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Pancreatic Cancer

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Abstract

The past two decades have witnessed an explosion in our understanding of pancreatic cancer, and it is now clear that pancreatic cancer is a disease of inherited (germ-line) and somatic gene mutations. The genes mutated in pancreatic cancer include *KRAS2*, *p16/CDKN2A*, *TP53*, and *SMAD4/DPC4*, and these are accompanied by a substantial compendium of genomic and transcriptomic alterations that facilitate cell cycle deregulation, cell survival, invasion, and metastases. Pancreatic cancers do not arise de novo, and three distinct precursor lesions have been identified. Experimental models of pancreatic cancer have been developed in genetically engineered mice, which recapitulate the multistep progression of the cognate human disease. Although the putative cell of origin for pancreatic cancer remains elusive, minor populations of cells with stem-like properties have been identified that appear responsible for tumor initiation, metastases, and resistance of pancreatic cancer to conventional therapies.

Keywords

pancreas; carcinoma; adenocarcinoma; precursor; genetics; stem cell; mouse model; targeted therapy

EPIDEMIOLOGY, RISK FACTORS, AND DEMOGRAPHICS

Pancreatic cancer is one of the deadliest of all of the solid malignancies. The five-year survival rate is only 4% (1). The American Cancer Society estimates that 37,170 Americans will be diagnosed with pancreatic cancer in the year 2007 and 33,370 will die from it, making pancreatic cancer the fourth leading cause of cancer death (1). In the United States, the age-adjusted incidence of pancreatic cancer is higher in blacks (14.9 cases per 100,000) than in whites (11.1 cases per 100,000), and it is higher in men (12.8 cases per 100,000) than in women (10.0 cases per 100,000) (http://seer.cancer.gov/).

A number of risk factors have been identified (2). Pancreatic cancer is predominantly a disease of the elderly. Pancreatic cancer is rare before the age of 40, and the median age at diagnosis is 73 years (http://seer.cancer.gov/). Cigarette smoking is by far the leading preventable cause of pancreatic cancer (3,4). Cigarette smoking doubles the risk of pancreatic cancer (Relative Risk = 2), and it is believed that as many as one in four cases of pancreatic cancer may be attributable to smoking (2). Other established risk factors include diets high in meats and fat, low serum folate levels, obesity, long-standing diabetes mellitus, and chronic pancreatitis (2,

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DISCLOSURE STATEMENT

Dr. Hruban has the potential to receive milestone payments and royalties from Cerus Corporation as a result of the mesothelin invention.

5–7). A well-publicized study linking coffee consumption with pancreatic cancer had methodological flaws, and more recent studies did not demonstrate any significant associations (2).

One of the more interesting risk factors for pancreatic cancer is a family history of this disease. Individuals with a strong family history of pancreatic cancer have a significantly increased risk of developing the disease themselves. For example, individuals with a first-degree relative with pancreatic cancer have a 2.3-fold increased risk of developing the malignancy (8). Klein et al. (9) have shown that this risk increases with the number of first-degree relatives one has with pancreatic cancer, and that the simplest model to explain this aggregation in families is an autosomal dominant inheritance of a rare allele. As summarized in Table 1, a number of germ-line genetic alterations have been associated with an increased risk of pancreatic cancer (10–19). These germ-line genetic alterations provide insight into the pathogenesis of pancreatic cancer.

Several features of the genetic syndromes associated with the familial clustering of pancreatic cancer deserve special note (20,21). First, the penetrance of cancer in the gene carriers is incomplete. For example, some individuals with germ-line *BRCA2* gene mutations do not have a strong family history of cancer (11). Goggins et al. (11) studied a series of 41 apparently sporadic pancreatic cancer patients and four (9.8%) of the 41 patients harbored a germ-line *BRCA2* gene mutation. Only one of the four had a family history of breast cancer and none had a family history of pancreatic cancer. Clearly, the absence of a strong family history of cancer cannot be used to rule out a germ-line mutation.

