Focus on pancreas cancer

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Epidemiology

About 30,000 Americans develop pancreatic cancer each year and 30,000 die from it. A large number of case-control and cohort studies have shown that there is a clustering of pancreatic cancer in some families and that this clustering occurs more frequently than one would expect by chance (Ghadirian et al., 2002; Tersmette et al., 2001; Coughlin et al., 2000). For example, Tersmette et al. followed over 340 kindreds enrolled in the National Familial Pancreas Tumor Registry (NFPTR) and found an 18-fold increased risk of pancreatic cancer in the kindreds in which at least a pair of first-degree relatives had been diagnosed with pancreatic cancer at the time the kindred enrolled in the NFPTR. More recently, segregation analyses have suggested that this clustering of pancreatic cancer has a genetic basis. In a complex segregation analysis on 287 families ascertained through an index case diagnosed with pancreatic cancer at the Johns Hopkins Medical Institutions between January 1, 1994, and December 31, 1999, nongenetic transmission models ($p < 0.0001$) were rejected and the most parsimonious models included autosomal dominant inheritance of a rare allele. Approximately 0.5% of the population is estimated to carry this allele. Five genetic syndromes associated with an increased risk of pancreatic cancer have already been identified. These five syndromes are listed in Table 1 and include (1) *BRCA2*; (2) familial atypical multiple mole melanoma (*p16/CDKN2A)* (Lynch et al., 2002); (3) Peutz-Jeghers Syndrome, which is characterized by melanocytic macules on the lips and bucal mucosa and numerous hamartomatous polyps of the gastrointestinal tract (Giardiello et al., 2000); (4) hereditary nonpolyposis colorectal syndrome (HNPCC) (Wilentz et al., 2000); and (5) familial pancreatitis (Lowenfels et al., 1997). These five syndromes only account for ~20% of the families in which there is an aggregation of pancreatic cancer. The hunt for the gene or genes responsible for the aggregation of pancreatic cancer in the majority of the families is one of the most exciting areas of pancreatic cancer research at the present time.

Disease mechanism and variant tumors

The pancreas is an extremely common site for the development of early neoplasms—noninvasive clonal epithelial expansions termed pancreatic intraepithelial neoplasia (PanIN) are extremely common in elderly persons (Hruban et al., 2000). In a minority of persons, these clones of cells serially acquire genetic changes that can lead to an invasive adenocarcinoma (Table 2). The epithelial cells in the advanced stage of this process are very aggressive, seemingly having an innate capability for metastasis that is exhibited rather soon after they invade beyond the duct structure into surrounding tissue. This invasion is nearly always accompanied by an exuberant nonneoplastic stromal response termed "desmoplasia" that generally accounts for the majority of the tumor volume (Wilentz, et al., 2000). In most cases, the cancer is a conventional ductal adenocarcinoma that arises from a high-grade PanIN lesion. There are also variants. The intraductal papillary mucinous neoplasm (IPMN) arises in the large pancreatic ducts and produces copious amounts of extracellular mucin. Medullary carcinomas have a distinct undifferentiated histologic appearance. Tumors with endocrine differentiation are usually treated as an entirely separate category of disease.

Key genes and pathways involved in the pathogenesis of pancreatic cancer

The conventional infiltrating ductal adenocarcinomas have all the accoutrements of widespread chromosomal instability (CIN), including a high rate of translocations and deletions (Hilgers and Kern, 1999). Nearly all have a mutation in the *KRAS2 (K-ras)* G protein involved in the transmission of growth factor signals and inactivation of the *CDKN2A* (*p16*) cyclin-dependent kinase inhibitor. Most also harbor mutations of the *TP53* (*p53*) gene and in *MADH4*, the common Smad protein involved in transduction of TGFβ and activin signals. Additional recurrent patterns of gene mutation are seen at lower frequencies (Table 2). *BRCA2* mutations are usually inherited, rather than acquired (Goggins et al., 1996), while *CDKN2A* and *LKB1* mutations can be germline or, much more often, acquired. Medullary cancers differ from the above pattern, often having a wild-type *KRAS2* gene and often with evidence of DNA mismatch-repair defects (Goggins et al., 1998; Yamamoto et al., 2001). The latter are due to mutation or methylation of DNA repair genes *MSH2* or *MLH1*, defects that can be inherited as a cause of genetic cancer susceptibility. These tumors lack the deletions that would be evidence of chromosomal instability,

