IV bolus 5-FU, 425 mg/m², days 1–5, and folinic acid, 20 mg/m², days 1–5, repeated monthly) for 6 months; (3) a combination of the two, with radiotherapy (with 5-FU as a chemosensitizer) plus follow-on 5-FU and folinic acid for 6 months; and (4) best supportive care. Radiation of 40-Gy EBRT was delivered in two doses, with 5-FU as a radiation sensitizer on the first 3 days of each 20-Gy split fraction. Randomization was stratified by resection margin involvement (negative or positive) and by randomization center (United Kingdom, Switzerland, Germany, and France). The trial needed to recruit 280 patients into the 2 × 2 factorial design, assuming 78% of these patients had negative resection margins and 22% had positive resection margins. This would allow detection of improvements in 2-year survival rates from 20% to 40% for those patients with negative resection margins and from 1% to 20% in patients with positive resection margins, with 90% power at the $\alpha = 0.05$ level of significance. The 2 × 2 factorial design would be able to answer two questions: (1) is there a role for chemoradiotherapy and (2) is there a role for chemotherapy? The trial design was expanded to provide a more pragmatic design to encompass centers unable to administer radiotherapy and to allow the use of adjuvant therapy while randomizing for chemotherapy. This allowed the choice of randomization between 2 × 2, no chemotherapy versus chemotherapy, and no chemoradiotherapy vs chemoradiotherapy designs. Consequently, the recruitment had to continue until 280 patients (140 in each arm) were recruited to answer the radiotherapy question and 280 patients (140 in each arm) were recruited to answer the chemotherapy question. The study commenced in February 1994 and had recruited over 560 patients by July 1999; it is thus the largest pancreatic cancer trial ever undertaken (Figure 22.3). Over 70 units participated from major centers in the United Kingdom and Ireland, France, Sweden (Lund), Spain (Barcelona), Italy (Verona), Germany (Ulm), Switzerland (Bern), Greece, Hungary, Belgium, and Austria. As anticipated, tumor grade, size, nodal status, and resection margin involvement were all associated significantly with survival. Five hundred and thirty patients with pancreatic ductal adenocarcinoma have been randomized. Randomization was stratified by resection margin status with 82% of patients having negative resection

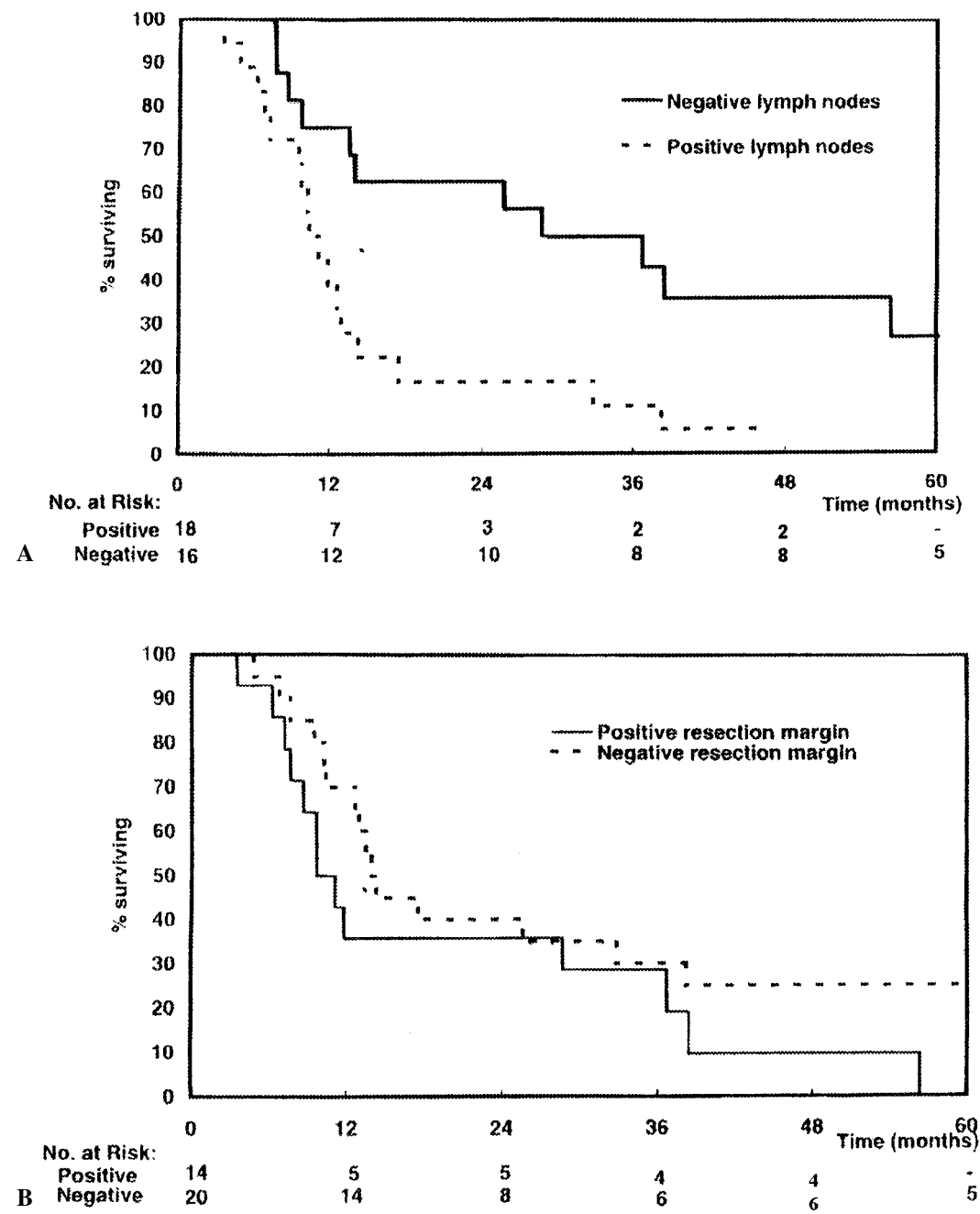


FIGURE 22.2. (A) Actuarial survival of patients with pancreatic ductal adenocarcinoma: 16 patients without lymph node involvement and 18 with lymph node involvement. (B) Actuarial survival of patients with pancreatic ductal adenocarcinoma: 20 patients with clear resection margins and 14 with involved resection margins. (from Neoptolemos,⁷¹ by permission of *GI Cancer*).

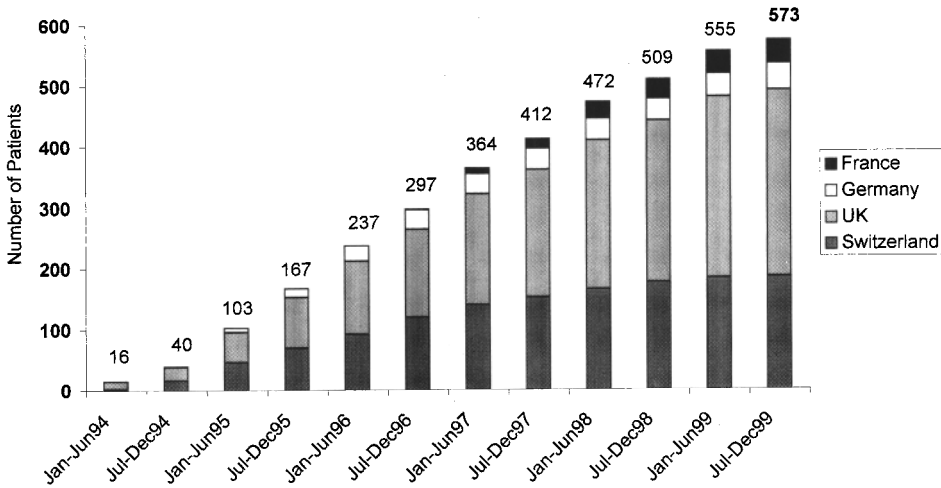


FIGURE 22.3. ESPAC-1 cumulative recruitment from February 1994 to December 1999.

margins. To date, 239 patients are alive with a median follow-up of 9 months. Preliminary results have shown no evidence of a survival benefit for chemoradiation treatment (median survival time of 14 months with chemoradiation vs 15.7 months without). There was some evidence of a survival benefit for patients having chemotherapy (median survival time of 19.5 months with chemotherapy vs 13.5 months without) and this effect was reduced when taking into account whether patients had received concomitant chemoradiation.⁷⁴ Thus, further randomization into the chemoradiotherapy option was stopped. The key findings of the study were as follows: chemoradiotherapy was of no benefit in the adjuvant setting; chemotherapy alone was suggestive of a significant survival benefit, even after all confounding factors were taken into account; chemoradiotherapy had a negative interactive effect on the chemotherapy benefit. In view of this, the degree of benefit from adjuvant chemotherapy was uncertain, which required the hypothesis to be reset around the question of chemotherapy alone without the confounding factor of chemoradiotherapy. The study will close with an anticipated 600 patients recruited immediately prior to commencement of the ESPAC-3 trial. The latter promises to be a major watershed in the investigation of adjuvant treatment in pancreatic cancer. There is considerable interest with major support from Holland, Norway, and Belgium, and also considerable interest from Australia and North America.

Conclusion

The results of large European randomized studies have been invaluable in determining a safe and standardized treatment protocol for patients with pancreatic cancer. It is clear that standard doses of postoperative adjuvant chemoradiotherapy have no survival advantage and may even be disadvantageous. Adjuvant chemotherapy, on the other hand, looks to be much more promising and warrants detailed evaluation. Further effective therapies can now be assessed within large cooperative organizations to improve the outlook, survival, expectancy, and quality of life for these patients. In this respect, ESPAC has been a major advance in clinical scientific investigation.

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Adjuvant Regional Infusion Therapy

A: Two-Channel Chemotherapy to Prevent Hepatic Metastasis After Extended Pancreatectomy for Adenocarcinoma of the Pancreas: The Osaka Experience

Osamu Ishikawa, Hiroaki Ohigashi, Terumasa Yamada, Yo Sasaki, Masao Kameyama, Masahiro Hiratsuka, Toshiyuki Kabuto, and Shingi Imaoka

Introduction

To date, surgical resection has been the only curative strategy available for the treatment of adenocarcinoma of the pancreas, but the long-term survival rate is still very poor. The 5-year survival rate was as low as 10% to 30% in most reports, and more than half of the patients died of locoregional recurrence, distant metastasis, or both within 2 years postoperatively. In 1976, Tepper et al¹ reported that locoregional recurrence was the most common cause of cancer death after pancreatectomy for this cancer. Therefore, to eradicate locoregional recurrence, an adjuvant chemoradiation (chemotherapy is used usually as a radiosensitizer)² and/or a wide range of lymphatic and connective tissue clearance (extended pancreatectomy)³ have been added during the last 2 decades. In our experience, an extended pancreatectomy has improved the 5-year survival rate from 8% to 24% by decreasing the incidence of locoregional recurrence. However, we failed to further improve the 5-year survival rate (23%), even by performing preoperative radiotherapy prior to extended pancreatectomy, because of an increased incidence of distant (hepatic) metastasis.⁴ In recent years, there has been an increasing number of reports of hepatic metastasis as the most common cause of cancer death in cases in which adjuvant radiation (locoregional) therapy had been performed in addition to

surgical resection.^{5–7} Thus, it is unlikely that we will obtain further improvement in patients' survival rate if we continue to devote ourselves to locoregional control alone without considering the prevention of hepatic metastasis. Although many chemotherapeutic regimens have been clinically tried to date, there is not as yet an effective method of systemic chemotherapy for this purpose.⁸ This may be because there were insufficient doses of anticancer drugs in the occult hepatic metastasis by systemic chemotherapy rather than a lack of drugs to which the pancreatic cancer cells are sensitive.

In 1985, Taylor et al⁹ reported that the incidence of hepatic metastasis was decreased when 5-FU (5-fluorouracil) was infused from the portal vein after curative resection of the colon cancer. We also found that hepatic metastasis was unlikely to develop during the period in which intra-arterial chemotherapy was performed, which was either weekly or biweekly for locally advanced and unresectable pancreatic cancer.¹⁰ It was subsequently confirmed that a high concentration of the anticancer drug methotrexate was repeatedly returned into the portal blood flow after the drug had been once delivered to the pancreas.¹¹ On the other hand, in Ackerman's¹² experimental study, the blood supply into the hepatic micrometastases was attributable more to the arterial blood flow than the portal blood flow as it grew in the hepatic parenchymal tissues. Likewise, Geoghegan and

Scheele¹³ suggested that hepatic artery chemotherapy was more effective than portal vein chemotherapy in the treatment of colorectal liver metastases that had already formed a macroscopically obvious tumor mass. However, in comparison with either hepatic artery chemotherapy or portal vein chemotherapy alone, we postulated that regional chemotherapy via both of these two routes would be more effective in eradicating possible micrometastases in the liver. Since we began to apply regional chemotherapy via these two routes (two-channel chemotherapy)¹⁴ to patients who had undergone extended pancreatectomy, we have successfully improved the long-term outcome. Here, we describe the surgical technique, adverse effects, and clinical benefits of our method of two-channel chemotherapy.

Techniques

A 1.5-cm length of the gastroduodenal artery from the branching point of the common hepatic artery was left intact during pancreatoduodenectomy. Af-

ter completion of the reconstruction procedures, the branching point of the common hepatic artery was clamped with a small bulldog clamp. A small incision was made on the anterior wall of the gastroduodenal artery close to the cut end. The anterior wall around this incision was gently elevated with a vein pick and a catheter (BardPort; Bard Co Ltd, Salt Lake City, Utah) was inserted retrogradely into the gastroduodenal artery. Care was taken not to advance the tip of this catheter into the common hepatic artery to prevent thrombus formation. To prevent dislocation of the catheter, which would cause massive arterial bleeding, two ligations were made on the gastroduodenal artery and one node on the catheter was placed between the two ligations. The gastroduodenal artery and the hepatic artery together with the catheter were coated with adhesive (Biobond; Yoshitomi Pharmaceutical Co Ltd, Japan). The other end of the catheter was connected to the port (reservoir), which was placed in the subcutaneous layer of the anterior abdominal wall. When intra-arterial infusion was initiated, the port was percutaneously punctured with a thin nee-

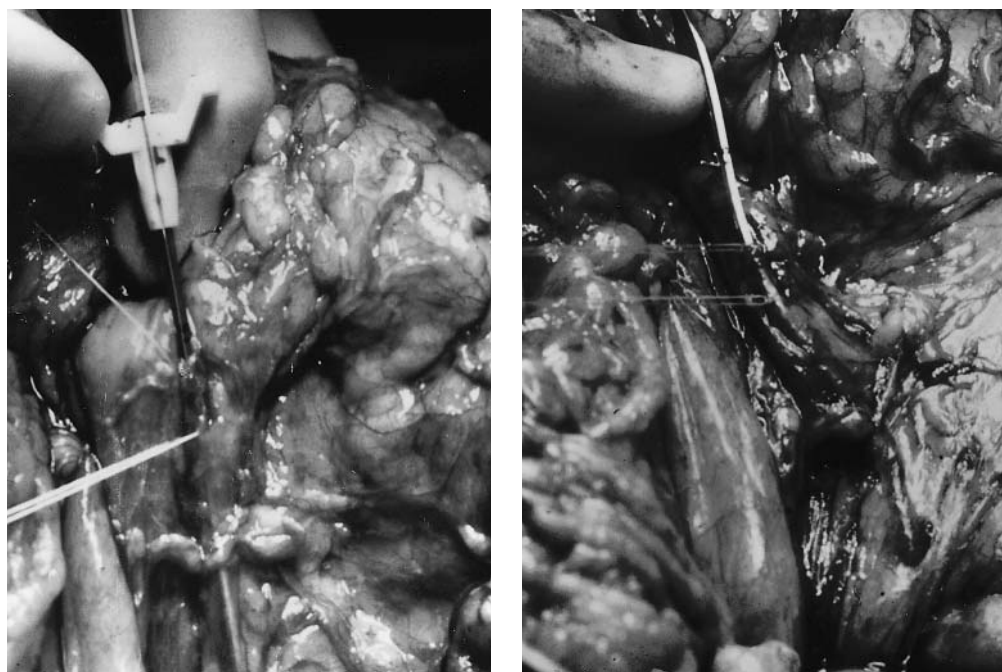


FIGURE 23.1. Catheterization into one of the branches of the superior mesenteric vein. One of the branches of the superior mesenteric vein was punctured using a metallic needle with Teflon sheath. When the metallic needle reached the lumen, the Teflon shield alone was advanced forward. After withdrawal of the metallic needle, another catheter was advanced into the Teflon shield. When this catheter arrived in the first or second branch of the superior mesenteric vein, the shield was withdrawn and discarded.

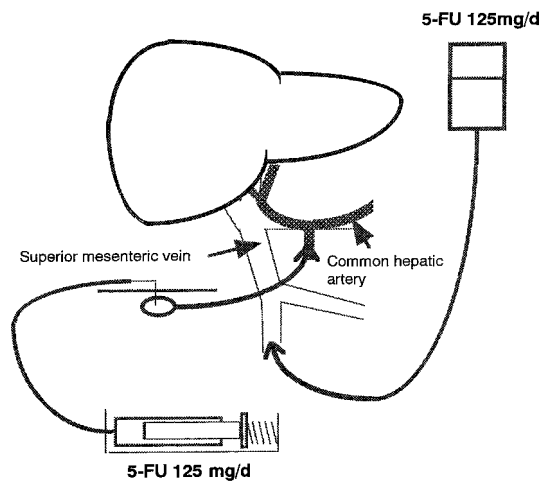


FIGURE 23.2. Schema of two-channel chemotherapy. A mixture of 5-FU (125 mg/d), heparin, and physiologic saline was infused continuously for more than 28 days via each of the two routes.

dle (Gripper; Deltec Co Ltd, St. Paul, Minn). For portal vein chemotherapy, one of the second or third branches of the superior mesenteric vein was selected. Using the “cut-down” technique, a catheter (Medicut UK-II catheter kit; Unitika Co Ltd, Japan) was inserted retrogradely into this vein (Figure 23.1). The tip of this catheter was advanced to the first or second branch of the superior mesenteric vein, and the other end was drawn out through the anterior abdominal wall.

Immediately after the operation, physiological saline containing both 125 mg/day of 5-FU and 2500 units/day of heparin was infused via each of the two routes using an infusion pump with a pressure-alarm system (Figure 23.2). Infusion was continued for 28 to 35 postoperative days (total dose of 5-FU administered by both routes was 7–8.75 g). After the period of continuous infusion, these two catheters were not used again for intermittent infusion.

Results

During the period of 1981–1998, pancreatectomies with a wide range of lymphatic and connective tissue clearance (extended pancreatectomy)³ were performed for 113 patients with adenocarcinoma of the pancreas at Osaka Medical Center for Cancer and Cardiovascular Diseases. The pancreatectomies did not include those for (1) low-grade malignant tu-

mors, such as intraductal papillary mucinous carcinoma, cystadenocarcinoma, or in situ carcinoma; (2) patients who had received preoperative treatment (irradiation or chemotherapy) prior to surgery; or (3) cases of macroscopically noncurative resection in which obvious residual cancer was left behind.

Excluding the 4 patients who died of postoperative complications, the remaining 109 patients tolerated the surgery well. Thirty-two patients received two-channel chemotherapy, while the remaining 77 patients did not (control). There were no significant differences in background factors (size of the tumor, location, status of nodal involvement, or invasion beyond the confines of the pancreas) between these two groups.

During two-channel chemotherapy, none of the patients developed leukocytopenia, thrombocytopenia, bleeding tendency, or liver dysfunction, and all of the patients tolerated the therapy well. The cumulative survival rates of the group treated with extended pancreatectomy alone and the group treated with extended pancreatectomy plus two-channel chemotherapy are compared in Figure 23.3. The 5-year survival rate was significantly different between the two groups (ie, 24% vs 40%, respectively). The cumulative rates of both local recurrence and hepatic metastasis are compared in Figure 23.4. Although the incidence of locoregional recurrence did not differ significantly between the

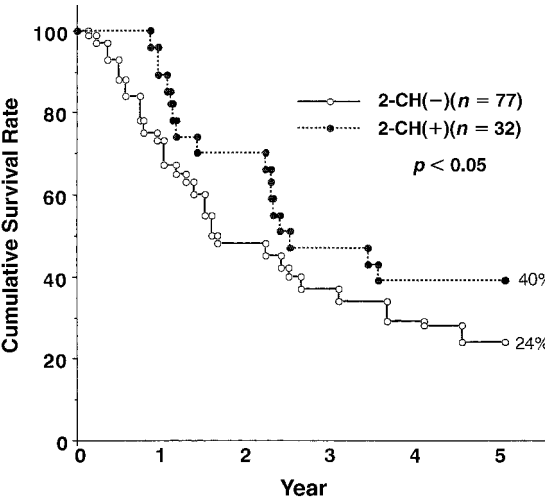


FIGURE 23.3. Comparison of cumulative survival rates after extended pancreatectomy. Two-channel chemotherapy significantly improved the 5-year survival rate from 24% to 40%.

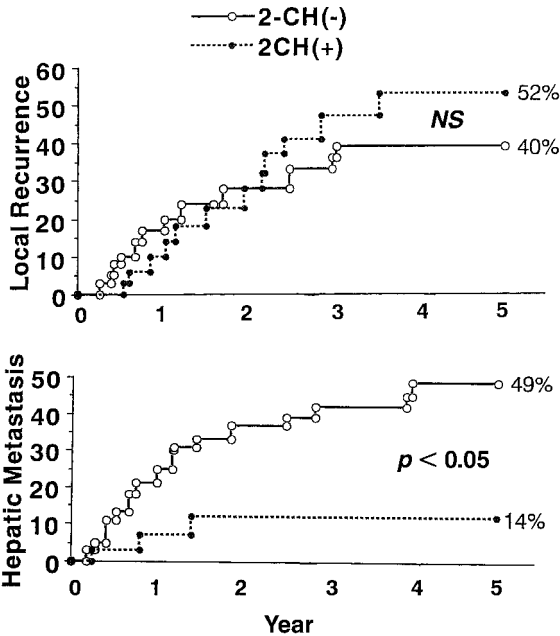


FIGURE 23.4. The cumulative rates of hepatic metastasis and local recurrence. 2-CH(–): two-channel chemotherapy was not performed ($n = 77$). 2-CH(+): two-channel chemotherapy was performed ($n = 32$). The cumulative rate of local recurrence did not differ between 2-CH(–) and 2-CH(+) groups (upper figure). The cumulative rate of hepatic metastasis in the 2-CH(+) group was significantly lower than that in the 2-CH(–) group (lower figure).

two groups, the incidence of hepatic metastasis was 49% versus 14%, respectively, and this difference was statistically significant.

Table 23.1 compares the 3- and 5-year survival rates for the subgroups, which were classified according to the size of the tumor, status of nodal in-

volvement, and the T factor in the Union Internationale Contre le Cancer, or UICC, classification¹⁵ (degree of direct invasion). Although the difference of survival rates between the two groups was not significant for tumors measuring 4.0 cm or less in diameter, it was statistically significant for tumors measuring 4.1 cm or more in diameter. The difference was small when the comparison was made for the node-positive subgroup, although greater when it was made for the node-positive subgroup ($P = .06$). Also, the difference was not large when the comparison was made with T1–3 cancers, although it became statistically significant when compared with T4 cancers.

Concentration of 5-FU in the Systemic Circulation and Intrahepatic Portal Vein

During chemotherapy, the concentration of 5-FU was determined by the bioassay method. It was always 0.01 to 0.02 $\mu\text{g/mL}$ in the systemic circulation. The 5-FU concentration was determined for the patients in whom another catheter had been placed into the intrahepatic portal vein by opening the umbilical vein. The 5-FU concentration showed wide variations, but it was greater than 0.14 $\mu\text{g/mL}$.

Comments

Anticancer drugs are more or less toxic for the vascular endothelium and its outer layers.¹⁶ Particularly when performing regional chemotherapy, the vascular lumen against which the tip of the catheter is placed would be exposed to the highest concentration of the drug. In addition, in our extended pancreatectomy procedure, the peripancreatic vessels

TABLE 23.1. Comparison of 3- and 5-year survival rates between 2CH(–) and 2CH(+) groups*

	Size of tumor (cm)			Nodal involvement		T factor (UICC)		
	<2.0	2.1–4.0	>4.1	Negative	Positive	T1 + 2	T3	T4
2CH(–)	59/53† ($n = 21$)	29/9 ($n = 39$)	22/14 ($n = 17$)	58/48 ($n = 26$)	25/12 ($n = 51$)	64/64 ($n = 17$)	32/10 ($n = 15$)	26/11 ($n = 45$)
2CH(+)	66/6 ($n = 6$)	32/19 ($n = 22$)	100/75 ($n = 4$)	50/50 ($n = 12$)	45/22 ($n = 20$)	66/66 ($n = 7$)	31/31 ($n = 9$)	43/21 ($n = 16$)
	NS	NS	$P < 0.05$	NS	$P = 0.06$	NS	NS	$P < 0.05$

*2CH(–) indicates two-channel chemotherapy was not performed; and 2CH(+), two-channel chemotherapy was performed; UICC, Union Internationale Contre le Cancer; and NS, not significant. Difference of patients' survival rates between the 2CH(+) group and the 2CH(–) group became greater, particularly when more advanced cancers were compared.

†3-y survival rate (%) / 5-y survival rate (%).

are skeletonized. Thus, before clinical use, we had paid a great deal of attention toward the possible risk of thrombus formation, aneurysm and rupture around the gastroduodenal artery, the hepatic artery, and some branches of the superior mesenteric vein. For this purpose, the anticancer drug 5-FU was selected because it is less toxic against the vascular endothelium and its outer layers than are mitomycin C, doxorubicin, and some other cytotoxic drugs.¹⁶ 5-FU was used for intraperitoneal chemotherapy, with no development of special complications. Likewise, the vessels into which the catheter had been placed were wrapped with adhesive, and heparin was also continuously infused together with 5-FU to prevent thrombus formation. As a result of these precautions, we have been able to perform regional infusion safely without any complications associated with placement of the catheter. Because our two-channel chemotherapy required neither skillful operative technique for the catheterization procedure nor special equipment for the postoperative management, this method can be performed easily in any institution.

In contrast to many other cytotoxic drugs, the anticancer effect of 5-FU is time-dependent. Shimoyama and Kimura¹⁷ reported that L-1210 cells, a mouse hepatoma cell line, were killed completely after culture in a medium containing more than 0.1 $\mu\text{g/mL}$ of 5-FU for 11 days. In our previous study,¹⁸ the number of PSN-1 cells (a cell line derived from a human hepatic metastatic lesion from the pancreatic cancer) cultured in the presence of 0.1 $\mu\text{g/mL}$ of 5-FU increased during the first 2 weeks of culture but decreased from 2–3 weeks. Our two-channel chemotherapy method maintained the concentration of 5-FU at 0.14 $\mu\text{g/mL}$ or more in the portal vein. Although it is impossible to determine the concentration of 5-FU in the hepatic artery, it was estimated as 0.33 $\mu\text{g/mL}$ or more, because the same dose of 5-FU was infused into each portal vein and the hepatic artery and the ratio of blood flow in the hepatic artery to that in the portal vein was 3 to 7 (the ratio of the 5-FU dilution was therefore 7:3). Thus, we postulated that a sufficient dose of 5-FU might have arrived at the occult hepatic metastases from each of the two routes. It seems to be almost impossible for systemic chemotherapy to maintain the 5-FU concentration at a level of more than 0.1 $\mu\text{g/mL}$ in the hepatic inflow for 28 days without developing any adverse effects.

All of our patients had undergone extended pancreatectomy; in such cases, hepatic metastasis has been shown to be the most common cause of cancer death. Our method of two-channel chemotherapy improved the 5-year survival rate from 24% to 40% by decreasing the incidence of hepatic metastasis. If we had performed two-channel chemotherapy in patients who had undergone nonextended (conventional) pancreatectomy alone, the prognostic benefit might have been canceled by the conventional pancreatectomy's higher incidence of locoregional recurrence.³ Recently, there has been an increase in the use of adjuvant radiation therapy of the pancreatic bed before or after nonextended pancreatectomy.^{2,5–7,19} Consequently, the main cause of cancer death was reported to have shifted from local recurrence to hepatic metastasis.^{5–7,20} Therefore, our method of two-channel chemotherapy seems to be recommended not only for patients treated by extended pancreatectomy alone, but also for those treated by conventional pancreatectomy plus adjuvant radiation therapy. In addition to pancreatic cancer, this chemotherapeutic method may also be applicable to other types of cancers, if associated with a high risk of liver metastasis.

It is generally accepted that pancreatectomy should not be performed in patients with either nodal involvement or direct invasion beyond the confines of the pancreas because of the limited survival rate.²⁰ However, as shown in Table 23.1, 3- and 5-year survival rates were improved by the combination of extended pancreatectomy and two-channel chemotherapy, even for locally advanced pancreatic cancer (eg, tumors measuring more than 4 cm in diameter, node-positive cases, and T4 cancers). Beger²¹ also developed a similar type of regional chemotherapy and successfully improved the 3-year survival rate from 5% to 30% for patients with nodal involvement. Thus, using well-balanced multidisciplinary treatment methods, the indication of pancreatectomy, even for locally advanced pancreatic cancer, will be gradually expanded, and our method of two-channel chemotherapy will play an important role.

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B: Adjuvant Intra-arterial Chemotherapy With and Without Radiation Therapy in Pancreatic Cancer: The Ulm Experience

Frank Gansauge, Karl H. Link, Andrea Formentini, Miriam Schatz, Erwin Röttinger, Johannes Görich, and Hans G. Beger

Introduction

The extremely poor prognosis of pancreatic cancer is still a great challenge for all clinicians. The overall 5-year survival rate is extremely low, ranging between 1% and 2%. To date, resection of the tumor at an early stage still offers the only chance for cure. However, even in patients who underwent resection of the primary tumor, median survival times are seldomly higher than 12 to 18 months, depending on the selection criteria for the patients investigated.¹⁻³ The overall 5-year survival rates following R0 resection range between 11% and 28%,³⁻⁵ and, in Union Internationale Contre le Cancer (UICC) stage I carcinomas with a tumor size less than 2 cm, the 5-year survival rate was 38%.⁶ In pancreatic cancer, the sites of recurrence following resection are mainly the liver (62%) and the pancreatic bed (73%).⁷ Postoperative radiochemotherapy or chemotherapy has improved the median survival times from 11 to 20 months^{5,8-10} or 11 to 23 months in curatively resected patients.¹¹ The disadvantages of radiochemotherapy are its limited effect on extraregional tumor progression and local toxicity, whereas systemic chemotherapy like the 5-fluorouracil (5-FU), adriamycin, mitomycin protocol is associated with considerable side effects.¹¹ Recently, Yeo and coworkers⁵ showed that radiochemotherapy according to a modified Gastrointestinal Tumor Study Group (GITSG) protocol increased median survival times, whereas radiochemotherapy according to an intensive protocol including prophylactic hepatic irradiation and long-term treatment with 5-FU had no effects with regard to median survival times.

Since celiac artery infusion (CAI) based on mitoxantrone, 5-FU, folinic acid, and cisplatin had beneficial effects in regard to increased median survival time and pain reduction in patients with unresectable pancreatic cancers,¹² we conducted a series of studies based on that treatment regimen in patients who underwent curative pancreaticoduodenectomy for pancreatic cancer. To evaluate the clinical benefit of additional radiation therapy (RT), we compared these patients with a second group who underwent additional external beam radiation therapy. The first treatment began 2 to 4 weeks after surgery.

Patients and Methods

Adjuvant CAI

For CAI, catheters were placed using Seldinger's technique with the tip into the celiac axis via the femoral artery and left in place for 5 consecutive days. The exact position was controlled on the 2nd and 5th day. Heparin (20,000 IU/day) was continuously given via the catheter, except during infusion of the drugs. One cycle consisted of mitoxantrone, 10 mg/m², and folinic acid, 170 mg/m² for 10 minutes (Day 1), followed by 5-FU, 600 mg/m² for 120 minutes (Days 2-4) and cisplatin, 60 mg/m², at Day 5.

Toxicity was evaluated, and each cycle was graded according to World Health Organization (WHO) criteria. After the third and sixth cycles, an upper abdomen computed tomography (CT) scan and a chest X-ray were performed to evaluate the remission status.

CAI-Group

From December 1992 to December 1997 we treated a total of 19 patients consecutively with CAI following curative resection (R0) for pancreatic cancer. The mean age was 60.2 years, ranging from 39 to 75 years. All patients had a pancreatic head carcinoma, and, in all cases, diagnosis was confirmed by histological examination. One patient had a UICC stage I pancreatic carcinoma, 2 patients had a locally advanced pancreatic head carcinoma with infiltration of the surrounding tissue (UICC stage II), and 16 patients had pancreatic carcinomas that had already spread into the regional lymph nodes (UICC stage III). In 3 patients, a partial duodenopancreatectomy was performed; 1 patient underwent a pancreatectomy; and, in 15 patients, a pylorus-preserving partial duodenopancreatectomy was performed. The mean number of cycles performed per patient was 5.8 (range, 2–15).

CAI + RT Group

From April 1997 to January 2000, we treated consecutively a total of 24 patients with CAI + RT following curative resection (R0) for pancreatic cancer. The mean age was 60 years, ranging from 35 to 78 years. Twenty-one patients had pancreatic head carcinoma, 3 patients had a carcinoma of the tail of the pancreas, and, in all patients, the diagnosis was confirmed by histological examination. Six patients were classified as UICC stage I, 3 patients were classified as UICC stage II, and 15 patients as UICC stage III. The following operations were performed: One partial duodenopancreatectomy, one pancreatectomy, three left resections, and 19 pylorus-preserving duodenopancreatectomies. The mean number of cycles performed was 4.45, ranging from one to six cycles. RT was performed between the first and second cycle of regional chemotherapy in a total dose of 54 Gy (2 Gy/day).

Results

Toxicity

CAI Group

No severe local side effects at the catheter insertion site occurred. During the treatment cycles, no severe systemic or abdominal complications were

observed. WHO I systemic side effects were seen in 28% of the cycles; WHO II side effects in 19%; and WHO III side effects in 8% of the cycles (mainly gastrointestinal ulcerations), whereas no WHO IV toxic events were observed (Table 23.2).

CAI + RT Group

Actually, 12 of 24 patients finished the sixth cycle. In 1 of 24 patients, occlusion of the celiac trunc made a locoregional infusion impossible. Four of 24 patients withdrew voluntarily prior to the end of the sixth cycle. Three of 24 patients died prior to the sixth cycle. WHO I systemic side effects were seen in 72% of the cycles, WHO II side effects in 42%, WHO III side effects in 5.6%, and WHO IV side effects in 3.6% of the cycles. One patient died due to thrombopenia-related hemorrhage (Table 23.2).

Relapse-free Survival and Relapse Pattern

CAI Group

After the sixth cycle, the restaging examinations revealed that 17 of 19 patients were disease free. One patient had died before the sixth cycle due to local relapse. One patient finished treatment prior to the sixth cycle. One patient has been disease free for more than 6 years. The remaining 18 patients died due to recurrence of the disease. Sixteen patients developed local relapses, 3 of these patients also developed liver metastases, and 2 also developed peritoneal metastases. Only in 2 patients was the liver the primary site of tumor recurrence (Table 23.3).

CAI + RT Group

After the sixth cycle, restaging revealed that 11 of 12 patients were disease free. One of 12 patients

TABLE 23.2. Side effects according to WHO criteria in CAI and CAI + RT groups.*

	CAI	CAI + RT
WHO I	28%	72%
WHO II	19%	42%
WHO III	8%	5.60%
WHO IV	0%	3.60%
Treatment-related deaths	0%	4%

*WHO indicates World Health Organization; CAI, celiac artery infusion; and RT, radiation therapy.

TABLE 23.3. Recurrence pattern and survival rates in CAI and CAI + RT groups.*

	CAI	CAI + RT
Local recurrence	84%	41%
Liver metastases	28%	47%
Peritoneal metastases	11%	29%
Pulmonary metastases	0%	12%
1-y survival rate	94%	78%
2-y survival rate	58%	34%

*CAI indicates celiac artery infusion; and RT, radiation therapy.

had developed local recurrence, 3 patients had died prior to the sixth cycle, and 4 patients withdrew from the study. During a median observation period of 17.3 months, 7 patients have been disease free, 7 of 17 patients (41%) developed local recurrence, and 4 of these patients also developed liver metastases; in 4 of 17 patients, the primary site of tumor recurrence was the liver, 5 patients developed peritoneal metastases, and 2 patients developed pulmonary metastases (Table 23.3).

Survival

CAI Group

In a Kaplan-Meier regression analysis of the 19 patients who received CAI following pancreatic head resection, the median survival time is 26.5 months (Figure 23.5A).

CAI + RT Group

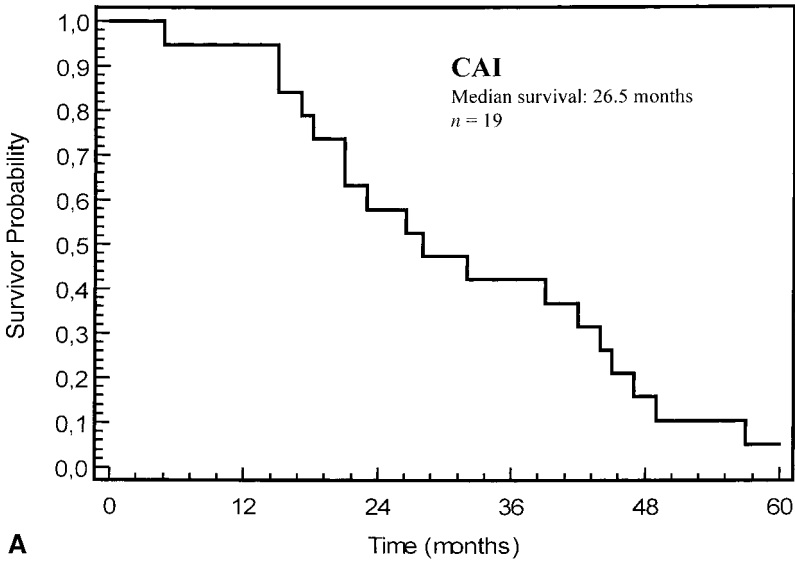
In a Kaplan-Meier regression analysis of the 24 patients who received CAI + RT, the median survival time is 20.4 months. 15 patients died, and 9 patients are alive, with an observation period between 9.6 and 30.8 months (Figure 23.5B).

Discussion

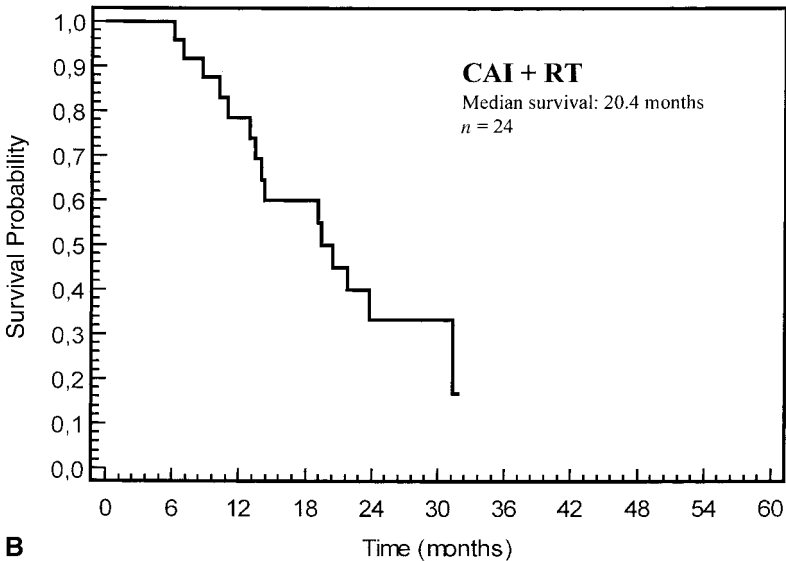
Systemic chemotherapy has not been accepted as a standard adjuvant procedure in patients who have had pancreatic resection for surgical treatment of pancreatic cancer.¹³ Postoperative radiochemotherapy based on a 5-FU chemotherapy was first introduced by GITSG, and recent studies have confirmed the beneficial effects of this treatment.⁵ However, confirmation of this treatment protocol

by phase III studies is needed before radiochemotherapy can be recommended as standard adjuvant therapy. These studies (the European Study Group for Pancreatic Cancer [ESPAC] and the Dutch multicenter trial) are currently under investigation. The potential positive effect of radiochemotherapy can contribute to better local disease control, which might be improved by increasing radiotherapeutic doses.^{10,14} However, increasing radiotherapeutic doses may also be associated with an increase in side effects.

Because our treatment protocol by CAI in the palliative situation in patients with unresectable pancreatic cancer led to an increase in median survival times,¹² we also used this treatment protocol in the adjuvant situation. In comparison to a historical control, we were able to show a dramatic increase in median survival times in patients receiving CAI.¹⁵ Although this treatment is inconvenient, as the patient is immobilized during the treatment for 5 consecutive days, the compliance was extremely high, which was probably due to the very low toxicity rates and the pain reduction as shown by reduced consumption of analgesics.¹⁶ The sites of recurrence in the CAI as well as in the CAI + RT patients were different to that normally observed in patients after resection for pancreatic cancer. Out of the 18 patients in the CAI group who developed recurrence of the tumor, only in 3 patients was the liver the primary site of recurrence (17%), whereas, in recently published studies, the rates of liver metastazation were much higher.^{7,15} These data imply that CAI provides hepatic protection, whereas local control of CAI remains questionable. With the idea that the control of local recurrence can be improved by additional radiation of the pancreatic bed, we treated the second group of patients with CAI + RT. Our relapse rate for local recurrence was 41%, which is not as low as presented in other studies using irradiation,¹⁴ but lower than in the group of patients who only received CAI. However, the median survival in the CAI + RT group was lower than in the CAI group. In the CAI + RT group, 12 patients died within 21 months after starting the therapy, whereas in the CAI group, median survival is 26.5 months. In the CAI + RT group, 9 patients are still alive, and 7 of these 9 patients are disease free. Due to the different observation periods in CAI and CAI + RT groups, comparison of long-term survival rates is not possible.



A



B

FIGURE 23.5. (A) Kaplan-Meier regression analysis of resected pancreatic cancer patients who received celiac artery infusion (CAI). (B) Kaplan-Meier regression analysis of the curatively resected patients who received CAI + radiation therapy (RT).

The most convincing result of CAI is the dramatic increase in median survival times. In patients treated with CAI in the adjuvant situation following pancreatic head resection for pancreatic cancer, the median survival time according to the Kaplan-Meier regression analysis is increased in comparison with other studies. Similar to our results, Ishikawa and coworkers¹⁷ also observed an increase in patients' survival when they treated patients with postoperative hepatic artery and portal

vein infusion. In Section C of this chapter, Lygidakis and colleagues¹⁸ report of the beneficial treatment of locoregional chemotherapy in combination with local interleukin (IL)2-based immunotherapy. Although in CAI + RT, the local relapse rates are lower than in the CAI group, the additional RT does not seem to improve survival.

In conclusion, this study provides strong evidence that adjuvant CAI in patients with pancreatic cancer prolongs survival with very low toxic-

ity rates. At this point, a significant benefit of additional RT, in view of lower median survival times in CAI and CAI + RT therapy groups, cannot be shown.