Second, individuals can be identified, tested for one of these mutations, and counseled on their pancreatic cancer risk. For example, the Peutz-Jeghers syndrome is characterized by melanocytic macules on the lips and buccal mucosa, and hamartomatous polyps of the gastrointestinal tract (13). Individuals with these stigmata of the Peutz-Jeghers syndrome can be clinically tested for germ-line mutations in the *STK11/LKB1* gene. Those found to carry a mutation may then benefit from screening for asymptomatic pancreatic neoplasia (22).

Third, most of these germ-line mutations, with the exception of those in the *PRSS1* gene, are also associated with an increased risk of extrapancreatic malignancies. For example, the risk of melanoma in *p16/CDKN2A* gene mutation carriers is approximately 28% (95% confidence interval = 18% to 40%) by age 80 (23). These extrapancreatic malignancies can also be screened for and, in the case of melanoma, preventative measures such as avoiding sun exposure can also be initiated.

Fourth, pancreatic cancers with microsatellite instability, the hallmark of DNA mismatch repair defects such as those produced by mutations in the *hMLH1* and *hMSH2* genes, often have a distinctive medullary histology (18,24). Medullary carcinomas are poorly differentiated; they have expanding borders and a prominent syncytial growth pattern (18,24). When a pancreatic cancer with this morphology is identified, the morphologic appearance of the cancer can be used to suggest that the patient may benefit from genetic counseling and possibly genetic testing.

Fifth, these germ-line genetic abnormalities may have therapeutic implications (20,21). For example, the protein product of the *BRCA2* gene interacts with the protein products of the Fanconi anemia complementation genes (the *FANC* genes) to promote homologous recombination (25). As discussed subsequently in further detail, van der Heijden et al. (26, 27) have shown that pancreatic cancers with *BRCA2* or other proximal *FANC* gene mutations are particularly sensitive to Mitomycin C and radiation—therapies known to cause DNA cross-linking and double strand breaks, respectively.

Finally, because of founder mutations, some of these genetic syndromes are more common in certain ethnic groups. For example, 1% of the Ashkenazi Jewish population carries the 6174delT *BRCA2* gene mutation (10). Knowledge of these associations can help guide genetic testing.

PATHOLOGY

Most cancers of the pancreas are infiltrating ductal adenocarcinomas, and we use the terms pancreatic cancer and infiltrating ductal adenocarcinoma of the pancreas synonymously (21). Pancreatic cancer grossly produces a firm, highly sclerotic mass (Figure 1***a***). The edges of these cancers are poorly defined, with long tongues of carcinoma extending well beyond the main tumor (21). At the light-microscopic level, pancreatic cancer is composed of an infiltrating gland-forming neoplastic epithelium with an intense desmoplastic reaction (Figure 1***b***) (21). This desmoplastic reaction is usually so intense that only a minority of the cells in the mass formed by pancreatic cancer are neoplastic cells (Figure 1***c***). As one might expect from their gross appearance, pancreatic cancers are extremely infiltrative neoplasms. Vascular and perineural invasion are present in the majority of surgically resected cancers, and metastases to regional lymph nodes, the liver, and distant sites are all too common (21).

The majority of pancreatic cancers express immunohistochemically detectable Cytokeratin (Cytokeratins 7, 8, 13, 18, and 19), carcinoembryonic antigen, carbohydrate antigen 19–9 (CA19–9), B72.3 (TAG-72), CA 125, and DUPAN 2 (21). Most pancreatic cancers also express a number of mucins, including MUC1, MUC3, MUC4, and MUC5AC (21,28). More recently described markers include claudin 4 and 18, sea urchin fascin homolog, several of the S-100 proteins (including S-100A4, S-100A6, and S-100P), and the glycosylphosphatidyl-inositol-anchored proteins mesothelin and prostate stem cell antigen (29–32).

A number of histologic variants of pancreatic cancer have been described. These include adenosquamous carcinoma, colloid carcinoma, hepatoid carcinoma, medullary carcinoma, signet-ring cell carcinoma, undifferentiated carcinoma, and undifferentiated carcinoma with osteoclast-like giant cells (21). These are important to recognize because some have distinct pathogenesis (medullary carcinoma), some have a significantly better prognosis (colloid, medullary), and some have a worse prognosis (adenosquamous carcinoma, undifferentiated carcinoma) than infiltrating ductal adenocarcinoma.