Table 1. Genetic disorders and germline genetic alterations associated with familial pancreatic cancer

Disorder	Gene location	Increased risk of pancreas cancer
Hereditary pancreatitis	PRSS1 (7q35)	50×
Hereditary nonpolyposis colorectal cancer lynch variant II	MSH2, MLH1	?
Hereditary breast and ovarian cancer	BRCA2 (13q12-q13)	3.5–20×
Familial atypical multiple mole melanoma syndrome (FAMMM)	p16 (9p21)	12–20×
Peutz-Jeghers syndrome	STK11/LKB1 (19p13)	130×

Table 2. Molecular alterations in pancreatic ductal neoplasia

Gene (chromosome)	Alteration[a]	Frequency in cancers[b]	Appearance in preinvasive lesions[c]
CDKN2A (9p)	Mutation, silencing	95%	Middle
KRAS2 (12p)	Activating mutation	95%	Early
TP53 (17p)	Mutation	50%–75%	Late
MADH4 (18q)	Mutation	55%	Late
EP300 (22q)	Mutation	25%	
AKT2 (19q)	Amplification	15%	
MYB (6q)	Amplification	10%	
BRCA2 (13q)	Mutation	7%	Late
LKB1/STK11 (19p)	Mutation	5%	
MKK4 (17p)	Mutation	4%	
RB1 (13q)	Mutation	rare	
TGFBR1 (9q)	Mutation, underexpression	2%	
TGFBR2 (3p)	Mutation, underexpression	4%–7%	
ACVR1B (12q)	Mutation	2%	
BAX (19q)	Mutation	6%	
MSH2 or MLH1	Mutation, silencing	3%–15%	
Mesothelin	Overexpression	95%	Rare
PSCA	Overexpression	75%	Early
Her2/neu	Overexpression		Middle

[a]"Mutation" refers to homozygous inactivating intragenic mutations and homozygous deletions, the exceptions being *KRAS2*, which has mutations of codons 12, 13, and 61 that activate its signaling function, and the amplified genes. "Silencing" refers to a total loss of gene expression in the absence of genetic mutation, usually associated with methylation of the gene promoter. Mutations that do not occur at a frequency higher than explicable by chance, and some unconfirmed gene mutations, are omitted.
[b]Most TGFBR2 and all Bax mutations reported represent microsatellite mutations in tumors having MSH2 or MLH1 alterations. Underexpression of TGFβ receptors is widespread; the mutational frequency alone is given here.
[c]In the case of BRCA2, the first mutation is germline, while the loss of the wild-type allele appears to be a late event in tumorigenesis.

although they commonly have mutations of genes that contain simple nucleotide repeats, such as in the *TGFBR1* and *BAX* genes.

Techniques are now available to comprehensively profile the gene expression patterns of pancreatic cancer. The use of serial analysis of gene expression (SAGE) and gene microarray technologies have identified a number of genes overexpressed by pancreatic cancers. For example, mesothelin is highly expressed in nearly all, and prostate stem cell antigen in most, ductal adenocarcinomas of the pancreas (Figure 1; Argani et al., 2001a, 2001b). Both are cell surface proteins that can also be secreted. These and other proteins secreted by the tumor, such as connective tissue growth factor, suggest a communication between the neoplastic epithelial cells and nonneoplastic stromal cells, perhaps accounting for the distinctive desmoplastic reaction present in conventional ductal pancreatic cancers (Iacobuzio-Donahue et al., 2002; Goggins et al., 2000).