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C: Locoregional Targeting Immunochemotherapy for Resectable and Unresectable Pancreatic Head Carcinoma: The Athens Experience

Nikolaos J. Lygidakis, Lobros Vlachos, Sotirios Raptis, George Rassidakis, and Christos Kittas

Introduction

Pancreatic head carcinoma remains an issue of controversy and conflict with regard to management, prognosis, and outcome.¹⁻⁴ The majority of patients (about 90%) at the time of diagnosis are considered not eligible for surgical resection, thus being condemned to die within an interval of 6 to 8 months.^{5,6} Furthermore, surgical resection, although carried out with a low early mortality and morbidity rate, is marred by the fact that long-term survival is limited because of a high incidence of locoregional recurrence and of secondary metastatic liver involvement.⁷

Since 1991, in an effort to reverse this situation, we developed an approach of locoregional application of combined-targeting immunochemotherapy for patients with both resectable and unresectable pancreatic head carcinoma. We used a variety of modalities. The present study represents the relevant results from a number of patients who had a standard management by use of a standard therapeutical modality from November 1994 to December 1999.

Material and Methods

In the present study, 227 patients underwent a standard treatment as an adjuvant regional infusion therapy from November 1994 to December 1999. They are divided into two groups. Group A ($n = 97$) had a standard type of pancreatic resection followed by adjuvant locoregional targeting immunochemotherapy, and group B ($n = 130$) had exploratory laparot-

omy with ($n = 37$) or without ($n = 93$) surgical palliative drainage (biliary or gastric) procedures, histological confirmation of diagnosis, and locoregional targeting immunochemotherapy.

At admission, all had standard screening tests, including blood tests, chest X-ray, upper abdominal computed tomography and magnetic angiography (during the last 3 years) to delineate the regional vascular status of each individual patient, as well as anatomical peculiarities. Table 23.4 gives the clinical characteristics of both groups.

Regional application of immunochemotherapy was carried out via two 4.2 Fr polyurethane arterial catheters introduced via a side arterial and venous branch of a jejunal loop 15 to 20 cm distal from the ligament of Treitz into the superior mesenteric artery (SMA) and into the portal vein under fluoroscopic control during the initial surgery (Figures 23.6, 23.7). Each catheter was connected to a corresponding implantable infusion port, which was positioned in a subcutaneous pocket in the anterior abdominal wall.

Adjuvant immunochemotherapy was carried out for both groups 20 days following initial surgery. For group A ($n = 97$), a 5-day course of chemotherapy followed by a 10-day course of immunotherapy was carried out on an outpatient basis. This was repeated for the first year every 2 months, for the second and third years every 4 months, and for the fourth and fifth years every 6 months. Table 23.5 gives the chemotherapy regimen.

Exactly the same procedure was followed for the patients with unresectable tumors. The only difference involved the frequency of treatment, which for the first year was every 2 months and for the

TABLE 23.4. Clinical characteristics of groups A and B

Group A (<i>n</i> = 97) Patients Who Underwent Resection for Pancreatic Head Carcinoma		
Age	23–82 y	
Mean age	62 ± 9 y	
M:F* ratio	2.46:1	
Stage I	6	
Stage II	50	
Stage III	41	
Group B (<i>n</i> = 130) Patients Who Underwent Locoregional Targeting		
Age	22–83 y	
Mean age	63 ± 8 y	
M:F* ratio	1.20:1	
Stage I	0	
Stage II	0	
Stage III	17	
Stage IV	113	

*M indicates male; F indicates female.

second and third years, every 3 months (Table 23.6). Blood screening tests were carried out before each treatment cycle. Upper abdominal computed tomography, chest X-rays, and serum CA 19-9 levels were evaluated every 3 months for the first and second years and every 6 months for the subsequent years.

Results

Treatment-related toxicity was minimal and included fever and chills in all patients, which was easily controlled via paracetamole administration. Incidence of treatment-related sequelae was similar in both groups. In group A, survival time ranged from 3 to 61 months (mean, 38 months (Table 23.7)). Incidence of locoregional recurrence was 14.4%, and incidence of liver secondaries was 10.3%. A total of 45.4% of group A patients developed peritoneal spread of disease. In group B (*n* = 130), survival time ranged from 4 to 37 months (mean, 18 months). From the total of 130 patients with unresectable tumors, in 28 patients (21.5%), the tumor mass shrunk in such a way that surgery was undertaken and the tumor was resected (Figures 23.8, 23.9).

Histologic examination confirmed the diagnosis of pancreatic carcinoma in all patients. The predominant histologic finding in treated patients who subsequently underwent pancreatic resection was a

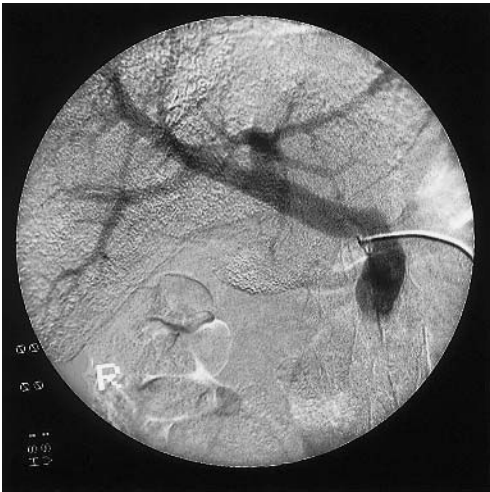


FIGURE 23.6. The catheter shown has been advanced to the portal vein via a jejunal vein.

significant vanishing of neoplastic cells in some areas, even in poorly differentiated tumors, and a subsequent extensive fibrosis, frequently with fibroblastic proliferation (Figure 23.10). A new focus of tumor necrosis was also observed in treated carcinoma sections.

A series of buffered formalin-fixed, paraffin-embedded tissue sections, including tumor and adjacent pancreatic parenchyma, were examined histologically using routine staining procedures. In addition, immunohistochemistry based on a three-step horseradish-peroxidase technique was performed

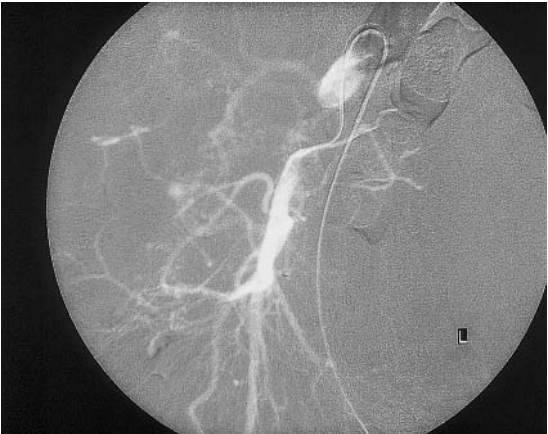


FIGURE 23.7. The catheter shown has been advanced to the superior mesenteric artery via a jejunal artery.

TABLE 23.6. Locoregional immunochemotherapy for unresectable pancreatic cancer.

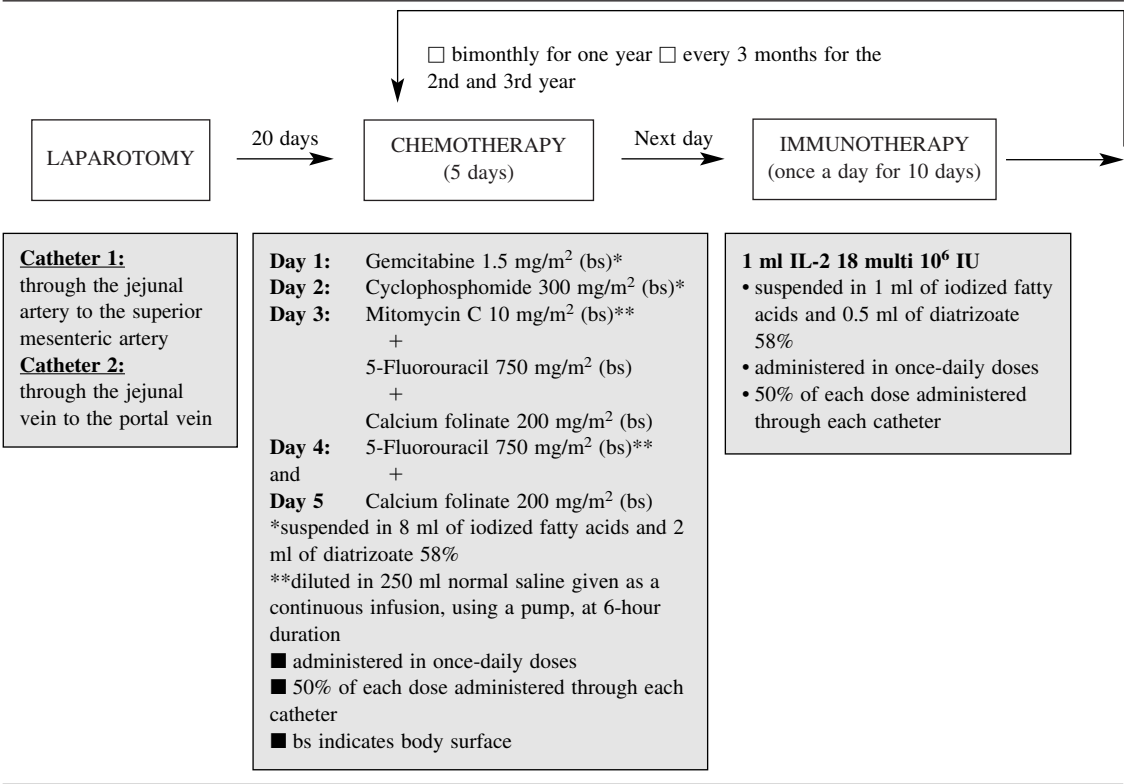


TABLE 23.7. Survival per year for groups A and B.

No of years	Group A* patients (%)		Group B† patients (%)
	Stages I & II (n = 56)	Stage III (n = 41)	
1	100	80.5	80.0
2	89.3	61.0	40.0
3	69.6	44.0	25.4‡
4	60.7	34.1	10.8‡
5	44.6	14.6	04.6‡

*Survival ranged from 3 to 69 months (mean, 38 mo).
†Survival ranged from 4 to 37 months (mean, 18 mo).
‡Patients who, although they were considered as bearing unresectable tumors after locoregional immunochemotherapy (average, 2–4 cycles of treatment), experienced tumor shrinkage that rendered the tumor resectable.

phocytes confined to the pancreatic space occupied by the tumor and activation of Kupffer cells, pitt cells, and macrophages that are harbored in the liver sinusoids. On the other hand, the chemotherapeutical dose is first entrapped in the pancreatic space occupied by the tumor, with the iodised fatty acids and diatrizoate 58% emulsion that includes droplets of 25 μ L retained in the vascular network in the organ space occupied by the tumor, including the newly developed vascular structure of the tumor itself. Furthermore, via a continuous 6-hour infusion, the chemotherapeutical dose is infused in both the pancreas and the liver, acting effectively against regional cancer cells.

The major pattern of failure of the current

chemotherapeutical approach is focused in the high incidence of peritoneal spread of the disease. This is due first to the advanced stage of the disease at the time of diagnosis and the high potentiality of disease dissemination during pancreatic resection. The second reason is the possibility that peritoneal seeding had been present and was overlooked during initial surgery. To reduce peritoneal spread, additional measures such as peritoneal chemotherapy or regional hypoxic (stop-flow) upper abdominal perfusion as neoadjuvant treatment might be of value in reducing the incidence of this event.^{9,10}

For group B (n = 130), locoregional immunochemotherapy not only contributed to significant prolongation of survival, which is, to our knowledge, unique and not comparable to any other kind of nonsurgical resectional management, but also led to a large increase in the resectability rate in a group of patients seen initially with unresectable tumors. Twenty-eight of 130 patients (21.5%) with unresectable tumors saw a significant decrease of their tumor size, which rendered the previously unresectable tumors resectable. This is a point of great interest and serious significance.

Moreover, the fact that all of those 28 patients underwent surgery successfully, the fact that they became free of their disease, and that 25%, 10%, and 5% of them have a 3-, 4-, and 5-year survival rate (Table 23.7), highlights the immense importance of combined locoregional infusion immunochemotherapy and its significant contribution

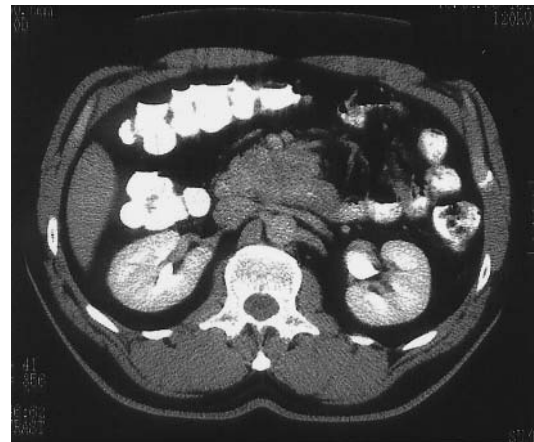


FIGURE 23.8. Tumor (diameter 7.2 cm) of 45-year-old man at the uncinate process of the pancreas.



FIGURE 23.9. An obvious reduction shown after two regional immunochemotherapeutical regimens (maximum tumor diameter, 5.2 cm).



FIGURE 23.10. An obvious vanishing of carcinoma cells and extensive fibrosis shown after regional therapy and subsequent pancreatic resection.

in the management of the average patient suffering from advanced pancreatic head carcinoma.

Conclusion

In conclusion, the experience from present series of patients favors the application of locoregional immunochemotherapy for both patients with resectable and those with unresectable pancreatic head carcinoma. We are convinced that combined immunochemotherapy applied regionally is a simple, safe, and effective means to deal with the problems associated with pancreatic cancer.

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24

Intraoperative Radiation for Pancreatic Cancer

Nora A. Janjan and Christopher H. Crane

Biological Considerations

There are many forms of radiation; each has specific biological properties and clinical advantages. Radiation can be administered through external-beam techniques or brachytherapy. External-beam radiation is given with a linear accelerator. Brachytherapy administers radiation by placing a radioactive source near the tumor bed. For both external beam radiation and brachytherapy, the biological effects are dictated by the total dose of radiation and the rate at which the radiation is administered.

A linear accelerator generates radiation from an electrical source, similar to the generation of kilovolt X-rays for diagnostic purposes. However, a linear accelerator delivers megavolt therapeutic radiation to a defined target. The radiation that is generated can be in the form of either photons or electrons. Photon radiation penetrates deeply through tissues and is used to treat tumors located deep within the body. By comparison, electron radiation penetrates only to the superficial tissue layers and is used to treat lymph nodes and skin cancers. Electron-beam radiation is also used during intraoperative radiation therapy (IORT).

The degree of penetration of both photon and electron radiation is dependent on the type of tissue that is being treated and the energy of the beam. As radiation passes through tissue, it loses energy; however, little radiation energy is lost as it passes through air. Therefore, as a radiation beam goes through viscera, such as the liver or kidney, it loses a substantial proportion of its energy. In contrast, little energy is lost from a radiation beam as it

passes through the lung, which contains a significantly higher proportion of air. Tissue inhomogeneity corrections are routinely performed to account for these differences when radiation doses are prescribed.

The energy of the radiation beam is also critical to localize the radiation dose. With photon radiation, higher energies penetrate the tissues to a greater degree and spare the superficial layers of skin. For example, when a 6-MV (megavolt) radiation beam is used, 100% of the prescribed radiation is received 1.5 cm below the skin surface, and 65% of the radiation is received 10 cm below the skin surface. With an 18-MV radiation beam, 100% of the prescribed radiation is received 3.5 cm below the skin surface, and 85% of the radiation reaches a depth of 10 cm below the skin surface. Therefore, higher energy photons, such as an 18-MV radiation beam, are routinely used to treat pancreatic cancer in order to localize the radiation dose in deep anatomic structures while sparing the skin from radiation effects.

With electron radiation, the dose is confined to the superficial tissues. As a general rule, 80% of the radiation is received at a tissue depth (in centimeters) that is equal to the electron beam energy divided by 3, and 95% of the radiation is received at a tissue depth that is equal to the electron beam energy divided by 2. Therefore, if a 9-MeV electron beam is used, 80% of the radiation is localized within 3 cm of the surface, and 95% of the radiation is localized within 4.5 cm of the surface.

Tumor cells are biologically similar to normal tissues that have rapid proliferation rates, such as the mucosa of the aerodigestive tract. Radiation ef-

fects on the tumor and mucosal surfaces are characterized by an inflammatory response, and they occur around the time of radiation. These are designated as acute radiation effects. Radiation effects that occur months to years after treatment—late radiation effects—are biologically dissociated from acute effects. Late radiation effects are characterized by fibrosis and vascular aberrations, such as vascular obliteration and telangiectasis. Late radiation effects occur most frequently in tissues that have slow rates of proliferation, such as muscle, liver, kidney, and neural tissue.¹⁻⁴

The increased effect of radiation on normal tissues relative to tumor cells is the limiting factor for single-fraction radiation and provides the justification for fractionated radiation. In fractionated radiation, small doses of radiation are given at frequent intervals over several weeks.¹⁻⁶ Between each dose of radiation, the normal tissues are able to repair the effects of treatment. Because the normal tissues have an opportunity to repair radiation damage in a manner that differs from the repair process of tumor cells, tumoricidal doses of radiation can be given with limited effects on normal tissues.

The total amount of radiation that can be given is influenced by the amount of radiation that is given with each fractional dose. When each fraction delivers a large dose of radiation, many cancer cells are killed. However, large fractions of radiation also can adversely affect the repair of normal tissues. Therefore, when large total dose of radiation are given, the total dose of radiation must be lowered because of the limited radiation tolerance of the adjacent normal tissues.¹⁻⁶

The single dose of radiation given in IORT usually ranges between 10 and 20 Gy. By comparison, conventionally fractionated radiation gives 25 to 35 fractions of 2 Gy each to total radiation doses of 50 to 70 Gy. Using an α - β calculation, which allows comparison of different radiation dose schedules, a single 10-Gy radiation dose given intraoperatively would be biologically equivalent to 17 Gy to the tumor and 26 Gy to normal tissues, such as muscle and nerve, given by external-beam radiation using conventional fractionation at 2 Gy per fraction.⁷ Using the same calculation, a single 20-Gy radiation dose given intraoperatively would be biologically equivalent to 50 Gy to the tumor and 92 Gy to normal tissues given by external beam radiation using conventional fractionation at 2 Gy per

fraction. Radiobiologically high doses of radiation can be administered intraoperatively without significant toxicity because only a small area is treated and adjacent normal structures are excluded from the intraoperative radiation field. IORT is usually combined with fractionated external-beam radiation.

Clinical Application of Radiobiological Principles

A prospective randomized trial conducted by the Gastrointestinal Tumor Study Group (GITSG) demonstrated a survival advantage when adjuvant chemotherapy and radiation were administered postoperatively to patients with pancreatic cancer.⁸ The chemotherapy consisted of a daily bolus of 5-fluorouracil (5-FU), 500 mg/m²/day, for 3 days at the start of weeks 1 and 5 of radiation. This was followed by weekly boluses of maintenance 5-FU. The radiation totaled 40 Gy, given in 2-Gy fractions. There was a planned 2-week interruption during the course of radiation ("split-course radiation") because of poor tolerance to abdominal radiation; the entire course of radiation therefore required 6 weeks. Furthermore, because of the prolonged time for recovery, 24% of patients in the GITSG trial were not able to begin receiving adjuvant therapy until more than 10 weeks after surgery.

A similar trial, which randomized pancreatic cancer patients to receive surgery alone versus surgery plus postoperative adjuvant chemoradiation, was performed by the European Organization for Research and Treatment of Cancer (EORTC).⁹ The chemoradiation regimen in the EORTC trial was the same as that used in the GITSG trial except that maintenance chemotherapy was not given. The EORTC trial found that 22% of patients did not receive adjuvant chemoradiation because of postoperative complications or patient refusal.

In recognition of the radiobiological disadvantage of split-course radiation, more aggressive postoperative treatment regimens have been used for pancreatic cancer. These regimens give high radiation doses (50.4 to 54 Gy, 1.8 Gy per fraction) in an uninterrupted course over 6 weeks concomitantly with 5-FU. Using this approach, the local recurrence rate ranges from 7% to 25%.¹⁰⁻¹² As in the EORTC trial, 24% of patients in one study did not receive postoperative

chemoradiation because of delayed recovery from surgery.¹² Even when adjuvant therapy can be completed, clinicopathologic factors such as tumor size, nodal involvement, positive margins of resection, and histologic grade can impact survival rates.^{13–19}

Because approximately one fourth of patients in the postoperative chemoradiation studies did not receive planned postoperative therapy, trials were initiated that gave chemoradiation prior to surgery. At The University of Texas M. D. Anderson Cancer Center, preoperative and postoperative adjuvant chemoradiation were retrospectively compared.¹² Three different radiotherapeutic regimens were used. Two of the regimens used conventional-fractionation radiation that gave 50.4 Gy in 2-Gy fractions before or after resection. The third radiation regimen, designated as rapid fractionation, administered 30 Gy in 3-Gy fractions before resection. The rapid-fractionation regimen was equivalent to 33 Gy in 2-Gy fractions in terms of acute radiation/tumor effects and 36 Gy in 2-Gy fractions in terms of late radiation effects. All patients who had resections also received 10 to 15 Gy of IORT. The median pancreatic tumor size was the same in the preoperative and postoperative chemoradiation groups. Although the differences were not statistically significant, the retroperitoneal margin was positive in 26% versus 12% of patients who had postoperative versus preoperative chemoradiation, respectively. Local control and survival rates were similar in the preoperative and postoperative chemoradiation groups.¹²

Although no differences were seen in local control and survival rates between conventional- and rapid-fractionation schemas, the time required for treatment did vary significantly. The median duration of treatment was 91 days for the group given 50.4 Gy preoperatively, 99 days for the group given 50.4 Gy postoperatively, and 63 days for the group given preoperative rapid-fractionation radiation ($P < 0.01$). The grade 3 toxicity rates among the preoperative and postoperative groups that received 50.4 Gy were 19% and 11%, respectively; the grade 3 toxicity rate with preoperative rapid-fractionation radiation was 7%. The corresponding rates of hospitalization because of treatment-related toxicity were 19%, 16%, and 7%, respectively.¹² The use of IORT did not result in higher complication rates.

Because of tumor cell repopulation,^{20–25} the dose of radiation necessary to achieve local tumor con-

trol may be influenced by overall treatment time. The advantage of giving smaller doses of radiation with each fraction (conventional fractionation) over an uninterrupted, protracted 6-week course of therapy is that the tumor receives a higher biological dose of radiation relative to the late radiation effects on the adjacent normal tissues. The more protracted course of radiation allows for repair of normal tissues between fractions and for higher total doses of radiation. However, higher doses of radiation are needed to overcome the repopulation of tumor cells that occurs over an extended treatment time.^{20–25} Furthermore, protracted courses of abdominal radiation in combination with chemotherapy are often poorly tolerated by patients with upper gastrointestinal malignancies.

The major disadvantage of giving large fractions of radiation in shorter courses is that the repair of normal tissues is limited. However, large fractions of radiation have many advantages. When large fractions of radiation are given, a proportionately greater number of tumor cells are killed. Although the total dose of radiation that can be given is limited, large fractions of radiation may be more biologically efficient. In addition, the radiation is given over a shorter period, reducing the possibility of tumor cell repopulation during the course of radiation. As shown in both experimental and clinical settings, tumor cell repopulation is accelerated after the first 2 weeks of radiation because of tumor reoxygenation.^{20–25} Therefore, an interruption in a protracted course of radiation after the first 2 weeks of treatment would result in accelerated repopulation of tumor cells and lower rates of local tumor control.

Theoretical concerns about tumor cell repopulation also exist when IORT is administered in conjunction with postoperative adjuvant chemoradiation. This is especially important because of the delays of up to 10 weeks between surgery and the initiation of postoperative chemoradiation. Furthermore, almost one fourth of patients do not receive intended postoperative adjuvant therapy.¹² In contrast, when chemoradiation is given first, the hiatus between preoperative chemoradiation and surgery is only 4 weeks, and tumoricidal doses given with the preoperative external-beam radiation make tumor cell repopulation less likely during the time between radiation and surgery.

TABLE 24.1. Radiobiologically equivalent doses of external-beam radiation achieved using IORT in conjunction with conventional-fractionation or rapid-fractionation external beam radiation.*

Dose of external beam radiation/IORT	Acute radiation effects	Late radiation effects
50.4 Gy/10 Gy	67 Gy	74 Gy
30 Gy/10 Gy	50 Gy	62 Gy
50.4 Gy/15 Gy	81 Gy	102 Gy
30 Gy/15 Gy	64 Gy	90 Gy

IORT indicates intraoperative radiation therapy. Conventional fractionation, 50.4 Gy at 1.8 Gy/fraction; rapid fractionation, 30 Gy at 3 Gy/fraction. The acute radiation effects are equivalent to the effects on the tumor.

The dose of radiation given with IORT is substantial and represents additional treatment to the area at highest risk for microscopic residual disease after preoperative external-beam radiation and surgery. Furthermore, IORT can efficiently administer biologically high doses of radiation without significant morbidity. In the M. D. Anderson experience,¹² high doses of IORT were localized to the areas at greatest risk for microscopic residual disease. As shown in Table 24.1, when a single fraction of IORT (10 Gy) is combined with conventional-fractionation radiation (50.4 Gy at 1.8 Gy per fraction), the tumor receives a dose radiobiologically equivalent to 67 Gy in terms of acute effects and 74 Gy in terms of late effects. When the IORT dose is increased to 15 Gy, the radiobiologically equivalent doses are 81 and 102 Gy, respectively. Although the total doses of radiation given by rapid fractionation were radiobiologically less than the doses given by conventional fractionation, equivalent clinical outcomes confirmed that the total dose continued to be in the therapeutic range. Furthermore, tolerance to therapy was better and treatment was completed in a shorter period with rapid fractionation. The use of IORT appears to have eliminated the need for a protracted course of external-beam radiation.

Clinical Experience With IORT

The initial experience with IORT for pancreatic cancer involved patients who were found to have unresectable tumors. An early publication from the Massachusetts General Hospital reported a 17-

month median survival time among patients who had unresectable disease and who received 15 to 20 Gy of IORT in conjunction with 45 to 50 Gy of external-beam radiation and chemotherapy. Therefore, the pancreatic tumor received a dose of radiation biologically equivalent to 76 to 100 Gy given externally in 2-Gy fractions. This radiation dose would not have been possible without the use of IORT. The median survival time was almost twice that seen in a historical cohort of patients who received a similar course of chemoradiation without IORT. Local control of the primary tumor was achieved in approximately half of the patients who received IORT.^{26,27}

In a Mayo Clinic experience, 20 Gy of IORT was given with 45 to 50 Gy of external-beam radiation. The overall median survival was 11 months, but disease progression occurred in 71% of patients who were treated with chemoradiation as compared with only 7% who were given IORT in addition to chemoradiation.²⁸ The Radiation Therapy Oncology Group conducted a trial in patients with locally advanced pancreatic cancer that delivered 17 to 22 Gy of IORT to the primary tumor followed by 50 Gy of postoperative external-beam radiation in conjunction with chemotherapy. The median survival time was 9 months, but 6% of patients with unresectable disease were alive at 2 years. A significant finding was the substantial levels of pain control achieved with IORT.²⁹

The tumor effects of IORT have been evaluated by [¹⁸F]fluorodeoxyglucose (FDG) positron emission tomography (PET) among patients with unresectable pancreatic cancer.³⁰ No external beam radiation or chemotherapy was given postoperatively. The results obtained with FDG-PET were compared with results obtained with conventional postoperative computed tomography (CT). Scans were obtained at 0 to 2 months, 2 to 4 months, and 4 or more months after surgery. CT demonstrated a slow decrease in tumor size over time. However, FDG-PET showed a significant decrease in the metabolism of the pancreatic tumor before a decrease in the tumor size could be detected by CT. It was concluded that FDG-PET may have a significant role in monitoring the effects of therapy, particularly among patients with unresectable disease.

Once the feasibility of combining IORT with pancreaticoduodenectomy was established, prospective randomized trials were conducted. Two large series

were published that showed improvements in local disease control with IORT. The first study involved 90 patients; 43 received 13 to 20 Gy of IORT at the time of resection.³¹ No adjuvant radiation or chemotherapy was administered postoperatively. The median time to relapse (13 months for IORT vs 8 months for surgery alone), local disease control rate (27% local recurrence rate with IORT vs 57% for surgery alone), and overall survival rate at 1 year (71% for IORT vs 49% for surgery alone) were improved with IORT.

A prospective randomized study among 24 patients with locally advanced but technically resectable pancreatic cancer was conducted by the National Cancer Institute.³² Although these cases were unresectable by conventional criteria, extensive surgical procedures were undertaken that included resection of portions of the portal vascular system. The perioperative mortality rate was 27%, and the morbidity rate was 71%; no difference in perioperative complication rates was seen, however, between the groups that did and did not receive IORT. During surgery, half of the patients received 20 Gy of IORT; all patients received 45 to 55 Gy of external-beam radiation postoperatively. The median survival time was 18 months with IORT and 12 months without IORT. Local recurrence occurred in only 33% of the IORT group as compared with 100% of the group that did not receive IORT.

Role of Gemcitabine

Recent therapeutic approaches to localized and metastatic pancreatic cancer have included the use of gemcitabine. Gemcitabine has been shown to be more effective than 5-FU in alleviating disease-related symptoms and has been associated with a small survival advantage.³³ The optimal therapeutic combination of gemcitabine and radiation still is not determined. It is known that gemcitabine has significant radiation-sensitizing properties.^{34,35} Gemcitabine inhibits cellular repair and repopulation, induces apoptosis, delays tumor growth, and enhances radiation-induced tumor growth delay.

There is evidence that the sequencing of radiation and gemcitabine is critical. When single doses of radiation and gemcitabine are given, the maximum enhancement of tumor response occurs when

gemcitabine precedes radiation by at least 24 hours. However, normal gastrointestinal epithelium may be protected from the effects of radiation when more than 24 hours elapses between radiation and gemcitabine administration.³⁵ In a study in mice, three different schedules of gemcitabine and radiation were evaluated. In all cases, five fractions of radiation were given. The first schedule administered gemcitabine 24 hours before the 5 days of radiation. The second administered gemcitabine 24 hours before the first and third fractions of radiation. The third schedule administered gemcitabine 24 hours before each fraction of radiation. The radiation dose enhancement factor for tumors ranged between 1.34 and 1.46 and was independent of the sequencing of gemcitabine and radiation. The enhancement was associated with tumor reoxygenation. However, normal tissue toxicity was highly dependent on the schedule of gemcitabine administration. The single dose of gemcitabine resulted in slight radiation protection of the mouse jejunum. Two and five doses of gemcitabine enhanced radiation toxicities by a factor of 1.09 and 1.23, respectively. Enhanced radiation toxicity to the jejunum resulted from apoptotic death of cells in S-phase and the synchronism of surviving cells.

Because normal tissues are excluded from treatment with IORT, much work is needed to determine the possible role of IORT with gemcitabine.

Summary

IORT has specific radiobiological advantages over other forms of radiation. Among these is the localization of the radiation dose only to the area of high risk, while excluding from treatment adjacent normal structures. Current evidence suggests that IORT can improve local control and palliation of symptoms in patients with nonmetastatic pancreatic cancer. With the introduction of novel systemic agents such as gemcitabine, the role of IORT must be redefined in terms of the timing of chemotherapy administration, efficacy, and toxicity. It is unclear whether the radiation doses currently used safely in IORT will be excessive with the newer radiation-sensitizing agents. It is also unknown how IORT can be best integrated with external-beam radiation. Further research is needed to best utilize the radiobiological properties of IORT with other therapeutic options.

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Conformal Radiation Therapy in Pancreatic Cancer

Christopher H. Crane and Nora A. Janjan

Three-Dimensional Conformal Radiation Therapy

Three-dimensional conformal radiation therapy (3DCRT) has been the most significant recent technical innovation in radiation oncology. It improves the distribution of the radiation dose to the tumor relative to the uninvolved normal tissues. Higher radiation doses, in general, are associated with better rates of tumor control. The dose that surrounding normal tissues receive limits the total tumor dose that can be given. 3DCRT utilizes multiple radiation beams in order to minimize the radiation dose to normal tissues. The adjacent normal tissues are spared, wherever possible, in the individual beams. Higher total doses of radiation are given to the tumor because the tumor receives the sum of all the radiation beams.

Three-dimensional (conformal) radiation treatment planning can be accomplished with a dosimetrist (treatment planner) selecting the treatment fields, or by a technique known as “inverse treatment planning.” Both are accomplished by obtaining multiple thin-section computed tomography (CT) slices that are 3 mm or less in thickness. These multiple CT cuts are then reconstructed to create a three-dimensional reconstructed volumetric analysis of the anatomy. The radiation oncologist defines the areas at risk for tumor involvement as well as the critical normal tissues on each CT cut. Once the anatomy is defined, the dosimetrist selects the fields and optimizes them using various beam angles, relative intensity, and customized beam shaping. In the case of inverse planning, the radiation

oncologist prescribes a radiation dose to the tumor and places a limit on the radiation dose that is allowed to each critical structure, such as the spinal cord, liver, kidneys, and bowel. A computer then determines the field orientations that lead to the optimal radiation dose distribution based on the clinical parameters that are prescribed.

A variety of approaches can be used to achieve the goal of giving high doses of radiation to the tumor while minimizing treatment of the normal tissues. The number of radiation fields that are needed depends on the complexity of the anatomy in the treated area. In general, a greater number of radiation fields are needed if the anatomy in the area to be treated has many radiation-sensitive critical structures. This is particularly true in the treatment of pancreatic cancer because of the limited radiation tolerance of adjacent normal tissues such as the liver, kidneys, bowel, and spinal cord. In addition to varying the number of radiation beams, the radiation dose also can be weighted and shaped and various beam energies can also be selected in order to alter the radiation dose distribution. Figure 25.1 is an example of a radiation dosimetry plan that has been optimized by a dosimetrist to reduce normal tissue irradiation. Therefore, 3DCRT localizes radiation to the tumor and minimizes the dose of radiation to critical structures by using multiple radiation beams, with customized weighting, blocking, and energies.

The technique of intensity-modulated radiation therapy (IMRT) goes a step beyond 3DCRT. With IMRT, the radiation dose can be varied to specific areas *within* a radiation beam. Inverse treatment planning is typically used, and treatment can be given with a beam that moves continuously around

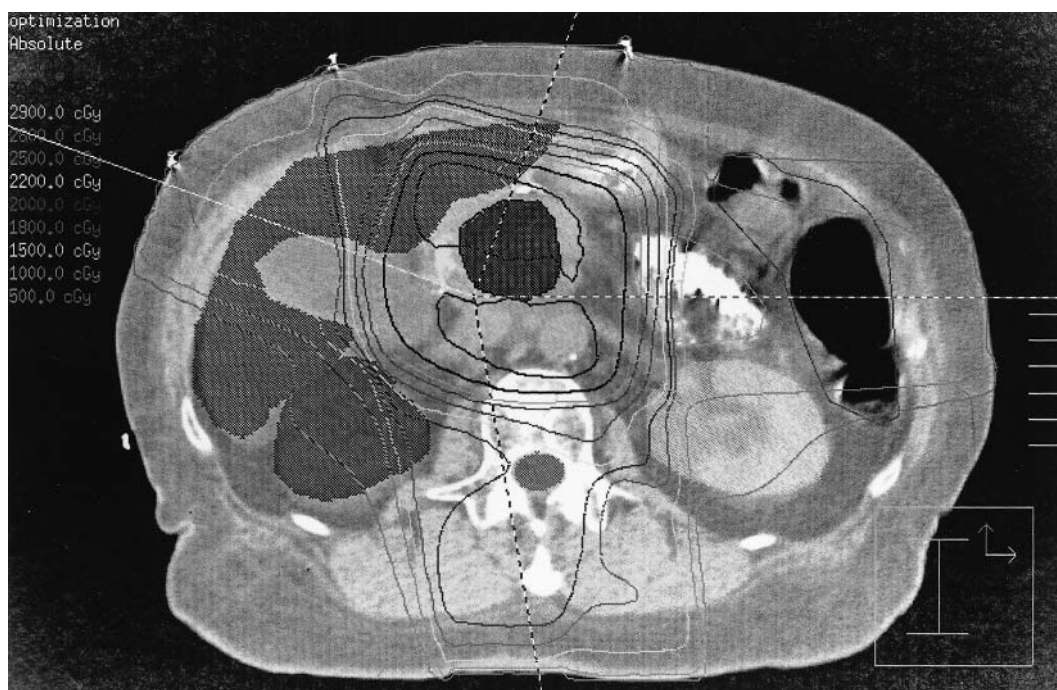


FIGURE 25.1. Radiation dosimetry plans optimized using 3DCRT. A dosimetrist has optimized this plan in order to reduce normal tissue irradiation. In this case, four fields were used with oblique angulation, customized weighting, and customized blocking to minimize the radiation dose to the kidneys and liver.

the patient. This added degree of freedom allows the possibility of shaping the radiation dose distribution like never before. IMRT also allows radiobiological flexibility in the prescription of radiation doses. The radiation dose can be specified for the tumor, for the lymph node areas at risk, and for critical structures such as the liver, kidneys, and spinal cord. For example, 2.8 Gy per fraction may be prescribed to the tumor and 2 Gy per fraction to the lymph node areas at risk for microscopic disease, and the radiation dose that the liver receives may be only 0.5 Gy per fraction. Therefore, the total radiation dose after 25 fractions would equal 70 Gy to the tumor, 50 Gy to the lymph nodes, and 12.5 Gy to the liver. Since higher radiation doses per fraction are biologically more effective, higher doses per fraction to the tumor would also provide a radiobiological advantage. Although the total dose to the tumor is 70 Gy when 25 fractions are given at 2.8 Gy per fraction, this is radiobiologically equal to giving 74.67 Gy at 2 Gy per fraction. An example of a dosimetry plan using IMRT in unresectable pancreatic cancer is shown in Figure 25.2.

The Clinical Application of Conformal Radiation Therapy

The majority of the investigation of 3DCRT has centered on optimizing the radiation dose distribution in patients treated with curative doses of radiotherapy. By more accurately targeting the tumor volume while sparing the normal tissues as much as possible, the maximum safe dose of radiotherapy has been redefined in some clinical situations. Based on clinical experience with 3DCRT in prostate cancer, higher total doses of radiation can now be prescribed, and a new standard of care has been established. Two prospective randomized clinical trials in prostate cancer have shown that 3DCRT reduces toxicity.^{1,2} Similar findings have prompted the escalation of radiation dose. In a phase I trial conducted at Memorial Sloan Kettering, the tumor dose was escalated using 3DCRT to 81 Gy. The prostate-specific antigen, relapse-free survival, and histologically negative biopsy rates were improved in the high-dose group.³ The higher

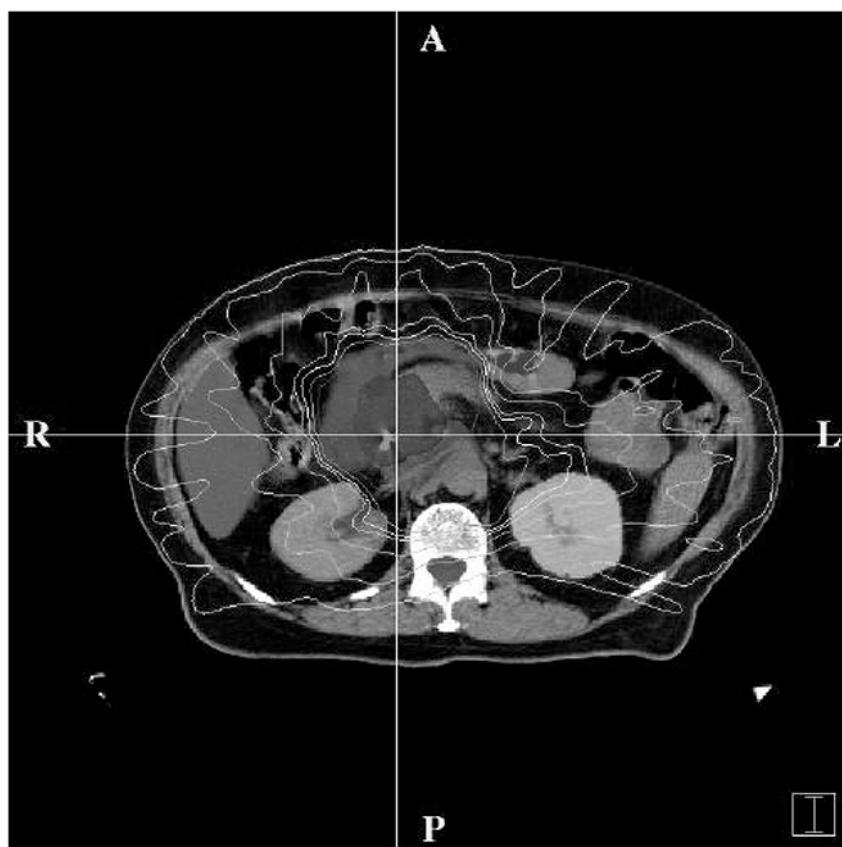


FIGURE 25.2. A maximal degree of radiation localization can be achieved with intensity-modulated radiation therapy (IMRT). In this example, the kidneys, liver, stomach, and spinal cord are all preferentially spared and the pancreatic head and surrounding lymphatics are treated.

doses have not resulted in increased toxicity.⁴ In fact, the overall grade 3 toxicity rate was 0.75% with 3DCRT. Investigators there have now implemented IMRT since it offers a higher degree of conformality of dose distribution. Clinical trials are now underway in the Radiation Therapy Oncology Group (RTOG), which will prospectively evaluate the role of 3DCRT in prostate cancer and lung cancer. Although no clinical data are available assessing the role of 3DCRT in pancreatic cancer, the dose to surrounding normal tissues has been shown to be significantly reduced in a phantom model.⁵

These encouraging results in prostate cancer have prompted ongoing clinical trials with IMRT in pancreatic cancer. Although results are not yet available, IMRT offers a significant theoretical advantage over conventional radiation techniques, especially when potent radiation-sensitizing chemo-

therapeutic agents, like gemcitabine, are combined with radiation.

Radiotherapy in Pancreatic Cancer: The Tolerance of the Upper Abdomen to Radiation

Adjacent critical structures limit the dose of conventional external beam radiation that can be administered. In the abdomen, the liver, kidneys, stomach, bowel, and spinal cord are the critical structures that are considered in radiation treatment planning. The toxicities of radiation to these critical structures are related to the radiation dose and the volume treated. In general, high radiation doses can be given to small areas. When the volume that

must be treated becomes larger, less radiation can be given to the normal tissues.

Chemoradiation in Pancreatic Cancer

Prolonged rates of survival were documented when chemotherapy was administered during radiation among patients with locally advanced pancreatic cancer in a trial conducted by the Gastrointestinal Tumor Study Group.⁶ Even though radiation therapy was interrupted (split-course radiation), which potentially allowed for tumor repopulation, the concurrent administration of 5-fluorouracil (5-FU) during the course of radiation improved the median survival rates when compared with the group given radiation alone.⁷ The toxicity rates for 5-FU and radiation were comparable to radiation alone. From this study, the concurrent administration of 5-FU as a radiation-sensitizing agent in pancreatic cancer became the standard of care. Although CT scans were not routinely used to document patterns of failure, fewer patients treated to a total radiation dose of 60 Gy had clinical findings of local recurrence as compared with patients who received only 40 Gy of radiation. Even though the local control rates were better among patients who received 60 Gy, overall survival rates were compromised by the high rates of distant metastases.⁶ Since that time, single institutional studies have addressed the role of escalating the radiotherapy dose using intraoperative radiotherapy. They have been able to show substantial improvements in local control, but only modest improvements in median survival rates.⁸⁻¹² Brachytherapy implantation as a boost has produced similar results with regard to enhanced local control.^{13,14} Therefore, a radiation dose-response relationship appears to exist in pancreatic cancer that translates to higher rates of local control, but survival is still limited by the development of distant metastases. Therefore, any improvement in outcome will have to involve treatment that maximizes local control and reduces distant failure. The logical choice is to investigate radiosensitizers that have demonstrated systemic activity.