PRECURSOR LESIONS

Histologically distinct precursor lesions have been described in the pancreas (33,34). These precursor lesions include pancreatic intraepithelial neoplasia (PanIN), the intraductal papillary mucinous neoplasm (IPMN), and the mucinous cystic neoplasm (MCN) (35). Lives can be saved if these noninvasive lesions can be detected and treated before they progress to an invasive carcinoma.

PanINs are microscopic lesions in the smaller (<5 mm) pancreatic ducts (33,34). PanINs can be papillary or flat, and they are composed of columnar to cuboidal cells with varying amounts of mucin. PanINs are subclassified into PanIN-1, PanIN-2, and PanIN-3 lesions, depending upon the degree of cytologic and architectural atypia (Figure 1 ***d***) (33,34). PanINs are often present in the pancreatic parenchyma adjacent to infiltrating adenocarcinomas, and several case reports have documented patients with PanINs who later developed an infiltrating pancreatic cancer (36). As illustrated in Figure 2, molecular analyses of PanINs have shown that PanINs harbor many of the same genetic alterations as are found in infiltrating pancreatic cancer (20,35). Activating point mutations in codon 12 of the *KRAS2* gene typically occur in early low-grade PanIN lesions (PanIN-1), whereas inactivating mutations in the *p16/CDKN2A* gene occur in intermediate lesions (PanIN-2), and inactivating mutations in

SMAD4, *TP53*, and *BRCA2* occur in late lesions (PanIN-3) (35). Telomere shortening is also an early event, occurring in PanIN-1 lesions, and telomere shortening may contribute to the accumulation of chromosomal abnormalities in PanINs (37). As discussed below, understanding of the genetics of PanINs was crucial for the development of genetically engineered mouse models of pancreatic cancer, and it forms a basis for early detection strategies.

IPMNs are mucin-producing epithelial neoplasms that arise within the main pancreatic duct or one of its branches and that often, although not always, have a papillary architecture (21, 34). IPMNs with an intestinal phenotype express the mucin MUC2, whereas most PanINs express MUC1 (21,34). The *SMAD4* gene is inactivated in only a small minority of IPMNs (38), whereas loss of the *STK11/LKB1* gene product is significantly more common (39). Because they are larger lesions, screening for IPMNs may be possible using currently available imaging technology (22,40).

The third histologically distinct precursor lesion in the pancreas is the MCN (21). The vast majority of MCNs arise in women. In contrast to IPMNs, MCNs do not communicate with the larger pancreatic ducts, and, in contrast to PanINs and IPMNs, MCNs have a distinctive ovarian-type stroma (21). The genetic alterations in MCN have not been extensively studied, but it appears that they can have many of the same abnormalities found in infiltrating ductal adenocarcinomas of the pancreas, but at a lower frequency (35).

It is clear that some noninvasive IPMNs and some MCNs of the pancreas progress to invasive adenocarcinoma over time (35). Patients with noninvasive IPMNs and MCNs are on average 3–5 years younger than patients with the corresponding invasive lesion (21). This latter observation suggests a significant window of opportunity during which these precursor lesions can be treated and the patient cured before an invasive cancer develops.

MOLECULAR GENETICS OF PANCREATIC CANCER

One of the most important paradigms to emerge from more than two decades of research in pancreatic cancer has been the understanding that pancreatic cancer is a disease of inherited and somatic mutations. A compendium of signature mutations defines pancreatic adenocarcinoma and differentiates this malignancy from other neoplasms of the pancreas.

KRAS2 and Other Oncogenes

Activating mutations of the *KRAS2* oncogene are possibly the single most common genetic abnormality in pancreatic cancer, present in ~90%–95% of cases (41). *KRAS2* encodes a member of the RAS family of guanosine triphosphate (GTP)-binding proteins that mediate a range of cellular functions, including proliferation, cell survival, cytoskeletal remodeling, and motility, among others. A variety of stimuli, such as binding of growth factor ligands to the cognate growth factor receptor, results in signal transduction via intermediary proteins that culminate in the activation of the Kras protein. The active protein is bound to GTP, and inactivation occurs through guanosine-triphosphatase-activating proteins, which promote GTP hydrolysis to the diphosphate GDP and attenuate Kras signaling. Activating mutations impair the intrinsic GTPase activity of the *KRAS2* gene product, resulting in a protein that is constitutively active in intracellular signal transduction (42). The spectrum of *KRAS2* gene mutations in pancreatic cancer is limited principally to alterations of codon 12, with occasional mutations of codons 13 or 61 (41). Mutations of the *KRAS2* gene are one of the earliest genetic abnormalities observed in the progression model of pancreatic cancer, demonstrable in approximately 36%, 44%, and 87% of cancer-associated PanIN-1A, PanIN-1B, and PanIN-2/3 lesions (35). PanIN lesions and an adenocarcinoma within the same pancreas may harbor different *KRAS2* gene mutations, suggesting that some precursors evolve as independent clones