Screening and diagnosis

Abdominal examination and standard radiologic imaging tests such as transabdominal ultrasonography, magnetic resonance imaging (MRI), and conventional spiral CT scan are not sufficiently sensitive for small early cancers, even in symptomatic patients (AGA, 1999). Therefore, routine pancreatic cancer screening is not recommended in asymptomatic persons (AGA, 1999). However, subgroups at increased risk (individuals with inherited genetic syndromes and those from familial pancreatic cancer kindreds) might benefit from screening. The most optimal method for screening is unknown and is currently under evaluation. Endoscopic ultrasonography (EUS) has been suggested as one radiologic imaging modality that may potentially detect pancreatic neoplasia. EUS is high-frequency, real-time ultrasonography combined with endoscopy. It is currently one of the best imaging modalities for the pancreas. It is associated with a very low risk of adverse effects (0%–0.5%) and very high sensitivity for detection of early pancreatic cancer (Grimm et al., 1990; Nakaizumi et al., 1995). Brentnall and colleagues first reported the use of EUS for screening relatives from familial pancreatic cancer kindreds. They found that EUS had a high positive predictive value for dysplasia in high-risk persons (Brentnall et al., 1999). Seven patients who had both EUS and endoscopic retrograde cholangiopancreography (ERCP) abnormalities underwent total pancreatectomy, and widespread epithelial ductal dysplasia (low-grade to high-grade) involving the small- and medium-sized ducts were found in all. The authors of this study recommend EUS as the preferred screening test because of the risk for pancreatitis associated with ERCP.

Prospective studies are currently underway to evaluate the feasibility and diagnostic yield of EUS- and spiral CT-based screening of high-risk individuals coupled with collection and banking of various tissue specimens for future molecular marker analyses. At Johns Hopkins, a screening and early detection program for high-risk individuals from kindreds with familial pancreatic cancer (National Familial Pancreas Tumor Registry) and patients with Peutz-Jeghers syndrome (Hereditary Colorectal Cancer Registry) is ongoing (Canto et al., 2002). EUS is performed and if EUS is abnormal, EUS-guided fine-needle aspiration (FNA), ERCP, and dual-phase, thin-section spiral CT are performed. Patients with a mass are referred for surgery, while those with other abnormalities undergo repeat EUS/FNA within 3–6 months. Thirty-seven patients with no symptoms referable to the pancreas or suggestive of cancer have been screened. Six pancreatic masses have been found by EUS (4/6 also detected by CT scan)—1 adenocarcinoma, 1 benign intraductal papillary mucinous tumor, and 4 nonneoplastic lesions. The one patient from a family with six pancreatic cancers who was diagnosed by EUS with pancreatic cancer was resected and is still alive and disease-free 3 years from diagnosis. The diagnostic yield of screening for a pancreatic neoplasm was 2/37 = 5.4%.

Current therapeutic strategies

Pancreaticoduodenectomy provides the only chance for cure.

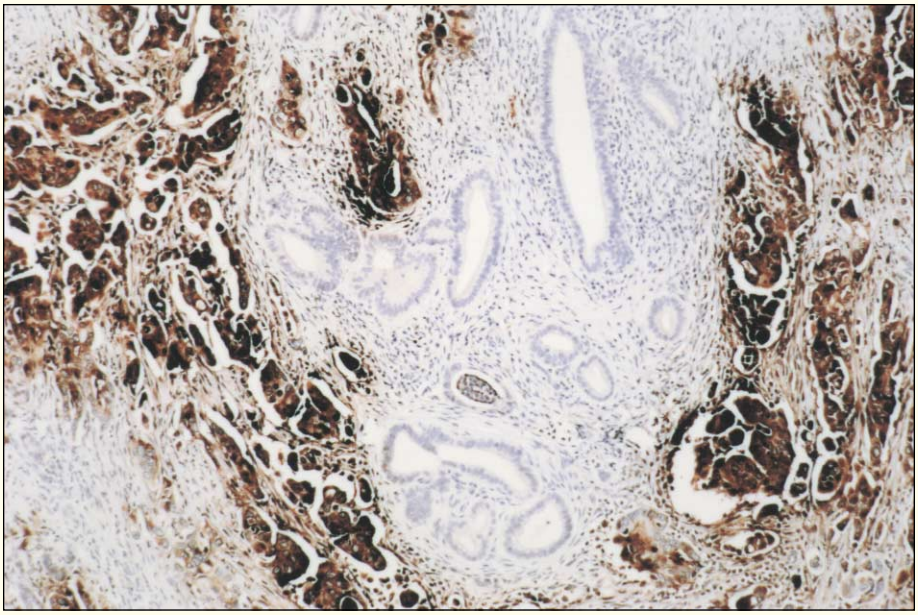

Figure 1. Immunohistochemistry staining of a pancreatic cancer for PSCA expression

Normal glands are shown in the center of the photo (unlabeled areas), and invasive cancer is on the sides (labeled areas).