Novel radiosensitizers, such as paclitaxel and gemcitabine, have been recently investigated in unresectable and resectable pancreatic cancer. A recent randomized trial of gemcitabine versus 5-FU

as first-line therapy in patients with advanced pancreatic adenocarcinoma demonstrated a survival benefit to those patients who received gemcitabine.¹⁵ The median survival time in gemcitabine-treated patients was 5.65 months, and for patients treated with 5-FU, 4.41 months ($P = 0.0025$). Twenty-four percent of patients treated with gemcitabine were alive at 9 months compared with 6% of patients treated with 5-FU. In addition, gemcitabine was shown to improve cancer-related symptoms and performance status as assessed by the quantitative clinical benefit scale in both untreated and previously treated patients with metastatic adenocarcinoma of the pancreas.^{15,16}

Preclinical data¹⁷ have shown that gemcitabine is a potent radiosensitizer of human pancreatic adenocarcinoma cells. Phase I clinical data have demonstrated that the combination of radiation and gemcitabine is a potentially very toxic one.¹⁸⁻²⁰ Phase I dose-escalation studies have been conducted evaluating both paclitaxel and gemcitabine with concurrent radiation, and the dose-limiting toxicity (nausea, vomiting, anorexia, abdominal pain, dehydration, and upper gastrointestinal bleeding) with these combinations is referable to acute normal tissue effects in the irradiated field.^{18,21} Based on the dose-escalation studies that have been done,¹⁸⁻²⁰ there appears to be an inverse relationship between the maximum tolerated combination (MTD) of gemcitabine and radiation and the radiation field size that is used. The groups at Fox Chase and at Michigan have used radiation fields confined to gross disease. The MTD in the Michigan study²⁰ is 42 Gy/15fx with 1000 mg/m² (C.J. McGinn, personal communication, June 2000) and the MTD in the Fox Chase study¹⁹ is 50.4 Gy/28fx with 700 mg/m². In the M. D. Anderson study, the regional lymphatics were treated and the field sizes were larger. The MTD was 350 mg/m² with 30 Gy/10fx.¹⁸ All of the previously described studies have used conventional treatment planning. The MTD therefore appears to be inversely related to the volume of normal tissue irradiated.

The phase I trial performed at M. D. Anderson¹⁸ in patients with inoperable pancreatic cancer has formed the basis of an ongoing phase II multi-institutional trial in resectable patients. The gemcitabine dose is 400 mg/m² per week for 7 weeks. Radiotherapy is given during the first 2 weeks at a dose of 30 Gy/10fx. Twenty-five patients com-

pleted protocol treatment. The severe toxicity rate was approximately 40%, again due to normal tissue effects in the irradiated field (R.A. Wolff, unpublished data, 2000). In an attempt to reduce toxicity by reducing the volume of normal tissues in the irradiation field, a subsequent phase I dose escalation trial is combining the administration of gemcitabine with IMRT in unresectable pancreatic cancer. More precise tumor targeting with sparing of normal tissue could allow higher doses of gemcitabine and/or radiation to be given with similar toxicity, or the same treatment to be given with less toxicity.

Summary

We are still learning how to best combine radiation with chemotherapy. Concurrent regimens with newer sensitizers have led to increased toxicity. One way of improving the therapeutic ratio is to reduce the toxicity of treatment. New radiation techniques allow improved localization of the radiation dose and a reduction in the volume of normal tissue irradiated. This could lead to reduced toxicity of treatment and possibly to radiation-dose escalation. The appropriate volume of irradiation with the concurrent use of newer, more toxic radiosensitizers in pancreatic cancer is controversial. Treatment of the regional lymphatics increases toxicity, but may be important. Concerns of toxicity have led some investigators to use smaller fields and to rely on chemotherapy for high-risk nodal areas. Newer radiotherapy techniques, such as 3DCRT and IMRT, improve the radiation-dose distribution and thus offer a theoretical advantage that may translate into a clinical benefit in this new era of more toxic combinations of radiation and chemotherapy. Improved radiation targeting may have a role in reducing toxicity and may allow escalation of the radiation dose, or the inclusion of additional cytotoxic agents into current regimens.

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Radiation Sensitizers, Fractionation Schedules, and Future Clinical Trials

A. William Blackstock

Introduction

Despite recent innovations in radiation fractionation, three-dimensional conformal therapy, and novel chemotherapeutic agents, pancreatic cancer remains a disease that is almost universally fatal. Conventional radiation therapy delivered in combination with chemotherapy currently represents the definitive therapy for the majority of patients presenting with locally advanced unresectable disease and is often utilized in the adjuvant setting for patients with resected disease. The combined modality approach utilizing concurrent radiation and 5-fluorouracil (5-FU) originated principally from the early experience at the Mayo Clinic and has further evolved as additional drugs with activity in pancreatic cancer have become available. This chapter will review recent efforts combining chemotherapy and radiation as well as altered fractionation schedules for the treatment of pancreatic cancer. The closing section will discuss innovative radiation-drug studies that are underway or are in development.

Conventional Radiation Alone for Unresectable Pancreatic Cancer

Although the median survival rate appears increased in selected patients with locally advanced unresectable pancreatic cancer treated with conventional radiation therapy, the fact remains that the majority of patients will succumb to their disease less than a year from diagnosis. Early studies

utilizing definitive radiation doses typically incorporated radiation schedules that were palliative in nature. In a retrospective analysis of 393 patients who were entered into pancreatic carcinoma protocols performed by the Gastrointestinal Tumor Study Group (GITSG), 79% presented with pain.¹ It is postulated this discomfort is related to bile duct obstruction, compression of the neuroplexus, extension of the tumor into other visceral organs, reflex ileus pain, or concurrent pancreatitis.² In an early study evaluating the palliative benefits of radiation, Green et al³ treated 22 patients with locally advanced disease utilizing a radiation schedule that delivered 1.5 Gy per day given 4 days per week for a total tumor dose of 50 Gy. While the efficacy of this regimen in terms of survival was unclear, the authors indicated that 77% of the patients had significant pain relief and a documented reduction in the potency and frequency of analgesic requirements. An early retrospective report from the Duke University Medical Center also suggested a benefit with radiation for patients with unresectable disease; in this study, good palliation was defined as complete relief of pain sustained for at least 4 weeks, any weight gain not due to ascites or edema, relief of obstructive symptoms and jaundice, or a decrease in the size of the tumor mass, if palpable. The radiation was delivered via an anterior/posterior (AP/PA) field to an average dose of 50 Gy over the course of 6 weeks (range, 42.5–66.9 Gy in 6–14 weeks). Of the 29 patients treated, good palliation was achieved in 45%.⁴ Recent studies incorporating chemoradiation and utilizing a prospective assessment of symptom relief are more encouraging. Kamthan et al,⁵ in a study of 35 patients receiving

54 Gy given as a split-course in conjunction with 5-FU-based chemotherapy, found that more than 90% of patients never developed symptoms of back pain or gastrointestinal obstruction. Similar encouraging data from a phase II study from Italy found an improvement in abdominal pain in 76% of patients treated with 5-FU/cisplatin chemotherapy and split-course radiation therapy.⁶ The reported improvement in symptoms lasted for a median of 5 months. Although these data are limited, it would appear that radiation delivered using a variety of schedules provides palliation in a significant cohort of patients with advanced disease. Further, the 30% to 40% survival rate observed at 1 year in these early pilot studies supported the resulting phase II and III trials, which were designed to elucidate the efficacy of chemoradiation for the treatment of unresectable pancreatic cancer.

The significance of radiation dose and schedule remains unclear. Komaki et al.⁷, in a retrospective study of 20 patients treated with radiation for unresectable disease, observed a median survival time of 13 months in patients receiving >45 Gy in 6 to 7 weeks versus 7 months in patients receiving <45 Gy in 3 to 6 weeks. A number of confounding factors were present in this study: the use of chemotherapy was inconsistent and a superior pretreatment performance status was associated with an improved median survival time, reflecting the importance of patient selection. Retrospective data from the University of Louisville and the University of Pittsburgh also found survival was improved for patients receiving higher doses of radiation.^{8,9} As with the Komaki data, however, patient selection likely explains the observed differences. Dobelbower et al.¹⁰ in a pilot study of 18 patients treated to a tumor minimum dose of 63–65 Gy (no split), observed no significant toxicity and an encouraging median survival time of 11.8 months. In contrast, a study involving 104 patients with advanced pancreatic cancer reported by Whittington et al.¹¹ using “precision high-dose” radiation therapy was less promising. The dose delivered was 50 Gy followed by a 15-Gy boost for a total dose of 65 Gy. Although 61% of patients had complete relief of their pain following treatment, the 10-month median survival rate suggests there is little additional benefit with this high-dose regimen. Definitive radiation delivered using standard fractionation, either split-course or continuous, to doses in

the range of 50 Gy results in good palliation in the majority of patients and will achieve a modest median survival time of 6 to 10 months for most patients with locally advanced unresectable disease.

Conventional Radiation and Chemotherapy for Unresectable Pancreatic Cancer

It has been demonstrated in a variety of in vitro and in vivo models that 5-FU has significant radiation-sensitizing properties and is an active agent in pancreatic cancer.^{12–18} One of the earliest studies evaluating chemoradiation in the management of advanced pancreatic cancer comes from the Mayo Clinic. Moertel and Reitemeier,¹⁹ in a randomized double-blind study, compared moderate doses of radiation (35–37.5 Gy) given over 4 weeks in combination with 5-FU (45 mg/kg) administered as a bolus injection during the first 3 days of therapy with the same radiation therapy in patients receiving saline placebo. The mean survival time for the radiation-only patients was 6.3 months compared to 10.4 months for patients treated with concurrent radiation and 5-FU. This result, although not always consistent as shown in Table 26.1, has been repeated in several subsequent nonrandomized and randomized trials evaluating radiation and concurrent 5-FU.^{20–22}

Most studies evaluating 5-FU and radiation have done so using either a 500 mg/m² bolus schedule or a 4-day 1000 mg/m² continuous infusion regi-

TABLE 26.1. Randomized trials of radiation with or without chemotherapy for unresected pancreatic cancer.*

Author	Radiation	Chemotherapy	Median survival, mo
Moertel et al ²⁷	60 Gy	None	5.5
	60 Gy	5-FU	10.0
	40 Gy	5-FU	10.0
GITSG ²⁰	54 Gy	5-FU/SMF	10.0
	No XRT	SMF	7.5
Klaassen et al ²²	40 Gy	5-FU	4.2
	No XRT	5-FU	4.4
GITSG ²¹	60 Gy	5-FU	8.5
	40 Gy	Doxorubicin	7.5

*5-FU indicates 5-fluorouracil; SMF, streptozocin, mitomycin C, and 5-FU; GITSG, Gastrointestinal Tumor Study Group; and XRT, external beam radiation.

men. With recent data in rectal cancer suggesting an advantage using a protracted venous infusion (PVI) 5-FU,²³ that strategy has recently undergone evaluation in unresectable pancreatic cancer. Whittington et al,²⁴ in a phase I study of patients with advanced pancreatic cancer, determined the maximum tolerated dose of PVI-5-FU to be 250 mg/m²/day, with oral mucositis representing the dose limiting toxicity. The median survival time for the group was an encouraging 11.9 months. Ishii et al,²⁵ in a phase II trial utilizing a similar PVI-5-FU regimen reported acceptable toxicity and a similar median survival time of 10.3 months. A third study of 74 patients reported by Poen et al,²⁶ compared the toxicity and efficacy of a 500-mg/m² bolus infusion with a PVI-5-FU regimen (200–250 mg/m²) concurrent with a standard continuous-course radiation therapy. The authors concluded that the PVI regimen was better tolerated and permitted an overall increase in the 5-FU dose compared with the bolus regimen. It would appear from the available data that 5-FU delivered as a prolonged venous infusion concurrent with standard radiation to the pancreas is a safe alternative to a bolus 5-FU administration, but no clear advantages in terms of efficacy have been demonstrated. Table 26.2 lists additional studies of combined modality therapy and shows the variety of chemotherapeutic agents that have been evaluated with radiation.^{27–30}

An alternative strategy piloted at the M. D. Anderson Cancer Center examined the use of prophylactic hepatic irradiation delivered in conjunction with chemoradiation for unresectable pancreatic cancer. Hepatic failure rates for patients with locally advanced disease range from 40% to 60%. In an effort to lower the hepatic failure rate, Komaki et al³¹ treated 16 patients with advanced unresectable pancreatic cancer with chemoradiation

to the primary tumor (61.2 Gy/5-FU) and 23.4 Gy directed to the liver. Hepatic failure as the first sight of relapse was seen in only 2 of 15 evaluable patients. A similar study in 21 patients reported by Wiley et al³² also revealed an apparent reduction in hepatic failures, but resulted in a modest median survival time of 11.5 months. Because the previously described pilot data were encouraging, a subsequent phase II study of 81 patients was initiated within the Radiation Therapy Oncology Group (RTOG). Unfortunately, the regimen resulted in a modest median survival time of 8.4 months and was associated with significant toxicity.³³ At present, there is little enthusiasm for additional studies of prophylactic hepatic irradiation.

Altered Radiation Fractionation and Chemotherapy for Unresectable Disease

Mathematical models derived from experimental data and verified by clinical experience support the importance of reducing the overall treatment time required to deliver a given dose of radiotherapy.³⁴ Based upon these data, it has been argued that accelerated tumor repopulation occurs during a course of radiotherapy after an average lag time of approximately 4 weeks. It is postulated that this increased rate of repopulation results from improved oxygenation and increased recruitment of clonogenic cells into the proliferating cell cycle during the course of radiotherapy. Thus, these clonogenic tumor cells are able to approach a theoretical maximum rate of multiplication, represented mathematically by a potential doubling time (TD_{pot}). TD_{pot} values have been analyzed experimentally in primary human pancreatic tumors and are reported

TABLE 26.2. Concurrent chemoradiation for unresected pancreatic cancer.*

Author	No. Patients	Radiation	Chemotherapy	Median survival, mo
Thomas et al ²⁸	16	60 Gy	Cisplatin†/5-FU	9.0
Boz et al ⁶	22	60 Gy	Cisplatin/5-FU	7.5
Kamthan et al ⁵	35	54 Gy	5-FU/streptozocin/cisplatin	15.0
Nguyen et al ²⁹	23	60 Gy	Daily cisplatin	10.0
McCracken et al ³⁰	68	60 Gy	Methyl-CCNU/5-FU ± T	9.0

*5-FU indicates 5-fluorouracil; CCNU, methyl-CCNU or semustine or lomustine methyl; and T, testolactone.
†Intra-arterial cisplatin.

to range from 16 to 70 hours.³⁵ While a very high cell loss factor likely accounts for the discrepancy between TD_{pot} and a prolonged radiographic tumor doubling time, prolongation of the radiotherapy course by a few days (late in a course of radiotherapy when oxygenation is optimal and cell loss is minimal), may allow several doublings of the surviving clonogens and adversely affect tumor control.³⁶

Several studies have attempted to discern the potential advantages of hyperfractionated radiation for patients with advanced pancreatic cancer (Table 26.3). In data from GITSG, patients received 50.4 Gy delivered in 1.2-Gy twice-daily fractions concurrent with 5-FU on the first and last 3 days of treatment. In the 18 patients with unresectable disease treated, a disappointing median survival time of 8 months was observed.³⁷ In a second study evaluating the advantages of hyperfractionated radiation therapy, Prott et al³⁸ evaluated a 1.6-Gy twice-daily radiation schedule to a total dose of 44.8 Gy with concurrent 5-FU and folinic acid given on days 1 to 3 of the radiation. While the acute toxicities, nausea, and diarrhea appeared tolerable, the median survival time was a modest 12.7 months. In a more recent report, Luderhoff et al³⁹ investigated a regimen of 1.1 Gy delivered in three fractions per day to a total dose of 45–50 Gy. Patients also received continuous infusion 5-FU during the first and third week of radiation. Although the regimen appeared to be well tolerated (11 of 18 patients completed treatment without modification), the observed median survival time of 8.5 months was not unlike that reported with more conventional radiation schedules. The results thus far with hyperfractionated radiation for locally advanced pancreatic cancer would indicate this treatment strategy remains investigational.

Preoperative Radiation and Chemotherapy for Pancreatic Cancer

Because of the high rate of positive resection margins and nodal involvement, the addition of preoperative therapy to augment surgical resection would appear to be a reasonable strategy for patients with locally advanced pancreatic cancer. Pilepich and Miller⁴⁰ reported one of the first experiences, evaluating 17 patients with either unresectable or borderline resectable disease treated with 40–50 Gy of radiation preoperatively. Six weeks posttherapy, all patients were evaluated for potential resection. Of the 17 patients treated, 11 went to laparotomy and 6 were able to undergo resection. A number of additional studies incorporating preoperative concurrent chemotherapy and radiation have been evaluated (Table 26.4).^{41–44} In an Eastern Cooperative Oncology Group study, 53 patients with localized resectable pancreatic cancer received 50.4 Gy concurrent with mitomycin C and 5-FU chemotherapy. Of the original 53 patients, 41 underwent surgery and 24 patients underwent resection. The median survival time was an encouraging 15.7 months for the cohort of patients resected but, perhaps more important, this study confirmed that preoperative chemoradiation could be tested in the setting of a multi-institutional cooperative group.⁴⁵ In data from the Fox Chase Cancer Center comparing preoperative chemoradiation to a similar regimen of postoperative adjuvant therapy, the investigators observed that the efficacy and toxicity of each regimen appeared to be similar. The ability to complete therapy, however, was significantly different; while all patients completed the preoperative regimen, 22% of patients intended to receive postop-

TABLE 26.3. Hyperfractionation for unresected pancreatic cancer.*

Author	No. Patients	Radiation	Chemotherapy	Medial survival, mo
Seydel et al ³⁷	18	50.4 Gy 1.2 Gy†	5-FU/streptozocin mitomycin C	8.0
Luderhoff et al ³⁹	13	45–50 Gy 1.1 Gy‡	5-FU	8.5
Prott et al ³⁸	32	44.8 Gy 1.6 Gy†	5-FU/folinic acid	12.7

*5-FU indicates 5-fluorouracil.
†Two times per day.
‡Three times per day.

TABLE 26.4. Preoperative chemoradiation trials for unresectable disease.

Author	No. patients	Radiation	Chemotherapy	Resected
White et al ⁴¹	25	45 Gy	5-FU/cisplatin/mitomycin C	20%
Jessup et al ⁴²	16		5-FU	12.5%
Yeung et al ⁴³	24	50.4 Gy	5-FU/mitomycin C	38%
Bajetta et al ⁴⁴	32	50 Gy	FUDR/leucovorin	16%

5-FU indicates 5-fluorouracil; and FUDR, floxuridine.

erative therapy did not recover adequately from surgery to receive the planned adjuvant treatment.⁴⁶ In an effort to reduce the toxicity associated with preoperative regimens that incorporate standard radiation fractionation schemes, Pisters et al⁴⁷ have evaluated a regimen of “rapid fractionation” preoperative chemoradiation. Seventy-two patients with resectable/marginally resectable disease received 30 Gy in ten 3-Gy fractions concurrent with either 5-FU or paclitaxel chemotherapy. At 4 weeks posttherapy, 39 patients were able to successfully undergo resection, with the authors reporting equivalent efficacy between the paclitaxel and 5-FU regimens. The experience reported thus far with preoperative chemoradiation clearly supports additional clinical studies evaluating this treatment strategy.

Novel Chemoradiation Trials in Unresectable Pancreatic Cancer

The concurrent administration of a number of novel compounds and radiation is currently under investigation. Preclinical and clinical data from a number of investigators have shown paclitaxel is an active agent in pancreatic cancer and possesses radiation-sensitizing properties.^{48–51} In an effort to discern the potential clinical advantages of concurrent radiation and paclitaxel in unresectable pancreatic cancer, Safran et al⁵² determined the maximum tolerated dose of paclitaxel to be 50 mg/m² given weekly with 50 Gy of radiation administered over 5 weeks. Preliminary data from 22 evaluable patients have revealed a response rate of 36%. The RTOG is currently conducting a phase II study based upon the pilot experience reported by Safran and colleagues.

The Cancer and Leukemia Group B (CALGB) is also conducting a phase II study for patients with

unresectable disease evaluating a regimen of radiation and concurrent twice-weekly gemcitabine. Gemcitabine has demonstrated significant activity in pancreatic cancer in a number of phase II and III clinical trials.^{53,54} The majority of the reported studies have administered gemcitabine in a single weekly dose of approximately 1000 mg/m². Yet there are intriguing preclinical and clinical data suggesting gemcitabine possesses equal if not greater cytotoxicity if given at a lower dose over several minutes or more frequently than once-weekly. The cellular pharmacology of gemcitabine supports this postulate. Phosphorylation by deoxycytidine kinase (dCK) is required to induce cytotoxicity upon incorporation of the difluorodeoxycytidine triphosphate of gemcitabine (dFdCTP) into DNA.⁵⁵ As suggested by Boven et al⁵⁶ and supported by data from Shewach et al,^{57,58} the phosphorylation of gemcitabine in tumor cells is a saturable process. There is also evidence that gemcitabine acts as a substrate inhibitor of dCK at high concentrations, which may be the basis for a demonstrable decline in the ability of cells to accumulate dFdCTP at gemcitabine concentrations above 20 μm/L.⁵⁹

Data from experiments performed in in vitro systems suggest an enhanced tumor response with longer exposures to gemcitabine. Cell culture experiments have clearly shown the incorporation of gemcitabine into DNA is time dependent, with several researchers having established a clear correlation between cellular DNA incorporation and cytotoxicity, indicating the intracellular accumulation and retention of gemcitabine is relevant.⁶⁰ Pharmacokinetic studies of mononuclear cells and leukemia cells, both in in vitro and clinical studies,^{55,61} have shown that dFdCTP accumulation reaches a plateau when the gemcitabine concentration in the medium or plasma exceeds 15–20 μmol/L. Pilot studies with leukemia patients suggest that a dose rate of 10 mg/m²/minute produces

plasma gemcitabine concentrations of greater than 20 $\mu\text{mol/L}$ and maximizes the rate of dFdCTP accumulation. Phase I data reported by Grunewald et al⁵⁹ further confirms the cellular accumulation of the active metabolite dFdCTP is maximal at lower doses of gemcitabine, during which plasma steady-state gemcitabine levels of 15–20 μM were recorded. A comparison of patients infused with 800 mg/m^2 over 60 minutes with those receiving the same dose over 30 minutes demonstrated that the gemcitabine steady-state concentrations were proportional to the dose rate, but that cellular dFdCTP accumulation rates were similar at each dose rate. These studies would suggest the cell's ability to convert gemcitabine to its cytotoxic metabolite dFdCTP is limited at intravenous doses greater than 350 mg/m^2 or continuous infusion rates of greater than 10 $\text{mg/m}^2/\text{minute}$.⁵⁹ It is likely that the enhanced ability of cells to accumulate gemcitabine nucleotides and retain them for a considerable duration after the end of the exposure is important to the clinical activity of gemcitabine and that this retention impacts the role of gemcitabine as a radiation sensitizer. The initial in vitro work demonstrating the radiation-sensitizing activity of gemcitabine was reported by Lawrence et al^{62–67} and Shewach and Lawrence,^{66–68} showing 24-hour cell exposures to noncytotoxic concentrations (10 nmol/L) of gemcitabine when combined with ionizing radiation resulted in increased cell kill. Additional work from the University of Michigan further demonstrated that equivalent levels of sensitization with a 4-hour exposure of gemcitabine

required a threefold increase (30 nmol/L) in drug concentration. The authors suggest the radiation sensitization observed is related to altered deoxynucleosidetriphosphate pools and have implemented further studies. Subsequent data from Wake Forest University and the University of North Carolina confirmed gemcitabine to be a potent radiation sensitizer. Cells treated initially with radiation followed by a 24-hour incubation at nontoxic gemcitabine levels (10 nmol/L) exhibited increased sensitivity to subsequent radiation-induced cytotoxicity.⁶⁹

A phase I study reported by Blackstock⁶⁹ combining radiation and gemcitabine has discerned the maximum tolerated dose of twice-weekly gemcitabine and concurrent radiation to the upper abdomen to be 40 mg/m^2 (80 $\text{mg/m}^2/\text{week}$). This same twice-weekly regimen is currently being tested in phase I/II studies at the Memorial Sloan Kettering Cancer Center and the Princess Margaret Hospital in Toronto.

Gemcitabine and CPT-11 as an induction to concurrent gemcitabine and radiation is in clinical trial at the Medical University of South Carolina, University of North Carolina and Wake Forest University Cancer Centers. The rationale for combining gemcitabine and irinotecan hydrochloride (CPT-11) includes the complementary toxicity profiles and differences in mechanisms of cytotoxicity as well as the overlapping antitumor activity spectra.^{70,71} Preclinical studies by Kanzawa and Saijo,⁷² evaluating in vitro combinations of gemcitabine with other cytotoxic agents, suggested a dose-de-

TABLE 26.5. Ongoing chemoradiation studies for unresected pancreatic cancer.*

Investigator	Phase	Induction	Chemoradiation	Adjuvant chemotherapy
NCCTG	I	NA	XRT 5–6 wk Gemcitabine/cisplatin	Gemcitabine/cisplatin
New York University	I/II	Gemcitabine/cisplatin	XRT 5–6 wk Gemcitabine/cisplatin	Gemcitabine/cisplatin
RTOG	II	NA	XRT 5–6 wk Paclitaxel	Surgery
University of Michigan	I	NA	XRT 3 wk Gemcitabine	Surgery
CALGB	II	NA	XRT 5–6 wk Gemcitabine	Gemcitabine
Dana Farber	I/II	Gemcitabine/5-FU	XRT 5–6 wk Gemcitabine/5-FU	NA

*NCCTG indicates North Central Cancer Treatment Group; RTOG, Radiation Therapy Oncology Group; CALGB, Cancer and Leukemia Group B; XRT, external beam radiation; 5-FU, 5-fluorouracil; and NA, not applicable. Physician Data Query (PDQ), National Cancer Institute, November 2000.

pendent interaction between gemcitabine and CPT-11. Supporting in vitro studies come from Bahaduri et al⁷³ using the MCF-7 breast cancer cell line and the SCOG small-cell lung cancer (SCLC) cell line. Gemcitabine and CPT-11 as single agents were found to be effective growth inhibitors in both cell lines, with subsequent isobologram analysis revealing synergy when cells were exposed to the compounds concurrently. The effect appeared to be dose dependent; at low concentrations, the combination of gemcitabine and CPT-11 was clearly synergistic in its growth inhibitory effect on MCF-7 cells, while the effect on SCOG cells became antagonistic at combination concentrations less than 1 μ M. These preclinical data provided an experimental basis for the subsequent phase I study performed at the Medical University of South Carolina, which evaluated this combination. The maximum tolerated dose of CPT-11 given intravenously over 90 minutes on Days 1 and 8 every 3 weeks, preceded by 1000 mg/m² of gemcitabine given intravenously on Days 1 and 8, was 100 mg/m²/dose. The dose-limiting toxicity was diarrhea at a CPT-11 dose of 115 mg/m², resulting in the recommendation that 1000 mg/m² of gemcitabine and 100 mg/m² of CPT-11 given on Days 1 and 8 and repeated every 3 weeks be the starting dose in subsequent phase II studies to determine efficacy.⁷⁴

As the toxicities and tolerability of single-agent chemotherapy and radiation are better defined, future studies will attempt to incorporate chemotherapy “doublets” and “triplets” with concurrent radiation therapy to the pancreas. Table 26.5 lists several phase I/II studies of chemoradiation listed with the National Cancer Institute that are actively accruing patients.

Strategies incorporating cytostatic agents are also being conducted. The guanine nucleotide-binding protein Ras plays a pivotal role in the control of both normal and transformed cell growth. Following stimulation by various growth factors and cytokines, Ras activates several down-stream effectors, leading to gene transcription and proliferation. In approximately 90% of all pancreatic cancers, *Ras* gene mutations produce mutated Ras that remains locked in the active state, thereby relaying uncontrolled proliferative signals. Farnesylation, the attachment of a 15-carbon farnesyl moiety by farnesyl protein transferase (FPTase), or Ras

is obligatory for Ras-mediated effects as it facilitates membrane localization. Preclinical studies evaluating farnesyl transferase inhibitors (FTIs) have confirmed these compounds have antiproliferative effects in vitro and in vivo and even possess radiation-sensitizing activity. A phase I/II study evaluating the combination of radiation and an FTI in advanced pancreatic cancer is currently under way.

Summary

Until very recently, patients with pancreatic cancer were allowed little optimism. Although radiation and concurrent 5-FU continues to represent the standard of care, we must now consider the increasing number of regimens incorporating novel chemotherapeutic agents and gene therapy-based treatment strategies. Today, the treatment of locally advanced pancreatic cancer requires a multimodality approach that includes input from the surgeon, radiation oncologist, and medical oncologist, as well as a clear understanding of the interactions between each modality.

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Cytotoxic Chemotherapy for Pancreatic Cancer: Past, Present, and Future

Robert A. Wolff

Introduction

For over 30 years, reports of cytotoxic therapy for pancreatic cancer have appeared in the medical literature. In the earliest trials of systemic therapy, published response rates were encouraging and spurred interest in chemotherapy for advanced disease, with particular emphasis on combination therapy. The explanation for the apparent early success with cytotoxic therapy may be attributed to the lack of accurate imaging modalities. In the 1960s through the early 1980s, objective responses to therapy relied heavily on liver–spleen scans, ultrasonography, and physical examination, since cross-sectional body imaging with computed tomography (CT) was not widely available.^{1,2} This led to a general overestimate of response to therapy without demonstrable impact on survival. More recent experience with cytotoxic chemotherapy has led to marginal advances in the treatment of pancreatic cancer. These advances represent the first small steps toward improved outcomes for patients with pancreatic cancer.

Past Experience

Fluorouracil and Fluorouracil Modulation

Previous cytotoxic therapy for pancreatic cancer has been based on the use of 5-fluorouracil (5-FU) either as a single agent or as part of combination therapy. In an often cited review article published in 1975, Carter and Comis³ reported their experi-

ence with chemotherapy for pancreatic cancer. At the time, 5-FU was the most extensively studied chemotherapeutic agent. The authors described the results of 15 separate trials of 5-FU in the treatment of pancreatic cancer. The trials included as few as 4 patients to as many as 50 patients with the median number being 11 patients per trial. Objective responses were based on physical examination and ultrasound. Liver–spleen scans were also employed in some cases. Response rates ranged from 0% to 67%. Using the compiled data, they reported an overall response rate to 5-FU of 28%, which they believed to be an overestimate of the drug's activity. They attributed the wide variation in response to patient selection, criteria for response, and the small patient numbers in uncontrolled studies.

Subsequent investigations of 5-FU using leucovorin as an activity-enhancing modulant have been disappointing. Prospective clinical trials in patients with advanced pancreatic cancer have evaluated high-dose leucovorin (500 mg/m²) administered daily or as a continuous infusion for 6 days in combination with 5-FU.^{4,5} The daily dose of 5-FU ranged from 375 to 600 mg/m². Objective response rates averaged less than 10%, and toxicity was often significant. In a more recent trial reported by Rubin et al,⁶ there were no responses to the Mayo Clinic regimen of 5-FU and leucovorin (5-FU, 425 mg/m², with 20 mg/m² of leucovorin daily for 5 days every 28 days). Other modulators of 5-FU have been tested in pancreatic cancer including *N*-(phosphonoacetyl)-L-aspartate disodium (PALA) and methotrexate. Neither agent appears to provide benefit over 5-FU alone.^{7,8}

Fluorouracil Combinations: FAM, SMF, and the Mallinson Regimen

Interest in 5-FU-based combination therapy began about 20 years ago. The combination of 5-FU, doxorubicin (Adriamycin), and mitomycin C (FAM) achieved notoriety as a regimen with significant activity in advanced disease. In one phase II trial of FAM, 39 patients were treated, and 27 of them had measurable disease, 10 of whom achieved a partial response (37%).⁹ These 10 patients had a median survival time of 12 months, compared with a median survival time of 3.5 months for those patients not responding to treatment. In another trial of FAM, Bitran et al¹⁰ reported similar results, with an objective response rate of 40%. At about the same time, a combination of streptozocin, mitomycin C, and 5-FU (SMF) was also described. This regimen also led to a high response rate (32%–43%) in advanced disease.^{11,12} Larger trials were therefore carried out by the cooperative groups comparing FAM and SMF. The Cancer and Leukemia Group B studied 184 patients and found response rates for FAM and SMF to be 14% and 4%, respectively.¹³ As is often observed, these combinations were much less active within larger patient populations than previously reported. Comparison of FAM to two schedules of SMF, to 5-FU alone, and to 5-FU and Adriamycin confirmed these low response rates.^{14,15}

While FAM and SMF were being developed, Mallinson et al¹⁶ reported on a multi-agent combination regimen of induction therapy with 5-FU, cyclophosphamide, methotrexate, and vincristine followed by maintenance treatment with 5-FU and mitomycin C. The median survival time was 44 weeks for patients who received cytotoxic therapy and only 9 weeks for patients offered supportive care. This led to a three-arm trial conducted through the North Central Cancer Treatment Group. In this trial, 187 patients were randomized to the Mallinson regimen, to 5-FU alone, or to 5-FU, Adriamycin, and cisplatin. Once again, results from this study failed to confirm any significant activity with the Mallinson regimen.¹⁷

Other Agents Previously Tested in Pancreatic Cancer

Most single agents contain little clinically reproducible activity in advanced pancreatic cancer.

These include the topoisomerase II inhibitor etoposide, the methotrexate analogs trimetrexate and edatrexate, and the platinum agents cisplatin and iproplatin.^{18–23} Other agents failing to show activity include fazarabine, diaziquone, mitoguazone, and amonafide.^{24–26} In the 1960s and 1970s, the nitrosoureas methyls lomustine (CCNU) and carmustine (BCNU) were tested for activity as were the alkylators cyclophosphamide, chlorambucil, and mechlorethamine. None of these alkylating agents have any meaningful activity in pancreatic cancer.³ In the mid-1980s, the alkylating agent ifosfamide showed promise as an active agent with a 21% response rate in 29 evaluable patients.²⁷ A subsequent phase II trial involved 25 evaluable patients and delivered ifosfamide as a continuous infusion with mesna. This yielded an overall response rate of 16% with 6 other patients achieving minor responses.²⁸ However, a study from the University of Texas M. D. Anderson Cancer Center did not confirm these favorable results.²⁹ An oral analog of ifosfamide and trophosphamide has also been investigated and is not active.³⁰ In the Comis and Carter report,³ mitomycin C was also reported as having activity in advanced pancreatic cancer with a response rate of 27%, similar to that reported for 5-FU. However, no recent studies using mitomycin C as a single agent have been reported. Other drugs with previously reported high response rates have included the anthracyclines epirubicin and Adriamycin.^{31,32} Unfortunately, reproducible activity with these agents has not been confirmed.³³

In summary, early trials of cytotoxic chemotherapy for advanced pancreatic cancer were initially encouraging, but larger trials failed to confirm activity that had any impact on survival. These trials typically included patients with locally advanced unresectable tumors and patients with metastatic disease. Criteria for response were defined by changes in palpable masses on physical examination, decreases in hepatomegaly, changes on serial liver–spleen scans, and ultrasounds. CT cross-sectional imaging, now considered standard,³⁴ was not widely available nor routinely used. Treatment with drug combinations, predominantly based on 5-FU, showed no significant improvement in survival over single-agent therapy. Recent trials of the past using newer agents have confirmed what many clinicians already knew: pancreatic cancer is very chemoresistant.

Cytotoxic Therapy for Pancreatic Cancer: The Present

The lessons of the past should be remembered as investigators look to improve therapy for pancreatic cancer. At this time, some basic tenets from the past can be applied to ongoing therapeutic trials in pancreatic cancer. First, pancreatic cancer is an inherently resistant neoplasm to conventional cytotoxic therapy, and response rates to therapy should be expected to be below 20%. Phase II trials of cytotoxic therapy should therefore accrue adequate numbers of patients to narrow the confidence limits on response rates.

Second, early studies of cytotoxic therapy demonstrated high objective response rates in an era before CT and magnetic resonance imaging (MRI) became widely available. It helps to read the early literature for insight. In one of the reports describing the activity of the SMF regimen, 19 of the 22 evaluable patients had an abdominal mass as their indicator lesion. These lesions were followed with echograms or delta scans. In the 2 patients with liver metastases, 1 patient was assessed with a liver-spleen scan, and 1 patient was followed up by a physical examination.¹² Although most physicians today continue to believe that technological advances have not replaced the need for thorough physical examination, the limitations of the physical exam in determining response to therapy in this disease should be obvious. Imaging tools now exist to more accurately stage patients with pancreatic cancer in regard to the resection of the primary tumor and the detection of metastatic disease to the liver, lung, and peritoneum. In addition, it is generally accepted that serial assessment of the primary tumor may not truly reflect the activity of cytotoxic therapy.³⁵ Thus, current clinical trial design of cytotoxic therapy should require serial cross-sectional imaging with either CT or MRI and include patients with measurable metastatic disease. Criteria for inclusion in a clinical trial should limit accrual to patients with adequate performance status (Eastern Cooperative Oncology Group [ECOG] score ≤ 2), measurable metastatic disease outside the primary tumor, and no prior cytotoxic therapy for metastatic disease.

Third, prognosis with metastatic pancreatic cancer has been dismal with median survival time

gravitating to 4 months. Although newer agents may induce higher objective responses, this needs to translate into appreciable improvements in survival. Therefore, trials of cytotoxic therapy should report median survival data for patients enrolled in the trial.

Finally, cytotoxic therapy is in fact toxic and may be detrimental to quality of life. Since pancreatic cancer is both debilitating and fatal, caution must be employed when using agents likely to worsen the situation. Thus, agents with potential palliative benefit and an acceptable toxicity profile should be given high priority for study. Current studies of chemotherapy for pancreatic cancer have begun to address the issue of palliation in a more formal way and will be discussed below.

Gemcitabine

The nucleoside analog 2',2'-difluorodeoxycytidine (gemcitabine) has recently demonstrated modest reproducible activity in advanced pancreatic cancer. Gemcitabine must be phosphorylated to its active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. Gemcitabine is an antimetabolite and, when phosphorylated to gemcitabine diphosphate, inhibits ribonucleotide reductase. This action reduces intracellular pools of deoxynucleotides and may impede DNA synthesis. More important, gemcitabine triphosphate can be directly incorporated into DNA, leading to chain termination and cell death.³⁶ In both preclinical and clinical testing, gemcitabine demonstrated activity in solid tumors greater than that of cytarabine.³⁷ This has been explained by its greater lipophilicity, its higher affinity for deoxycytidine kinase, and the greater intracellular retention of difluorodeoxycytidine triphosphate of gemcitabine, or dFdCTP, compared with cytarabine.³⁸ In a phase I study, gemcitabine demonstrated activity in patients with solid tumors.³⁹ Gemcitabine was subsequently used in a multicenter trial of 44 patients with advanced pancreatic cancer.⁴⁰ Objective responses were documented in 5 patients (11%). Of note, the investigators reported frequent subjective symptomatic benefit, often in the absence of objective response. In another phase II trial reported by Carmichael et al,⁴¹ similar results were obtained. In this study, the objective response rate was slightly lower at 6.7%, but, again, the investigators

observed palliative benefits to the therapy with 17% of patients having improvement in performance status and 28.6% having improvement in their pain scores. Importantly, gemcitabine was well tolerated with primary toxicities, including myelosuppression, which was mild, and nausea and vomiting, which were described as modest. Flu-like symptoms and peripheral edema were also observed. Most toxicities were manageable. Based on the observations of subjective improvement with gemcitabine therapy, two subsequent trials of gemcitabine in advanced pancreatic cancer were completed. In both of these trials, clinical benefit response was defined. The parameters for clinical benefit response included improvement in pain scores or analgesic consumption, improvement in performance status, and weight maintenance or weight gain. In one of the trials, gemcitabine was compared with 5-FU in previously untreated patients. Patients treated with gemcitabine achieved a modest but statistically significant improvement in median survival time (5.65 months vs 4.41 months, $P = 0.0025$) compared with those treated with 5-FU. The 1-year survival rate for patients treated with gemcitabine was 18%, but only 2% for those treated with 5-FU. Of note, more clinically meaningful effects on disease-related symptoms were recorded with gemcitabine than with 5-FU (23.8% vs 4.8%, $P = 0.0022$).⁴² Objective response rate to therapy was very low, with gemcitabine producing a response rate of 5.4%. There were no objective responses to 5-FU, confirming its lack of activity as a systemic agent in advanced disease. In a separate trial, the clinical benefit observed with gemcitabine was also documented in patients treated after experiencing disease progression on 5-FU.⁴³ Given the poor prognosis of patients with advanced pancreatic cancer and the low likelihood of objective response, some authorities have advocated using clinical benefit as a primary endpoint in future trials of therapy for pancreatic cancer.^{44,45}

Other Cytotoxic Agents

Currently, several other agents continue to be investigated, including the semisynthetic taxane, docetaxel, and some of the camptothecin analogs. In vitro docetaxel has demonstrated significant activity against human pancreatic cancer cell lines.⁴⁶ In one of the initial clinical trials, Rougier and

coworkers⁴⁷ at the Institut Gustave Roussy reported 5 objective responses (29%) in 17 patients with hepatic metastases. Toxic effects were substantial and included grade 3 or 4 neutropenia in all patients and severe edema in 13%. Three patients discontinued therapy based on edema. Other authors have reported on less impressive results with docetaxel,⁴⁸ even when delivered in conjunction with hematopoietic growth factor support.⁴⁹ Nevertheless, docetaxel continues to be investigated in pancreatic cancer, but, given its toxicity profile, an improved therapeutic index will be necessary for widespread acceptance. Novel analogs of a topoisomerase I inhibitor, camptothecin, have undergone limited evaluation in patients with advanced pancreatic cancer. Topotecan, administered once daily for 5 days or using a 21-day continuous infusion schedule, has been ineffective.^{50–53} Irinotecan, or CPT-11, has also been studied. In a study by the European Organization for the Research and Treatment of Cancer Early Clinical Trials Group, irinotecan was delivered to 34 patients at a dose of 350 mg/m² every 3 weeks; objective partial responses were documented in only 3 patients (8%), with a median survival time of 5 months for the entire group.⁵⁴ Despite these discouraging results, the recognized schedule dependence of these drugs supports further study of topoisomerase I agents using protracted intravenous schedules or daily oral administration. One such orally bioavailable drug is 9-nitrocamptothecin (9-NC), also known as RFS2000.⁵⁵ In a small phase II trial of 9-NC given orally (1–1.5 mg/m²/day, 5 days/week), response rates were reported to be 32%. Caution is required to interpret this result since only 60 of the initial 107 patients enrolled were evaluable for response, and response rates were defined using a number of parameters including serum tumor markers, clinical benefit, or objective radiographic response.⁵⁶ Of interest, 1 patient has survived beyond 44 months. This compound is currently undergoing further clinical evaluation in a large multicenter phase III trial in which patients with advanced pancreatic cancer are randomized to receive either 9-NC or gemcitabine.