from the one that eventually progresses to the invasive cancer (43). The high frequency of *KRAS2* gene mutations in human PanINs supports its role as an initiating event for pancreatic cancer formation, and this has been reiterated in several recent animal models, where expression of mutant Kras is a prerequisite for the development of ductal preneoplasia and cancer (see below) (44–48). In addition to its role in pancreatic cancer initiation, constitutive RAS signaling appears to be required for pancreatic cancer maintenance as well (49).

Activated Kras engages a number of downstream effector pathways, including RAF–mitogen-activated protein kinase (RAF-MAPK), phosphoinositide-3-kinase (PI3K), and RalGDS pathways. Several lines of evidence point toward a critical role for RAF-MAPK signaling in pancreatic carcinogenesis. One-third of pancreatic cancers with wild-type *KRAS2* harbor mutations of the *BRAF* oncogene, thereby resulting in the activation of RAF-MAPK signaling even in the absence of *KRAS2* mutations (50). Second abrogation of RAK-MAPK signaling with small molecule inhibitors, or with antisense inhibition of an essential transducer of RAS signaling to RAF, the *kinase suppressor of ras 1* (*KSR1*), results in growth inhibition of pancreatic cancer xenografts (51). The PI3K-AKT pathway is an essential cell survival pathway, with diverse roles in tumorigenesis across multiple solid malignancies. The PI3K-AKT pathway is constitutively activated in most pancreatic cancers, and targeting this pathway with small molecule inhibitors or genetic strategies results in growth inhibition in vitro and in vivo (52). Although RAS certainly contributes to PI3K-AKT signaling in pancreatic cancer, independent genomic events can also activate this pathway, including amplification of the *AKT2* gene on chromosome 19q (~10%–15% of pancreatic cancers) (53) and activating mutations of the *PIK3CA* gene in a subset of IPMNs (54). Recently, Ral A, a downstream moiety in the RalGDS effector pathway, has emerged as another key player in RAS-mediated transformation, as knockdown of Ral A in pancreatic cancer cells essentially abolishes their tumorigenic phenotype (55). Whether these signaling moieties can be utilized as therapeutic targets remains to be determined.

Additional candidates that contribute to oncogenesis have been identified (56–58). For example, the gene encoding the oncogenic transcription factor C-Myc on chromosome 8q is amplified in 20%–30% of pancreatic cancers, and *CMYC* transcripts are overexpressed in nearly all cases (56). Other oncogenes that are targets of amplification in pancreatic cancer include *MYB* (chromosome 6q), *AIB1/NCOA3* (chromosome 20q), and *EGFR* (chromosome 7p) (56–58). A number of recurrent chromosomal amplifications have also been identified in the high-resolution copy number experiments (e.g., localized amplicons on chromosomes 18q and 7q), wherein the putative targets are under investigation or simply unknown (56–58). Elucidation of these oncogenic events will undoubtedly provide additional insights into the biology and therapy of pancreatic cancer.

Tumor Suppressor Genes (*p16/CDKN2A*, *TP53*, and *DPC4/SMAD4*)

The gene *p16/CDKN2A*, also known as *INK4A*, is the most commonly inactivated tumor suppressor gene in pancreatic cancers (59). The encoded protein belongs to the cyclin-dependent kinase (CDK) inhibitor family and inhibits cell cycle progression through the G1-S checkpoint mediated by CDKs such as CDK4 and -6. Loss of *p16/CDKN2A* function is seen in approximately 90% of pancreatic cancers. This loss occurs by several different mechanisms, including homozygous deletion (40%), intragenic mutation with loss of the second allele (40%), and epigenetic silencing by promoter methylation (10%–15%) (20).