However, few options exist for the 80% of patients with locally advanced or metastatic disease at diagnosis. Prolonged survival has been observed in a small subset of pancreatic cancer patients treated with Gemcitabine either alone or in combination with other chemotherapeutic agents such as Cisplatin and Paclitaxel (Rosenberg, 2000, Laheru et al., 2001).

Experimental therapeutic approaches

Molecular technology advances have ushered in a new age of targeted novel approaches that should translate rapidly into new therapeutic options for patients with pancreatic cancer. Some of these new approaches aim to specifically inhibit tumor growth and metastases by targeting specific components of the tumor's microenvironment and selected signal transduction pathways. Other approaches aim to augment antitumor immunity to specific antigenic targets.

Targets in the tumor microenvironment

Evidence suggests that matrix metalloproteinases (MMP) facilitate early pancreatic tumor invasion and metastasis. Several MMPs have been shown to be overexpressed by neoplastic pancreatic epithelium and the desmoplastic stromal cells. A small molecule inhibitor of MMP, Marimastat, is currently undergoing phase II testing in inoperable pancreatic cancer patients (Rosenberg, 2000). Angiogenesis is also critical for the growth of primary tumors and is essential to the metastatic process. Several pancreatic cancer-associated genes have been linked to angiogenesis. DPC4 upregulates vascular endothelial growth factor expression, and mutated K-ras expression is associated with increased microvessel density (Schwarte-Waldhoff et al., 2000). These angiogenesis pathways provide new targets for therapeutic intervention that are currently being explored. One major challenge will be to identify targets that are specifically altered relative to the normal counterpart so as to avoid significant treatment-related toxicities.

Signal transduction inhibition

The *K-ras* oncogene product and its signaling pathway has been a major focus of pancreatic research. Some ras proteins require a posttranslational 15-carbon farnesyl group addition to bind to the cell membrane for signaling to occur. Inhibitors designed to block the addition of the farnesyl group have been demonstrated to inhibit pancreatic cancer growth in preclinical in vitro and in vivo models. However, early clinical trials have not demonstrated significant activity against pancreatic cancers (Hao and Rowinsky, 2002). Interestingly, other signaling molecules rather than mutated *KRAS2* may turn out to be the target of the farnesyltransferase inhibitors, since these inhibitors appear to have activity in nonmutated ras-expressing tumors. The identification of additional signaling pathways specifically upregulated in pancreatic cancers should be forthcoming from the gene discovery studies described earlier.

Immunotherapy

Immunotherapy represents a noncrossresistant mechanism of antitumor activity that can be integrated with surgery, radiation therapy, and chemotherapy. A number of preclinical studies have been published demonstrating synergistic activity between immune-based therapy and other cancer treatment modalities (Emens et al., 2001). Antibody-based therapy has been studied against a few pancreatic cancer antigens and has demonstrated some activity (Laheru et al., 2001). Active immunization with whole tumor cells that have been genetically modified to express cytokines or other costimulatory molecules are an attractive approach that has demonstrated significant potency in animal models. Recently, an allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine was tested in 14 stage 2 or 3 patients who underwent a Whipple procedure. The vaccine was demonstrated to be safe and to induce tumor-specific immunity in 3 of 8 patients receiving 10^8 and 5×10^8 vaccine cells (Jaffee et al., 2001). Immunization with heat shock proteins (HSP) isolated from pancreatic tumor cells is another important approach that is undergoing clinical testing (Laheru et al., 2001). Vaccine and antibody approaches targeting pancreatic cancer-associated tumor antigens (CEA, MUC-1, and mutated KRAS) have also undergone clinical testing. Although toxicities were minimal, these approaches so far have failed to generate clinical responses (Laheru et al., 2001).