At the present time, gemcitabine is considered to be accepted first-line therapy for advanced pancreatic cancer, primarily for its palliative benefit and its modest cytotoxic or cytostatic activity in this highly resistant neoplasm. The future of cytotoxic

therapy is likely to focus on the use of gemcitabine as a foundation to advance therapy.

The Future of Cytotoxic Chemotherapy

Patients with advanced pancreatic cancer suffer with significant symptoms, including pain, fatigue, jaundice, and intestinal dysfunction. Given the poor prognosis, the palliative benefits of cytotoxic therapy must continue to be emphasized. However, if progress is to continue, chemotherapeutic trials should also include objective response rates and survival data. Future studies of cytotoxic therapy will most likely focus on improving response and survival rates, but clinical benefit will continue to be a meaningful endpoint. Therapies that have poor tolerance are not likely to be accepted. Since gemcitabine has demonstrated reproducible albeit modest palliative and objective activity in pancreatic cancer with an acceptable toxicity profile, this drug will receive further attention, alone and in combination with other agents.

Gemcitabine Infusion

One area of recent interest in the use of gemcitabine includes improving its therapeutic index. In the early phase II trials of weekly 30-minute infusions of gemcitabine, it was noted that chemotherapy-naïve patients could tolerate higher doses compared with patients who had received prior chemotherapy. However, it was also discovered that simply increasing the dose administered over 30 minutes might not increase cytotoxicity nor improve gemcitabine's therapeutic index. During early clinical trials in patients with solid tumors, gemcitabine triphosphate levels were measured in peripheral blood mononuclear cells and revealed that the rate of gemcitabine phosphorylation, like that of cytarabine, was subject to saturation kinetics.^{39,57} The concentration of gemcitabine triphosphate in the circulating mononuclear cells increased proportionately when the gemcitabine dose was between 35 and 250 mg/m². However, as the gemcitabine dose rose above 350 mg/m², gemcitabine concentration in plasma increased, while intracellular levels of gemcitabine triphosphate did not, suggesting saturation of gemcitabine 5'-phosphate accumula-

tion. The rate of gemcitabine triphosphate accumulation and the peak cellular concentration were highest at a dose rate of 350 mg/m² per 30 minutes (~10 mg/m²/minute), during which steady-state plasma gemcitabine levels of 15 to 20 nmol/L were achieved.⁵⁷ Similar results were found in a pilot trial of gemcitabine in leukemia patients.⁵⁸ A more comprehensive phase I trial in leukemia patients showed that a gemcitabine dose rate of 10 mg/m²/minute achieved a mean steady-state gemcitabine level of 26.5 nmol/L and was sufficient to maximize the rate of gemcitabine triphosphate accumulation in circulating blasts.⁵⁹ The pharmacokinetic relationships documented through these studies suggest that increasing the infusion time while holding the dose rate constant at 10 mg/m²/minute will increase intracellular levels of the active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. Thus, intracellular dose intensification will be achieved. To establish the feasibility of this approach, phase I studies of gemcitabine were conducted in patients with advanced solid tumors whereby dose escalation was achieved by increasing the duration of the weekly gemcitabine infusions while maintaining the dose rate at 10 mg/m²/minute.^{60,61} A subsequent randomized phase II trial in patients with metastatic pancreatic cancer suggested that infusional schedules of gemcitabine (10 mg/m²/minute) may be more effective than the standard 30-minute bolus technique. Preliminary results from this trial demonstrated that compared with 2300 mg/m² over 30 minutes, the 1500 mg/m² dose delivered over 150 minutes (10 mg/m²/minute) led to a higher objective response rate (16.2% vs 2.7%), and a longer median survival time (6.1 vs 4.7 months).⁶² It is anticipated that future clinical studies of gemcitabine as a single agent and in combination with other drugs will focus on the delivery of the drug at 10 mg/m²/minute. However, it remains to be determined whether gemcitabine has a clinically relevant dose-response curve. Although the infusion rate of 10 mg/m²/minute may optimize gemcitabine's conversion to its active metabolites, it is not clear whether delivering maximal tolerated doses of the drug by this schedule is more active than lower doses given at the same dose rate. Based on observations in selected patients, gemcitabine is active in pancreatic cancer at doses substantially below 1000 mg/m² (Figure 27.1).



FIGURE 27.1. Pretreatment (A) and posttreatment (B) computed tomography images of the abdomen from a 78-year-old man who presented with a large pancreatic tail lesion (large arrow) and biopsy-proven adenocarcinoma in a periumbilical nodule (small arrow). The patient was treated with gemcitabine at a dose of 600 mg/m² delivered over 60 minutes (10 mg/m²/min) weekly $\times$ 3, with 1 week off, for a total of four cycles. Of note, therapy was well tolerated with no doses withheld for toxicity.

Gemcitabine Combinations with Other Cytotoxic Agents

Given the growing acceptance of gemcitabine as first-line therapy in advanced disease, gemcitabine-based combinations are also under active evaluation.^{63,64} Early trials have combined gemcitabine with 5-FU,^{65,66} cisplatin,^{67,68} docetaxel,^{69–71} paclitaxel,⁷² and epirubicin.⁷³ Randomized trials of gemcitabine versus gemcitabine-based combinations are now being performed. In one study, patients with advanced pancreatic cancer were randomized to receive weekly gemcitabine given at 1000 mg/m² over 7 weeks or gemcitabine at the same dose combined with cisplatin given at 25 mg/m² weekly for 6 of 7 weeks. The overall response rate for the patients receiving gemcitabine alone was 10%, similar to that reported in phase II trials. Those patients receiving

the combination of gemcitabine plus cisplatin had a response rate of 40%.⁶⁸ The results of an ECOG trial comparing gemcitabine with or without weekly bolus 5-FU are awaited with interest.

In the future, a number of gemcitabine combinations will be reported. Many of these trials are likely to demonstrate higher response rates than those historically reported for gemcitabine alone or for other agents. To be convincing, large phase III trials of gemcitabine combinations will need to be compared with single-agent gemcitabine with improved survival time as the primary endpoint. As discussed previously, it is expected that administering gemcitabine at a dose rate of 10 mg/m²/minute may enhance its activity and, in fact, lead to lower doses of gemcitabine given in combination with other cytotoxic drugs. Thus far, minimal attention has been paid to this concept.

Cytotoxic Therapy in Combination with Novel Agents

Although gemcitabine has demonstrated modest activity in pancreatic cancer, median survival time for patients with metastatic disease continues to be less than 6 months. While development of alternative gemcitabine schedules and combinations continues, investigation of novel agents alone or in combination with traditional cytotoxic therapy should receive priority. The foundation of such therapies stands on the recent elucidation of molecular events implicated in pancreatic carcinogenesis, chemoresistance, and metastases. Inhibition of receptor tyrosine kinases exemplifies this concept and is described below.

Trastuzumab (Herceptin)

The HER2/neu oncoprotein is a receptor tyrosine kinase that transduces growth-promoting signals. Binding the HER2/neu oncoprotein with specific antibodies leads to growth inhibitory signaling and promotes apoptosis. Trastuzumab (herceptin) is a humanized monoclonal antibody developed in mice that binds the HER-2 receptor. In preclinical studies, herceptin led to growth inhibition in cell lines overexpressing HER2/neu. In combination with most cytotoxic agents, herceptin appears to be synergistic. Clinical trials in patients with metastatic breast cancer whose tumors overexpress HER2/neu demonstrated activity of herceptin as a single agent, with objective response rates ranging from 11% to 26%.^{74,75} In a subsequent landmark trial by Norton et al,⁷⁶ response and survival rates were both improved in women receiving both herceptin and paclitaxel compared with paclitaxel alone for metastatic breast cancer. (All patients had tumors with overexpression of HER2/neu.)

In pancreatic cancer, 30% to 40% of human tumors overexpress HER2/neu; however, the role of herceptin has not been defined. Currently, a phase II trial of herceptin and gemcitabine is underway in patients with measurable metastatic pancreatic cancer.

Epidermal Growth Factor-Receptor (EGF-R) Monoclonal Antibody (C225)

Antibodies to EGF-R have been shown to compete with EGF and transforming growth factor α (TGF-

α), the growth stimulatory ligands for binding to this receptor. Binding with specific antibodies leads to growth inhibition and, in some cases, to apoptosis. Recently, a humanized monoclonal antibody to EGF-R (C225) has demonstrated potent competitive binding to the receptor leading to growth inhibition.⁷⁷ In vitro studies suggest an additive effect of C225 with cytotoxic agents. However, data from laboratories at The University of Texas M. D. Anderson Cancer Center suggest a synergistic interaction in animal models of metastasis when gemcitabine and C225 are combined.⁷⁸ A phase II trial studying the toxicity and efficacy of gemcitabine combined with C225 is now beginning for patients with advanced pancreatic cancer.

Summary

Progress in the treatment of pancreatic cancer has moved at a glacial pace. Continued investigation of conventional cytotoxic agents is warranted and may result in better palliation and survival. Efforts to improve therapy should concentrate on further elucidation of the molecular mechanisms of invasion, metastases, and resistance. It is expected that as therapy for metastatic disease improves, treatment for patients with resectable and locally advanced disease will also evolve.

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28

Animal Models of Pancreatic Adenocarcinoma

Ramon E. Jimenez, Andrew L. Warshaw, and Carlos Fernandez-del Castillo

Introduction

Pancreatic adenocarcinoma is a poorly understood disease that ranks fifth among the leading causes of cancer mortality.¹ Precursor lesions remain largely unknown, and metastases are common at the time of presentation for most patients. Surgery provides the only chance for cure, but only a minority of patients are candidates for such treatment. Overall, median survival time after diagnosis is 6 months, and minimal improvement has been observed within the past 30 years.² Clearly, a better understanding of the biology of adenocarcinoma of the exocrine pancreas is needed as a foundation for more successful treatment modalities.

Perhaps one of the reasons for the slow progress in the medical management of pancreatic cancer is a lack of appropriate animal models. Relevant animal models of pancreatic cancer must re-create the major characteristics of this disease in humans. In general, these characteristics include the following: (1) a ductal phenotype and presumed cell of origin; (2) intense desmoplastic reaction; (3) disease progression resulting in biliary and gastric obstruction; (4) aggressive disease characterized by perineural invasion and early metastases to the peritoneum and liver; and (5) frequent mutations of the *K-ras*, *p16*, and *p53* genes among others.³ In this chapter, we describe most of the existing animal models of pancreatic cancer (Table 28.1). An evaluation of each model ultimately depends on how accurately it reproduces the biology found in human disease.

Chemical Carcinogenesis

Azaserine

The azaserine-induced cancer model in rats was discovered and extensively characterized by Daniel S. Longnecker.⁴ Carcinogenesis is induced by intraperitoneal injections of azaserine administered to young (2-week-old) rats. A variety of pancreatic lesions are generated, ranging from adenomas to invasive carcinomas. In general, carcinomas are found in 40% to 50% of animals autopsied at 18 months. Neoplasms have produced biliary obstruction when occurring in the head of the pancreas, and metastases have been described to periaortic lymph nodes, liver, and lungs. Transplantable tumors have been grown in Lewis rats, and multiple cell lines are now commercially available.

The major drawback of the azaserine-induced cancer model is that it results in acinar-cell carcinomas, as opposed to the ductal phenotype of more than 90% of tumors in man.⁵ No hyperplasia or dysplasia is found within pancreatic ducts, and precursor lesions, such as tubular complexes, are only rarely found. In addition, mutations in the *K-ras* or *H-ras* oncogenes have not been identified.⁶ Interestingly, one of the cell lines developed from these tumors (DSL-6A) has undergone metaplastic transformation and produces ductlike structures surrounded by fibrous tissue upon transplantation.⁷ These findings are thought to suggest that ductlike carcinomas can arise from neoplastic acinar cells.

TABLE 28.1. Animal models of pancreatic carcinoma.

Chemical carcinogenesis
Azaserine (rats)
Dimethylbenzanthracene (rats)
Nitrosamines (hamster)
Orthotopic models in nude mice
Transgenic mice

Dimethylbenzanthracene

In 1975, dimethylbenzanthracene (DMBA) was originally shown to induce pancreatic adenocarcinomas in rats.⁸ Carcinomas with a ductal phenotype and their precursor lesions, known as tubular complexes, were noted to occur after DMBA exposure. Most of the initial characterization of tumors induced by DMBA was done by Dale E. Bockman.⁹ His evaluation consisted of plain histology, electron microscopy, and a complex series of morphological studies involving three-dimensional reconstructions after silicone injection into the pancreatic ducts. Bockman concluded that DMBA tumors were not ductal in origin but resulted from “dedifferentiation” of acinar cells, a process involving halting of cellular synthesis and zymogen granule disappearance. Through dedifferentiation, acinar cells were thought to take on the appearance of ducts (tubular complexes), which then underwent neoplastic transformation.¹⁰ The presumed acinar cell of origin of DMBA rat tumors led to a loss of interest as a model of human pancreatic cancer.

The DMBA model has recently resurfaced due mostly to our studies at the Massachusetts General Hospital Pancreatic Research Laboratory. Pilot studies testing three different carcinogens, administered by either parenchymal implantation or intraductal infusion, demonstrated that only DMBA reliably induced pancreatic adenocarcinomas.¹¹ DMBA requires surgical implantation into the head of the pancreas. Approximately 40% of animals show tumors at 9 months. A high-fat, high-protein diet has been shown to increase the yield of adenocarcinomas.¹² Grossly, these tumors cause biliary and gastric outlet obstruction resulting in jaundice and weight loss (Figure 28.1). Copious mucin secretion is often noted, which contributes to the large size of some of these masses. Six percent of animals demonstrate peritoneal dissemination. Histologically, tumors show a ductal phenotype with

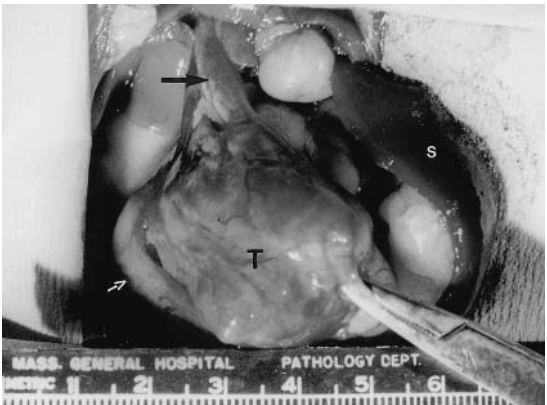


FIGURE 28.1. Gross photograph of dimethylbenzanthracene-induced adenocarcinoma in a rat. Note common bile duct dilatation and obstruction from mass effect. T indicates tumor in head of the pancreas; S, spleen; black arrow, common bile duct; and white arrow, duodenum.

irregular invasive glands within a dense desmoplastic stroma (Figure 28.2). Importantly, changes consistent with ductal hyperplasia, dysplasia, and carcinoma-in-situ can be found, suggesting a gradual transition from normal epithelium to invasive carcinoma.¹¹

To further characterize DMBA-induced neoplasms, we studied the expression of ductal and acinar cell markers in these tumors using immunohistochemical techniques.¹³ Carcinomas showed strong expression of ductal cell markers (cytokeratins 19 and 20) and no expression of acinar cell

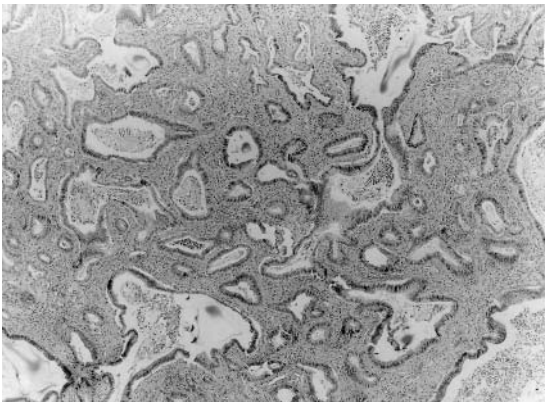


FIGURE 28.2. Ductal pancreatic adenocarcinoma induced by dimethylbenzanthracene in rats. Note the presence of invasive, irregular neoplastic glands within a desmoplastic stroma (hematoxylin-eosin stain).

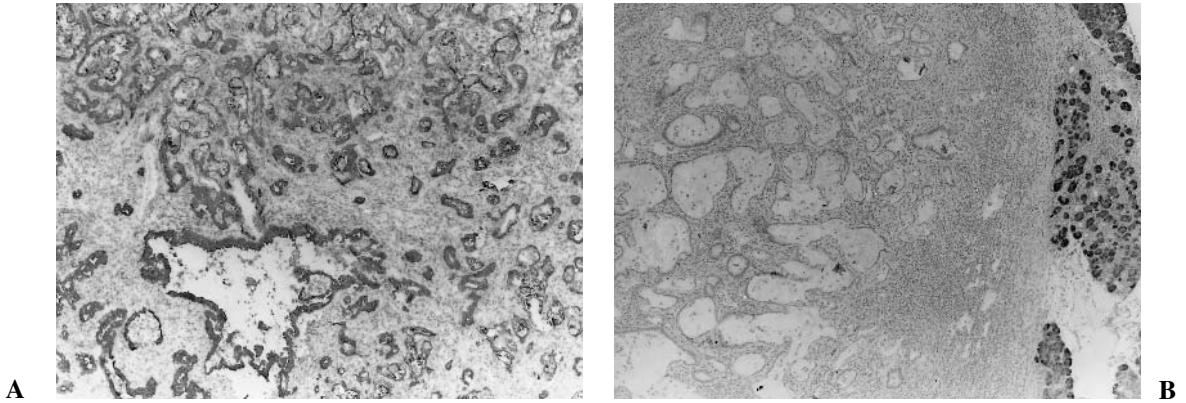


FIGURE 28.3. Ductal and acinar cell markers in dimethylbenzanthracene-induced pancreatic adenocarcinomas. A, immunohistochemical stain with antibodies against cytokeratin 19 (ductal cell marker) shows labeling of neoplastic epithelium. ($\times 100$). B, immunohistochemical stain with antibodies against chymotrypsin (acinar cell marker) shows no labeling within tumor tissue (left), but strong labeling in adjacent normal pancreas (right) ($\times 50$).

markers (chymotrypsin), a pattern identical to that found in human pancreatic ductal adenocarcinomas (Figure 28.3). Moreover, we found the same prevalence of ductal markers in precursor lesions (tubular complexes) as early as 2 weeks after DMBA implantation, without any evidence of acinar cell protein expression (Figure 28.4). These findings challenge Bockman's results, which suggested an acinar cell of origin and indicated that DMBA neoplasms may indeed be of ductal origin.

Some of the genetic mutations present in DMBA-induced carcinomas have also been stud-

ied. Using fine microdissection techniques combined with a very sensitive, nested polymerase chain reaction assay, we investigated the prevalence of *K-ras* and *H-ras* mutations in these tumors.¹⁴ The data revealed that more than 90% of tumors carry *K-ras* mutations, while maintaining a wild-type *H-ras* gene. Additional experiments, which searched for *p53* mutations by immunohistochemistry, have shown negative results.

DMBA carcinogenesis remains the only model of pancreatic ductal adenocarcinoma in the common laboratory rat. At the present time, major lim-

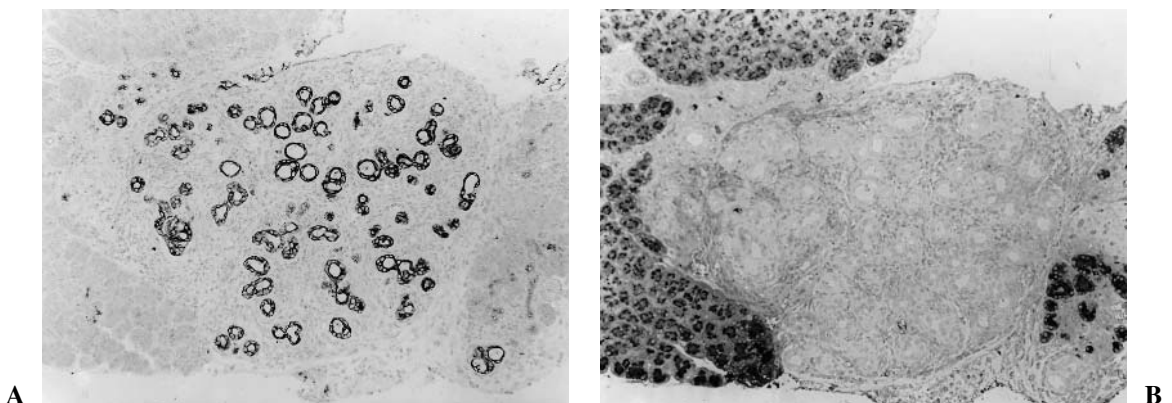


FIGURE 28.4. Ductal and acinar cell markers in precursor lesions of dimethylbenzanthracene pancreatic adenocarcinomas. A, immunohistochemical section of tubular complex stained with antibodies against cytokeratin 19 (ductal cell marker) demonstrates strong labeling of preneoplastic gland epithelium. B, staining of similar section with antibodies against chymotrypsin (acinar cell marker) shows absence of labeling within tubular complex, but positive labeling in surrounding normal pancreas ($\times 100$).

itations of the model include relatively low tumor yields and a long latency period before the appearance of malignancy. As opposed to other models, no stable transplantable tumors or cell lines are currently available. These are topics of ongoing research at our institution.

Nitrosamines

Pioneering work by Parviz M. Pour et al¹⁵ demonstrated that a variety of nitrosamines could induce pancreatic adenocarcinomas in Syrian golden hamsters. The most potent of these agents, *N*-nitrosobis(2-oxopropyl)amine (BOP), is administered by subcutaneous injection and causes pancreatic neoplasia in greater than 75% of animals autopsied at 1 year.¹⁶ Carcinogenesis can be accelerated by cycles of "augmentation pressure" (ethionine plus a choline-deficient diet) following exposure to BOP, resulting in high tumor yields by 10 weeks.¹⁷ Representative tumors extend locally to invade the stomach and peritoneum, and metastases to lymph nodes, liver, and lungs may occur. Transplantable tumors as well as cell lines have been developed,^{18,19} and orthotopic transplantation of neoplastic cells can be used to produce a "rapid" version of the model.²⁰ Orthotopically transplanted cell lines retain the differentiation and growth features of their parent tumors.

Histologically, BOP-induced neoplasms display a ductal phenotype. Carcinoma-in-situ is observed in the epithelium of medium- and large-sized ducts, and well-differentiated carcinomas frequently show copious mucin secretion.¹⁶ In addition, precursor lesions and carcinomas express ductal cytokeratin markers, confirming their ductal phenotype.²¹ Despite the preponderance of ductal features, Pour and Schmied²² believe these tumors arise from cells within the islets of Langerhans. These previously described studies raise significant questions about the contribution of the endocrine pancreas to neoplastic transformation.

Several studies have now conclusively demonstrated that the majority of BOP-induced neoplasms have mutated *K-ras* genes.^{23,24} These mutations appear clustered at codon 12, as is the case in *K-ras* mutations found in humans. In contrast, mutations in the *p53* gene are rarely found in primary tumors, but have been observed in several cell lines.¹⁸ Mutations of the *p53* gene are currently believed to be

late events associated with tumor transplantation and cell culture establishment.

The BOP-induced model of pancreatic carcinoma is the best characterized and most widely used model of pancreatic carcinogenesis. It is easy to reproduce and results in tumors bearing a close resemblance to their human counterparts. Criticisms have centered mostly around the lack of selective targeting of the pancreas, with synchronous induction of malignancies in other organs, such as the liver and bile ducts.²⁵ Hamster neoplasms are also less aggressive than human tumors, remaining mostly well-differentiated and showing low frequency of metastases.

Orthotopic Models of Pancreatic Cancer in Nude Mice

Following the first report of successful transplantation of human colon adenocarcinoma in nude mice by Rygaard and Povlsen,²⁶ multiple investigators have used this model to study cancer biology and response to treatment. The athymic or nude mouse is deficient in T-cell function and as such is incapable of rejecting foreign tissue transplants. Nude mice technology facilitated the establishment of human tumor tissue banks from which cancer cell lines could be developed. Currently, multiple human pancreatic cancer cell lines are commercially available (eg, the American Tissue Culture Collection, Rockville, MD) that can be used in nude mice models. The diversity of cell lines includes well- to poorly differentiated tumors, and lines derived from primary or metastatic tumors can be found.

Heterotopic transplantation of pancreatic cancer can be easily achieved by implantation of tissue or cell lines in the subcutaneous tissues of nude mice. Tumors usually show extensive local growth but rarely metastasize, in contrast to pancreatic cancer behavior in humans.²⁷ Although heterotopic transplantation models bear minimal resemblance to human disease, they have been instrumental in the study of genetic events involved in pancreatic carcinogenesis. Dissemination of tumors in nude mice allows enrichment in neoplastic cells and elimination of stromal cells, facilitating DNA analysis in neoplastic tissues. The absence of contaminating stromal cells has also allowed identification of al-

elic losses and homozygous deletions, which would not have been detectable in primary tumors. Hahn and colleagues²⁸ at Johns Hopkins Hospital have exploited this system to describe new genetic mutations found in human pancreatic tumors.

To circumvent the shortcomings of subcutaneous growth of pancreatic tumors, orthotopic transplantation models have also been developed.²⁹ Some models involve injection of tumor cell suspensions directly into pancreatic tissue using a fine 30-gauge needle.²⁷ These models are technically demanding given the thin and fragile nature of the mouse pancreas and, in many instances, result in intra-abdominal spillage of tumor cells. Improved models of orthotopic implantation have used solid tissue fragments grafted directly into the pancreas.^{30–32} These models have shown take rates of 66% to 100% approximately 1 month after tumor implantation. When grown in the mouse pancreas, human tumors preserve their histologic appearance and maintain expression of tumor markers (Figure 28.5). Depending on the tumor, a desmoplastic reaction may or may not be reproduced. Lymphatic, hematogenous, and peritoneal metastases have been observed, albeit with less frequency than in human neoplasms. However, perineural invasion has been mostly absent.³¹

Xenograft models of pancreatic carcinoma have been useful in the study of tumor behavior in vivo. However, several factors have emerged as real weaknesses inherent in these model systems. Nude

mice models ignore the contribution of the host's immune system in tumor growth modulation. Interactions between cancer cells and the immune system are currently the focus of intense research in the development of potential vaccines against pancreatic cancer. Other significant drawbacks are related to the number and frequency of genetic alterations found in human primary tumors versus those found in tumors perpetuated in nude mice. Tumor cell selection inevitably occurs during xenotransplantation and may introduce bias towards specific mutations that confer growth advantage in mice. By this process, particular mutations may turn out to be overrepresented in nude mice models, as has been noted for *p53* and *p16* genes.^{31,33}

Last, advanced passages of tumors or cell lines in nude mice must be analyzed with caution. Multiple studies have demonstrated increasing genetic instability developing with repeated tumor passages, with the appearance of new mutations not necessarily present within primary tumors.³⁴ In general, only early passages of transplantable tumors can be considered to retain the major characteristics of their tumors of origin.

Transgenic Mouse Models

Most of the transgenic mouse models of pancreatic neoplasia have been created by Ralph L. Brinster. These models utilize the elastase-1 promoter to tar-

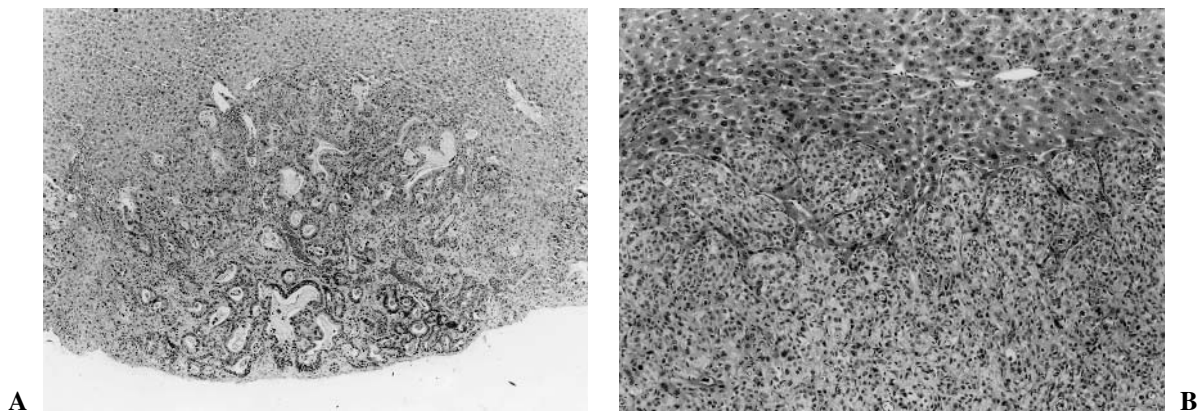


FIGURE 28.5. Human pancreatic cancer cell lines preserve their histologic differentiation when grown in nude mice. (A), liver metastasis of well-to-moderately differentiated cell line (Capan 1) forms neoplastic glands (tumor in lower half of photomicrograph). (B), liver metastasis of poorly differentiated cell line (AsPC-1) shows solid tumor nest without gland formation (tumor in lower half of photomicrograph) ($\times 150$).

get expression of oncogenes or growth factors to the pancreas.³⁵ Transgenic mice models have been mostly of interest in the study of neoplastic transformation and tumor progression in pancreatic tissue, but have not been used to any extent in treatment trials.

Some of the first transgenic mice models created carried the elastase-1 promoter linked to the SV40 early genes (referred to as ELSV mice).³⁶ ELSV mice develop acinar cell dysplasia and hyperplasia that progress to acinar cell carcinoma. No preneoplastic or neoplastic lesions occur in pancreatic ducts. Carcinomas appear between 3 and 8 months of age, and their acinar cell differentiation is reminiscent of azaserine-induced tumors in rats. Curiously, a number of islet cell tumors have also been observed in certain strains of these animals.

Other transgenic mice have been designed to target *H-ras* or *myc* overexpression to the pancreas. Expression of *ras* in acinar cells induces massive acinar hyperplasia in the fetal pancreas, leading to neonatal death.³⁷ In contrast, transgenic mice with pancreatic *myc* expression show acinar cell hyperplasia followed by acinar cell carcinomas.³⁸ Interestingly, up to half of these acinar cell tumors show areas of ductal differentiation with associated desmoplasia. These ductal areas remain limited in comparison to acinar elements, and no pure ductal carcinomas have been observed. Observations in the *Ela-1-myc* model are often quoted in discussions about the potential of transformed acinar cells to generate ductal neoplasms.

Recent excitement has centered around the transforming growth factor α (TGF- α) transgenic mouse (EL-TGF α). Initially described in 1990, the model was thought to be nontumorigenic in the pancreas.³⁹ However, multiple changes were noted in the pancreatic parenchyma, including acinar cell hyperplasia, formation of ductal tubular complexes, and formation of tremendous interstitial fibrosis. Reevaluation of these animals during the past 3 years has revealed that a very small percentage of them (6%) develop pancreatic papillary to cystic tumors when observed for greater than 280 days.⁴⁰ Malignant transformation occurs within dysplastic tubular complexes that are thought to originate from acinar cells that subsequently gain ductal features. Tumors express ductal markers, such as carbonic anhydrase, and overexpress the epidermal growth factor receptor and *p53*. Unfortunately,

these tumors lack *K-ras* mutations. For the most part, these experiments have reopened debates on a possible acinar cell origin for carcinoma of the exocrine pancreas.

The major drawback of current transgenic mouse models of pancreatic carcinoma is that genetic alterations are targeted to acinar cells as a result of their elastase-promoter design. Improved models will need to focus on concentrating genetic abnormalities within ductal cells, as seems to be the case in human neoplasms.

Conclusion

Multiple animal models for pancreatic carcinoma are currently available, each with its own strengths and weaknesses. From a biological standpoint, these models have raised important questions about the cell of origin of pancreatic exocrine cancer and have helped elucidate some of the genetic changes involved in carcinogenesis. In addition, the models provide systems on which to test new anticancer agents and study cancer cell-host interactions. In the near future, animal models may be instrumental in the development of vaccines or gene therapies that may improve the outcome of patients with pancreatic carcinoma.

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29

Strategies for Gene Therapy

Eugene A. Choi and Francis R. Spitz

Introduction

Due to pancreatic cancer's local aggressive nature, high metastatic potential, and late symptom presentation, approximately 80% of pancreatic cancer cases are unresectable at the time of diagnosis. The 5-year survival rate is approximately less than 3%, despite advances in chemotherapy and surgical and radiation treatment.^{1,2} With such dismal outcomes, novel treatment modalities are being investigated. The recent developments of molecular biology, the mapping of the human genome, and discoveries in cancer biology have made gene therapy a potential treatment modality for pancreatic cancer. This chapter will present a brief description of the gene delivery systems and a broad overview of the strategies of cancer gene therapy in general. We will present a review of gene transfer *in vitro* and *in vivo* studies as it relates to pancreatic cancer. The strategies discussed will include immunotherapeutic gene therapy, suicide gene therapy, and replacement/inactivation of tumor suppressor genes and proto-oncogenes evaluated against pancreatic cancer.

Gene Therapy Vectors

Viral Vectors

Gene therapy involves the transfer of genetic material into target cells. The process of transfer can be done through several delivery systems, which include viral vectors, cationic liposomes, and protein-conjugated DNA, either *in vivo* or *ex vivo*. The

major challenge in the field of gene therapy is the development of an efficient and safe vector system. Each particular viral vector has advantages and disadvantages, and the preference of a particular vector is based on various factors such as the nature of the disease, the size of the transgene, and the desired period of time for gene expression (Table 29.1). Retroviruses deliver genes in the range of 10 kilobases (kb) to dividing/proliferating cells and complete stable integration into the host genome with stable expression.^{3,4} Moreover, the virus has broad tropism that allows targeting of different tissue. The application of retroviruses is not 100% predictable; there is a theoretical possibility of random insertion of viral genome causing mutagenesis and the development of a replication-competent virus by homologous recombination events. Furthermore, the retrovirus vectors have low transduction efficiencies.

Lentiviruses behave similarly to the retroviruses, but the major advantage of the HIV-based vector is their ability to infect nondividing cells.⁵ Studies have demonstrated efficient transduction and expression of the transgene in terminally differentiated tissue, including neurons and macrophages.⁶ Lentiviruses can carry large DNA inserts (10 kb) to a wide range of tissue. Because the lentivirus is an HIV-1-based vector, there is the possibility of recombination events that may result in genetic mutations via random host integration in dividing as well as nondividing cells.

The adenovirus can be produced in very high titers and can facilitate the transfer of large DNA inserts (7–8 kb). The adenovirus can also infect nondividing cells and produce a high-level expres-

TABLE 29.1. Gene therapy vectors in current application: advantages and disadvantages.

Gene therapy vectors	Advantages	Disadvantages
Retrovirus	Delivers large DNA segments (10 kb) Stable integration into host genome Stable transgene expression Available in high titers	Mutagenesis Possibility of replication-competent virus
Adenovirus	Delivers large DNA segments (8 kb) Infects dividing and nondividing cells No integration into host DNA Available in high titers	Transient transgene expression Activation of immune response
Adeno-associated virus (AAV)	Stable integration into host genome Stable transgene expression	Can only transfer small DNA segments High titers of pure virus difficult to obtain Mutagenesis Mutagenesis
Lentivirus	Infects dividing and nondividing cells Stable integration into host genome Stable transgene expression	
Cationic lipid/DNA complexes	Delivers large DNA segments Broad tropism Relatively low toxicities No viral vector-associated complications	Relatively low transduction efficiencies Relatively low transgene expression
DNA/protein ligand/receptor	Target specificity No viral vector-associated complications	Relatively low transduction efficiencies Relatively low transgene expression Limited application

sion of the transgene.^{7,8} In addition, the delivered transgene does not undergo native DNA integration and thus gene expression is transient. One potential drawback of adenovirus delivery is the acute activation of the host nonspecific immune system. In addition, neutralizing antibodies may develop after the initial administration of the vector, limiting effective readministration.^{9–11}

Adeno-associated viruses (AAV) have been used less frequently than retroviruses and adenoviruses because high titers of pure virus are difficult to obtain and this vector can only transfer small genes (4 kb). In addition to the low titers, AAV vectors have low transduction efficiencies. AAV infection results in the stable site-specific integration into the cell genome with long-term expression, extending into months in dividing and nondividing cells.¹² The virus is stable at room temperature and is relatively nonpathogenic. There have been recent advances in AAV production, resulting in more consistent and higher titer production of the vector. This may ultimately lead to wider use of this vector system.

Cationic Lipid/DNA Complexes

DNA material can also be delivered through DNA-liposome complexes. Polycationic lipids can be delivered systemically, intraperitoneally, or through

direct tissue injection. The DNA-liposome complexes can deliver large segments of DNA, and have relatively low toxicities. Moreover, the complex has broad tropism. Experiments using DNA-liposome complexes in vivo delivered through the intra-arterial system demonstrated universal transfection of heart, lung liver, kidney, spleen, and skeletal muscle.¹³ Transduction efficiency is relatively low, and expression is transient.

DNA/Protein Ligand/Receptors

Protein-conjugated DNA utilizes specific receptors on cell surfaces. DNA has been conjugated to a carrier with a ligand domain for a specific receptor and a DNA-binding domain (poly-L-lysine peptide). The ligand domain binds to the receptor, activating internalization of the extracellular receptor/ligand complex through endocytosis. Using the ligand-DNA complex, Wu et al¹⁴ first demonstrated gene transfer and expression in hepatoma and liver cells by using asialo-orosomucoid protein as the ligand for the asialoglycoprotein receptor. The major disadvantages are the low transduction and expression efficiencies. The plasmid DNA may not be bound in a conformation optimal for endocytosis and, once inside the cell, may not leave the endosome efficiently to enter the nucleus for replication and transcription.

Naked DNA may also be used without a carrier protein, a liposome complex, or viral delivery vectors. Studies have demonstrated that injection of DNA plasmid into regenerating muscle can result in stable transduction with limited toxicities.¹⁵

In Vitro and In Vivo Gene Transfer to the Exocrine and Endocrine Pancreas

There have been many recent studies utilizing various strategies and delivery systems that have demonstrated gene transfer to the pancreas or pancreatic cancer cell. All this work has been accomplished using in vitro cell culture and/or in vivo animal models. Experiments demonstrating effective gene transfer to pancreatic tissue have been a catalyst for the application of gene therapy for the treatment of pancreatic cancer. A majority of the studies have involved the use of adenovirus. Leibowitz et al¹⁶ examined factors such as hepatocyte growth factor/scatter factor and cell dispersion that may affect the gene transfer efficiency of human fetal and adult pancreatic endocrine cells with adenovirus, retrovirus, and lentivirus vectors containing the green fluorescent protein. Results demonstrate the adenovirus vector delivery had the greatest percentage of transduction with high transient production of the transgene. In addition to the islet cells, other studies have demonstrated gene transfer to acinar cells and ductal epithelia of the pancreas.^{17,18} O'Brien et al¹⁹ delivered an adenoviral vector containing the β -galactosidase gene at doses of multiplicity of infections (MOI) of 0, 10, 100, and 1000 plaque-forming units (pfu) per rat islet cell. Results demonstrated that the efficiency of gene transfer is greatest between Days 1 and 4, and dependent on MOI. At an MOI of 1000, there was a 58.6% transduction rate, compared with 34.1% and 8.3% at MOIs of 100 and 10, respectively. Toxicity was assessed by measuring insulin levels in conditioned medium from transduced islet cells at each time point, as well as measuring the release of insulin in response to high-glucose concentrations. Insulin levels from both assays are comparable between nontransduced and transduced cells, suggesting limited toxicity to the islet-cell function.

Gene transfer to the pancreas has also been successful with polycationic liposomes. Saldeen et al²⁰ demonstrated in vitro that among the different delivery methods, including calcium phosphate pre-

cipitation, the monocationic liposome Lipofectin, the polycationic liposome Lipofectamine, and adenovirus-polylysine DNA complexes, polycationic liposomes are the most efficient in the transduction of islet cells of humans, mice, and rats. Lakey et al²¹ also demonstrated that the transduction efficiency of islet cells isolated from the canine pancreas with different lipid-based transfection reagents are different, and some liposome mixtures may blunt the islet-cell insulin response after DNA transduction. Cells were infected with a plasmid vector containing the *Escherichia (E) coli* β -galactosidase gene (pCMV β gal) with varied DNA/liposome ratios. The highest expression of β -galactosidase varied with the specific liposome reagent, and peaked after 48 hours of incubation. Lipofectamine, Dotap, and Dosper required 1:1, 1:5, and 1:2 ratios, respectively. Lipofectamine and Dosper blunted islet-cell insulin response during static glucose incubation after infection. This group also demonstrated that the efficiency of liposome transduction is also dependent on the DNA concentration in the liposome mixture.

In vivo, Schmid et al²² delivered a replication-deficient adenovirus vector containing the reporter *E. coli* β -galactosidase gene with a Rous sarcoma virus (RSV) long-terminal repeat promoter mixed with liposomes directly via the celiac trunk and common pancreatic duct of Wistar rats. Tissue sections of the pancreas were obtained from animals sacrificed at 5, 14, and 28 days after gene transfer. The histological data demonstrated that the intra-arterial injection resulted in the transduction of the vessels of the pancreas as well as a few acinar cells, and intraductal injection resulted in expression of β -galactosidase in the cells lining the pancreatic duct. Although serum amylase levels were slightly elevated compared with controls, there was no histological evidence of pancreatitis.

Vickers et al¹⁷ have demonstrated gene transfer of the human exocrine pancreas using an adenoviral vector. The authors used a human pancreas procured for transplantation, but subsequently found unsuitable, and a heat-controlled organ procurement perfusion system. A recombinant adenovirus vector containing the β -galactosidase gene driven by the cytomegalovirus (CMV) promoter was injected into the distal pancreatic duct via a balloon occlusion catheter at a concentration of 1×10^{10} pfu/mL. X-gal staining was performed on pancreatic tissue sections. Results of the chromogenic

substrate assay demonstrated efficient adenovirus transduction of the pancreatic ductal cells. Using a similar adenovirus vector containing the β -galactosidase gene, Padfield et al¹⁸ have shown in vitro gene transfer of primary culture of both isolated pancreatic acini and individual acinar cells. The results demonstrated approximately 100% infection and β -galactosidase expression when the acinar and acinar cell suspension were infected with 5×10^6 and 1×10^6 pfu of virus, respectively. Beginning at 6 hours, β -galactosidase expression increased, and peaked at 20 hours in this study.

Adenovirus vector has been demonstrated to be an effective delivery system for the pancreas. The disadvantage is the immune response against the adenovirus. Using a murine in vivo model, McClane et al²³ demonstrated that the immune response was partially directed at the early viral gene antigens 2 and 4. Adenovirus constructs containing early gene deletions were associated with longer β -galactosidase expression (approximately 28 days). β -galactosidase expression is also longer in immunodeficient mice. Adenovirus vector injected directly into the pancreas can elicit a local inflammatory response characterized by edema and cell necrosis and inflammatory cell migration, as well as a decrease in amylase production.²⁴ The administration of adenovirus leads to the formation of neutralizing antibodies that prevents additional effective dosing of adenovirus vector.