Inactivation of the *TP53* gene on chromosome 17p is present in approximately 50%–75% of pancreatic cancers, and inactivation of the *TP53* gene almost always occurs via intra-genic mutation combined with loss of the second allele (60). The p53 protein has a number of important functions in the cell, including regulation of the G1-S cell cycle checkpoint, maintenance of G2-M arrest, and the induction of apoptosis. Loss of p53 function allows cells

to survive and divide despite the presence of damaged DNA, leading to the accumulation of additional genetic abnormalities (61).

The *deleted in pancreatic carcinoma* 4 gene, or *DPC4* (also known as *SMAD4*), on chromosome 18q21 is inactivated in approximately 55% of pancreatic cancers, either by homozygous deletion (30%) or by intragenic mutations and loss of the second allele (25%) (62). The Smad4 protein plays a critical role in signaling through the transforming growth factor-β (TGF-β) pathway. The TGF-β pathway is activated when the TGF-β protein binds to specific cell surface receptors. This triggers an intracellular cascade that results in phosphorylation and nuclear localization of the Smad transcription factors Smad 2/3, complexed with Smad4. The TGF-β pathway has profound growth-inhibitory effects by regulating the expression of specific target genes; therefore, loss of Smad4 in pancreatic cancer cells, which abrogates Smad-dependent TGF-β signaling, provides a selective growth advantage (63). Immunohistochemical labeling for Smad4 protein expression mirrors *DPC4/SMAD4* gene status in pancreatic cancers with rare exceptions (64). Loss of Smad4 labeling is seen only at the stage of PanIN-3 lesions, whereas virtually all noninvasive IPMNs and MCNs retain expression (38,65,66). Inactivation of *DPC4/SMAD4* is uncommon in nonductal neoplasms of the pancreas (21) and is rare in most extrapancreatic malignancies (67). Therefore, immunolabeling for loss of Smad4 is a convenient ancillary diagnostic marker in clinical specimens, including suspected metastases from an occult pancreatic primary.

Several tumor suppressor genes are inactivated in smaller proportions (5%–10%) of pancreatic cancers, including the serine-threonine kinase *LKB1/STK11* (chromosome 19p) (16) and the TGF-β/activin signaling pathway receptors such as *TGFBR1* (chromosome 9q), *TGFBR2* (chromosome 3p) (68), and *ACVR1B* (chromosome 12q) (69). The gene *MKK4* on chromosome 17p, which encodes for a stress-activated protein kinase, is preferentially inactivated in subsets of pancreatic cancer metastases, suggesting that the protein product may function as a metastases suppressor (70). In addition to classic oncogenes and tumor suppressor genes, several so-called caretaker or genome maintenance genes are inactivated in pancreatic cancer (61). These genes do not directly influence cell growth and proliferation, but rather prevent the accumulation of DNA damage and maintain genomic fidelity. We have already discussed the role of such caretaker genes—specifically the DNA mismatch repair genes *hMLH1* and *hMSH2*, *BRCA2*, and related Fanconi anemia (*FANC*) genes—in the pathogenesis of familial pancreatic cancer (see above). However, somatic inactivation of these genes is also observed in pancreatic cancer outside the familial context.

Telomere Length Abnormalities

Telomeres are hexameric TTAGGG repeats at the ends of chromosome arms that confer stability to chromosomes during cell division and prevent the ends from becoming sticky (71). Loss of telomeres renders chromosome ends highly recombinogenic, with the formation of end-to-end fusions that result in chromosome breakage during anaphase (72). In fact, lagging chromosomal material in dividing cancer cells is often visible as anaphase bridges under the microscope. The continuous cycles of breakage-fusion-bridge that form between chromosomes result in regions of amplifications and deletions within the daughter cell genome. A recent study using an in situ fluorescent probe to evaluate telomere length demonstrated that telomere attrition is one of the earliest demonstrable genetic aberrations in pancreatic cancer, with >90% of even the low-grade PanIN lesions demonstrating marked shortening of telomeres, as compared with normal ductal epithelium (37).