Future challenges

Cancer research focused on deciphering the human genome sequence is dissecting the pathobiology of pancreatic cancer and is leading to new targets for diagnosis, prevention, and therapy. This new era raises new challenges for translating these new discoveries. New animal models are required for understanding the biologic significance of identified genetic pathways, for rapidly evaluating potential new therapies, and for revealing potential synergistic interactions among interventions that target multiple biologic pathways. In addition, innovative clinical trial designs are required that incorporate new end-

points for evaluating these new therapeutic interventions that are cancer specific. To this end, noninvasive methods for repetitive sampling of tissues from treated patients and for imaging biologic responses are also essential.

References

AGA (American Gastroenterological Association) (1999). American gastroenterological association medical position statement: epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. Gastroenterology *117*, 1463–1484.

Argani, P., Iacobuzio-Donahue, C.A., Ryu, R., Goggins, M., Rosty, C., Wilentz, R.E., Murugesan, S., Kaushal, M., Leach, S.D., Jaffee, E.M., et al. (2001a). Mesothelin is expressed in the vast majority of adenocarcinomas of the pancreas: identification of a new cancer marker by serial analysis of gene expression (SAGE). Clin. Cancer Res. *7*, 3862–3868.

Argani, P., Rosty, C., Reiter, R.E., Wilentz, R.E., Murugesan, S., Leach, S.B., Ryu, B., Goggins, M., Yeo, C.J., Cameron, J.L., et al. (2001b). Discovery of new markers of cancer through serial analysis of gene expression (SAGE): prostate stem cell antigen (PSCA) is overexpressed in pancreatic adenocarcinoma. Cancer Res. *61*, 4320–4324.

Brentnall, T.A., Bronner, M.P., Byrd, D.R., Haggitt, R.C., and Kimmey, M.B. (1999). Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. Ann. Intern. Med. *131*, 247–255.

Canto, M., Wroblewski, L., Goggins, M., Brun, K., Peterson, G., Giardello, F., Yeo, C., and Hruban, R. (2002). Screening for pancreatic neoplasia in high-risk individuals: the Johns Hopkins experience. Gastroenterology *122* (Suppl. 1), A17.

Coughlin, S.S., Calle, E.E., Patel, A.V., and Thun, M.J. (2000). Predictors of pancreatic cancer mortality among a large cohort of United States adults. Cancer Causes Control *11*, 915–923.

Emens, L.A., Machiels, J.-P., Reilly, R.T., and Jaffee, E.M. (2001). Chemotherapy: friend or foe to cancer vaccines? Curr. Opin. Mol. Ther. *3*, 77–84.

Ghadirian, P., Liu, G., Gallinger, S., Schmocker, B., Paradis, A.J., Lal, G., Brunet, J.S., Foulkes, W.D., and Narod, S.A. (2002). Risk of pancreatic cancer among individuals with a family history of cancer of the pancreas. Int. J. Cancer *97*, 807–810.

Giardiello, F.M., Brensinger, J., Tersmette, A.C., Goodman, S.N., Petersen, G.M., Booker, S.V., Cruz-Correa, M., and Offerhaus, J.A. (2000). Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology *119*, 1447–1453.

Goggins, M., Schutte, M., Lu, J., Moskaluk, C.A., Weinstein, C.L., Petersen, G.M., Yeo, C.J., Jackson, C.E., Lynch, H.T., Hruban, R.H., and Kern, S.E. (1996). Germline *BRCA2* gene mutations in patients with apparently sporadic pancreatic carcinomas. Cancer Res. *56*, 5360–5364.

Goggins, M., Offerhaus, G.J.A., Hilgers, W., Griffin, C.A., Shekher, M., Tang, D., Sohn, T., Yeo, C.J., Kern, S.E., and Hruban, R.H. (1998). Adenocarcinomas of the pancreas with DNA replication errors (RER+) are associated with wild-type K-ras and characteristic histopathology: poor differentiation, a syncytial growth pattern, and pushing borders suggest RER+. Am. J. Pathol. *152*, 1501–1507.