Therapeutic Approaches

Immunotherapeutic Gene Therapy

The basic principle of immunotherapeutic gene therapy is to bolster the immune system to help recognize the malignant transformed cell, which originally evaded the immune system. Initial immunotherapeutic strategies utilized vaccination with unmodified tumor cells. More recently, autologous tumor cells have been engineered to secrete various cytokines including interleukin 2, (IL-2), IL-4, IL-6, and IL-7; interferon- γ (IFN- γ); tumor necrosis factor (TNF- α); and granulocyte macrophage-colony stimulating factor (GM-CSF).²⁵⁻²⁹ Cytokines are involved in the activation of the immune system. For example, IFN- γ induces the expression of class I and class II ma-

jor histocompatibility complex (MHC) antigens, activates macrophages and the development of cytotoxic CD8+ T cells, and has a possible role in the inflammatory process.²⁷ TNF- α (cachectin) is a cytokine produced by activated macrophages as well as T and B lymphocytes and activates the immune system in response to infections and tumors. Various cell types produce GM-CSF in response to inflammatory stimuli; GM-CSF is a growth factor for granulocyte-macrophage, erythroid and eosinophil progenitors. Ironically, it is known to stimulate the growth of certain tumor cells. Immunotherapeutic gene therapy may not only initiate an innate tumor response, but also initiate long-term memory.

Immunotherapeutic gene therapy has been aggressively used to study animal models of pancreatic cancer. Results have demonstrated that tumor cells engineered to produce cytokines inhibit the growth and proliferation of tumors; the mechanism for this phenomenon is based on short- and long-term activation of the immune system. Kimura et al²⁵ demonstrated the loss of tumorigenicity of human pancreatic carcinoma cells engineered to produce IL-2 and IL-4 in nude mice. AsPC-1, a human pancreatic carcinoma cell, was infected with a retrovirus containing the human IL-2, IL-6, and mouse IL-4 and GM-CSF gene with a lipofectin reagent. The IL-2- and IL-4-expressing clones (1×10^6) were cultured and injected in BALB/c nu/nu mice subcutaneously. Of the animals injected with cells secreting IL-2 and IL-4, small tumors had developed, but subsequently regressed completely. Animals injected with autologous cells secreting IL-6 and GM-CSF demonstrated smaller tumor's compared with controls. In addition, animals injected with cells secreting IL-2 and IL-4 demonstrated no tumor growth after additional injections with the parental pancreatic cancer cells, demonstrating the establishment of long-term immunity against the tumor. The rejection of the pancreatic cancer cells after injection of autologous cancer cells engineered to secrete cytokines suggests a memory response, which was thymus independent. Histological examination of the tumor specimens demonstrated monocyte and macrophage infiltration.

Various studies have also reported the antitumor effect of cell transduction with IFN- γ , TNF- α , IL-6, and IL-7 in addition to IL-2 and IL-4. Clary

et al²⁹ studied the effect of cells secreting IL-2 and IFN- γ against pancreatic cancer, using a novel AAV plasmid-based gene delivery system. Panc02 cells, a highly tumorigenic murine pancreatic cell line derived from a methylcholanthrene-induced tumor in a C57BL/6 mouse, were transfected ex vivo. Uninfected Panc02 cells were subcutaneously injected with 5×10^5 tumor cells and subsequently (3 days later) given weekly injections of irradiated Panc02 cells capable of secreting IL-2, IFN- γ , or both. To demonstrate the long-lasting immunity, animals free of tumor at Day 130 after tumor inoculation were given a single vaccination of Panc02/IL-2 cells and, 3 days later, a subcutaneous injection of Panc02 cells. The results of the study demonstrated treatment with IL-2 and IFN- γ results in smaller tumors or complete tumor regression (40% vs 30%, respectively) compared with controls. Combined treatment with IL-2 and IFN- γ resulted in 79% tumor regression, suggesting a synergistic effect. Moreover, all animals challenged with a reinjection of native Panc02 cells demonstrated no tumor growth, providing evidence of the development of long-term memory (CD4+) T cells against the tumor cells. These results represent exciting progress toward a therapeutic approach in pancreatic cancer. Clearly, a strategy that requires transduction of only a small population of cancer cells has broad potential application.

In addition to cytokine expression in tumor cells, another potential immunotherapeutic strategy against pancreatic cancer is the transduction of DNA-expressing tumor-specific antigens. Pancreatic cancer expresses several antigens, including erbB-2 (HER-2/*neu*), a 185-kilodalton (kDa) transmembrane glycoprotein that is homologous to the epidermal growth factor receptor, carcinoembryonic antigen (CEA), and MUC1, a type I membrane-bound glycoprotein expressed by ductal epithelia. These tumor antigens are all potential targets for immunotherapy.^{30–33}

Directed-Enzyme Prodrug Therapy (Suicide Gene Therapy)

Directed-enzyme prodrug therapy, or suicide gene therapy, involves the delivery of a transgene that converts an inactive nontoxic drug into an active cytotoxic agent. The herpes simplex virus (HSV) thymidine kinase and the *E. coli* cytosine deami-

nase are the two most frequently studied and applied for enzyme prodrug therapies.^{34–36} The HSV thymidine kinase phosphorylates ganciclovir, converting the nontoxic drug into ganciclovir-triphosphate, which is a DNA nucleotide precursor that terminates DNA replication and activates cellular apoptosis. The *E. coli* cytosine deaminase converts a nontoxic antifungal agent, 5-fluorocytosine (5-FC), into a conventional chemotherapeutic agent, 5-fluorouracil, or 5-FU, which is used against various gastrointestinal malignancies. The directed transfer of enzymes to the tumor and neighboring cells permits a high peritumoral concentration of active chemotherapeutic metabolites to potentially avoid the limiting toxicities associated with conventional chemotherapy and dosing. Varying amounts of tumor cell transduction are required for a therapeutic effect; however, 100% transduction of tumor cells is not required for the complete elimination of the tumor. This strategy demonstrates a phenomenon termed the “bystander effect.”³⁷ The transduced cells metabolize the prodrug and may release the active metabolite into the extracellular space via exocytosis or cell lysis, or adjacent cells may transfer the metabolites via cell channels or gap junctions. This therapeutic approach does require the transduction of tumor cells for specificity. In an attempt to improve specificity, Ohashi et al³⁸ constructed an adenoviral-based HSV-TK vector with a specific promoter for the oncofetal protein, CEA, which is produced in various tumors, including gastric, colon, and pancreas. The study demonstrated that the tumoricidal effect of ganciclovir is increased in only CEA-producing cells and introduced a method to increase the specificity of directed-enzyme prodrug therapy. Suicide gene therapy can also be combined with other conventional cancer treatment modalities to increase treatment effect. Khil et al³⁹ demonstrated that human colorectal carcinoma cells transduced with the cytosine deaminase gene were more sensitive to radiation. WiDR cells, a human colorectal carcinoma cell line, were cultured and infected with a CD2 retrovirus vector containing the cytosine deaminase gene. Infected cells were incubated for 1 day and given increasing concentrations of FC (0–30 $\mu\text{g/mL}$). After 72 hours of FC exposure, a single 8-Gy dose of radiation was given to the cells. Results demonstrated that the colon cancer cell line with the CD gene demonstrated a significantly

lower percentage of survival with radiation than without radiation, which was dependent not only on the FC dose, but the time of FC exposure prior to radiation treatment.

Suicide gene therapy has been applied against pancreatic cancer. A majority of studies used in vivo models of pancreatic cancer. Evoy et al⁴⁰ demonstrated the efficacy of the cytosine deaminase gene in a subcutaneous tumor model. The pancreatic cell line Panc02 was injected near the hind leg of C57BL/6 mice. On Day 7 after tumor inoculation, 10^7 pfu of adenovirus containing the cytosine deaminase gene was injected directly into the tumor; FC was administered intraperitoneally for 10 consecutive days. An additional virus was given on Day 11. Results demonstrated a reduction of tumor size in the treatment group compared with controls.

Aoki et al³⁴ evaluated the HSV thymidine kinase against a carcinomatosis model of pancreatic cancer. The pancreatic cell line PSN-1 (1×10^5) was injected into the peritoneal cavity. (Animals were humanely euthanized to confirm peritoneal tumor dissemination.) On Day 8 after tumor inoculation, animals were given an intraperitoneal injection of DNA plasmid containing the herpes simplex thymidine kinase gene mixed with a cationic lipopolyamine diotadecylamidoglycylsperamine, and subsequent daily injections of ganciclovir for 8 days. At 24 days, after tumor inoculation, 8 of 14 animals in the treatment group were tumor free. Control groups given an empty vector and liposome alone or in combination demonstrated tumor growth in all the group animals. Block et al³⁵ evaluated this therapy in a liver metastasis model of pancreatic cancer. Panc02 cells were injected into the left lobe of the liver of C57BL/6 mice and given adenovirus containing a Rous sarcoma virus (RSV) promoter and the thymidine kinase gene directly into the tumor. Similar to previous studies, a significant reduction in tumor size was observed in the treatment group.

Tumor Suppressor Gene Replacement/Oncogene Inactivation

The malignant transformation of cells is dependent upon specific mutations of tumor suppressor genes and dominant oncogenes. Genetic analysis of various tissue tumors has demonstrated specific mul-

tiples deletions, mutations, or both of proto-oncogenes and tumor suppressor genes. Genetic analysis of pancreatic cancer has demonstrated mutations in the *K-ras*, *p16*, *p53*, and *DP4* gene (detected in pancreatic cancer, locus 4) in approximately 95%, 90%, 75%, and 55% of tumors, respectively.⁴¹ The tumor suppressor gene *p53* and the oncogene *K-ras* are the two most frequent mutations found in pancreatic cancer. All of the genes are involved in regulating the cell cycle and proliferation. *p16* is an inhibitor of cyclin-dependent kinase 4 that regulates cell cycle progression to the G1/S phase, and is a mutation found in other tumors. *DP4* is a gene that undergoes homozygous deletion or point mutations in approximately 50% of pancreatic cancers. The protein belongs to a family of genes, and its precise function is unknown; however, it may be involved with TGF- β signaling. In addition, genetic analysis of family individuals with pancreatic cancer has demonstrated germline mutations of *p16* and *BRCA2* genes, suggesting a hereditary component of pancreatic cancer.

Tumor suppressor genes, such as *p53* and retinoblastoma (*Rb1*) genes, function at cell cycle checkpoints to maintain controlled cellular proliferation and high-fidelity DNA replication.⁴² Mutation or deletions of both copies of the tumor suppressor gene may result in cell transformation due to the loss of cell cycle regulation and continued proliferation despite DNA mutations. Oncogene mutations, translocation, and amplification may have the same end result. The mutation of proto-oncogenes results in an active protein. For example, the *ras* protein is a cytoplasmic membrane G-protein that is activated via phosphorylation after mitogenic cell stimulation and involved in signal transduction.⁴³ Mutations of the *ras* gene result in a constitutively activated form of the protein, which signals cell proliferation regardless of the cell environment and in the absence of mitogenic signals. Transcription factors activate and translocate to the nucleus for transcription of genes required for cell survival.

Gene therapy has been used to replace tumor suppressor genes or to inactivate oncogenes. Wild-type tumor suppressor genes such as *p53* have been reintroduced into various different cancer cell lines, including lung, prostate, head and neck, colon, and pancreas.^{44–48} Results have demonstrated that cells transduced by the various delivery systems with the

wild-type *p53* have significant reductions in cell proliferation and an increase in cellular apoptosis.

Tumor suppressor gene transfer has been demonstrated against pancreatic cancer cell. Lang et al⁴⁹ used a temperature-sensitive mouse plasmid under the control of a metallothionein promoter containing the wild-type *p53* gene against a poorly differentiated pancreatic carcinoma cell line, Panc-1 with a *p53* mutation in vitro. The introduction and stable expression of wild-type *p53* resulted in up-regulation of the *p21/WAF1* gene, G1 cell arrest, and increased apoptosis. However, 30% of the cells survived, and displayed a neuroendocrine-like phenotype. This study demonstrates the anti-tumorigenic effect of *p53*, but also demonstrates that all the effects of *p53* transfer are unpredictable. This latter phenomenon may be dependent on the differentiation of the pancreatic carcinoma cell. Several groups have transferred wild-type *p53*, using different in vivo animal models of pancreatic cancer. Hwang et al⁵⁰ injected Bxpc3 cells (8×10^6), a human pancreatic cell line, subcutaneously into nude mice. Once gross tumors were measurable, a retroviral vector containing the wild-type *p53* gene (G1153SvNa) driven by a cytomegalovirus (CMV) promoter was injected directly into the tumors. An orthotopic in vivo tumor model was also treated; tumor cells were injected into the tail of the pancreas, which produced large primary tumors and peritoneal dissemination. On Day 3 after tumor inoculation, animals received an intraperitoneal injection of the *p53* retrovirus (10^6 pfu/mL) for 10 days and, subsequently, were killed. Results demonstrated the treatment group had smaller primary, as well as fewer peritoneal, tumors. Furthermore, immunohistochemical analysis demonstrated the up-regulation of *p21*.

Bouvet et al⁴⁸ also evaluated the effect of wild-type *p53* in several *p53* mutant pancreatic cell lines, including AsPC-1, BxPC-3, Capan-1, MIA PaCa-2, and PANC-1 in vitro, as well as in an in vivo murine subcutaneous tumor model. Cells were infected with an adenovirus vector containing the *E. coli* β -galactosidase gene (Ad5/CMV/*p53*) with different MOIs (10, 25, 50, 100, and 500). Transduction efficiencies were determined by X-gal staining; cell proliferation was determined by tritiated thymidine incorporation, and apoptosis was quantified by propidium iodide staining and fluorescence-activated cell sorting. Their results

demonstrated that the MIA PaCa-2 had the highest transduction efficiency among all the pancreatic cell lines (65% at an MOI of 50). Furthermore, the introduction of *p53* inhibited cell proliferation in an MOI-dependent manner and induced tumor apoptosis at 48 and 72 hours after tumor infection. In an in vivo model, female nude mice were given an injection of MIA PaCa-2 (5×10^6) cells subcutaneously. Three injections of virus (6×10^9 pfu) were given every other day after tumors had reached a volume of 200 mm³. The in vitro results were confirmed in vivo, with a significant inhibition of tumor growth seen in the *p53* treatment mice compared with controls. In situ terminal nucleotidyl transferase-mediated uridine 5'-triphosphate biotin nick end labeling (TUNEL) staining of animal tumors demonstrated apoptosis in the Ad5/CMV/*p53* group.

Other tumor suppressor genes, such as retinoblastoma gene (*Rb1*) and *tob* (transducer of ErbB-2), have been evaluated in pancreatic cancer.⁵¹ The *Rb1* gene is a regulator of G₁-S phase transition. Hyperphosphorylation of *Rb1* results in cell cycle transition and cell proliferation. One group tested the hypothesis that overexpression of the nonphosphorylated form of *Rb1* will prevent cell proliferation, regardless of the other upstream mutations of tumor suppressor genes or oncogenes in several different pancreatic cancer cell lines, such as bxPC-2, Panc-1, and MIA PaCa-2.⁵² Adenoviral-mediated delivery of *Rb1*, under the control of the Rous sarcoma virus (RSV) promoter, resulted in efficient transduction of the virus and decreased DNA synthesis as measured by thymidine uptake in all three different pancreatic cell lines. However, TUNEL staining demonstrated that the antiproliferative effect was not due to apoptosis. Yanagie et al⁵³ evaluated another tumor suppressor gene specific to pancreatic cancer. The *tob* protein interacts with ErbB-2, which is a protein tyrosine kinase that is involved in the *ras* signaling pathway and overexpressed in pancreatic cancer cell lines. Pancreatic cell lines, AsPC-1, BxPC, and SOJ, were infected in vitro with an adenovirus containing the *tob* gene. Northern blot analysis demonstrated that cells expressing the exogenous *tob* messenger RNA (mRNA) were enlarged and megakaryocytic, suggesting senescent cells, and Western blot analysis demonstrated an inverse correlation between *tob* protein expression and cell proliferation. Although the results of *Rb1*

and *tob* gene transfer were of a lesser magnitude than *p53*, they represent additional targets for gene therapy specific to pancreatic cancer, and warrant further investigation.

In addition to *p53* mutations, pancreatic cancer also has a high rate of *K-ras* mutations.⁵⁴ Gene therapy vectors expressing antisense DNA or RNA complementary to the proto-oncogene encoding mRNA have been used to inactivate oncogenes. The transduced DNA sequences may prevent transcription and/or translation. Mukhopadhyay et al⁵⁵ delivered antisense DNA (2-kb DNA fragment containing the second and third exon sequences of the *K-ras* gene) and inhibited proliferation of lung cancer cells in vitro by down regulation of the *ras p21* protein expression. Despite the absence of *K-ras* expression, cells remained viable.

Aoki et al⁵⁶ evaluated the application of antisense RNA against the *K-ras* mutation in three pancreatic cell lines, AsPC-1, MIAPaCA-2, and BxPC-2. All the cell lines except for BxPC-3 have transition mutations in the *K-ras* gene; they were infected with a retrovirus-based plasmid with the *K-ras* cDNA fragment in antisense orientation using cationic liposomes. Cells with intrinsic *K-ras* mutations demonstrated a decrease in cell proliferation; the BxPC-3 cell line predictably did not. In addition, AsPC-1 cells (6×10^5) were injected intraperitoneally, which resulted in tumor dissemination and formation of tumors. Three days later, animals received intraperitoneal injections of liposome/antisense *K-ras* DNA. Ten of 12 treatment animals demonstrated no evidence of tumor compared with 9 of 10 control animals, which demonstrated gross, disseminated tumor. In an alternate strategy, Shichinohe et al⁵⁷ transfected eight different human pancreatic cell lines containing *K-ras* mutations with a plasmid carrying a dominant negative *H-ras* mutant gene. The expression of the mutant was confirmed by reverse-transcription polymerase chain reaction. Transfected cells (1×10^6 or 2×10^6) were subcutaneously injected into nude mice and observed for tumor formation. Results were similar to the antisense *K-ras* DNA treatment. The dominant-negative mutant inhibited tumor growth compared with controls; N166Y-expressing clones exhibited morphological similarities with cells treated with antisense *K-ras* DNA. These previously described studies demonstrated that *K-ras* and other oncogenes are possible targets.

Additional Targets

Additional targets for gene therapy include proteins that activate cell death or apoptosis directly. Kawaguchi et al⁵⁸ have demonstrated that transduction and expression of Fas, a type I membrane protein that mediates apoptotic death, can induce cell death. During malignant transformation, cells undergo selective changes that increase their tumorigenicity. For example, cells may increase expression of glypican-1, which belongs to a family of glycosylphosphatidylinositol-anchored heparan sulfate proteoglycans and may play a role in mitogen signal transduction. Studies demonstrate that gene therapy with antisense DNA against glypican-1 can inhibit pancreatic cell growth.⁵⁹

Summary

Pancreatic cancer offers an appealing target for the development of novel therapeutic strategies, including the developing field of cancer gene therapy. Most clinical trials have utilized viral gene vectors because of their relative improved efficiency over nonviral vectors. However, with ongoing improvements in nonviral vector technology, these vectors may become a more appealing option as their efficiency improves and the overall toxicity of these vectors decreases. This field of cancer gene therapy offers many potential therapeutic approaches. These include tumor suppressor gene therapy, suicide gene therapy, and the marriage of immunotherapy with gene therapy. Each strategy offers potential benefits and pitfalls. Tumor suppressor gene therapy has been demonstrated to effectively induce tumor death in transduced cells and, to a limited extent, their bystanders. At this time, this therapy requires a direct injection strategy for effectiveness and to limit toxicities. The direct injection of vector into pancreatic tumors may be a feasible cancer gene therapy approach. However, the limitations of this approach would be the potential toxicities related to the inflammatory changes in the pancreas and its limited clinical role, given that most patients with pancreatic cancer develop metastatic disease. Cancer gene therapy utilizing immunotherapy has the benefit of not requiring transduction of a substantial population of cancer cells in order to engender an effect. The lim-

itation of this approach relates mainly to the limitations of the field of immunotherapy. Given our present technology, this therapy holds the potential for a clinical impact on patients with this disease. Ultimately, improvements in vector technology, including improved efficiency and targeting, will be required prior to any additional advances in the field of pancreatic cancer gene therapy.

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Photodynamic Therapy and Endoscopic Ultrasound-Guided Therapy for Pancreatic Cancer

Sandeep Lahoti

Introduction

Pancreatic carcinoma afflicts an estimated 75,000 patients annually in developed countries in Europe, North America, and the Far East. In the United States, the annual incidence is estimated at 29,000 cases, with 28,900 associated deaths.¹ Due to our inability to detect pancreatic cancer at an early stage, and the lack of effective systemic therapies, only 1% to 4% of patients with adenocarcinoma of the pancreas will be alive 5 years after diagnosis.² The primary therapy for localized, potentially resectable disease is surgery (Whipple procedure). Unfortunately, only roughly 10% of all patients with pancreatic carcinoma will have tumors that are potentially resectable. In addition, up to 50% of all patients taken to laparotomy for potential resection have unresectable disease. Thus, only between 5% and 10% of all pancreatic cancer patients can be cured surgically.

For patients with locally advanced pancreatic cancer, chemotherapy alone provides minimum benefit.^{3–5} In one multicenter randomized trial with 126 patients, gemcitabine was compared with 5-fluorouracil (5-FU) in previously untreated patients.⁶ Patients treated with gemcitabine had a median survival time of 5.65 months compared with 4.41 months in those treated with 5-FU. Twenty-four percent of patients treated with gemcitabine were alive at 9 months compared with 6% of patients treated with 5-FU.

Today, multimodality schedules (radiation and chemotherapy) show the best results with regard to tumor growth control and survival.^{7–10} While chemoradiotherapy is more efficacious than radia-

tion therapy alone in patients with locally advanced pancreatic cancer, survival times for multimodality patients only range from 8 to 15 months. Moreover, severe complications such as complete liver failure,⁸ gastric hemorrhage and ulcer,⁹ and hematopoietic toxicity,⁸ and a high morbidity for minor complications such as nausea, vomiting, pancreatic insufficiency, and dermatitis (ranging up to 86%)⁹ limit the potential use of these therapeutic schedules. There remains an urgent need for more effective therapies for these terminally ill patients.

Photodynamic Therapy

In 1981, Holyoke¹¹ proposed an investigational use of photodynamic therapy as an alternative treatment for pancreatic carcinoma. The effectiveness of this treatment modality has been proven experimentally for a large variety of tumors and, in the last decade, has been clinically confirmed.^{12–15} Photodynamic therapy (PDT) is highly tumor selective, mainly by two different pathways^{16,17} as follows: (1) the tumor tissue retains the photosensitizer at higher concentrations than normal tissue and (2) the drug is only cytotoxic when activated by appropriate light. Normal tissue thus can be avoided by careful dosimetry.¹⁸

A third pathway of selectivity exists for the pancreas. There have been several investigations that have ruled out a significant accumulation of photosensitizer in experimental pancreatic carcinoma tissue compared with normal pancreatic tissue.^{17,19} Nevertheless, pancreatic tumor response has been seen in animals.^{19–21} This appears to occur with-

out significantly affecting normal pancreatic tissue.^{20,21} An increase of serum amylase has been observed when treating rat pancreas with very high PDT doses, although there was still no microscopic or macroscopic evidence of pancreatitis.²² One out of 7 animals treated by Schroder et al¹⁹ died of necrotizing pancreatitis. This may have resulted from pancreatic duct obstruction or papillary edema since microscopic damage to a normal pancreas has not been seen in other experiments. The selective effect on pancreatic tumor as opposed to normal tissue may be due to a preferentially lymphatic localization of the photosensitizer within the normal pancreatic tissue.²²

PDT for pancreatic cancer has been evaluated in a clinical pilot study.²³ Seven patients with inoperable cancer underwent PDT after sensitization with metatetrahydroxyphenylchlorin (m-THPC). Under guidance of computed tomography (CT), four optical fibers were positioned percutaneously in each tumor and 20 J of red light (652 nm) was delivered to between 4 and 16 sites. All patients had nonenhancing lesions on CT 4 days posttreatment, with volumes of 16 to 49 cm³ corresponding to the treated tumor. Four patients experienced destruction of $\geq 90\%$ of their tumor bulk, as seen on CT, at 1 month. No pancreatitis or life-threatening complications occurred but exocrine pancreatic function worsened in 6 patients. Four patients were living 3 to 12 months after PDT. Three died at 16, 6, and 4 months post-PDT (2 patients died of liver metastases that appeared before 3 months). Two patients developed PDT-induced duodenal ulceration and, ultimately, duodenal strictures.

Endoscopic Ultrasound–Guided Therapy

Endoscopic ultrasound (EUS) is currently the best modality available for the detection of pancreatic neoplasms.^{24,25} It allows for precise localization of instruments within tumors under real-time imaging. Because of these properties, several studies have investigated EUS-guided therapy for pancreatic tumors. In a recent preliminary study,²⁶ local immunotherapy with activated T cells, allogeneic mixed lymphocyte culture (Cytoimplant), was performed in patients with advanced pancreatic can-

cer. The Cytoimplant was administered by EUS-guided intralesional injection at a single tumor site. A total of 8 patients (stage II, 4; stage III, 3; and stage IV, 1) were treated. Tumor response was measured by serial CT scans or by EUS. Two patients experienced a partial response; 1 patient, a minor response; 3 patients had no change; and 2 patients had progressive disease. Median survival time was 13.2 months. There were no procedural complications.

At the M. D. Anderson Cancer Center, we recently completed a phase I/II multicenter study investigating EUS-guided injection therapy for unresectable pancreatic cancer utilizing an attenuated adenovirus.²⁷ The attenuated adenovirus used will only replicate in p53-negative cells. The virus was injected into the tumors under EUS-guidance. Unlike the previously described study where only one depot injection was performed, multiple injections in different planes were performed in order to distribute the virus throughout the tumor. The total number of injections was calculated by a formula based on the volume of tumor present. The patients underwent EUS-guided therapy on Days 1, 5, 8, 15, 36, 43, 50, and 57, with the last four sessions using concurrent gemcitabine therapy. A total of 21 patients were treated, with over 100 sessions of virus injection performed. Three patients received 10 pfu per session, and 18 patients received 19 pfu per session. Preliminary results show 2 patients had a partial response, with 57% and 51% tumor regression. Two patients had a minor response, 6 patients had stable disease, and 11 had progressive disease or treatment failure. Complications included 2 patients with sepsis that occurred before oral perioperative antibiotics were used. One patient had an asymptomatic cystic fluid collection. Several patients had elevations of amylase and lipase but no episodes of clinical pancreatitis. Two patients had duodenal perforations from the rigid echoendoscope tip, highlighting the fact that further refinements in this technique are needed.

In another trial, EUS-guided radiofrequency ablation of a portion of the pancreas was attempted in a pig model.²⁸ The pancreas was localized by linear array EUS and a specially modified 19-gauge needle was advanced transgastrically into the pig pancreas. A radio-frequency current (285 ± 120 mA) was delivered for 6 minutes. The pathology exam revealed 8- to 10-mm foci of coagulation

necrosis in pigs examined immediately, and 1 to 2 days after ablation. In 4 of 6 pigs examined on Day 14, retraction of the coagulated focus was observed. A 1- to 3-mm fibrotic capsule surrounded the coagulated tissue in the remaining 2 pigs. A focal zone of pancreatitis followed by a pancreatic fluid collection occurred in 1 pig, while biochemical parameters were normal in the other pigs. Other complications included three gastric and one intestinal burn due to improper electrode placement. The authors hypothesized that this technology could be used for the management of small neuroendocrine tumors and possibly for palliation of unresectable pancreatic tumors.

Conclusions

The results of the few preliminary studies make it difficult to assess the utility of PDT or EUS-guided therapy for the treatment of pancreatic cancer. However, these modalities have tremendous potential and deserve further investigation.

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31

Vaccine Therapy for Pancreatic Cancer

Eileen M. O'Reilly

Introduction

The principles of cancer immunotherapy are derived in large part from the infectious disease world where immunization has a long established precedent. Cancer immunotherapy can be broadly divided into active and passive immunization. In turn, each may be subdivided into specific and non-specific approaches (Figure 31.1). Passive immunotherapy does not require that the host immune system be activated. An example of passive specific immunity is the use of antibodies in the treatment of various cancer types, eg, colon cancer with a Mab 17-1A monoclonal antibody and breast cancer with trastuzumab. Nonspecific immunotherapy approaches in cancer have met with limited success, with the exception of the regional use of BCG for superficial or in situ bladder cancer. Active immunotherapy requires activation of the host immune system, resulting in up-regulation of specific immune cells, eg, CD4+ and CD8+ T cells.

The key element necessary for successful immunotherapy is a tumor antigen, or antigens, sufficiently different from normal tissue antigens so that it can be recognized as foreign by the immune system. This concept allows selective exploitation of these antigens as potential tumor targets. These antigens must also be immunogenic, ie, proficient at inciting an immune response. Most tumor antigens are poorly immunogenic and do not incite a host immune response resulting in immune tolerance, which is part of the reason why tumors may develop in the first place. The host immune system must also be able to respond immunologically to the challenge of stimulated antigens. Cell-mediated

immunity, T lymphocytes, natural killer cells, and macrophages are integral to host immunity. In addition, a minimal tumor burden increases the likelihood that an immunotherapy strategy will be successful.¹ The limitations of immune strategies include difficulties in identifying isolated tumor-specific antigens and poor host response, as often tumor antigens are not sufficiently immunogenic, in part because of immune tolerance.

Immunotherapy for pancreatic cancer is an emerging field. Many of the issues surrounding vaccine development in pancreatic cancer are common to cancers where vaccine therapy has an established role. Pancreatic adenocarcinomas have been shown to be susceptible to immune enhancement, and there are several targets on pancreatic cancer cells that are open to immune exploitation, including glycoproteins, epidermal growth factor receptors, mucins, and *ras* peptides.² A variety of vaccine approaches have been examined in pancreatic cancer, including tumor cell extracts, purified extracts, whole tumor cells, and peptides.³ The two strategies that have been most extensively assessed in pancreatic cancer are *ras* vaccines and genetically modified cytokine vaccines, both of which will be reviewed in some depth later in this chapter.

A brief review of key components of the immune system will be helpful for an understanding of vaccine strategies. T cells, both T helper (CD4+) and cytotoxic T cells (CD8+) are essential components of antitumor cell-mediated immunity. Almost any component of a tumor cell can be a tumor antigen. Antigens may be processed and presented on the surface of tumor cells by the major histocompatibility complex (MHC). Small peptide fragments, 8 to 10

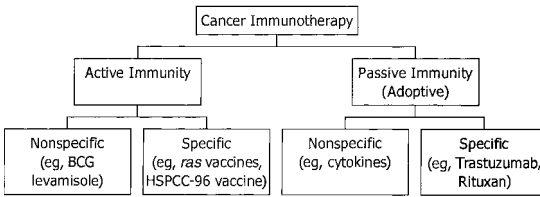


FIGURE 31.1. Divisions of cancer immunotherapy.

amino acids, are presented by MHC class I molecules. Larger peptides, 12 to 20 amino acids, are presented by MHC class II molecules. Activated CD4+ T cells can help activate the immune cascade and can cross-amplify CD8+ T cells. Activated CD8+ T cells can result in tumor cell destruction. CD4+ memory cells can lead to protective immunity. Co-stimulatory molecules are essential to activation of the immune system and are one method of maximizing the success of a particular vaccine strategy. For example, using antigen-presenting cells (APCs) may be a potent way of enhancing immunity. Cytotoxic T lymphocytes (CTLs) are an essential component of anti-tumor defense and their specific up-regulation is a desired goal, as CTLs may kill tumor cells.

Peptide Vaccines

Certain cell peptides can be processed intracellularly and expressed on the cell surface bound by the MHC. A peptide vaccine strategy is based on the peptide being processed and presented by MHC molecules of APCs. Several in vitro studies have demonstrated the feasibility of this concept.^{4,5} Most clinical work using a peptide vaccination approach has been conducted in melanoma patients. Several responses and protracted progression-free survival have been demonstrated with a MAGE3 peptide administered with Freund's adjuvant and with a MART-1, Melan A, tyrosinase vaccine.^{6,7} In pancreatic cancer, *ras* peptide vaccines exemplify this approach.

Ras Mutations and Pancreatic Cancer

The three human *ras* genes encode for four homologous 21 kDa proteins: H-*Ras*, K-*Ras4A*, K-*Ras4B*, and N-*Ras*. These proteins are members

of a large family of guanosine triphosphatases (GTPases) that have varied cellular functions. One of their key functions is regulating a guanosine diphosphate (GDP)-GTP switch that controls various signal transduction pathways between the Raf kinase/MAP kinase cascade and cell surface receptors. In pancreatic cancer, mutations of the *K-ras* gene occur in over 85% to 90% of pancreatic cancers.^{8–11} Typically, when genes are mutated they are inactivated; however, the converse is true for *ras* mutations. In the mutated state, the GTP is bound and *ras* signaling is permanently activated. The role of *ras* mutations in pancreatic cancer tumorigenesis is not fully known; however, *ras* mutations are identifiable relatively early on in the cumulative genetic mutation cascade leading to invasive cancer and are thought to be integral to this process. The *ras* gene is 188 amino acids long. *Ras* mutations in pancreatic cancer occur primarily as a single amino acid substitution at codon 12. In the native state, a glycine residue is present at codon 12, and in mutated *ras*, aspartic acid, valine, and arginine substitutions are most common, with serine, threonine, cysteine, and other amino acids being rarely identified. Even less commonly, substitutions occur at codons 13 and 61. The key issue in relation to mutated *ras* and vaccine therapy, is that mutant *ras* is unique to tumor cells and does not occur in normal pancreatic cancer epithelium. This allows an opportunity for a selective tumor target vaccination strategy in that the K-*ras* products are unique epitopes for T-cell (CD4+ and CD8+) recognition in cancer immunotherapy.¹²

Ras Vaccines in Pancreatic Cancer

A number of investigators have demonstrated that in normal subjects^{13–16} and in cancer patients^{17–19} there are CD4 T cells capable of recognizing codon 12 *ras* mutations. A key issue is whether a patient's T cells could recognize the same *ras* mutation expressed by the patient's own tumor. Sun and colleagues²⁰ typed 29 patients with pancreatic cancer for codon 12 mutations by cloning and sequencing polymerase chain reaction-amplified DNA and/or by multiplex competitive oligonucleotide probe ligation. Forty-one percent of the patients had codon 12 mutations. In several of these patients, T cells were tested for thymidine incorporation and tumor

necrosis factor release in response to codon 12-substituted K-*ras* peptides. Despite the low sensitivity of this assay, patients with certain *ras* mutations were found to recognize only the specific *ras* mutation corresponding to the mutation in their tumor and did not cross-react with other *ras* peptides or wild-type *ras*. These data demonstrate that patients with pancreatic carcinoma do possess circulating T cells that can recognize mutant *ras* in their own tumor cells. Similar findings have also been observed in other cancers.^{16,21,22}

Gjertsen and colleagues^{23–26} vaccinated pancreatic cancer patients with a synthetic mutant *ras* vaccine with interesting results. Five patients with either locoregionally advanced or metastatic pancreatic cancer had their tumors typed for *ras* mutational status. The patients underwent leucapheresis for peripheral blood mononuclear cell (PBMC) harvesting. These PBMCs were incubated with a synthetic *ras* peptide (the vaccine) corresponding to the mutation in the patient's tumor. Patients were vaccinated by administering the PBMC-peptide suspension at $t = 0$, Day 14, and Day 35 with the option for booster vaccines at 4 to 6 weeks. PBMCs were screened for T-cell proliferation against the immunizing peptide. At baseline, none of the patients had T-cell responsivity against any of the *ras* peptides, including their own tumor *ras* mutation. In two of the 5 patients it was possible to induce a transient proliferative T-cell response, evident about 6 to 7 weeks after vaccination. One patient had T cells specific for mutated *ras* (Val12) and the second patient had a T-cell response against both mutated and wild-type (gly12) *ras*. It is notable that in both patients the T-cell response was transient, despite booster vaccinations. Several theories have been offered to explain this phenomenon. T-cell sequestration at the tumor site has been offered as an explanation for this finding, as well as the relatively insensitive techniques used for T-cell detections. While serial tumor biopsies were not performed as part of this study, the 2 patients who demonstrated an immune response underwent autopsy examination. In both patients, there was significant T lymphocytic infiltration in the tumor sites, possibly explaining the difficulty in detecting these T cells by conventional techniques. In the 2 patients who responded, there was a suggestion of delayed time to tumor progression compared with the nonresponders, 10.4 versus 4.5 months, al-

though there was no objective change in tumor burden in any patient as a result of *ras* vaccination. The vaccinations were well tolerated with no significant side effects encountered. While clearly the patient numbers in these experiments are small, the observations are encouraging and pave the way for development of further *ras* peptide vaccination strategies in pancreatic cancer. This experiment demonstrates that even with just a single amino acid substitution or point mutation, T cells, both CD4+ and CD8+, can be specifically induced as a result of *ras* peptide vaccination. More recently, Gjertsen and colleagues²⁷ demonstrated that following cloning of responsive Cys12 or specific cytotoxic T cells after intradermal *ras* peptide vaccination with granulocyte macrophage-colony stimulating factor (GM-CSF) in a patient with pancreatic cancer, cytotoxic T lymphocytes were processed and presented in vivo and were capable of killing target cells that expressed the same *ras* mutation and haplotype.

Khleif and colleagues at the National Cancer Institute, conducted a phase I pilot trial in patients with advanced malignancies using a 13-amino acid peptide (13 mer) vaccine containing a codon 12 mutation.²⁸ Patients were vaccinated with a peptide vaccine corresponding to their own tumor *ras* mutation. Three of the 10 patients had detectable mutant *ras*-specific CD4+ and/or CD8+ T-cell immunity. In 1 patient, the CD8+ cytotoxic T cells were found to be cytotoxic in vitro to a tumor cell line harboring the corresponding *ras* mutation. Side effects were minimal. Notably, there was no correlation between peptide vaccine dose and immunity development and side effects. The authors concluded that this was a safe approach and offered a unique way of directing tumor-specific therapy and of developing adoptive immunotherapy approaches.

Z'graggen and colleagues²⁹ have reported preliminary results on a *ras* peptide vaccination study conducted in a multinational setting in locally advanced unresectable pancreatic cancer. The vaccine consisted of a 7-amino acid (7 mer) *ras* peptide. The vaccine was administered intradermally weekly for 4 weeks followed by two additional vaccinations at Weeks 6 and 10. GM-CSF was co-administered intradermally. Of 82 vaccinated patients, immunity results are available for 71 patients, of which 42% showed a positive immune re-

sponse determined by a delayed-type hypersensitivity (DTH) reaction measured at 4 weeks. In the DTH-positive group of patients, 12 deaths (40%) were observed, with 20 deaths (48.8%) occurring in the DTH-negative group. All deaths were disease related. The therapy was well tolerated with few serious adverse effects, only one instance of fever, leucopenia, and vomiting. It remains to be seen whether developing a DTH response protects against relapse.

Ongoing Clinical Trials With Peptide Vaccines in Pancreatic Cancer

At Memorial Sloan-Kettering Cancer Center, a *ras* peptide vaccination protocol is underway for patients with pancreatic cancer, as well as other malignancies where *ras* mutations are relatively common (nonsmall cell lung cancer, refractory leukemia). For pancreatic cancer patients, we have chosen to target patients with a minimal residual tumor volume, so vaccination is offered as an adjuvant therapy. To enhance the likelihood of development of an immune response, the cytokine GM-CSF is used as an immune adjuvant. While GM-CSF is normally known for its myeloid maturation effects, it also potentiates antigen presentation and keratinocyte growth and can function as an immune adjuvant in peptide vaccines.³⁰ Kaplan and colleagues³¹ showed that in a series of leprosy patients GM-CSF was chemotactic for Langerhan cells and that a local DTH response could be attributed to local GM-CSF production. Jager et al⁷ demonstrated in patients with melanoma that co-administration of GM-CSF with intradermal peptides enhanced antigen (peptide) presentation to the immune system by up-regulation of the key APCs, dendritic cells, in the dermis. Thus, GM-CSF is recognized to be one of the most potent immune adjuvants. In our protocol, patients are thought to self-administer GM-CSF intradermally, in an attempt to overcome one of the major limitations of vaccine strategies, ie, inadequate antigen exposure to the host immune system. Three monthly *ras* peptide vaccines are planned. Each *ras* peptide vaccine is administered into the same skin site as the GM-CSF. The vaccine itself is a synthetically derived

16-amino acid peptide spanning codon 12. The primary aim of the study is to determine the specific T-cell response to *ras* peptide vaccination. A secondary aim includes an assessment of whether baseline skin test reactivity or HLA type correlates with the induction of anti-K-*ras* immunity; a third aim is to characterize the toxicity. Several methods of immune assessment will be utilized including DTH response, interferon- γ -release, Elispot and cytotoxicity assays. Patients who have undergone a gross complete tumor resection are eligible for vaccination. To date, a small number of patients have been vaccinated and immune results are awaited.

Wojtowicz and colleagues³² at the National Cancer Institute are vaccinating patients with codon 12 mutations and advanced disease to one of two treatments. Patients receive either mutant *ras* peptide administered subcutaneously every 4 weeks with GM-CSF or the same peptide dose and schedule but with interleukin 2 (IL-2) instead of GM-CSF. To date, treatment has been well tolerated with some mild typical IL-2 toxicities, maximum grade II, on the second arm of the study. The GM-CSF-treated patients had local skin reactions and flu-like symptoms. No objective tumor regressions have been observed on either arm of the study in patients with bulk disease. Three of 9 patients on the IL-2 arm, and 2 of 10 patients on the GM-CSF arm, with known subradiologic metastases, remain without progression; 1 patient is now at 15 months after initiation of vaccination therapy. Further characterization of the immune responses and longer follow-up are awaited.

Whole-Cell Vaccines

Methods of enhancing tumor-specific immunity include administering the vaccine with immune adjuvants, such as cytokines or co-stimulatory molecules. Advantages of having high local production of a cytokine at the tumor site include enhancement of antigen presentation to APCs and the activation of tumor-specific lymphocytes³³ and induction of a local DTH response. A DTH response is accepted as a surrogate of a cell-mediated immune response to an antigen. A variety of cytokines have been assessed including IL-2, IL-3, IL-4, IL-6, IL-7, TNF, G-CSF, GM-CSF, γ -interferon, and others. In pa-

tients with advanced renal cell carcinoma using a tumor vaccine with autologous GM-CSF secretion, the feasibility of the concept was demonstrated, and also the technical challenges associated with such an approach, including the labor-intensiveness, the need for an individual vaccine, and the difficulty in expanding primary tumors.³⁴ In vitro data are compelling in that autologous or allogeneic tumor cells genetically modified to secrete GM-CSF induce effective immune responses that can cure tumors in mice, leading the way to treatment of pancreatic cancer.

Jaffee and colleagues are now employing a different strategy for pancreatic cancer vaccination by using autologously derived tumor cells that are genetically modified to express immune-stimulatory molecules, such as GM-CSF.³⁵ More recently, antigen-specific, rather than the whole tumor cell, vaccine strategies are being used.³⁶ In vitro stable cell lines from primary pancreatic tumors are hard to come by. One of the byproducts of vaccine research has been the development of successful tissue culture techniques that result in stable cell lines of pancreatic cancers, which can be used to generate genetically modified human allogeneic tumor vaccines.³⁷ Initial studies with cytokine-secreting allogeneic vaccines have demonstrated that these vaccines are safe. Typical side effects include a local inflammatory reaction, including some erythema and skin induration, and mild pyrexias. Adjuvant studies are underway at the Johns Hopkins Cancer Center, integrating this vaccine strategy with adjuvant chemoradiotherapy. A phase I study of an allogeneic-irradiated, GM-CSF-secreting tumor vaccine in pancreatic cancer has been preliminarily reported.³⁸ The study's primary objectives were to assess safety and the antitumor immune response. Fourteen patients who had undergone a pancreaticoduodenectomy received an initial vaccine, in a dose-escalating fashion, 6 to 8 weeks following surgery. Adjuvant chemoradiation was subsequently delivered. Thereafter, patients were eligible to receive three additional monthly vaccines. Treatment was well tolerated. DTH responses were evident in patients at the higher dose levels. There was a suggestion that DTH response correlated with progression-free survival. Skin biopsies from the vaccine tumor sites demonstrated eosinophil and macrophage infiltration comparable to what had been observed in preclinical models.

Clearly, these results are provocative, but further follow-up is awaited.

Heat Shock Protein Vaccines

Heat shock proteins (HSPs) are molecular chaperones that assist proteins in maintaining their three-dimensional conformation. They also have other diverse cellular functions, including mopping up cellular detritus. There are over 50 types of HSPs, classified on the basis of size. In animal models, vaccination with an HSP-70 complex can render the animals immune to subsequent tumor challenge.³⁹ The antigenicity was determined to be derived from the complexed antigens rather than the HSP-70. Similar observations have been made for HSP-96 and HSP-90.⁴⁰ Several advantages of HSPs used as vaccines are that they do not require a precise definition of the complexed tumor antigenic epitopes, nor do they depend upon cell lines or specific cytotoxic T lymphocytes.^{41,42} Suto and Srivastava⁴³ have shown that HSPs can be presented via the MHC class I pathway and that they can be recognized by CD8⁺ lymphocytes.⁴³ Other putative mechanisms of action of HSPs are the activation of cytotoxic T lymphocytes via the MHC class II pathway, as well as by binding to macrophages and inducing proinflammatory cytokines. In pancreatic cancer, the gene expression pattern for HSP has been partially defined, suggesting that HSPs may play an indirect role in tumor pathogenesis.^{44,45}

HSPCC-96 is an HSP of 96 kDa that is complexed to many antigenic peptides. The tumor antigens are unique to the patient from which the HSPCC is derived, and it is therefore autologous. The 96-kDa backbone is stable between patients, termed gp96, whereas the complexed peptides constitute the unique antigenic fingerprint. Gp96 preparations are capable of eliciting an immune response against a tumor, whereas intact tumor cells may not be capable of doing so.⁴⁶ Gp96 preparations can also elicit a memory T-cell response capable of being recalled.⁴⁷ The inducible immune response is specific to the tumor from which the gp96 is derived.⁴⁸ In Germany, a pilot phase I study was conducted in patients with refractory solid tumors, demonstrating the feasibility of using an HSPCC-96 vaccine approach. The vaccine was

found to be safe and well tolerated. At Memorial Sloan-Kettering Cancer Center, Brannan et al are conducting a small study of HSPCC-96 vaccination in patients with resectable pancreatic adenocarcinoma. Major technical difficulties were encountered in regard to vaccine preparation for the critical few patients. Pancreatic cancers contain a high level of catalytic enzymes, making it difficult to isolate adequate amounts of HSPCC-96 to make a vaccine. However, some of these issues have now been overcome, and a pilot HSPCC-96 trial is in progress to determine the feasibility of this approach. Ongoing trials are also assessing HSPCC-96 vaccination in patients with nonresectable sarcomas and other solid tumors.

Carbohydrate Antigen Vaccines

Peptide vaccines induce cell-mediated immunity. Another method of immune activation is antibody or humoral immunity, which can result from ganglioside vaccination. Gangliosides are neuraminic acid-containing glycolipids situated in the lipid bilayer of the plasma membrane. The carbohydrate portion of the gangliosides is located on the extracellular border of the plasma membrane and is accessible for antibody targeting. In melanoma, GM2 is immunogenic and can induce anti-GM2 antibodies that can lead to complement-activated cell lysis of melanoma cells bearing GM2. Gangliosides are also expressed on the surface of pancreatic cancer cells. Chu et al⁴⁹ examined the expression of gangliosides in 20 controls and 20 patients with either surgically resected ($n = 5$) or locally advanced ($n = 15$) adenocarcinoma of the pancreas.⁴⁹ Serum ganglioside levels were above normal in 16 of 20 patients (75%) with pancreatic cancer and 1 of the 20 control subjects (5%). Higher serum expression of gangliosides (>25 mg/dL) was also observed in patients with a more advanced stage of disease, a finding that has been observed in other malignancies.⁵⁰ In addition, serum ganglioside levels were found to correlate with survival ($P < 0.05$). The authors noted that some of these findings were similar to *K-ras* expression in pancreatic cancer, in that *K-ras* expression also reflects tumor burden and may be correlated with survival. On the basis of observed high levels of immunoglobulin M (IgM) antibody levels against GM₂ and GD_{1b}, these antigens are immunogenic in pancreatic cancer patients and are thus potential immunotherapeutic targets.

Other Antigen Vaccines

A variety of other vaccine target antigens are being assessed in pancreatic cancer. As several of these approaches have been discussed in other chapters, only brief details will be presented here. MUC-1 is a hypoglycosylated transmembrane immunogenic protein that is found throughout epithelial cells of the body, particularly in the gastrointestinal tract. MUC-1 is overexpressed in pancreatic, gastric, breast, prostate, and other malignancies. Preclinical data and early phase I studies demonstrate that specific T cells can recognize selective epitopes and yield a DTH response.^{51,52} A phase I study of MUC-1 vaccination has been preliminarily reported⁵³ in a small population of patients with resected and locally advanced pancreatic cancer. Patients were vaccinated with 100 to 300 μ g of a 100-amino acid synthetic mucin peptide with five repeated immunodominant epitopes. Immune responses were assessed with DTH, T-cell, and immunoglobulin G (IgG) levels. All 8 patients developed a MUC-1-specific DTH response. T-cell responders had mostly CD8+ T cells and were detectable in the peripheral blood. Proliferative T-cell responses as well as MUC-1-specific IgG responses were observed. Thus far, no correlation has been observed between the strength of the immune response and the dose of the vaccine. Other MUC-1 vaccination strategies in development in pancreatic cancer include the use of MUC-1 gene transfected dendritic cells as the tumor vaccine and the use of recombinant adenoviral vectors expressing MUC-1 or carcinoembryonic antigen epitopes.^{54,55}

Conclusions

Vaccine therapy for pancreatic cancer is a rapidly developing field. Vaccine therapy typifies translational research in that the basics of immunology, molecular biology, genetics, and pharmacology are applied in a clinical arena. Vaccine types assessed thus far in pancreatic cancer include autologous whole-cell vaccines, genetically modified cytokine-secreting allogeneic tumor cell vaccines, as well as *ras* peptide and HSP peptide vaccines (Table 31.1). It is clear that no one strategy is going to be entirely successful, and many issues remain to be resolved, including the optimal compo-

TABLE 31.1. Selected vaccine approaches in pancreatic cancer.*

Vaccine type	Disease stage	Reference
Peptide		
Ras peptide	Advanced	Khleif et al ²⁸
Ras peptide	Advanced	Gjertsen et al ^{23,25,26}
Ras peptide + GM-CSF	Advanced	Wojtowicz et al ³²
Ras peptide + IL-2	Advanced	Wojtowicz et al ³²
Ras peptide	Locally advanced	Z'graggen et al ²⁹
Ras peptide + GM-CSF	Adjuvant	Memorial Sloan-Kettering Cancer Center
Allogeneic whole cell		
GM-CSF gene	Adjuvant	Jaffee et al ³⁸

*GM-CSF indicates granulocyte macrophage-colony stimulating factor.

sition of the vaccine, the best immune adjuvant, the dose, route, and the ideal frequency of vaccine administration. During tumor evolution, cancer cells may lose their genetic profile and/or develop immune tolerance, rendering a vaccine approach ineffective. Ways of trying to mitigate against the emergence of immune tolerance and to overcome the heterogeneity of tumor antigen expression include using multiple tumor antigens as a polyvalent vaccine or multiple types of different vaccines, engendering both cell-mediated and humoral immunity.

In pancreatic cancer, it would appear that early stage disease offers the opportunity in which to examine a vaccine approach as, theoretically, the tumor cells are relatively antigenically homogeneous. Therefore, presumably adjuvant vaccine strategies are most likely to be effective in inducing immunity. In the setting of bulk disease, it would not appear that an immunization strategy on its own is going to have significant utility, particularly so in pancreatic cancer where the course of the disease is often rapid. Moreover, in pancreatic cancer, vaccine approaches are going to need to be integrated with traditional cytotoxic and radiotherapeutic treatments.

Indeed, at several institutions, such approaches are underway and will tell us the feasibility and safety of chemo- and radioimmunotherapy and whether the effects of any of the modalities are compromised or enhanced. Other areas of ongoing refinement include determining the optimal tumor antigens to target, as well as the optimal methods of characterizing immune responses.

As the molecular genetics of pancreatic cancer continue to be defined, it is anticipated that new tumor-specific targets will be identified for novel

vaccine approaches. However, we already have one gene, *ras*, that is ubiquitously expressed in pancreatic adenocarcinoma and is uniquely tumor selective, offering a very potent selective target for vaccination, which remains to be exploited to its full potential.

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Antiangiogenic Strategies in Pancreatic Cancer

Christiane J. Bruns, Lee M. Ellis, and Robert Radinsky

Pancreatic Cancer Biology

Cancer of the exocrine pancreas is characterized by extensive local invasion and early lymphatic and hematogenous metastasis.^{1,2} At the time of diagnosis, more than 80% of patients present with either locally advanced or metastatic disease.¹⁻³ The inability to detect pancreatic cancer at an early stage, its aggressiveness, and the lack of effective systemic therapy are responsible for rapid death from this disease. In fact, only 1% to 4% of all patients with adenocarcinoma of the pancreas survive 5 years after diagnosis.^{4,5} For patients with advanced pancreatic cancer, even the recent introduction of the deoxycytidine analogue gemcitabine does not extend median survival duration beyond 6 months.⁶

Research efforts using archival human pancreatic tumor tissue or human pancreatic cancer cell lines have identified a number of characteristic biochemical and genetic abnormalities. These include: point mutations at codon 12 in the *K-ras* oncogene in 75% to 90% of pancreatic adenocarcinoma specimens;^{7,8} allelic deletions involving the cyclin-dependent kinase (CDK)-inhibitory protein p16, found in 85% of human pancreatic cancer xenografts;⁹ mutation, truncation, or deletion of Smad 4 (DPC4), a signal transduction molecule mediating the antiproliferative effects of transforming growth factor β (TGF- β);^{10,11} and mutations in the tumor suppressor gene, *p53*.^{8,12} Other investigators have also demonstrated that receptor protein tyrosine kinases, such as the epidermal growth factor receptor (EGF-R),¹³ c-erbB2,¹⁴ insulin-like growth factor 1 receptor,¹⁵ and fibroblast

growth factor receptor¹⁶ are highly expressed in human pancreatic cancer tissues and pancreatic cancer cell lines.

Tyrosine kinase growth factor receptors and their corresponding ligands act to influence tumor cell growth, differentiation, invasion, metastasis, and angiogenesis. For example, extensive work has shown that cultured human pancreatic cancer cells express high levels of EGF-R and produce one of its stimulatory ligands, transforming growth factor α (TGF- α), and that human pancreatic cancers overexpress EGF-R and all five known ligands.^{13,17-19} The concomitant presence of EGF-R and its ligands EGF, TGF- α , and/or amphiregulin is associated with enhanced tumor aggressiveness and shorter survival periods following tumor resection.^{17,18} Expression of a truncated mutant EGF-R is associated with inhibition of pancreatic cancer cell growth and enhanced sensitivity to cisplatin.²⁰ Furthermore, the growth inhibitory effects of the TGF- β superfamily of serine-threonine kinase receptors and their ligands are often blocked in pancreatic cancer cells. Comparison of the mRNA/protein expression of a variety of growth factors and their receptors between normal pancreatic ductal epithelial cells and five different ductal carcinoma cell lines demonstrated that all ductal carcinoma cell lines overexpressed vascular endothelial growth factor (VEGF), keratinocyte growth factor, and Met/hepatocyte growth factor receptor, whereas cultured immortalized normal pancreatic ductal epithelial cells expressed low levels of these growth factors and receptors.²¹ Taken together, the abundance of growth-promoting factors, the disturbance of growth inhibitory pathways,

tumor-induced angiogenesis, and the presence of gene mutations combine to give pancreatic cancer cells a distinct growth advantage, which clinically contributes to rapid tumor progression, early metastasis, and poor survival.

Biology of Angiogenesis

Definition of Angiogenesis

Angiogenesis is the establishment of a neovascular blood supply derived from pre-existing blood vessels. Newly derived blood vessels are derived from postcapillary venules. In contrast, vasculogenesis is the embryonic establishment of a blood supply from mesodermal precursors, such as angioblasts or hemangioblasts. Recently, it has become apparent that the process of tumor angiogenesis is, in reality, a combination of the above; ie, the main blood supply to a tumor is derived from pre-existing blood vessels, but circulating endothelial cell precursors may contribute to the growing endothelial cell mass.

Pioneering work from Folkman²²⁻²⁴ over the last 30 years has established that angiogenesis is an essential step in the growth of both primary tumors and metastases. Direct observation of tumor growth demonstrates that the rapid exponential growth of a tumor does not result until neovascularization occurs, because growth of tumors in organs where blood vessels do not proliferate is limited to the distance of oxygen diffusion (1-2 mm). Angiogenesis is driven by the production of angiogenic factors by the host, the tumor cells, or both.^{22,24} Increased vascularity may allow not only an increase in tumor growth but also a greater risk of hematogenous metastasis.²⁵ For example, Weidner and colleagues^{26,27} demonstrated a correlation between the incidence of metastasis and microvessel count in invasive breast carcinomas. Similar studies have confirmed this finding in other malignancies, including lung cancer, prostate cancer, melanoma, and colon cancer.²⁸⁻³³ Our data from animal experiments suggest similar trends in pancreatic cancer. The incidence of metastasis correlates with an increased microvessel density. Following in vivo orthotopic selection, a highly metastatic human pancreatic cancer cell variant gave rise to highly

vascularized pancreatic tumors.³⁴ The presence or absence of angiogenesis is related to the balance of pro- and antiangiogenic factors. For example, ASPC1, a human pancreatic cancer cell line, produces in vitro enzymes capable of producing a potent angiogenesis inhibitor, angiostatin. The in vitro generation of angiostatin by ASPC1 cells is related to the constitutive production of large amounts of the serine protease, urokinase plasminogen activator (uPA) and can be inhibited by TGF- β .^{35,36}

Proangiogenic Factors in Pancreatic Cancer

The process of angiogenesis is driven by the production of positive angiogenic factors that override the effect of inhibitory angiogenic factors (Table 32.1). Some of the factors listed bind specifically to receptors on endothelial cells; however, there are pro-angiogenic factors that not only bind to endothelial cells, but may affect the function of other cell types as well. Furthermore, the development of a neovascular blood supply is a series of interlinked processes that eventually leads to new blood vessel formation. Therefore, it is likely that the process of angiogenesis is not driven by a single molecule or family of molecules, but is dependent upon the co-operation and integration of various factors that lead

TABLE 32.1. Regulators of angiogenesis.*

Stimulatory	Inhibitory
Acidic and basic FGF	Angiostatin
Angiogenin	Endostatin
ENA-78	Interferons
GRO α , β , γ	IP-10
Hepatocyte growth factor	MIG
Interleukin 8	Platelet factor 4
Placenta growth factor	Prolactin fragment
Platelet-derived endothelial cell growth factor	Thrombospondin
Transforming growth factor α , β	TIMP
Tumor necrosis factor α	Vasculostatin
Vascular endothelial growth factor/ vascular permeability factor	Angiopietin 2
Angiopietin 1	Others
Others	

*FGF indicates fibroblast growth factor; ENA, Epithelial Neutrophil Activating Peptide-78; IP, inducible protein; MIG, monokine induced by interferon-gamma; and TIMP, tissue inhibitor of metalloproteinase.

to endothelial cell proliferation, migration, invasion, differentiation, and capillary tube formation.

Studies have demonstrated that different processes in the overall process of angiogenesis may be regulated by different angiogenic factors.³⁷ The most well characterized family is the VEGF family. The VEGF family is composed of five VEGF molecules designated A, B, C, D, and E, and placenta growth factor.^{38,39} The best characterized of these factors is VEGF-A (VEGF-A will be referred to as VEGF in the remainder of this review). The importance of VEGF in tumor progression and angiogenesis is supported by the fact that numerous anti-angiogenic strategies target VEGF activity. VEGF was initially discovered as vascular permeability factor, which was subsequently found to be homologous to VEGF⁴⁰, since it induces vascular permeability 50,000-fold that of histamine, the gold standard for induction of permeability. Permeability induction by VEGF allows for diffusion of proteins into the interstitium that form the lattice network on which endothelial cells migrate. The receptors for VEGF are expressed almost exclusively on endothelial cells. The current nomenclature for the VEGF receptors lists three receptors entitled VEGFR-1 (Flt-1), VEGFR-2 (KdR/Flk-1), and VEGFR-3 (Flt-4).

In addition to angiogenic factors that may induce new blood vessel formation, it is also important to note that once blood vessels develop, it is critical that they survive under adverse conditions. The tumor microenvironment is one of low oxygen tension and acidity. VEGF has been shown to be a survival factor for endothelial cells under various stress conditions, including serum starvation and hypoxia.^{41,42} There are numerous nonspecific angiogenic molecules where these factors affect not only the growth of endothelial cells but other cell types as well. These factors include the fibroblast growth factors (acidic and basic) TGF- α and EGF (both of which bind to the EGF-R), platelet-derived growth factor (PDGF), platelet derived-endothelial cell growth factor (PD-ECGF),⁴³ angiogenin,⁴⁴ and the CXC chemokines, interleukin 8 (IL-8),⁴⁵ MIP,⁴⁶ PF-4,⁴⁷ and GRO. These factors are known to be angiogenic in *in vivo* models, but are not specific for endothelial cells.

Recent data from our laboratory have shown that human pancreatic cancer cells secrete proangiogenic molecules including VEGF, IL-8, and basic

fibroblast growth factor (bFGF) in accordance with their metastatic capacity.^{34,48} The higher the metastatic potential of the pancreatic tumor cell variant, the higher the production levels of all three proangiogenic factors.³⁴ In a study of 40 human pancreatic cancer specimens, Ikeda et al⁴⁹ correlated VEGF and PD-ECGF expression and intratumoral microvessel density with patient survival. The median survival time of patients positive for either VEGF or PD-ECGF gene expression was significantly shorter than the median survival time of patients without VEGF or PD-ECGF gene expression. Moreover, VEGF gene expression was moderately associated with an increase in intratumoral microvessel density. Cox multivariate analysis indicated that intratumoral microvessel density and VEGF expression were independent prognostic factors for pancreatic cancer.⁴⁹ Fujimoto et al⁵⁰ identified VEGF121 and VEGF165 as the predominant variants of VEGF produced in pancreatic cancer. However, with 50 human pancreatic tumor specimens, no significant association was found between the expression of VEGF, PD-ECGF, and clinicopathological features, except for tumor histology. The expression of PD-ECGF correlated with poor survival, but microvessel density and VEGF expression were not found to be useful for the prediction of overall survival.⁵⁰ Similar results were observed by Ellis et al.⁵¹

Yamazaki et al⁵² analyzed bFGF expression levels in 20 selected cases of human pancreatic cancer using immunohistochemistry and *in situ* mRNA hybridization and reported that bFGF expression levels are strongly associated with proliferation of tumor cells and endothelial cells. Shimoyama et al⁵³ observed an up-regulation of angiogenin in human pancreatic cancers as compared with normal pancreas. Increased angiogenin mRNA expression as well as elevated serum angiogenin concentrations correlated with poor survival times. In another study, Kuehn et al⁵⁴ described elevated expression of intracellular adhesion molecule 1, vascular cell adhesion molecule 1, and VEGF in surgical specimens from pancreatic cancer patients and in patients with chronic pancreatitis; however, microvessel density was significantly higher in patients with pancreatic cancer versus those with chronic pancreatitis.

Another family of endothelial cell-specific mole-

cules is the angiopoietin family. At the present time, the members of this family are designated angiopoietins 1 through 4. The best characterized of these factors are Ang-1 and Ang-2. Ang-1 and Ang-2 bind to the specific tyrosine kinase receptor, Tie-2, on endothelial cells.⁵⁵ Ang-1 acts as an agonist and is involved in endothelial cell differentiation and stabilization. In contrast, Ang-2 binds to Tie-2 and blocks the binding of Ang-1 to this receptor, which leads to endothelial cell destabilization and vascular regression.⁵⁶ The more recent recognition of Ang-1 and Ang-2 and their role in endothelial cell stabilization suggests that these molecules are most important in endothelial cell survival.⁵⁷ At this time, the role angiopoietins perform in pancreatic cancer development, progression, and metastasis is unclear.

External signals that lead to induction of angiogenic factor expression include environmental stimuli, such as hypoxia or decreasing pH.^{58–60} In fact, hypoxia is the most potent stimulus for angiogenic factor induction, especially VEGF. For pancreatic cancer, Shi et al⁶¹ reported that hypoxia and acidosis contribute to the overexpression of IL-8 in metastatic human pancreatic cancer cells. Other external factors that increase the angiogenic response include various cytokines and growth factors. Cytokines such as insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), EGF, hepatocyte growth factor, IL-1, and PDGF can either act directly on endothelial cells or indirectly in a proangiogenic fashion by up-regulation of VEGF (or other proangiogenic molecules) by tumor cells.^{62–64} In addition, activated *ras* and *src* oncogenes have both been shown to be associated with increased VEGF production and angiogenesis in *in vivo* models.^{65,66} Protein products of tumor suppressor genes, such as the Von Hippel-Lindau (VHL) or p53, also regulate angiogenesis. The wild-type VHL protein is a repressor of transcriptional regulation of the VEGF gene.⁶⁷ A loss of heterozygosity with a mutation in the remaining VHL allele will lead to loss of transcriptional control of the VEGF gene and overexpression of VEGF. Mutant p53 has also been associated with an increase in angiogenesis.^{68,69} Reinsertion of the wild-type *p53* gene into cells with mutant p53 can down-regulate VEGF expression and angiogenesis.⁷⁰ As mentioned previously, mutations in the *p53* tumor suppressor gene and *K-ras* oncogene are common in pancreatic tumor specimens.

Antiangiogenic Strategies

Concept of Antiangiogenic Therapy

Antiangiogenic therapy is an area of active basic and clinical research.⁷¹ Both endogenous and pharmacological antiangiogenic agents have been investigated. Since primary tumor growth is often controlled with surgery or irradiation, antiangiogenic agents may be most beneficial in the treatment of widespread metastatic disease. However, several principles must be considered if this therapy is to be effective. First, because antiangiogenic agents are not tumoricidal but rather tumoristatic, this type of therapy will need to be delivered chronically. Thus, the agent must be well tolerated with minimal side effects. Second, the endpoint of antiangiogenic therapy may not be tumor shrinkage, but rather tumor stabilization. Achieving tumor stabilization over a given period, contrary to traditional endpoints in clinical trials, would be considered a desirable event rather than a clinical failure. Third, since antiangiogenic therapy may be chronic, normal physiologic processes that require angiogenesis may be impaired. In addition to wound healing and cycling of the uterine lining, these processes would also include the physiologic response to cardiac ischemia or peripheral vascular disease. For optimal clinical results, antiangiogenic therapy should perhaps be used in combination with other antineoplastic drugs.⁷²

Numerous antiangiogenic strategies are currently in phase I or phase II clinical trials to treat patients with various malignancies (Table 32.2). Such agents are listed on the National Cancer Institute's cancer trials Web site at <http://cancertrials.nci.nih.gov/>. Trials in progress when this chapter was written are listed in Table 32.2. Typically, tumors secrete angiogenic factor(s) that bind to specific receptors on endothelial cells. One strategy to prevent the angiogenic factor from inducing new blood vessels is to neutralize the factor with specific antibodies. Another strategy would be to block the binding of this angiogenic factor to its receptor by using either antibodies specific to the receptor or small molecules such as kinase inhibitors. Many of the receptors for angiogenic factors have intracellular tyrosine kinase domains; small molecules that inhibit this kinase and hence signaling by these receptors may also inhibit the angiogenic response.

TABLE 32.2. Antiangiogenic agents in clinical trials.*

Drug	Sponsor	Trial	Mechanism
Marimastat	British Biotech, Annapolis, MD	Phase III against pancreas, NSC lung, breast cancers	Synthetic MMP inhibitor
Bay 12-9566	Bayer, West Haven, CT	Phase III against lung and pancreatic cancers	Synthetic inhibitor of tumor growth
AG3340	Agouron, La Jolla, CA	2 trials—phase III against lung and prostate cancers	Synthetic MMP inhibitor
MM1270	Novartis, East Hanover, NJ	Phase I	Synthetic MMP inhibitor
COL-3	Collagenex, Newtown, PA	Phase I	Synthetic MMP inhibitor, tetracycline derivative
Vitaxin	Ixsys, Inc, La Jolla CA	Phase II enrollment begins in early 1999	Antibody to integrin on endothelial cell surface
Interleukin 12	Genetics Institute, Cambridge, MA	Phase I/II	Inhibits endothelial cell growth
Rhu-Mab VEGF Anti-VEGF	Genentech, San Francisco, CA	Phase II/III against lung, breast, prostate, colorectal cancers	Monoclonal antibody to VEGF
SU5416	Sugen, Inc, Redwood City, CA	Phase I and Phase I/II for Kaposi's sarcoma	Molecule that blocks VEGF receptor signaling
SU101	Sugen, Inc, Redwood City, CA	Phase III against glioblastoma and brain cancers; phase II combination against glioblastoma, and phase II against ovarian cancer.	Molecule inhibitor that blocks the signaling of the PDGF receptor
Interferon α	Commercially available	Phase II/III	Molecule that blocks bFGF expression
TNP-470	TAP Pharmaceuticals, Deerfield, IL	Phase II against advanced cancer for adults with solid tumors	Synthetic analogue of fungal protein; inhibits endothelial cell growth
TNP-470 + gemcitabine-based chemoradiation	TAP Pharmaceuticals, Deerfield, IL	Phase II against advanced, nonmetastatic pancreatic cancer	Synthetic analogue of fungal protein; inhibits endothelial cell growth
Intratumoral injection of Onyx-015		Phase I/II against unresectable pancreatic cancer	Chimeric human group C adenovirus with wt p53

*NSC indicates nonsmall cell; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; wt, wild type; and bFGF, basic fibroblast growth factor.

It is also possible that antiangiogenic therapy may involve down-regulation of upstream targets of the angiogenic factors rather than targeting the angiogenic factors themselves.

Preclinical and Clinical Studies for Antiangiogenic Therapy in Pancreatic Cancer

Our own results demonstrated an inhibition of primary pancreatic tumor growth and spontaneous metastasis following biweekly intraperitoneal treatment with a monoclonal rat antimouse flk-1/KDR antibody (DC101) 8 days after orthotopic injection

of highly metastatic human pancreatic cancer cells in nude mice (C.J. Bruns et al, unpublished data, 2001; Table 32.3). Earlier reports by Witte et al⁷³ demonstrated that DC101 potently blocks ligand binding and inhibits VEGF-induced signaling in vitro as well as strongly inhibiting angiogenesis and subcutaneous tumor growth in mice. Using immunohistochemistry, we observed a significant decrease in microvessel density and an increase in the amount of hypoxic areas per total tumor area in pancreatic tumor specimens after 3 to 4 week's treatment with DC101 (C.J. Bruns, et al, unpublished data, 2001). As measured by immunofluorescent double staining for CD31/PECAM-1 (vessels) and TUNEL

TABLE 32.3. Efficacy of antimouse Flk-1/KDR receptor DC101 antibody therapy for human pancreatic cancer growing orthotopically in nude mice.*

Therapy	Incidence [†]			Tumor volume, [‡] mm ³ (range)	Body weight, g (range)
	Pancreas tumor	Liver metastasis	Lymph node metastasis		
Control	8/8	4/8	8/8	395.7 (266–562)	26.7 (18.5–30.5)
Gemcitabine	9/9	3/9	8/9	137 (120–548) [§]	27.6 (25.6–28.6)
DC101	9/9	1/9	9/10	115 (37–285) [§]	27.8 (19.8–30.4)
Gemcitabine + DC101	10/10	2/10	4/10	24.9 (1.8–93.3) [§]	22.3 (20.9–30.9)

*Ten animals per group were treated biweekly with 850 μ g/mouse DC101 and/or 125 mg/kg gemcitabine by intraperitoneal injection.

[†]Visible (>1 mm in diameter) nodules were scored as liver metastases. Macroscopically enlarged regional lymph nodes counted as lymph node metastases and confirmed by histology.

[‡]Tumor volume was calculated by the formula $V = ab^2/2$ where a is the longest diameter and b is the shortest diameter of the tumor.

[§]Control vs gemcitabine, $P < .002$; control vs DC101, $P < .01$; control vs gemcitabine + DC101, $P < .00001$.

Adapted from C.J. Bruns et al, unpublished data, 2001.

(apoptosis), we confirmed in temporal studies a significant increase of apoptotic endothelial cells starting as early as 16 to 23 days after initiation of therapy with DC101 (C.J. Bruns et al, unpublished data, 2001).

In two other preclinical studies using either an antihuman EGF-4 monoclonal antibody (Mab C225,⁷⁴ or a specific EGF-R tyrosine kinase in-

hibitor PKI166),⁷⁵ we demonstrated a significant therapeutic response characterized by primary pancreatic tumor regression and abrogation of metastasis in nude mice. Furthermore, treatment with Mab C225 (biweekly intraperitoneal application) resulted in suppression of tumor cell production of VEGF and IL-8, both in cell culture and in tumors growing orthotopically in nude mice (Fig-

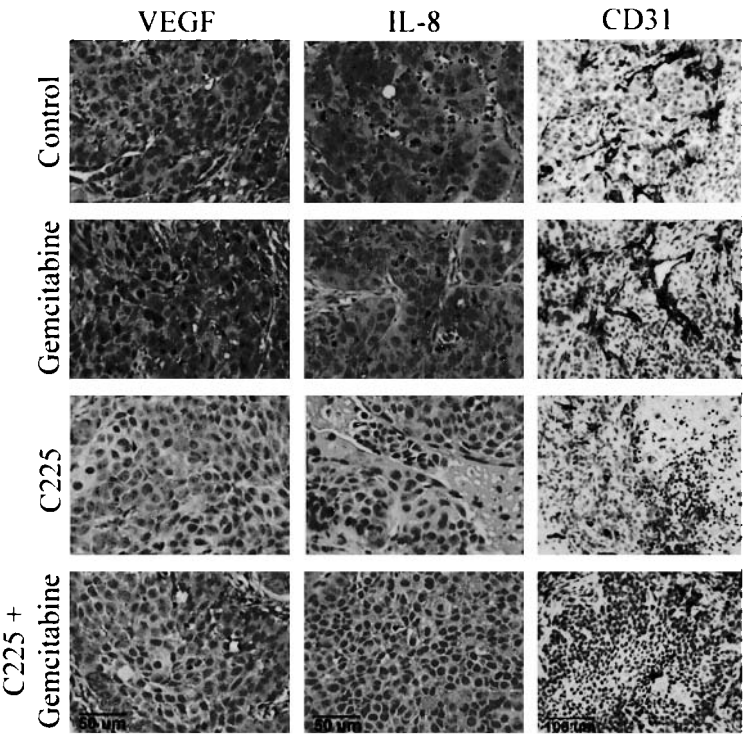


FIGURE 32.1. Immunohistochemical staining of L3.6pl pancreatic tumors for vascular endothelial growth factor (VEGF), IL-8, and CD31 after 18 days of therapy. Tumors from control, gemcitabine-, C225; and C225 + gemcitabine-treated animals (18 days after therapy) were evaluated using immunohistochemistry with anti-VEGF and anti-IL-8 antibodies demonstrating higher immunoreactivity in tumors from control and gemcitabine-treated animals vs C225- and C225 + gemcitabine-treated animals. These tumors were also analyzed with anti-CD31 antibodies directed against mouse endothelial cells. Note the increased vessel density in the control and gemcitabine-treated tumors compared with that of the C225- and C225 + gemcitabine-treated specimens. Adapted from Bruns et al.⁷⁴, with permission.

TABLE 32.4. Efficacy of antihuman EGF-R antibody Mab C225 for human pancreatic cancer growing orthotopically in nude mice.*

Therapy	Incidence*			Tumor volume,‡ mm³ (range)	Body weight, g (range)
	Pancreas tumor	Liver metastasis†	Lymph node metastasis†		
Saline	10/10	5/10	10/10	538.7 (253.7–859.6)	21.7 (16.2–23.8)
Gemcitabine	10/10	3/10	6/10	152.4 (58.8–364.5)§	22.4 (18.1–28.9)
C225	5/10	2/10	8/10	0.3 (0–13.4)§	25.3 (21.3–27.5)
C225 + gemcitabine	0/9	0/9	1/9	0 (0)§	22.7 (20.0–26.8)

*Each therapy group contained 10 animals. Animals were treated biweekly with 1 mg/mouse Mab C225 and 250 mg/kg gemcitabine by intraperitoneal injection.

†All nodules visible under the dissecting microscope (>1 mm in diameter) were considered as liver metastases; macroscopically enlarged regional lymph nodes counted as lymph node metastases when confirmed by histology.

‡Median (range) tumor volume was calculated by the formula $V = ab^2/2$ in which a is the longest diameter and b is the shortest diameter of the tumor.

§ $P < .0001$ (unpaired Student t test), significant difference between control and all three therapy groups.

|| $P < .0001$, between C225 and C225 + gemcitabine groups.

Adapted from Bruns et al.⁷⁴, with permission.

ure 32.1).⁷⁴ The down-regulation of these angiogenic factors preceded the involution of blood vessels as shown by double immunofluorescence microscopy for apoptotic endothelial cells in temporal studies, suggesting a cause and effect relationship (Table 32.4; Figure 32.2).⁷⁴ Similar results were obtained with PTK166 by daily oral administration over 4 weeks (Figure 32.2).⁷⁵

Combination therapy with gemcitabine and either antimouse flk-1/KDR antibody (DC101) (C.J. Bruns et al, unpublished data, 2001) or antihuman EGF-R antibody (Mab C225)⁷⁴ or the EGF-R tyrosine kinase inhibitor PTK166⁷⁵ resulted in further inhibition of primary pancreatic tumor growth and metastasis in an orthotopic nude mouse model. In addition, these animals had a significantly longer survival time when treated with either compound alone.^{74,75}

Shishido et al⁷⁶ reported that TNP-470 (*O*-(chloroacetyl-carbamoyl) fumagillol) in combination with cisplatin displayed a significant inhibitory effect on liver metastasis after intrasplenic injection of a metastatic human pancreatic cancer cell line in nude mice.⁷⁶ In vitro experiments demonstrated that the growth of human pancreatic cancer cells (HPC-3H4) was mildly inhibited, whereas the tumor cell production of VEGF was clearly inhibited.⁷⁶ For patients with locally advanced, nonmetastatic adenocarcinoma of the pancreas, a phase II clinical study using gemcitabine-based chemoradiation in combination with TNP-470 has been initiated.

Since endothelial cells invade basement membranes in order to establish the neovasculature, compounds that inhibit invasion, such as matrix metalloproteinase (MMP) inhibitors, may also inhibit angiogenesis. A multi-institutional phase III study comparing marimastat, a synthetic MMP inhibitor, with placebo as adjuvant therapy in patients after extended pancreaticoduodenectomy has been initiated. Alternatively, since it is reported that mutant p53 is associated with an increase in angiogenesis and reinsertion of the wild-type *p53* gene into cells with mutant p53 can down-regulate VEGF expression and angiogenesis,⁷⁰ clinical trials have been initiated with local application of *p53* adenoviral gene therapy as a putative indirect antiangiogenic therapy. For unresectable pancreatic cancer, a phase I/II study is ongoing using intratumoral injection of Onyx-015, a chimeric human group C adenovirus for wild-type *p53* gene delivery.

Future Goals of Antiangiogenic Therapy in Pancreatic Cancer

Accumulating experimental evidence demonstrates that tumors in animals can be limited to a dormant microscopic lesion when the growth of vascular endothelial cells is suppressed. The most recent evidence that vascular endothelial cells exert potent growth control over tumor cells was established from the following experiments: (1) administration of an angiogenesis inhibitor specific for the prolif-

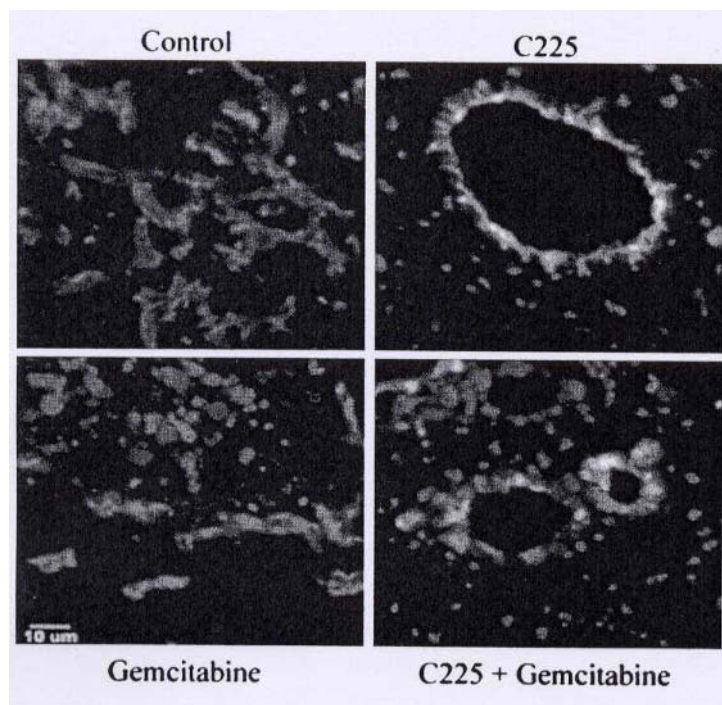


FIGURE 32.2. Immunofluorescence double-staining for CD31 (endothelial cells) and terminal deoxynucleotidyl transferase-mediated NICK end labeling (TUNEL) (apoptotic cells) in L3.6pl human pancreatic tumors after 18 days of therapy. Frozen tissue sections were fixed, treated with a rat anti-CD31 antibody, and then incubated with goat anti-rat IgG conjugated to Texas Red. TUNEL was performed using a commercial kit with modifications. Immunofluorescence microscopy was performed using 400 $\times$ magnification. In a color reproduction, endothelial cells would be identified by red fluorescence, and DNA fragmentation would be detected by localized green fluorescence within the nucleus of apoptotic cells. Overlaying the two results is a yellow fluorescence that would indicate apoptotic endothelial cells. Adapted from Bruns et al.⁷⁴, with permission. (Figure printed in black-and-white to expedite publication.)

erating vascular endothelium in the tumor bed; (2) optimization of the dose and schedule of conventional cytotoxic chemotherapy for vascular endothelium; and (3) targeting of low-dose cytotoxic chemotherapy only to the vascular endothelium in the tumor bed or IV sensitization of vascular endothelium in the tumor bed to radiotherapy by coadministration of an angiogenesis inhibitor.⁷⁷ In the future, antiangiogenic therapy will be added to conventional chemotherapy, radiotherapy, immunotherapy, or even other novel modalities, such as gene therapy. Also, different angiogenesis inhibitors may be administered together for increased efficacy. The overall goal of antiangiogenic therapy is to reduce toxicity, reduce the risk of drug resistance, and to increase the anticancer efficacy. Based on preclinical studies, different antiangiogenic therapeutic approaches have been chosen for the clinical trials in pancreatic cancer. Our own data as well as other preclinical results suggest that combination of conventional cytotoxic chemotherapy (gemcitabine) with direct or indirect antiangiogenic strategies significantly increases the anticancer efficacy and prolongs survival in human pancreatic cancer growing orthotopically in nude mice. Ultimately, the inhibition of tumor-associated angio-

genesis and related processes may form the foundation for a less toxic and more effective systemic treatment of pancreatic cancer.

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Role of Matrix Metalloproteinase Inhibition in the Treatment of Pancreatic Cancer

Alexander S. Rosemurgy II and Mahmudul Haq

Introduction

Pancreatic cancer is a deadly cancer. It is one of the few cancers whose incidence equals its death rate.¹ Despite advances in diagnostic and operative techniques, only a small percentage of patients with pancreatic cancer survive more than 1 year.¹ Because of this bleak prognosis, new therapies for this cancer are being sought in hope of improving survival.

A variety of solid organ cancers, including pancreatic cancer, contain relatively high levels of matrix metalloproteinases (MMPs).² MMPs are proteolytic enzymes that disrupt and destroy intercellular bonds. In this way, MMPs degrade basement membranes, a critical step in invasion and metastasis of cancer cells. As well, these enzymes participate in peritumoral stromal degradation and angiogenesis, also essential components of cancer growth, invasion, and metastasis.³⁻⁵ Using animal models, several investigators have reported the potential therapeutic role of MMP inhibition in the treatment of solid tumors, including breast cancer,^{6,7} colon cancer,^{8,9} ovarian cancer,^{10,11} and cutaneous melanoma.¹² Clinical trials have promoted beneficial effects of MMP inhibition in patients with advanced lung cancer¹³ and in patients with malignant ascites.¹⁴

The role of MMP in the growth and dissemination of pancreatic cancer has received interest because conventional cytotoxic therapy has produced disappointing results with pancreatic cancer. Implicit in understanding the role of MMP in the behavior of pancreatic cancer is the possibility of therapeutic MMP inhibition.

MMP in Pancreatic Cancer

MMPs have been found to be present in high levels in a host of solid organ cancers. There are 19 different recognized MMPs. We have found MMP production to be increased in pancreatic cancers, relative to normal pancreas (Figure 33.1). Furthermore, pancreatic cancer MMP gene expression is differentially increased for different types of MMPs, including MMP-2 and MMP-9 (Figure 33.1). Of these, MMP-2 seems particularly important in the growth and dissemination, or aggressiveness, of pancreatic cancer, with MMP-9 playing a lesser role^{15,16} (Figure 33.2).