Goggins, M., Canto, M., and Hruban, R. (2000). Can we screen high-risk individuals to detect early pancreatic carcinoma? J. Surg. Oncol. *74*, 243–248.

Grimm, H., Maydeo, A., and Soehendra, N. (1990). Endoluminal ultrasound for the diagnosis and staging of pancreatic cancer. Baillieres Clin. Gastroenterol. *4*, 869–888.

Hao, D., and Rowinsky, E.K. (2002). Inhibiting signal transduction: recent advances in the development of receptor tyrosine kinase and Ras inhibitors. Cancer Invest. *20*, 387–404.

Hilgers, W., and Kern, S.E. (1999). Molecular genetic basis of pancreatic adenocarcinoma. Genes Chromosomes Cancer *26*, 1–12.

Hruban, R.H., Wilentz, R., and Kern, S.E. (2000). Genetic progression in the pancreatic ducts. Am. J. Pathol. *156*, 1821–1825.

Iacobuzio-Donahue, C.A., Ryu, B., Hruban, R.H., and Kern, S.E. (2002). Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. Am. J. Pathol. *160*, 91–99.

Jaffee, E.M., Hruban, R.H., Biedrzycki, B., Laheru, D., Schepers, K., Sauter, P.R., Goemann, M., Coleman, J., Grochow, L., Donehower, R.C., et al. (2001). Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J. Clin. Oncol. *19*, 145–156.

Laheru, D., Biedrzycki, B., and Jaffee, E.M. (2001). Immunologic approaches to the management of pancreatic cancer. Cancer J. *7*, 324–337.

Lowenfels, A.B., Maisonneuve, E.P., Dimagno, Y.E., Gates, L.K., Perrault, J., and Whitcomb, D.C. (1997). International hereditary pancreatitis study group. hereditary pancreatitis and the risk of pancreatic cancer. J. Natl. Cancer Inst. *89*, 442–446.

Lynch, H.T., Brand, R.E., Hogg, D., Deters, C.A., Fusaro, R.M., Lynch, J.F., Liu, L., Knezetic, J., Lassam, N.J., Goggins, M., et al. (2002). Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families: the familial atypical mole melanoma-pancreatic carcinoma syndrome. Cancer *94*, 84–96.

Nakaizumi, A., Uehara, H., Iishi, H., Tatsuta, M., Kitamura, T., Kuroda, C., Ohigashi, H., Ishikawa, O., and Okuda, S. (1995). Endoscopic ultrasonography in diagnosis and staging of pancreatic cancer. Dig. Dis. Sci. *40*, 696–700.

Rosenberg, L. (2000). Pancreatic cancer. A review of emerging therapies. Drugs *59*, 1071–1089.

Schwarte-Waldhoff, I., Volpert, O.V., and Bouck, N.P. (2000). SMAD4/DPC4-mediated tumor suppression through suppression of angiogenesis. Proc. Natl. Acad. Sci. USA *97*, 9624–9629.

Tersmette, A.C., Petersen, G.M., Offerhaus, G.J.A., Falatko, F.C., Goggins, M., Rosenblum, E., Wilentz, R.E., Yeo, C.J., Cameron, J.L., Kern, S.E., et al. (2001). Increased risk of incident pancreatic cancer among first-degree relatives of patients with familial pancreatic cancer. Clin. Cancer Res. *7*, 738–744.

Wilentz, R.E., Goggins, M., Redston, M., Marcus, V.A., Adsay, N.V., Sohn, T.A., Kadkol, S.S., Yeo, C.J., Choti, M., Zahurak, M., et al. (2000). Genetic immunohistochemical, and clinical features of medullary carcinomas of the pancreas: a newly described and characterized entity. Am. J. Pathol. *156*, 1641–1651.

Yamamoto, H., Itoh, F., Nakamura, H., Fukushima, H., Sasaki, S., Perucho, M., and Imai, K. (2001). Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. Cancer Res. *61*, 3139–3144.