Twenty-one human pancreatic cancers resected for cure were studied for MMP content. For these 21 cancers, no significant correlation among conventional measures of prognosis, including tumor grade, nodal status, and histological grade, was noted. We did find that tumor levels of the active form of MMP-2 (MMP-2a) correlated extremely well with nodal status and tumor stage (Table 33.1). To a lesser extent, in the active form, MMP-9 correlated with nodal status and stage. Tumors high in MMP-2a and/or MMP-9a were more likely to have nodal metastases and be of advanced stage. Tumor levels of MMP-9a and MMP-2a did not correlate with tumor grade, implying that tumors with low MMP levels can grow to a larger size prior to contiguous invasion or dissemination. Active MMP levels, particularly MMP-2, significantly correlated with apoptosis within the tumors, denoting that increased tumor cell growth rates occurred with and superseded increased programmed cell death. Notably, tumor histology inversely correlated with tumor levels of naturally oc-

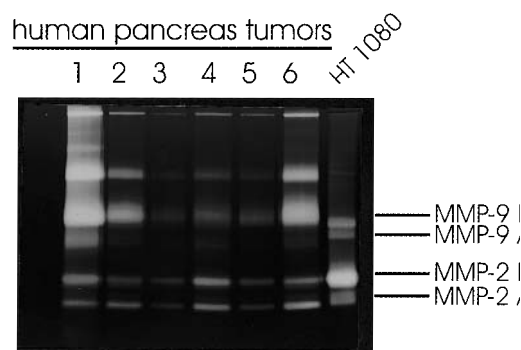


FIGURE 33.1. Western blot of purified samples of human pancreatic tumor tissue showing matrix metalloproteinase (MMP) production. HT 1080 cells (known producer of MMP) were used as a control. Purified proteins were blotted with active (A) and latent (L) MMP-2 (M, 72, 000) and MMP-9 (M, 92, 000) antibodies. Higher levels of active MMP-2 were found in poorly differentiated cancer (lanes 1, 2, 4, 6) and moderately differentiated cancer (lane 5) than in normal pancreatic tissue (lane 3).

curing inhibitors of MMP (TIMP-1 levels). Moreover, aggressive histological grades were associated with lesser levels of tumor TIMP-1. There was no TIMP-1 in peritumoral stroma, documenting that, in peritumoral boundaries, MMP activity was unimpeded by this naturally occurring inhibitor. These findings promote a complex role for MMP in the biological behavior of pancreatic cancer.

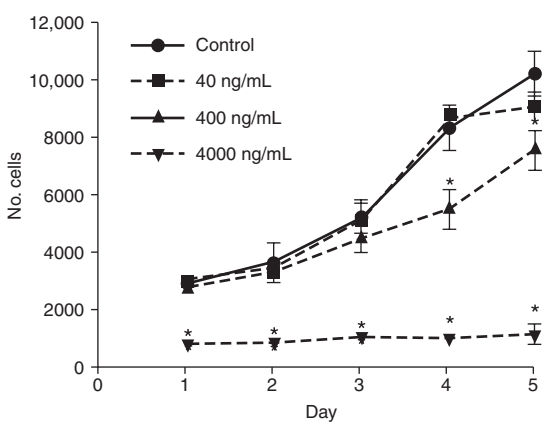


FIGURE 33.2. Toxicity profile of BB-94 on pancreatic cancer cell line as determined by MTT assay. Significant inhibition of cell proliferation occurred at 400 ng/mL on Days 4 and 5, while near complete inhibition occurred at 4000 ng/mL. * $P < 0.05$, analysis of variance.

TABLE 33.1. Correlation between MMP* production and tumor grade, nodal status, and tumor histological grade TNM stage in resected human pancreatic cancers. (Data shown as correlation coefficients.)

	Tumor grade	Nodal status	Stage	Histological grade
MMP-2	0.19	0.51 ($P = 0.02$)	0.60 ($P = 0.01$)	0.07
MMP-9	0.17	0.40 ($P = 0.08$)	0.46 ($P = 0.04$)	0.03

*MMP indicates matrix metalloproteinase.

MMP Inhibition in Treating Pancreatic Cancer

MMP inhibitors have been available for a considerable time. Various inhibitors have varying selectivity and efficacy. BB-94 (Batimastat) is a parenteral broad-spectrum inhibitor, which binds to a zinc-binding site on MMP, thereby changing MMPs' three dimensional anatomy and action. BB-94's IC_{50} of MMP activity occurs at a low nanomolar range. Once an oral MMP inhibitor became available, increased enthusiasm came to the concept of MMP inhibition. The oral inhibitor BB-2156 (Marimastat) has a spectrum of action very much like BB-94 and was well tolerated in phase 1 trials.

To study the role of MMP inhibition in the growth and dissemination of human pancreatic cancer, we first utilized the in vitro 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.¹⁷ This assay allows reliable and reproducible determination of cancer cell growth curves. By comparing a vehicle control with various concentrations of BB-94, we were able to determine the effect of MMP inhibition in vitro (Figure 33.2). In these studies, only when the concentration of BB-94 was more than 100 times the therapeutic goal of serum concentrations did any reduction in cancer cell proliferation occur. With these extremely high concentrations of BB-94, reductions in cancer cell proliferation are not due to any specific action of MMP inhibition but, rather, due to nonspecific extreme changes in the growth medium due to the high concentrations of BB-94 or its vehicle soap.

Effects of MMP inhibition on cancer cell invasiveness in vitro were studied using the matrigel assay. This assay involves a chamber divided by a reconstituted basement membrane. The intent of

the assay is to determine the ability of cancer cells to cross the basement membrane, this ability being equated to cancer cell invasiveness *in vivo*. In this assay, MMP inhibition had minimal effects on invasiveness. *In vivo*, reductions in cancer cell proliferation and invasion through MMP inhibition require factors and mediators not present *in vitro*.

For *in vivo* studies of MMP inhibition, we have utilized an athymic T-cell depleted (nude) mouse model of human pancreatic cancer. In these studies, commercially available cancer cell lines and cancers resected from patients have been grown in cell culture. Cancer cells were then heterotopically implanted into the flanks of mice or orthotopically implanted into the head of the pancreata of the mice at celiotomy. Serial evaluations of tumor growth and survival studies have been undertaken. Necropsies have allowed us to study the rate of cancer implantation, tumor growth, and the role of MMP in cancer progression.

It seems clear that MMP inhibition *in vivo*, by the inhibitor Batimastat (BB-94), reduces implantation, delays tumor growth, and prolongs survival (Figure 33.3).¹⁹ Generally, compared with mice receiving vehicle control, implantation is decreased by 15% in mice receiving BB-94, even when therapy is started as long as 1 week following tumor cell implantation. The specific cause of reduced implantation is not known, though it seems that the efficacy of the metalloproteinase inhibitor is such that the immune status of the mouse is sufficient to eliminate all traces of the injected cancer cells.^{17,18}

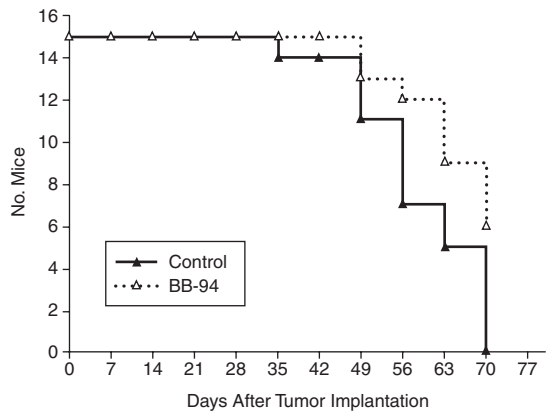


FIGURE 33.3. Survival curve demonstrating improved survival in animals receiving BB-94 vs animals receiving vehicle control. $P < 0.05$, Wilcoxon test.

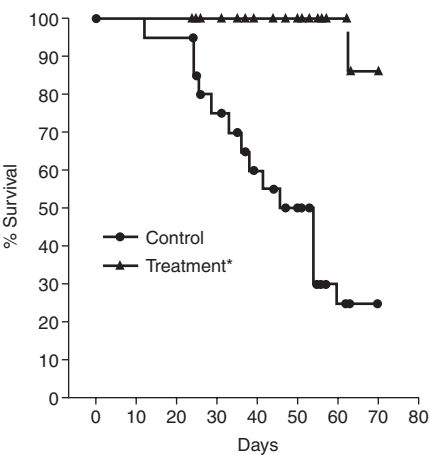


FIGURE 33.4. Survival curve beginning on implantation date (Day 0) and continuing to sacrifice (Day 70). Treated animals survived significantly longer than controls. $*P < 0.05$, Wilcoxon test.

Mice receiving the parenteral inhibitor BB-94 have been shown in a number of ways to have smaller tumors. Tumors appear later in mice receiving BB-94. The inhibitor reduces tumor weights and tumor volumes and, consistent with these, Ca 19-9 levels are dramatically reduced in mice receiving BB-94. MMP inhibition is also associated with less tumor invasion and fewer metastases in fewer mice in this mouse model. Survival times in mice receiving BB-94 significantly surpass survival times in mice receiving vehicle control (Figure 33.3), further promoting the efficacy of metalloproteinase inhibition.¹⁷ We have found improvements in survival of mice in study after study, even when therapy is initiated at a time distant to implantation.¹⁸ Survival time is particularly improved when therapy is initiated *prior* to tumor implantation¹⁷ (Figure 33.4). The survival time of mice starting BB-94 therapy prior to tumor implantation is almost indefinite, indistinguishable from mice receiving sham injections.

Combining Cytotoxic Therapy With Metalloproteinase Inhibition

Gemcitabine is the standard cytotoxic drug used in patients with unresectable pancreatic cancer. Its activity is due to interference with DNA replication.

Complications or side effects are generally hematologic.

Gemcitabine and metalloproteinase inhibitors obviously have different mechanisms of action. The potential added efficacy of combination therapy is naturally considered, and synergistic activity may be possible. Unfortunately, given these drugs' differences in action, different and varied complications may be expected with such combination therapy, and accentuated or exaggerated complications may be seen as well. To investigate the possibility of added efficacy with combination therapy, we have studied the combination of gemcitabine and BB-94 in the nude mouse model of human pancreatic cancer utilizing a well-established well-differentiated human pancreatic adenocarcinoma cell line called HPAC.¹⁹

As seen in our previous *in vivo* nude mouse studies, cancer cell implantation in mice receiving vehicle control was not uniform. Approximately 10% of mice receiving the vehicle control beginning 1 week following cancer cell injection did not harbor malignancy at necropsy. As stated earlier with similar studies, diminished rates of implantation

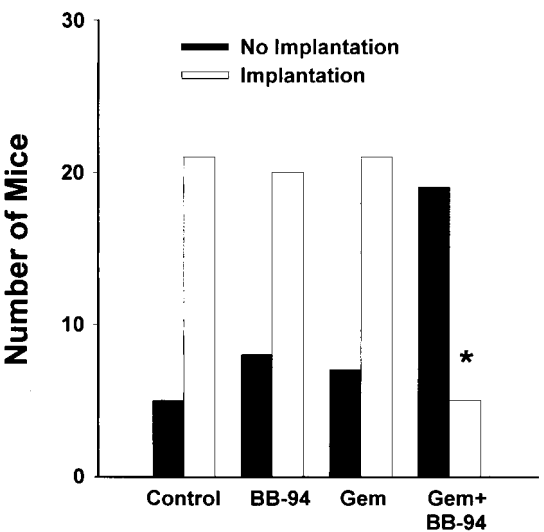


FIGURE 33.5. Rate of implantation in the groups of mice. There was significant reduction of implantation in mice treated with combination therapy (gemcitabine [Gem] + BB-94), $P < 0.0001$, Wilcoxon test. Control group (implantation in 21 of 26); BB-94 (implantation in 19 of 27); Gem (implantation in 21 of 28); and Gem + BB-94 (implantation in 5 of 24).

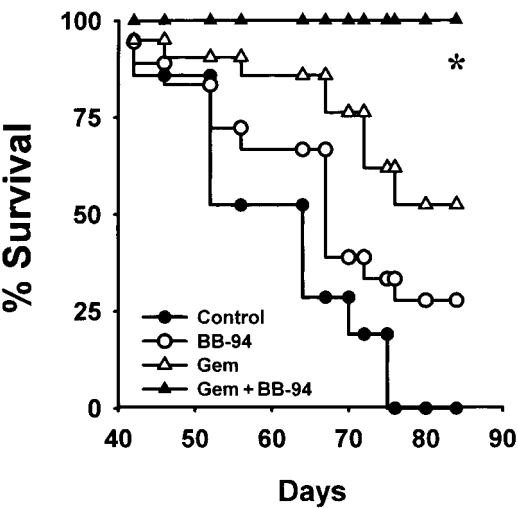


FIGURE 33.6. Survival curves of all groups beginning at 40 days after tumor injection and continuing up to 84 days until all mice were dead or sacrificed. Treated mice with combination (gemcitabine [Gem] + BB-94) therapy survived significantly longer than mice receiving other therapies or control vehicle. * $P < 0.001$, Wilcoxon test.

were seen in mice receiving BB-94 alone, and, as well, slightly diminished rates of implantation were seen in mice receiving gemcitabine alone. Mice receiving BB-94 and gemcitabine had greatly diminished rates of implantation (Figure 33.5). The vast majority of mice receiving such combination therapy did not experience successful implantation of cancer cells, even though therapy was started 1 week following operative orthotopic injection of cancer cells at celiotomy.¹⁹ While such low rates of implantation make it difficult to study the growth of cancer in mice receiving this combination therapy, it certainly does document that combination therapy offers new hope of controlling pancreatic cancer, particularly in circumstances of minimal cancer. This certainly suggests a role for such combination therapy for patients in need of adjuvant therapy.

Since not all mice had successful implantation of cancer cells, we eliminated mice without cancer from further analysis and study. Studying only mice that had cancer, survival was significantly prolonged by combination therapy of BB-94 and gemcitabine¹⁹ (Figure 33.6). With combination therapy, mice with cancer experienced indefinite survival times, which ended only with sacrifice. Similar to our other stud-

ies, mice receiving the vehicle control had progressive deterioration in survival beginning at 40 days following implantation. This survival curve was relatively poorer than, though not statistically different from, survival curves associated with BB-94 or gemcitabine alone. While other previous studies of ours have shown significant enhancement in survival times by BB-94,¹⁸ this specific trial of combination therapy did not, because the number of mice receiving BB-94 alone was small.

Similar to improvements in survival times brought about by combined BB-94 and gemcitabine therapy, mice receiving gemcitabine in combination with BB-94 had lower tumor levels of active and latent MMP-2 as well as lower levels of active MMP-9. Similarly, serum MMP levels were lowest in mice receiving combination therapy.¹⁹

From this preclinical study, it seems that combination therapy of metalloproteinase inhibition and cytotoxic therapy holds promise in the treatment of pancreatic cancer. This seems especially true for circumstances of relatively minimal tumor burden.

Preclinical studies give hope that MMP inhibition, especially in combination with cytotoxic therapy, will significantly prolong survival of patients with pancreatic cancer receiving cytotoxic therapy alone, as well as begin to elucidate mechanisms that may result in prolonged survival.

Metalloproteinase Inhibition in Patients with Pancreatic Cancer

There is a growing body of preclinical and clinical data documenting the efficacy of metalloproteinase inhibition for patients with cancer. Patients diagnosed with small cell lung cancer¹³ and ovarian cancer¹⁴ have been studied.

A survival study involving patients in the United States and the United Kingdom with inoperable pancreatic cancer was undertaken in the early 1990s.²⁰ This trial was designed, in many ways, to mimic the inclusion criteria utilized when 5-fluorouracil was compared with gemcitabine in patients with inoperable pancreatic cancer.²¹ CA 19-9 levels were used as a surrogate marker of efficacy in this trial, as the mechanism of MMP inhibition is noncytotoxic and efficacy seems to be optimally monitored through such a surrogate

marker. To enter into this trial, patients had to have unresectable pancreatic cancer and have an increasing CA 19-9 of 25% or more in the month prior to inclusion in the trial. Given this patient population, 74 patients were studied and only 3 patients were later excluded. Median survival time was 125 days upon conclusion of the trial, with a number of patients still alive. Patient survival to 365 days was estimated to be approximately 30%. With all the caveats of using historical controls, this number does seem to be superior to the 18% 1-year survival commonly associated with gemcitabine.

Patients in this trial could be divided into two groups based on the rate of rise of CA 19-9 following initiation of therapy. Retrospectively, it was seen that patients who continued to have a rise in CA 19-9 of more than 25% in the month following initiation of therapy had poorer outcomes, with a projected survival rate of approximately 22% at 1 year. Patients with a decrease in the rate of rise of CA 19-9 or patients that had an actual decrease in CA 19-9 within 1 month following initiation of therapy had a much better survival rate, which approached 60% at 1 year.²⁰ This latter group was not small, comprising approximately 40% of the patients in the trial. The correlation between survival and CA 19-9 response to therapy gives hope that patients early in the course of their therapy could be evaluated for response, and therapy could be continued for patients with a high probability of long-term survival.

Gemcitabine has been compared to an oral broad spectrum metalloproteinase inhibitor, Marimastat (BB-2516), in a prospective randomized clinical trial, which was presented at the American Society of Clinical Oncology in 1999.²² This randomized trial compared Marimastat to gemcitabine as first-line therapy for patients with unresectable pancreatic cancer. The randomized comparison involved three-dose levels of Marimastat and a standard regimen of intravenous gemcitabine. Marimastat was dosed at 5 mg twice per day, 10 mg twice per day, and 25 mg twice per day. Gemcitabine was dosed at 1000 mg per meter squared. One hundred patients were enrolled to receive each dose of Marimastat or gemcitabine. The objectives of this trial were to evaluate survival, as a primary endpoint, and time to disease progression, as a secondary endpoint. In addition, quality of life, safety, and tolerability were secondary endpoints.²²

All patients entering into the trial had histological or cytological diagnoses of unresectable pancreatic cancer. All patients were more than 18 years old and all had Karnofsky performance scores of more than 50%. Patients with prior malignancies were excluded, and all patients had adequate liver and renal function and adequate bone marrow reserve. Patients entered into the trial underwent minimization to ensure all groups were comparable in makeup. Minimization criteria included tumor stage, Karnofsky score, recurrent versus newly diagnosed pancreatic cancer, gender, and clinical center.

Mean age, mean weight, gender distribution, Karnofsky performance score, stage of cancer, and the presence of liver metastasis were equivalent in all groups.

Primary mortality analysis documented that patients receiving 5 mg of Marimastat twice daily or 10 mg of Marimastat twice daily had similar survival curves. These curves were significantly inferior to patients receiving Marimastat 25 mg twice daily or gemcitabine. Patient survival with twice-daily doses of 25-mg Marimastat was not different than with gemcitabine.

The median time for disease progression was 56, 59, and 57 days for twice-daily Marimastat doses of 5 mg, 10 mg, and 25 mg, respectively. The median time to disease progression with gemcitabine was significantly longer (115 days; $P < 0.001$).²² Patients with liver metastases seemed to survive longer with gemcitabine, while patients with extensive locoregional disease seemed to survive longer with the twice-daily 25-mg dose of Marimastat.²²

Studies of safety and tolerability documented few casualties among patients receiving either gemcitabine or Marimastat. Patients receiving either of the therapies had similar numbers of adverse events. Most of the adverse events were very mild and many of them were more consistent with the underlying cancer than the therapy being tested, such as vomiting, nausea, and constipation. Of significant complications, gemcitabine seemed to be most associated with hematologic complications, such as leukopenia and thrombocytopenia. The metalloproteinase inhibitors, as expected, seemed most associated with musculoskeletal complaints, the majority of which were self-limiting. Musculoskeletal complaints with metalloproteinase inhi-

bition have been particularly noted by us to occur in the dominant hand, the hands more than the shoulders, and more in the shoulders than in the lower extremities (Table 33.2). Most of the musculoskeletal complaints were minor, but they were the primary reason for dose reduction and drug holiday for patients receiving Marimastat.

This randomized study comparing Marimastat with gemcitabine as a first-line therapy in patients with unresectable pancreatic cancer documents no significant difference between the survival curves associated with twice-daily 25-mg doses of Marimastat or gemcitabine. One-year survival associated with twice-daily 25-mg of Marimastat was the same as that for patients receiving gemcitabine. In regards to survival, this study did not meet its endpoint. The Cox model of proportional hazards documented a dose-dependent effect of Marimastat, with patients receiving 25 mg twice daily surviving longer than patients receiving lesser doses. Although there were differences in tolerability, gemcitabine and Marimastat were generally well tolerated by all patients, with no marked differences in safety. Expected hematologic complications (gemcitabine) and musculoskeletal complications (Marimastat) were seen. Complications associated

TABLE 33.2. Safety and tolerability—grade 3 or 4 NCI-CTCs.*

	5 mg	10 mg	25 mg	Gemcitabine
All causalities, % of patients: >5% incidence in any group				
Any adverse event	51	55	53	52
Musculoskeletal	9	8	13	1
Vomiting	5	7	11	10
Pulmonary	14	9	11	18
Neurocortical	17	12	10	13
Neuromotor	14	7	9	10
Nausea	12	9	8	6
Infection	7	7	8	12
Neuroconstipation	6	8	5	2
Bilirubin	18	27	21	15
Alk. phosphatase	17	15	17	7
Hyperglycemia	11	11	12	11
Transaminases	4	9	6	4
Granulocytes	1	0	0	6
Treatment-related, % of patients: >5% incidence in any age group				
Any adverse event	14	10	15	20
Musculoskeletal	7	7	12	0
Pulmonary	3	0	1	7

*Data from Rosemurgy et al.²⁰

with rapid disease progression were noted and did not seem to complicate this trial.²²

A study comparing gemcitabine to gemcitabine with Marimastat is currently in progress and the data from this trial should be available in the near future. Preliminary results suggest that there is no added benefit in survival with Marimastat. Unfortunately, despite minimization, homogeneous group comparisons are generally not possible in such trials and negative results must be taken with some trepidation. Furthermore, subgroup analysis may not be possible, thereby obscuring a role for MMP inhibition for patients with more minimal disease. Final results of this trial are eagerly awaited.

A prospective double-blind randomized placebo-controlled trial studying the efficacy of Marimastat after pancreatic resection of curative intent is in progress. Given the preclinical work we have generated and the subgroup analyses undertaken in the preceding clinical trials, we remain hopeful that effective adjuvant therapy will be documented in this trial. It seems logical, knowing how MMP inhibition works and knowing it seems most efficacious in settings of minimal disease, that MMP inhibition is best suited to be used as adjuvant therapy.

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Regulation of Pancreatic Cancer Growth by Gastrointestinal Hormones: A Clinically Useful Strategy?

William E. Fisher and David H. Berger

Introduction

The concept that hormonal manipulation could be used to control the growth of cancers originating from organs normally under hormonal control was proposed over 100 years ago. In 1895, the Scottish surgeon George T. Beatson performed bilateral oophorectomy on a woman with advanced breast carcinoma, which resulted in dramatic regression of her disease and demonstrated that hormonal control of cancer was possible.¹ Another surgeon, Charles B. Huggins, eventually won the Nobel prize in 1966 for demonstrating regression of cancers by endocrine manipulation. He demonstrated that antiandrogenic treatment consisting of orchiectomy or the administration of estrogens could produce long-term regression in patients with advanced disseminated prostatic carcinoma.² Today, dramatic results are not uncommon after hormonal treatment of prostatic, breast, endometrial, and ovarian cancer, even in patients with advanced disease.^{3,4} A similar approach to pancreatic cancer, a malignancy that usually presents in an advanced stage, may hold great promise.

The fact that many gastrointestinal hormones affect the growth of the normal exocrine pancreas suggests that these peptides could be used to slow the growth of pancreatic cancer. Gastrointestinal hormones, such as somatostatin, that normally inhibit the division of the exocrine pancreatic cells, may also slow the growth of exocrine pancreatic cancer and provide a relatively nontoxic therapeutic option. It has also been postulated that antagonists of growth-promoting hormones, such as cholecystokinin, may be beneficial in the adjuvant treatment of pancreatic cancer. Alternatively, growth-promoting hormones, by

increasing tumor cell division, may increase the sensitivity of a pancreatic tumor to adjuvant cytotoxic treatment. To provide a more substantial rationale for gastrointestinal hormonal therapy for pancreatic cancer, it must be proven that these hormones can indeed affect the growth of autologous malignant cells.

In the laboratory, several experimental steps are commonly taken to demonstrate the potential of a hormone in manipulating the growth of pancreatic cancer. The growth effect of the hormone must be shown *in vitro* using proliferation assays. The growth effect should also be demonstrated *in vivo*, commonly by studying the effect of the hormone on the growth of established tumor xenografts in athymic nude mice or by studying the effect of the hormone on carcinogenesis. Evidence that the tumors produce receptors for the hormone are important in clarifying the mechanism of altered growth. Finally, neutralization of the hormone or the receptor with an antibody or specific antagonist should inhibit the growth effect. By summarizing the results of many such experiments, this chapter provides a substantial rationale for further study into the use of gastrointestinal hormones as adjuvant treatment of pancreatic cancer.

Gastrointestinal Hormones That May Inhibit Pancreatic Cancer Growth

Somatostatin

Somatostatin is a naturally occurring 28-amino-acid peptide secreted by the D cells in the pancre-

atic islets. It has been characterized as the “universal off switch” because it inhibits the release of growth hormone and essentially all gastrointestinal hormones. Somatostatin is known to inhibit not only pancreatic endocrine and exocrine secretion but also DNA synthesis.⁵ Somatostatin has been shown to inhibit carcinogenesis in animal models (Table 34.1). Somatostatin receptors have been

TABLE 34.1. Gastrointestinal hormones that may inhibit pancreatic cancer growth.

Somatostatin	References
Somatostatin and its analog, RC-160, decreased preneoplastic changes and tumor formation in hamsters exposed to a pancreatic carcinogen.	30–31
RC-160 decreased tumor weight and prolonged survival of hamsters with pancreatic cancer.	32
Somatostatin and 5-FU inhibited preneoplastic changes and tumor formation in hamsters.	33
Somatostatin receptors were demonstrated in the normal exocrine pancreas and in pancreatic cancer specimen.	34
Somatostatin receptors were demonstrated on normal human pancreas and pancreatic cancer cells.	35
Growth of human pancreatic cancer (MIA PaCa-2) cells was inhibited in cell culture by somatostatin-14.	36
Growth of human pancreatic cancer (MIA PaCa-2) cells was inhibited in cell culture by three long-acting analogs of somatostatin.	37
Colony formation of human pancreatic cancer BxPc-3, but not SOJ-6, cells was decreased by somatostatin.	38
Growth of human pancreatic cancer (MIA PaCa-2) cells implanted in nude mice was inhibited by octreotide.	39
A somatostatin analog, RC-160, inhibited the growth of human pancreatic cancer (MIA PaCa-2) tumors in nude mice.	40
A somatostatin analog containing methotrexate enhanced inhibition of human pancreatic cancer (MIA PaCa-2) growth.	41
Human pancreatic cancer (MIA PaCa-2 & Panc-1) cells did not have somatostatin receptors & somatostatin-14 did not affect growth of the cells in culture.	42
Somatostatin inhibited the growth of human pancreatic cancer cells, SKI, in nude mice but not PGER. The SKI, but not PER, cell line was shown to have somatostatin receptors.	43–44
Somatostatin inhibited the growth of human pancreatic cancer cells (SKI & CAV) in nude mice.	45
Sandostatin, a long acting somatostatin analog, had no effect on 14 patients with metastatic pancreatic cancer treated for 7 weeks.	46
No survival benefit was seen in 19 patients with advanced exocrine pancreatic carcinoma given the somatostatin analog BIM 23014 for 2 months.	47
No benefit was seen in a randomized prospective trial of somatostatin vs. placebo in 86 pancreatic cancer patients.	48
The human pancreatic cancer cell line, MIA PaCa-2 was inhibited by somatostatin & octreotide in vitro and in vivo. Other human pancreatic cancer cell lines (Capan-1, Capan-2, CAV, & Panc-1) were not inhibited.	49
Vasoactive Intestinal Peptide (VIP)	
VIP receptors were demonstrated on human pancreatic cancer cells.	50
VIP receptors were demonstrated on human pancreatic cells (Capan-1) but no inhibition of cell proliferation in culture was seen.	51
VIP inhibited growth of hamster pancreatic cancer (H2T) in nude mice but not human pancreatic cancer (MIA PaCa-2). VIP receptors were demonstrated on H2T but not MIA PaCa-2.	52
Tumor masses were visualized by gamma-camera scanning after IV, ¹²³ I-VIP in 10 of 12 patients with pancreatic adenocarcinoma.	53
Pancreatic Polypeptide Family	
Peptide YY (PYY) & its synthetic analog BIM-43004-1 inhibited human pancreatic cancer cell growth (MIA PaCa-2) in vitro.	54
PYY receptors were demonstrated on human pancreatic cancer cells (MIA PaCa-2). Decreased intracellular cAMP levels were associated with suppressed tumor growth in vivo.	55
NPY & PYY increased the incorporation of ³ H-thymidine in MIA PaCa-2 and hamster (H2T) pancreatic cancer cells.	56
Pancreastatin	
Pancreastatin inhibited the growth of the normal pancreas and human pancreatic cancer cells (MIA PaCa-2 & SW-1990). Pancreastatin caused only a transient decrease in tumor volume but did not alter final tumor weight of MIA PaCa-2 xenografts in nude mice.	57

demonstrated on some animal and human pancreatic cancer cells, and the growth of these cell lines is inhibited by somatostatin and its analogs in vitro and in vivo (Table 34.1). However, a role for somatostatin and its analogs in pancreatic cancer therapy has yet to be established. Initial studies suggest that these agents could provide a useful and relatively nontoxic adjuvant therapy (Table 34.1).

The exact mechanism of the antiproliferative effect of somatostatin is not known. The inhibitory effect could be mediated through specific, high-affinity somatostatin receptors on the tumor cell surface. Alternatively, the antitumor effect of somatostatin could be independent of receptors. Somatostatin could inhibit tumor proliferation by inhibiting the secretion of other gastrointestinal hormones thought to be important in pancreatic cancer growth. In addition, somatostatin has also been shown to selectively decrease splanchnic blood flow and inhibit angiogenesis, either of which might interfere with pancreatic tumor growth.

Five different somatostatin receptors have recently been cloned and we are just beginning to unravel the biological properties of each. The mechanism of receptor-dependent growth inhibition of cancer cells can either be cytotoxic or cytostatic. Some groups believe that cytotoxicity leading to apoptosis is signaled solely via the somatostatin receptor subtype 3 (SSR-3), whereas the other four receptor subtypes initiate a cytostatic response leading to growth inhibition. It has been reported that activation of SSR-3 causes the induction of wild-type p53 and apoptosis.⁶ Activation of SSR-1, 2, 4, and 5 results in induction of the retinoblastoma protein Rb and G1 cell cycle arrest.⁷ Of these receptors, SSR-5 had the most potent growth inhibitory effect. Another group has shown that binding of somatostatin to SSR-2 up-regulates p27, thus leading to cell cycle arrest in the G0-G1 phase, and subsequently to apoptosis.⁸

Despite promising cell culture and animal studies, recent clinical trials of somatostatin analogs in the adjuvant treatment of pancreatic cancer have failed to demonstrate a response (Table 34.1). All of these studies examined patients with advanced pancreatic cancer. None of these clinical studies have examined the tumors being treated for somatostatin receptors, but other studies have shown that most human pancreatic cancers do not express

somatostatin receptors. Loss of somatostatin receptor expression may be an important step in the process of pancreatic carcinogenesis. Many important questions remain to be answered regarding the role of somatostatin receptors in pancreatic cancer growth. The molecular mechanism causing loss of receptor expression by pancreatic adenocarcinoma cells remains unknown. Transfer of somatostatin receptor genes may be a viable treatment strategy for receptor-negative tumors. However, this strategy is currently unproven, and the most effective receptor or combination of receptors has yet to be determined.

Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is a 28-amino-acid peptide involved in the regulation of the exocrine pancreas. VIP may inhibit the replication of epithelium by acting through cyclic adenosine monophosphate. Several studies have demonstrated functional receptors for VIP on animal and human pancreatic cancer cells and inhibition of cancer growth in vitro and in vivo (Table 34.1). We have been unable to demonstrate VIP receptors or an antiproliferative effect in cell culture by VIP with the MIA PaCa-2, Panc-1, Capan-1 and CAV human pancreatic cancer cell lines. In addition, when we examined 20 histologically proven human pancreatic adenocarcinomas from our patients undergoing laparotomy, none were found to express VIP receptors by reverse transcriptase polymerase chain reaction (RT-PCR) (Fisher, WE, unpublished data, 1996). Our data suggest that VIP would not be useful as adjuvant therapy for pancreatic cancer.

Pancreatic Polypeptide Family

Pancreatic polypeptide, first isolated by Kimmel and associates in 1968, is a 36-amino-acid peptide secreted from the F cells, which are most prominently found in the periphery of the islets in the head of the pancreas.⁹ Pancreatic polypeptide binds to specific receptors and inhibits exocrine pancreatic secretion of enzyme, bicarbonate, and water and decreases pancreatic blood flow.¹⁰ In 1980, Tatemoto and Mutt¹¹ isolated peptide YY (PYY) and neuropeptide Y (NPY), also 36-amino acid peptides sharing about 50% homology with pancreatic polypeptide and having similar actions. The

effect of these peptides on human pancreatic cancer growth has been variable (Table 34.1). We have examined the effect of pancreatic polypeptide on the growth of the Capan-2 and H2T cell lines and examined the cells for pancreatic polypeptide receptors using competitive binding assays with ^{125}I -VIP. Increasing concentrations of cold pancreatic polypeptide displaced the hot peptide from the surface of the H2T cells, indicating the presence of specific cell surface receptors for pancreatic polypeptide. In contrast, there was no specific binding of pancreatic polypeptide on the tumor cell surface of the Capan-2 cells, indicating the absence of receptors. Dose-dependent inhibition of tumor cell proliferation was observed when the H2T cells were cultured with increasing concentrations of pancreatic polypeptide from 10^{-10} – 10^{-7} mol/L. However, in the receptor negative cell line, Capan-2, no growth effect was detected. Pancreatic polypeptide, as well as other members of this family of related peptides, may exert an inhibitory effect on pancreatic ductal adenocarcinoma cells. Specific receptors appear to be involved in the mechanism of growth inhibition. Further studies are warranted to determine the usefulness of these peptides and their analogs, either alone or in combination with chemotherapy and radiation in the adjuvant treatment of pancreatic cancer.

Pancreastatin

Pancreastatin is a recently discovered pancreatic islet peptide product that inhibits insulin, and possibly somatostatin release, and augments glucagon release.^{12,13} In addition to its effects on the endocrine pancreas, pancreastatin inhibits pancreatic exocrine secretion.¹⁴ Pancreastatin inhibits growth of the normal human pancreas. Pancreastatin has also been shown to inhibit the growth of human pancreatic cancer cells in vitro and in vivo (Table 34.1). We have examined a number of human pancreatic carcinoma cell lines (BxPC-3, Capan-1, Capan-2, CAV, HS766T, MIA PaCa-2, and Panc-1) and the hamster pancreatic adenocarcinoma cell line, H2T, for pancreastatin receptors by competitive binding assays on whole-cell preparations. In our study, none of these cell lines were found to have pancreastatin receptors, and pancreastatin had no effect on the growth of these cells in culture.

Gastrointestinal Hormones That May Promote Pancreatic Cancer Growth

Cholecystokinin

Cholecystokinin (CCK), a peptide hormone produced in the upper small intestine, is known to stimulate the secretion and growth of the normal exocrine pancreas.¹⁵ Many studies have been conducted to examine the role of CCK in the development of pancreatic adenocarcinoma and promotion of its growth once established (Table 34.2).

Ductal adenocarcinoma of the pancreas develops in hamsters after administration of nitrosamines, such as *N*-nitrosobis(2-oxopropyl)amine (BOP). The effect of CCK on the pancreatic carcinogenicity of BOP has been studied by several groups of investigators in the hamster model with mixed results. These contradictory results may be due to the sequence of the carcinogen and CCK administration. In the studies with an inhibitory effect, the CCK was given before or simultaneously with the BOP, whereas in the other studies, CCK was given after BOP. The inhibitory effects of CCK on the carcinogenicity of BOP observed in these studies could be caused by CCK's ability to modify DNA alkylation.¹⁶ In the rat, azaserine has been used to induce acinar pancreatic cancers. Rats treated with CCK and the pancreatic carcinogen, azaserine, develop more adenocarcinomas than control animals treated with the carcinogen alone. The majority of the experimental data suggest that CCK modulates the induction of experimental pancreatic cancer.

The effect of CCK on the growth of established pancreatic cancer has also been extensively studied (Table 34.2). CCK can promote the growth of rodent and human pancreatic cancer cells in vitro and in vivo. CCK antagonists have been shown to inhibit pancreatic cancer growth. In addition, CCK receptors have been demonstrated on rodent and human pancreatic cancer cells. Early clinical trials involving small numbers of patients with advanced pancreatic cancer have failed to demonstrate a survival advantage. However, the CCK receptor status of the tumors in these patients was not examined.¹⁷

CCK, by promoting tumor cell division, may increase the sensitivity of a pancreatic cancer to cytotoxic treatment. In a phase I study, 14 patients with

pancreatic cancer were given FAM (5-fluorouracil, Adriamycin, and mitomycin C) chemotherapy along with CCK. There was no increase in side effects including pancreatitis, and there was no evidence of increased tumor growth induced by CCK and not compensated for by FAM.¹⁸ Further studies are warranted to determine the role of CCK and its antagonists in the adjuvant treatment of pancreatic cancer.

Bombesin

Bombesin is a gastrointestinal hormone originally isolated from the skin of a frog (*Bombina bombina*) and subsequently found to be homologous with human gastrin-releasing peptide (GRP). As its name implies, GRP stimulates the release of gastrin. It also stimulates pancreatic polypeptide and CCK release and promotes pancreatic exocrine secretion and growth.¹⁹

Studies have shown that growth of the pancreas and the development of preneoplastic lesions and carcinoma are increased by bombesin administration in azaserine-treated rats (Table 34.2). However, bombesin has an opposite effect on the development of BOP-induced pancreatic carcinoma in the Syrian hamster. The effect of GRP on the growth of established pancreatic cancer has also been studied in the hamster model and in human pancreatic cancer cell lines with mixed results (Table 34.2).

Secretin

Whereas CCK acts primarily as a stimulator of the acinar cells, secretin is a stimulant of the ductal and ductular cells.²⁰ This suggests that secretin may act as a more potent cocarcinogen than CCK in the de-

velopment of ductal adenocarcinoma. However, secretin does not seem to promote pancreatic carcinogenesis in the hamster-BOP model (Table 34.2). Secretin has been shown to increase the growth of cultured human pancreatic cancer cells in vitro and in vivo and secretin receptors have been demonstrated on several human pancreatic cancers (Table 34.2).

Epidermal Growth Factor and Transforming Growth Factor α

The mitogenic polypeptide epidermal growth factor (EGF) exerts a trophic effect on the exocrine pancreas, which has been shown to have EGF receptors.^{21,22} The incidence of pancreatic cancer induced by BOP in EGF-treated hamsters is significantly increased (Table 34.2).

The EGF receptor is similar to the *v-erbB* oncogene product. Korc et al²³ demonstrated that four human pancreatic carcinoma cell lines (Panc-1, T₃M₄, COLO 357, and UACC-462) overexpress the EGF receptor gene. The overexpression of EGF receptors was associated with structural or numerical alterations of chromosome 7, the chromosomal locus of the *v-erbB* oncogene. An overabundance of EGF receptors caused by these chromosomal alterations may be involved in human pancreatic carcinogenesis.

Transforming growth factor α (TGF- α) is a 50-amino-acid polypeptide that is structurally similar to EGF, whose actions are mediated through the EGF receptor.²⁴ Smith et al²⁵ examined five human pancreatic cancer cell lines (ASPC-1, T₃M₄, Panc-1, COLO 357, and MIA PaCa-2) for TGF- α peptide and mRNA. All five cell lines were found

TABLE 34.2. Gastrointestinal hormones that may promote pancreatic cancer growth.

Cholecystokinin (CCK)	References
CCK given prior to or simultaneously with the pancreatic carcinogen BOP inhibited pancreatic cancer induction.	58
CCK administered after or simultaneously with BOP did not alter the incidence of pancreatic tumors.	59
CCK enhanced pancreatic carcinogenesis in BOP-treated hamsters.	60
CCK had no effect on pancreatic carcinogenesis in BOP-treated hamsters.	61
CCK enhanced the effect of the pancreatic carcinogen azaserine in rats. Lorglumide, a CCK antagonist, inhibited the effect.	62
CCK in combination with secretion promoted the growth of hamster pancreatic cancer cell (H2T) implants.	63
The CCK receptor antagonist, L-364, 718, reduced pancreatic tumor implant growth in hamsters.	64
CCK promoted the growth of established pancreatic acinar cell carcinoma in rats. The effect was reversed by the CCK antagonist, CR 1409, which by itself also reduced tumor growth.	65
CCK-8 receptors are overexpressed in premalignant and malignant azaserine-induced tumors in the rat.	66

Continued on next page

TABLE 34.2. *Continued.*

Cholecystokinin (CCK)	References
CCK stimulates the growth of human pancreatic cancer cells (SW-1990, Panc-1, MIA PaCa-2, BxPC-3, RWP-2, and Capan-2) in tissue culture.	67
CCK receptors were demonstrated on human pancreatic cancer (Panc-1) cells.	68
CCK had no effect on the growth of human pancreatic cancer (Panc-1, MIA PaCa-2) xenografts in nude mice.	69
CCK stimulated the growth of SKI, a CCK receptor positive human pancreatic cancer, in vivo. The effect was blocked by a CCK receptor antagonist. CCK had no effect on CCK receptor negative xenografts (CAV).	70–71
Growth of the human pancreatic carcinoma cell line KP-IN was stimulated by CCK in tissue culture and this effect was blocked by the CCK receptor antagonist CR 1505. CR 1505 also diminished tumor colonies in the liver after intrasplenic injection of tumor cells in nude mice.	72
CCK promoted the growth of (SW-1990) human pancreatic cancer cells in vitro and in vivo.	73
CCK receptor antagonist, L-364, 718, inhibited the growth of SW-1990 xenografts in nude mice.	74
The combination of CCK antagonist, MK-329, and cisplatin has a synergistic cytotoxic effect on MIA PaCa-2 cells.	75
MK-329 reduced the growth of PGER, a CCK receptor positive cell line, in athymic mice.	76
Bombesin and Gastrin-releasing Peptide (GRP)	
Bombesin increased the growth of the normal pancreas, preneoplastic lesions, and pancreatic carcinomas in azaserine-treated rats.	77
GRP increased the growth of azaserine-induced pancreatic cancer cells in vitro and in vivo. Cell-surface GRP receptors were demonstrated.	78
Bombesin increased the growth of the normal pancreas but decreased preneoplastic and neoplastic ductular pancreatic lesions in BOP-treated hamsters.	79
The CCK antagonist, lorglumide (CR-1409), did not influence the effect of bombesin on pancreatic carcinogenesis.	80
The bombesin antagonist, RC-3095, inhibited the induction of pancreatic cancer in the hamster and the effect was augmented by concomitant treatment with RC-160, a somatostatin analog.	81
GRP did not stimulate growth, but the GRP antagonist, RC-3095, inhibited growth of established BOP-induced pancreatic ductal adenocarcinoma in the hamster.	82
Bombesin inhibited the growth of human pancreatic cancer cells (SKI) implanted in nude mice.	83
Secretin	
Secretin given before or simultaneously with BOP (but not after) inhibited pancreatic carcinogenesis in hamsters.	84
Secretin reduced the latency and increased the induction rate of pancreatic cancer in hamsters treated with BOP.	85
Secretin increased the growth of SW-1990 cultured human pancreatic cancer cells.	74
Secretin receptors were demonstrated on several human pancreatic cancers established as xenografts in athymic mice.	86
Gastrin	
Gastrin receptors were demonstrated in a hamster ductal pancreatic adenocarcinoma cell line (H2T).	87
Gastrin receptors were demonstrated on AR42J, a rat pancreatic acinar carcinoma. Gastrin promoted pancreatic tumor growth. CR2093, a gastrin antagonist, had no effect.	88
EGF TGF- α	
EGF treatment increased the incidence of pancreatic cancer in BOP-treated hamsters.	89
Pancreatic cancer cell lines, Panc-1, T ₃ M ₄ COLO 357, and UACC-462 over-express the EGF receptor gene.	23
ASPC-1, T ₃ M ₄ , Panc-1, COLO 357, and MIA PaCa-2 express TGF- α mRNA and cell surface EGF and TGF- α receptors. EGF and TGF- α increased colony formation in soft agar.	24–25
Insulin	
Capan-1, Capan-2, CFPAC-1, HS766T, MIA PaCa-1, and Panc-1 possess specific cell surface insulin receptors.	90
Insulin promoted the growth and colony formation of the human pancreatic cancer cell lines BxPC-3 and SOJ-6.	26
IGF-1	
MIA PaCa-2 human pancreatic cancer cells produce IGF-1, which stimulates the growth of this cell line in an autocrine fashion.	27
The human MIA PaCa-1, but not Capan-1, Capan-2, or CAV cell lines have IGF-1 receptors and are stimulated by IGF-1 in cell culture.	91

to express TGF- α mRNA. Picogram quantities of TGF- α were detected only in media conditioned for 48 hours by 10^6 ASPC-1 and T₃M₄ cells. Specific competitive binding was demonstrated for EGF and TGF- α on all of the five cell lines, indicating the presence of the EGF receptor. The effect of EGF and TGF- α on cell growth were tested in three of the cell lines. EGF and TGF- α (1-10 ng/mL) exerted a dose-dependent increase in colony formation of the ASPC-1, T₃M₄ and Panc-1 cell lines in soft agar. The authors concluded that production of TGF- α may be a mechanism whereby pancreatic cancer cells that overexpress the EGF receptor obtain a growth advantage.

Insulin

There is a paucity of data evaluating the effect of insulin on the growth of pancreatic cancer. This may be due to the fact that insulin is known to be trophic for many cell types. Type 2 diabetes is a risk factor for pancreatic carcinoma. We have hypothesized that the hyperinsulinemia in type 2 diabetes promotes pancreatic cancer growth. Takeda and Escribano²⁶ found that insulin promoted the growth and colony formation of two human pancreatic carcinoma cell lines (BxPC-3 and SOJ-6). We have examined six human pancreatic cancer cell lines (Capan-1, Capan-2, CFPAC-1, HS766T, MIA PaCa-1 and Panc-1) for insulin receptors by competitive binding with ¹²⁵I-insulin. All six cell lines were found to possess specific cell surface insulin receptors. Dose-dependent increases in DNA synthesis, as measured by incorporation of ³H-thymidine, were caused by adding insulin (10^{-12} – 10^{-8} mol/L) to the cell culture media (Table 34.2).

Insulin-like Growth Factor 1

Insulin-like growth factor 1 (IGF-1) is a polypeptide similar in structure to insulin, which has been shown to stimulate cell growth directly. The MIA PaCa-2 human pancreatic cancer cell line has been shown to produce IGF-1, which stimulates the growth of this cell line in an autocrine fashion.²⁷

We have examined five human pancreatic cancer cell lines (Capan-1, Capan-2, CAV, MIA PaCa-2, and Panc-1) for the presence of IGF-1 receptors using ¹²⁵I-IGF-1 and determined the response of

these cell lines to IGF-1 in tissue culture. We were able to demonstrate IGF-1 receptors on the MIA PaCa-2 and Panc-1 cell lines and observed a dose-dependent increase in the incorporation of ³H-thymidine when these cells were cultured in the presence of IGF-1 (10^{-11} – 10^{-7} mol/L). The other three cell lines did not have IGF-1 receptors and IGF-1 did not affect their growth in cell culture (Table 34.2).

Conclusions

Although much work is still required, this impressive body of experimental data generated by many investigators over decades provides compelling evidence that hormonal therapy for pancreatic cancer may be a clinically useful option in the future. It is clear that polypeptide hormones and their antagonists can promote and inhibit pancreatic carcinogenesis and the growth of some established pancreatic cancers in vitro and in vivo. The subset of pancreatic adenocarcinomas that may respond to gastrointestinal hormonal therapy is unknown. Although some studies in which no effect was observed have been published, countless other “negative” studies may have gone unreported and perhaps repeated because of the lack of interest typically given to such data.

There are multiple reasons for the apparent discrepancies in the published literature summarized. Different doses and timing of administration of the same hormone may have different effects on the cancer cell. The expression of receptors may be related to the degree of differentiation of the cancer cell, and all pancreatic cancer cells clearly do not possess the same receptors. Passage of cells in culture may alter this receptor expression. In addition, there may be several forms of a receptor for a given hormone that function differently. Hormones may act directly on cancer cells or indirectly by suppression or promotion of the release of other growth factors, making the total effect complicated. Although it is unlikely that these obstacles will be easily overcome, the studies summarized here represent early steps of major importance in our understanding of the tremendously complex regulation of pancreatic cancer growth by gastrointestinal hormones.

A strong foundation for the gastrointestinal hormonal therapy of pancreatic cancer has been laid by the data summarized. However, much more must be accomplished before clinical application is possible. Optimal clinical application of this approach will require a method to determine which patients have responsive tumors. The evaluation of excised pancreatic cancers for gastrointestinal hormone receptors may help define the responsive subset of patients in a manner similar to current strategies employed successfully in the treatment of patients with breast cancer. Many therapeutic strategies deserve further inquiry. If gastrointestinal hormones bind to tumor cell surface receptors, can cytotoxic agents be linked to these peptides and thus carried directly to the target cell? Can receptor-negative pancreatic cancer cells be rendered responsive to the growth-modulating effects of gastrointestinal hormones by transfecting the genes for their receptors? The experimental models presently established will certainly help answer these and other questions and contribute to the development of new therapeutic approaches with eventual clinical utility.

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Farnesyltransferase Inhibitors: Biological Considerations for Future Therapeutics

Adrienne D. Cox and L. Gerard Toussaint III

Introduction

A striking feature of pancreatic cancer is the finding of mutated forms of the *K-ras* oncogene in nearly 90% of cases, the highest association of any tumor type. These findings suggested Ras as an important target for novel anticancer drugs, and pancreatic cancer as a potentially important tumor target amenable to treatment with such agents. Since the identification and cloning of farnesyltransferase (FTase), a critical enzyme that post-translationally modifies Ras and other proteins with a farnesyl isoprenoid lipid, tremendous effort has brought FTase inhibitors (FTIs) on their way to clinical practice for cancer therapy. FTIs can inhibit the growth of tumor cells in culture and in animal models and are now in clinical trials for a variety of tumor types, including pancreatic cancer. Interestingly, the mechanism of FTI action is not as simple as originally envisioned, and Ras is probably not the most important farnesylated protein whose modification is inhibited as a result of FTI treatment. Moreover, K-Ras proteins can escape FTI inhibition of processing. Nevertheless, some tumors are inhibitable by FTIs. This chapter delineates the evolution of FTI development and understanding of their mechanism of action, and summarizes prospects for the eventual use of such agents in pancreatic cancer.

Ras and Pancreatic Cancer

Ras proteins are guanine nucleotide triphosphate (GTP)-regulated switches that regulate signal transduction pathways controlling a tremendously diverse group of biological processes, including cellular proliferation, differentiation, and apoptosis (reviewed in

Reuther and Der¹). Oncogenic mutations are those that result in Ras proteins that are constitutively GTP-bound and therefore chronically stimulatory; typically, these mutations occur at hot spots, such as codons 12, 13, or 61. *Ras* is the most frequently mutated oncogene in human tumors, with a wide variation depending on tumor type. In a strikingly high percentage of pancreatic tumors, up to 90% or more depending on the study, mutated *ras* genes are present (reviewed in Hruban et al²). Of the three *ras* genes, *H-ras*, *N-ras*, and *K-ras*, only mutated *K-ras* is typically associated with pancreatic cancer, and those mutations are almost entirely at codon 12.³ Thus, a very particular subset of *ras* mutations is associated with a very high percentage of pancreatic tumors. It is not clear at present whether this dramatic ubiquity of mutated *K-ras* reflects a requirement for this type of mutation for tumor initiation or progression, or rather, reflects a special sensitivity of *K-ras* genomic DNA sequences to the particular genetic and environmental insults that result in pancreatic cancer. For example, more *K-ras* mutations are found in pancreatic cancer patients who are also smokers.⁴ Regardless of the etiology, the high association of *K-ras* mutations with pancreatic cancer has pointed to anti-Ras therapy as a potential treatment for this difficult disease.

FTase as a Target for Anticancer Drug Design

All Ras proteins absolutely require covalent modification by a farnesyl isoprenoid lipid for their proper membrane localization and biological activity (reviewed in Cox and Der⁵ and Casey⁶).

FTase modifies newly synthesized Ras proteins by the transfer of a farnesyl pyrophosphate donor to the cysteine of the carboxyterminal CAAX motif (where C = cysteine, A = aliphatic, and X = any amino acid) of the substrate protein; this is the first and obligate processing step that ultimately results in membrane localization of the mature protein.^{5,6} These facts led to the idea that enzymes important for post-translational modification ("processing") of Ras would provide novel targets for drug design. Because farnesol is an obligate intermediate in the cholesterol biosynthetic pathway,⁷ it was initially thought that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, such as the statins, which block the synthesis of mevalonic acid that is the precursor for all the isoprenoid groups, might act as useful anticancer agents by interfering in the processing of Ras. Unfortunately, this did not turn out to be the case, as the level of lovastatin required for blockade of Ras processing is approximately 100-fold higher than that required to inhibit cholesterol biosynthesis.⁸ Thus, a more specific means of interfering in Ras processing would be required.

Development of FTase Inhibitors

The cloning of the FTase enzyme, and the finding that a tetrapeptide sequence (ie, the CAAX sequence) was both necessary and sufficient to serve as its substrate, drew the attention of pharmaceutical companies and academic researchers to designing small molecule inhibitors of FTase (recently reviewed in Cox et al,⁹ Oliff,¹⁰ Rowinsky et al,¹¹ and Gibbs¹²). The earliest generation of rationally designed FTIs were peptidomimetics of CVIM, the CAAX sequence of K-*ras*4B. Such peptidomimetic inhibitors could selectively block FTase compared to the related enzyme geranylgeranyltransferase I (GGTase I), which attaches a geranylgeranyl isoprenoid to proteins, and were good inhibitors of enzymatic activity in vitro. Current generation FTIs are largely nonpeptide, non-thiol-containing compounds with nanomolar potency.

Preclinical studies done in animal tumor models, using both H-Ras-transformed fibroblasts grown as xenografts in nude mice, as well as in transgenic mice that stochastically developed mammary and salivary tumors due to the H-*ras* transgene, showed the ability of FTIs to inhibit growth of such tumors.

In the transgenic H-*ras* models, FTI treatment even caused tumor regression,^{13,14} a surprising feat for compounds that were expected to be cytostatic. A wide variety of structurally distinct FTIs all showed similar results, suggesting that the tumor inhibition was mechanism-based and a consequence of inhibiting FTase. These results were highly encouraging to those interested in using FTIs as anticancer drugs for Ras-containing tumors. However, because all the early tests were done using H-Ras, rather than N-Ras or K-Ras, certain surprises that did not emerge until later suggested a less simple mechanism of action than originally envisioned.

K-Ras Is Resistant to FTI Inhibition of Processing and Becomes Alternatively Prenylated

Due to alternate splicing, there are four forms of Ras proteins: H-Ras, N-Ras, K-Ras4A, and K-Ras4B. Although it was very easy and straightforward to inhibit both processing of and transformation due to oncogenic H-Ras, this was not the case with K-Ras. Reasons for the resistance of K-Ras to FTIs were not only that its affinity for the FTase enzyme was high, but also that in the presence of FTIs, it could become a substrate for alternative prenylation by the related enzyme, GGTase I in vivo.^{15,16} This provides a mechanism whereby K-Ras processing could escape FTI inhibition; nevertheless, tumors containing mutated K-Ras could be inhibited by FTIs.

Ras Mutation Status Does Not Predict FTI Sensitivity or Resistance

Although some preclinical studies on the response of cultured human tumor cells to FTI treatment found that the type of mutant Ras protein present was predictive of relative tumor cell responsiveness,¹⁷ others showed the totally unexpected result that *ras* mutation status did not predict FTI sensitivity or resistance to blockade of both anchorage-dependent and anchorage-independent growth.¹⁸ All possible combinations could be found, such that

tumor cell lines with mutated *ras* genes were both sensitive and resistant, and the same was true of tumor cell lines containing only wild type *ras*. These results forced consideration of the possibility that Ras might not be the most important farnesylated protein whose function was altered by inhibiting the FTase enzyme. Other results also suggested this possibility. For example, the dramatic tumor regression seen in the H-*ras* transgenic animals^{13,14} was a surprise, given that FTIs were expected to be cytostatic, rather than cytotoxic, based on their ability to cause a loss of function in newly synthesized Ras proteins. However, if non-Ras targets were important, then FTIs might be able to be cytotoxic after all. Although widely accepted today, the possibility that Ras might not be the most important downstream target of FTase inhibitors was a tremendous paradigm shift, considering that the push for FTIs had originally been seen as a logical way in which to block oncogenic Ras proteins associated with a wide spectrum of cancers. Indeed, given that K-Ras processing has turned out to be so resistant to FTI inhibition, the existence of a target potentially more important than Ras might turn out to be consoling, rather than devastating.

The Concept of “Target X”

All the data described previously have now led to the concept that the FTIs under development are true inhibitors of FTase, but that the most important consequence of this inhibition is altering the processing not of Ras proteins but of some other unidentified farnesylated protein(s).^{5,9–12,19} Vigorous efforts are underway to identify such a “Target X” in order to improve FTI design, to improve the targeting of potential patient populations that may benefit from FTI treatment, and possibly also to identify one or more targets for a new round of drug discovery and development.

Currently, attention is focused on several proteins known to be farnesylated, including members of the Ras-related family Rho (RhoB,¹⁹ RhoE, and others) and proteins important for cell cycle progression (centromere-binding proteins, CenP-E and CenP-F, and a nuclear phosphatase, PTP-CAAX). In particular, there is evidence that RhoB, an immediate-early protein, functions differently depending on whether it is modified by a farnesyl or

geranylgeranyl isoprenoid, and that geranylgeranylated RhoB, the only type of prenylated RhoB present under conditions of FTI treatment, is growth inhibitory.²⁰ There also is very recent functional evidence that one or more farnesylated proteins controlling the PI3-K/Akt lipid kinase survival pathway are important for regulating apoptosis in response to FTIs.^{20,21} It is not clear whether there will be multiple Target X proteins, depending on the tumor type treated or the phenotype inhibited (eg, proliferation vs metastasis vs radiation sensitization). Identification of these targets, however, is not required to move forward with the development of FTIs for clinical use.

FTIs Inhibit Growth of Tumor Cells in Preclinical Studies When Used as Single Agents

Regardless of the mechanism, FTIs are indeed capable of inhibiting, and, in some cases, even causing regression of, a wide variety of tumor cells both in culture and in various animal models. Tumor cells include those derived from model cell lines, such as transfected rodent fibroblast or epithelial cells, as well as diverse human tumor cell lines grown in monolayer culture, in soft agar, or as xenografts in nude mice. Mice transgenic for H-*ras* alone¹³ or in combination with p53 or *myc*²² have shown tumor shrinkage or growth inhibition, respectively, upon FTI treatment. In addition, transgenic mice overexpressing N-*ras* have shown both reduction in tumor growth rates and decreased incidence of lymphoma upon FTI treatment.²³ Impressively, recent work with newly developed transgenic K-*ras* mouse models shows that the growth of their tumors (mammary tumors resulting from expression of Ras from the murine mammary tumor virus (MMTV) promoter) also becomes static upon FTI treatment.²⁴ Therefore, there is reason to hope that even tumors such as pancreatic cancers that routinely bear mutated K-*ras* can be targeted successfully by FTIs. Overall, nearly 60% of human tumor cell lines demonstrate some sensitivity to FTI growth inhibition, suggesting that a variety of different tumors may also prove to be clinically amenable to FTI treatment. Even pancreatic carcinomas can be sensitive to growth inhibition by

FTIs,¹⁸ despite their poor response to conventional anticancer therapies.

FTIs Can Be Radiosensitizers

It seems likely that FTIs will find their greatest utility in combination with other treatment modalities. Some of the very first combination studies were done on the basis that, because Ras proteins induce radioresistance,²⁵ inhibiting Ras function by means of FTIs might produce important radiosensitizing effects. These results are potentially of great significance to pancreatic cancer, as the first-line therapy for this disease remains resection followed by postoperative radiation therapy with concurrent 5-fluorouracil (5-FU). The first study to test the potential of FTIs as radiosensitizers showed that FTIs could modestly radiosensitize H-Ras-transformed rodent fibroblasts,²⁶ and that this effect correlated with the ability to block H-Ras processing. A follow-up study showed that FTIs could also radiosensitize human tumor cell lines, including those derived from pancreatic cancers, although it suggested that for tumors with oncogenic K-Ras mutations, the addition of an inhibitor of GGTase I was also required.²⁷ This result is in conflict with that seen for inhibition of tumor growth, and may reflect a difference in the roles of Ras in transformation and radioresistance. However, an FTI was also shown to be a radiosensitizer even in transformed cells containing only wild-type Ras,²⁸ suggesting that FTIs may influence multiple pathways regulating radioresistance. Interestingly, long pretreatment with FTI prior to irradiation does not appear to be required, which bodes well for combination therapy.

FTI Synergy With Standard Anticancer Agents

To test whether FTIs could be used in conjunction with standard cytotoxic agents, comparisons were made between FTIs alone and in combination with different classes of standard chemotherapies, including antimetabolites (5-FU), alkylating agents such as cytoxan and cisplatin, DNA intercalators (doxorubicin), and various microtubule-binding agents (vinblastine and vincristine, taxanes and

epothilones).^{14,29–32} In general, FTIs did not interfere with the action of cytotoxic drugs. Instead, FTIs exhibit additive effects when used in combination with most cytotoxics, and even can exhibit synergistic effects when used in combination with taxanes. Several different cell types treated with both FTI and taxol, for example, display increased G1 or G2/M arrest, and are apparently sensitized to mitotic block by the combination treatment. The mechanism behind this synergy is unclear, but points to the importance of one or more farnesylated proteins in controlling mitotic checkpoints.

Finally, as described previously, apoptosis can be induced by FTIs in preclinical models when an additional stressor, such as growth factor or anchorage deprivation, is involved.^{33,34} Apoptosis may be increased as a consequence of up-regulating proapoptotic Bcl family proteins and activating caspase-3 by undefined mechanisms,³³ and/or by blocking the farnesylation of a protein(s) regulating the PI3-K/Akt lipid kinase-mediated survival pathway.^{20,21} Interestingly, the FTI BMS-214662 promotes regression and increased levels of apoptosis in preclinical xenograft models where others do not.³⁵ It is unclear at present whether this is a unique response due to additional activities in this particular compound not possessed by others.

Clinical Trials Begin—Phase I/II

The earliest phase I clinical trials using FTIs as single agents have successfully identified doses appropriate for the phase II and phase III trials that are now underway. For the most part, phase I trials have not concentrated on any particular tumor type, but have covered a variety of cancers. An early phase I study tested the Janssen FTI compound R115777 in combination with 5-FU and leucovorin in patients with advanced pancreatic or colorectal cancers; dose-limiting toxicity of myelosuppression but no efficacy was reported.³⁶ A mixed response for 1 pancreatic cancer patient was reported for the Schering-Plough compound SCH66336 in a phase I study.³⁷ R115777 and SCH 66336 are also currently in a variety of open phase II studies. Other FTIs in the clinic include those from Merck (L778,123)³⁸ and Bristol-Myers Squibb (BMS-214662).³⁵ Overall, delivery by both oral and intravenous routes has been accomplished in these trials, and pharmacokinetic and pharmacody-

dynamic studies have shown that it is possible to achieve plasma levels of drug that are in the target range for inhibiting FTase activity using either of these methods.

Still to be determined are the optimal surrogate markers for monitoring the efficacy of FTase inhibition.³⁹ Ideally, tumor samples would be taken for monitoring purposes. In the absence of good assays for monitoring FTase activity directly in patient samples, currently the emphasis is to analyze inhibition of processing of farnesylated proteins, such as H-Ras, hDNAJ2, or prelaminA, in peripheral blood mononuclear cells or buccal mucosa cells, and, although this is still problematic, also to monitor FTase levels directly.

Preclinical testing suggested that FTIs were likely to be extremely nontoxic; surprisingly, the therapeutic index has been narrower than expected from preclinical models. Reversible myelosuppression, largely grade 3 neutropenia and thrombocytopenia, is the most commonly reported toxicity, and is presumably mechanism-based.^{36–38} Other toxicities reported have been unique to each structurally distinct FTI, for example, diarrhea with SCH 66336,⁴⁰ and therefore are likely not to be mechanism-based.

Although not the goal of phase I studies, it is always hoped that some efficacy will be seen even at this stage. To date, only one partial response has been reported, lending additional support to the possibility that FTIs will best be used in combination therapy. The fact that some investigators have reported additive effects and some have reported synergistic effects with combination FTI and taxol in preclinical models is also relevant to a clinical issue that must still be addressed in analyzing results of trials already underway and in planning new trials. Different dosing, scheduling, and sequencing parameters are only now beginning to be compared. It is possible that such differences may account for whether a given schedule provides additive effects or synergy, and these must be taken into account when planning new trials.

FTI-Related Terpenes in Pancreatic Cancer

Crowell⁴¹ showed that dietary isoprenoids, as well as perillyl alcohol and *d*-limonene, isoprenoid-related monoterpenes, have chemotherapeutic ac-

tivity as inhibitors of pancreatic cancer growth in vitro using human pancreatic tumor cell lines, such as MiaPaCa2, and in Syrian hamster models of pancreatic tumors. The terpenes cause tumor regression, increase apoptosis, and induce proapoptotic proteins, such as Bak, preferentially in pancreatic adenocarcinoma cells compared with nonmalignant pancreatic ductal cells, and they also block prenylation of cell growth-regulating proteins that are likely to include not only Ras but Ras-related proteins of the Rho family. As more is learned about FTIs and regulation of the isoprenoid metabolic pathways that they control, testing of the terpenes in clinical trials may be warranted.

Potential Role of FTIs in Pancreatic Cancer Treatment

The potential role of FTIs as single agents in cancer treatment should become clearer in the relatively near term as results of phase II/III trials become available. However, more extensive use of FTIs in different contexts will be required to learn whether chronic administration will be required, whether the development of resistance will be a problem, and what schedules for administration should be used. Historically, when FTIs were viewed as likely anti-Ras agents, it was thought that pancreatic cancers would be the most likely tumor types to respond to FTIs due to their extremely high percentage of *K-ras* mutations. More recently, with the additional information that H-Ras processing is easily blocked, whereas K-Ras becomes alternatively prenylated in the presence of FTIs, the pendulum of thought has swung again such that conventional wisdom now says that head and neck tumors may be the most susceptible to FTI growth inhibition. However, until the most important farnesylated target of FTase inhibition has been identified in a rational and mechanistic manner, it remains critical to study different types of tumors to learn empirically which tumors are susceptible and which are not. Clearly, preclinical studies with some pancreatic tumors and others where mutated *K-ras* is present have shown responses, so it is important that clinical trials currently underway continue to include all types of diseases. In addition, the transgenic mouse data have shown that FTIs cause different effects on cell cycle and apoptosis depending upon the context of the mutated *ras* gene. Thus,

it will be important to investigate the context of genetic mutations of the treated tumors as thoroughly as practical, so that combinations may be assembled more rationally. In pancreatic cancers, the presence of additional mutations, such as loss of function of p53, p16, and MTS-1, may influence the potential utility of FTIs in ways as yet undetermined.

The Future

Investigators are awaiting the results of future clinical trials with cautious optimism. Because of the variety of trials planned or underway, it seems reasonable to expect that appropriate target populations and schedules may be identified within the next 2 or 3 years. To aid in the identification of appropriate target populations, it will be important to collect data not only on clinical response but also on degree of inhibition of FTase, as defined by surrogate markers, and on genetic mutations, including not only Ras status but also p53 and other tumor suppressor genes. With the advent of gene array chips, it would also be desirable to bank tumor biopsies for future investigations, although this certainly will not be practical for most trials now supported. Meanwhile, it will be important to obtain as much information as possible regarding the efficacy of FTIs in combination with different conventional and novel treatment modalities to learn how best to use this potentially promising new class of antitumor agent.

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36

Novel Therapeutic Targets for Drug Development

Daniel D. Von Hoff, Elizabeth R. Campbell, and David J. Bearss

Introduction

Pancreatic cancer has the worst prognosis of any malignancy in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program database.¹ It is estimated that more than 29,200 people in the United States will develop pancreatic cancer in the year 2001 with 28,900 patients dying of the disease.² In addition, the vast majority of patients with pancreatic cancer experience pain, weight loss, severe fatigue, and a declining performance status. Not only is the human toll of pancreatic cancer very high, but Gudjonsson³ has estimated that the cost of carcinoma of the pancreas in the United States alone in 1995 was \$3.73 billion. Clearly, more has to be done to try to prevent and treat the disease.

In this chapter, we will discuss some promising targets for treatment and prevention of pancreatic cancer, as well as for treatment of the symptoms associated with pancreatic cancer.

Identifying Targets for Treatment of Pancreatic Cancer

Tissue Taken Directly from Patients

There is no question that new molecular biology tools have given us tremendous ability to find genetic abnormalities in tumor cells which are not present in normal cells.^{4,5} However, finding suitable targets for therapy in pancreatic cancer represents a particular challenge for several reasons. These reasons include the following:

1. In the clinical care of patients with pancreatic cancer, surgery is performed infrequently because the disease is commonly advanced at the time of diagnosis. Instead of a tumor resection (where sizeable amounts of tumor could be obtained), the diagnosis of pancreatic cancer is frequently made by fine-needle biopsy, which yields very few cells—clearly not enough for the usual investigation of gene expression.
2. When tissue is obtained, unless it is immediately processed/frozen, the enzymes released (including RNase) rapidly destroy nucleic acids.
3. When tissue is obtained at postmortem examination, tissue autolysis is usually so complete that the specimen is destroyed.

If human pancreatic tumor tissue is not available, as it has been for other types of tumors (eg, breast cancer), progress in the molecular approach to finding new therapeutic targets will be slow. Optimal use and distribution of human pancreatic specimens is critical for progress in finding new targets, since it is clear that cell lines are not as desirable as tissue (pancreatic cancer and normal pancreas) taken directly from patients.

Human Pancreatic Cancer Cell Lines

Most investigators would agree that pancreatic cancer cell lines are not ideal models for the disease.⁶ However, there are a number of cell lines available to allow the identification of new targets. One can then probe human tumors taken directly from patients to determine if these primary or metastatic tumors contain the target of interest. Table 36-1

TABLE 36.1. Attributes of some human pancreatic cancer cell lines.*

Cell line	ATCC No.	Origin	Histology/morphology	Karyotype	Markers/mutations	Tumorigenic in nude mice	Reference
MIA PaCa-2	CRL-1420	Adenocarcinoma of the pancreas	Epithelial		hCSF/ K-ras, p16, p53	Yes	7
PANC-1	CRL-1469	Epithelioid carcinoma of the pancreas	Epithelial	Hyperdiploid; modal no. = 63	K-ras, AKT, p16, p53	Yes	8
ASPC-1	CRL-1682	Ascites fluid from adenocarcinoma of the pancreas	Epithelial		CEA, mucin/K-ras, AKT, p16, MIKK4, DPC4, p53	Yes	9
BXPC-3	CRL-1687	Adenocarcinoma of the pancreas	Epithelial		CEA, mucin/AKT, p16, DPC4, p53	Yes	10
HPAF-II	CRL-1997	From peritoneal ascitic fluid adenocarcinoma of the pancreas	Epithelial		Mucin	Yes	11
Capan-1	HTB-79	Adenocarcinoma of the pancreas	Epithelial	Hypotriploid	CFTR, mucin/K-ras, p16, BRCA2 MKK4, DPC4, p53	Yes	12
Capan-2	HTB-80	Adenocarcinoma of the pancreas	Well-differentiated/polygonal		Mucin/K-ras, p16	Yes	12
SU.86.86	CRL-1837	Liver metastasis of adenocarcinoma of the pancreas	Epithelial		CEA/K-ras, AKT, p16, p53	Yes	13
CFPAC-1	CRL-1918	Liver metastasis of adenocarcinoma of the pancreas	Epithelial	Hyperdiploid; modal no. = 73	CEA, CA 19-9/K-ras, p16, MKK4, DPC4, p53	Yes	14
COLO 357		Metastasis of a pancreatic adenocarcinoma	Epithelial	Hyperdiploid; modal no. = 53	CEA, HCG/DPC4	Yes	15
L3.6 sl		A variant of COLO 357	Epithelial		CEA, HCG	Yes	16

* ATCC indicates American type culture collection; CEA, carcinoembryonic antigen; AKT, the human homolog of the oncogene *v-akt*; and HCG, human chorionic gonadotropin.

outlines the human pancreatic cancer cell lines available.⁷⁻¹⁶ This table is not an exhaustive list, but there are some attributes of each of these lines that are of interest. The molecular characterization of many of these lines is just beginning. Our own team is performing microarray analyses of the mRNA from these cell lines compared with mRNA from normal pancreas to determine which genes have increased or decreased expression.

Human Pancreatic Carcinoma Cell Lines Growing in Nude Mice

There has been an extensive literature in this area, particularly in the area of evaluation of new anticancer agents. Since we now have an anticancer agent that has demonstrated some clinical effectiveness against the disease, we at least have a positive control. Burris and colleagues¹⁷ demonstrated that the nucleoside analog gemcitabine (when compared in a randomized trial with 5-fluorouracil) significantly improved clinical benefit (eg, pain, weight, and performance status) and median and 1-year survival rates for patients with advanced pancreatic cancer. This work followed on the heels of excellent phase II work by Casper and colleagues,¹⁸ Rothenberg and colleagues,¹⁹ and Carmichael and colleagues.²⁰

Schultz and colleagues²¹ have examined a variety of agents against a variety of human tumor cell lines grown in nude mice. They found that gemcitabine had modest activity against the MiaPaCa and the PANC-1 cell lines grown in mice. The activity was modest in these models, which mimics the modest activity of gemcitabine in the clinic. If a new agent were to have activity greater than gemcitabine in the MiaPaCa model, then it would certainly be worthy of consideration for bringing forward into clinical trials in patients with advanced pancreatic cancer. Some investigators²² are exploring several agents utilizing this strategy.

Most recently, Bruns and colleagues¹⁶ have described a most interesting cell line, which was selected utilizing an orthotopic implantation technique. This cell line, called the L3.6p1 (pancreas to liver) line (see Table 36-1), is a variant of the COLO 357.^{17,23} The L3.6p1 is of great interest, because it has a high incidence of metastases from the pancreas to the liver. Bruns and colleagues¹⁶ have also described the L3.6s1 sub-

line of COLO 357, which causes a high incidence of metastases from spleen to liver. Probably, the most important part of their work was the demonstration that orthotopic implantation can produce a model that more closely mimics the metastatic pattern of human pancreatic cancer. The utility of the model for evaluation of new therapies or as a model for discovery of new targets is as yet untested.

Targets That Have Been Identified

Cyclooxygenase-2

There is considerable evidence that cyclooxygenase-2 (COX-2) is elevated in human pancreatic cancer. Nakamori and colleagues²⁴ utilized immunohistochemistry (IHC) to determine expression of COX-2 in six human pancreatic cancer cell lines and 48 ductal carcinomas of the pancreas, taken directly from patients. Forty-three of 48 tumors taken directly from patients strongly or moderately expressed the COX-2 protein and five faintly expressed it. Four of the six cell lines had strong or moderate expression of COX-2. Of importance is that the growth of these cell lines was inhibited in a dose-dependent manner by the addition of a COX-2 inhibitor. Yip-Schneider and colleagues,²⁵ using immunoblot analysis, analyzed COX-2 protein expression in 23 primary pancreatic cancers and in 11 normal adjacent tissues. They noted a significant increase in COX-2 expression in pancreatic tumor specimens compared with normal pancreatic tissue. Koshiba et al,²⁶ Molina et al,²⁷ Tucker et al,²⁸ and Okami et al²⁹ noted increased expression (moderate to strong) of COX-2 in 67% to 90% of primary pancreatic adenocarcinomas.

Based on the previously described work, there are ongoing clinical trials with COX-2 inhibitors alone and in combination with gemcitabine for patients with advanced pancreatic carcinoma.

HER-2/*neu*

Safran³⁰ has tested thin sections from 123 patients with paraffin-archived tumor tissue, utilizing IHC and the DAKO Hercep test monoclonal antibody system. HER-2/*neu* overexpression (2+ or 3+) was noted in 21 of 123 patients (17%). Dugan and

colleagues³¹ noted that 46 of 79 cases of pancreatic cancer (58%) had moderate to strong HER-2/*neu* expression. There was lower expression of HER-2/*neu* in poorly differentiated portions of the tumor. They found no difference in survival rates of patients with HER-2/*neu* positive versus HER-2/*neu* negative tumors. Similar findings were described by Day and colleagues.³² Based on these findings, phase II trials of Herceptin (the monoclonal antibody to HER-2/*neu*) and gemcitabine are ongoing.

Glutathione-S-Transferase

The pancreas is exposed to many molecules, including many carcinogens. The pancreas is therefore likely equipped with protective enzymes to detoxify these molecules. Of note is that pancreatic cancer specimens have been shown to have an elevation of expression of glutathione-S-transferase (GST). Collier and colleagues³³ noted that 70% of 26 adenocarcinomas of the pancreas and 0% of 12 normal pancreas samples had an elevation in the expression of GST. This is an important finding because there is now a chemotherapeutic agent that is actually activated by GST and therefore would be activated by elevated GST present in disseminated pancreatic carcinoma. The agent activated by GST is TLK286 (see Figure 36.1), which has pre-clinical activity in GST-containing cell lines and is currently undergoing phase I trials in patients with advanced malignancies.^{34–36}

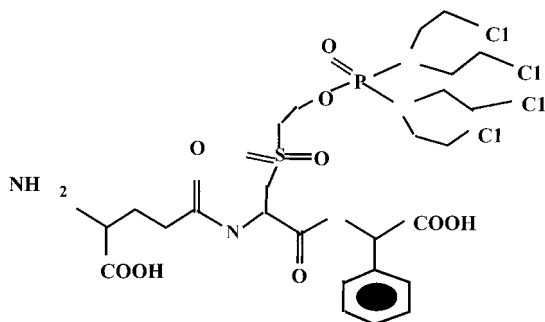


FIGURE 36.1. Structure of TLK286.

The Androgen Receptor

In early studies, there was a predominance in the risk of developing pancreatic cancer for men versus women at a ratio of 1.7 to 1.0.³⁷ In more recent studies, this risk has fallen slightly to a ratio of 1.3 to 1.0.³⁸ In addition, two investigators found that male animals fed carcinogens were more likely to develop pancreatic cancer than were female animals.^{39,40} Androgen receptors have been found in some pancreatic cancer specimens.⁴¹ Antiandrogens, such as cyproterone acetate, have been utilized in nude mouse models and have been shown to decrease tumor growth.⁴² However, in a randomized clinical trial of cyproterone acetate versus best supportive care, the median survival time was 4.3 months for cyproterone acetate treatment versus 3.0 months for best supportive care (P = not significant).⁴³ Recently, interest was rekindled in this approach by Greenway,⁴⁴ who conducted a clinical trial that involved 29 patients and demonstrated a significant improvement in survival for those receiving the antiandrogen flutamide versus best supportive care. In addition, a recent phase II study by Shaw and colleagues⁴⁵ noted that 3 of 27 patients experienced a partial response (duration, 1+, 3+, and 7+ months) with flutamide (250 mg 3×/day). Median survival time was 3.4 months (range, 0.1–25.2) with 8 patients still alive at the time of publication. These results suggest that researchers should continue to pursue the androgen receptor as a target for pancreatic cancer treatment.

EGF Receptor

There are multiple reports of the increased expression of the epidermal growth factor receptor (EGF-R) in human pancreatic cancers.^{46–48} Of additional interest is the finding by Schmiegel and colleagues⁴⁹ that tumor necrosis factor alpha (TNF- α) increases the expression of the EGF-R and that treatment of patients with a monoclonal antibody to EGF-R plus TNF- α can result in tumor response. Recently, the EGF-R interactive monoclonal antibody C225 and the small molecule EGF-R kinase inhibitors, ZD1839 and OSI774, have entered phase II trials in patients with advanced pancreatic cancer. The design of these trials is critical, given the cytostatic nature of these small molecules. Investigators must examine

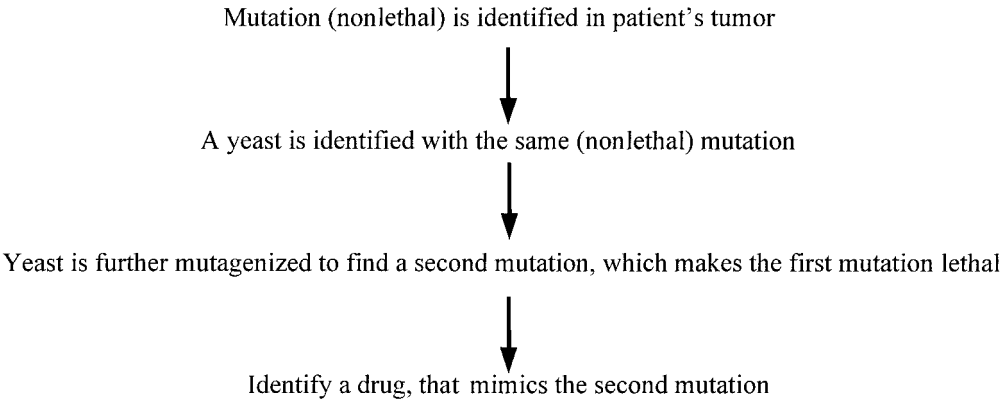


FIGURE 36.2. Schema for synthetic lethal screening.

6-month and 1-year survival rates as endpoints, rather than radiographic measurement of tumor response.

A New Method to Take Advantage of Genetic Abnormalities in Individual Patients' Pancreatic Cancer Specimens

Synthetic Lethal Screening Technique

As noted earlier, ongoing research will likely discover more genetic abnormalities in human pancreatic cancer. It is obvious that these genetic alternations are not lethal. However, nonlethal genetic alternations can be very helpful in finding new targets if we utilize a relatively new drug development technique called synthetic lethal screening (SLS).^{50,51} The schema for the SLS technique is outlined in Figure 36.2. In the SLS, a nonlethal

mutation is identified in a patient's tumor. Then, a model organism (eg, yeast, *c. elegans*, zebra fish, etc.) is identified that has the same nonlethal mutation. This model organism is then further mutagenized (usually with ultraviolet light in the yeast example) to find a second mutation that makes that first mutation lethal. Work is then done to identify a drug that mimics that second mutation.

Table 36.2 details the genetic alterations in pancreatic cancer and their yeast homologs. As more molecular work is done on primary tumors, we should be able to find additional homologs in yeast.

To date, only one case has validated this SLS approach to finding new targets and new agents. In yeast with abnormalities in the mismatch, repair genes MLH1 or MSH₂, an agent that affects delta or epsilon polymerase can be lethal.^{52,53} Our group has shown that the agent gemcitabine, which is a weak inhibitor of delta and epsilon polymerase, is more toxic to cells with mutations in mismatch repair genes (D. Bearss, oral communication). Mismatch repair abnormalities are present in a small percentage (4%–18%) of patients with pancreatic

TABLE 36.2. Genetic alterations in pancreatic cancer and their yeast homologs.

Gene	Alterations	Yeast homolog
MLH1, MSH ₂	Deletions, promoter methylation	MLH1, MSH ₂
BRCA2	Deletions, insertions	RAD51, RAD54
MKK4	Homozygous deletions	MKK1, MKK2
PTEN	LOH*	Cdc14, YNL128W
MKP-3	LOH on chromosome 12q21	MSG5

*LOH; loss of heterozygosity.

cancer. Our finding that gemcitabine is effective against tumor cells with abnormalities in mismatch repair genes may explain why gemcitabine is effective in only a small percentage of patients' tumors (perhaps only in those with abnormalities in mismatch repair). A prospective test of our findings will determine whether that is true. As more nonlethal genetic abnormalities are identified in pancreatic cancers, it is likely that SLS will become a valuable tool in finding both new therapeutic targets and agents that hit those targets.

Targets for Supportive Care

Asthenia

Asthenia is a profound fatigue, which affects many patients with pancreatic cancer.⁵⁴ It is commonly felt that the asthenia and the cachexia associated with advanced pancreatic cancer are mediated by proinflammatory cytokines secreted either by the tumor cells or by immunocytes, such as macrophages that are stimulated by cytokines. There is also evidence to indicate that a proinflammatory cytokine, such as C-reactive protein, is secreted by liver cells in response to the interleukin 8 (IL-8) being secreted by tumor cells. Proinflammatory cytokines implicated in pancreatic cancer-induced asthenia and cachexia include TNF- α , IL-6, IL-8, C-reactive protein, IL-2, IL-1, and others.⁵⁴

Agents That Suppress Proinflammatory Cytokines

Eicosapentaenoic Acid and Docosahexaenoic Acid

There are n-3 polyunsaturated fish oils that suppress the production of the proinflammatory cy-

tokines, TNF- α , IL-1, and IL-6, by peripheral blood mononuclear cells. Of note is a recent clinical trial where patients acted as their own control.⁵⁵ In that study of a nutritional supplement enriched with fish oil, patients had a gain in weight (compared with a run-in period without the fish oil supplement), a rise in lean body mass, an increase in appetite, and a positive change in Karnofsky performance status. There was no change in mid-arm muscle circumference, triceps skin fold, or total body water.⁵⁵ These results are promising and should be tested in a well-controlled, randomized trial. Table 36.3 outlines other cytokines, which are thought to mediate asthenia and cachexia, and possible ways to modulate the production of these cytokines.^{56–58} As can be seen in that table, the modulating agents are common agents used for other clinical conditions. Clinical trials, testing each of these agents, are either ongoing or will be started soon.

Summary

There are multiple new therapeutic targets in pancreatic cancer against which new therapies can be directed. There are many targets that have not been discussed in this chapter, including farnesyl transferase,⁵⁹ asparagine synthase, thymidylate synthase, PTEN/MMAC (phosphatase and tension homolog deleted on chromosome *ten*/mutated in multiple advanced cancers),⁶⁰ and others. As we continue to examine fresh human tumors taken at the time of operation, with new molecular biology techniques, many more targets will emerge. These new targets will challenge our drug development efforts as well as our clinical trial designs.

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TABLE 36.3. Other candidates to decrease cytokines.*

Cytokine target	Agent mechanism	Reference
C-reactive Protein	Pravachol (decreases the production of C-reactive protein)	Ridker et al. ⁵⁶
TNF	Enbrel (the soluble receptor for TNF—it complexes TNF- α)	Mohler et al. ⁵⁷
IL-6	Thalomid (decreases production of IL-6)	Rowland et al. ⁵⁸

*TNF indicates tumor necrosis factor alpha.

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