The likely secondary consequence of telomere erosion in PanIN lesions is the creation of an environ that is permissive to acquisition of chromosomal rearrangements. Indirect evidence from concurrent allelotype and mutation analysis of microdissected PanIN lesions implies this to be true, as allelic deletions often precede point mutations at selected tumor suppressor gene

loci (73). In most instances, cells harboring this degree of genomic instability are eliminated through activation of p53. However, chromosomal rearrangements likely persist in cells with *TP53* gene mutations (as observed in higher-grade PanIN lesions), and these cells will then quickly accrue further genomic alterations (71). Paradoxically, telomerase reactivation, observed in the vast majority of invasive pancreatic cancers (74), is postulated to attenuate ongoing genomic instability and stabilize the neoplastic cells against potentially catastrophic rearrangements.

Epigenetic Abnormalities

It is now evident that epigenetic abnormalities are extremely common in cancers, and these abnormalities provide an alternative mechanism of transcriptional silencing (75). Epigenetic abnormalities in cancer predominantly encompass methylation of CG dinucleotides (CpG islands) in the 5′ regulatory region of tumor suppressor genes, which abrogates RNA polymerase from binding and initiating transcription. In cancers, there is preferential methylation of the gene promoter in the neoplastic cells, but not in the corresponding normal cells within the tissue of origin.

Epigenetic silencing is frequently observed in pancreatic cancers and tends to involve genes that function in tumor suppression and/or critical homeostatic pathways (e.g., *p16/CDKN2A, E-cadherin, retinoic acid beta, SOCS-1, TSLC1,* and *reprimo*, among others) (76). Epigenetic silencing is unlikely to be a bystander effect, but has tangible functional consequences on the biology of neoplastic pancreatic cells. For example, the gene *reelin* (*RELN*), whose product is involved in regulating neuronal migration, is frequently methylated in pancreatic cancer. Downregulation of *RELN* transcripts in pancreatic cancer cells confers upon these cells properties of increased motility, invasiveness, and clonogenicity, whereas re-expression of *RELN* in cell lines with epigenetic silencing of this gene inhibits their migration (77). Similarly, aberrant methylation at certain gene promoters (e.g., *reprimo*, whose gene product is associated with p53-induced G2-M arrest) is associated with increased genetic instability and adverse prognosis in established pancreatic cancer (78). A subset of genes that undergoes epigenetic modification in pancreatic cancer is also silenced by promoter methylation in PanIN, IPMN, and MCN, the three precursor lesions of adenocarcinoma (76,79–81). This finding suggests that epigenetic abnormalities are an early event in the multistep progression of pancreatic neoplasia.

One important confounding factor gleaned from these studies has been the age-related methylation at multiple gene promoters observed in normal duodenal mucosa (82). Pure pancreatic juice free of contamination by small intestinal secretions is therefore needed to specifically identify differentially methylated DNA shed into the pancreatic duct by pancreatic cancer cells. As further discussed in the section on translational studies, quantitative analysis of promoter methylation in a limited panel of genes has emerged as a promising new biomarker strategy, with demonstrable superiority to previous qualitative analyses (82).

Although there has been extensive work on the role of promoter hypermethylation in the pathogenesis of cancers, recent data suggest that promoter hypomethylation in candidate genes may also be important in the development and progression of cancer. Genes demonstrating preferential hypomethylation in pancreatic cancers are typically overexpressed in the cancers versus non-neoplastic pancreas (e.g., *Maspin, S100A4, mesothelin, prostate stem cell antigen,* and *claudin4*), suggesting a putative oncogenic role for these proteins (83). This has been functionally demonstrated in the case of *VAV1,* a Rho–guanine exchange factor in the RAS superfamily, which is overexpressed in pancreatic cancers as a consequence of promoter demethylation (84). Even in the presence of oncogenic *KRAS2* mutations, downregulation of *VAV1* by RNA-mediated interference abrogates the transformed phenotype of pancreatic

cancer cell lines, whereas overexpression of the Vav1 protein in primary cancers is associated with an adverse prognosis compared with Vav1-negative cases.

Expression Abnormalities, Including Aberrant MicroRNA Expression in Pancreatic Cancer

Global expression platforms, such as cDNA and oligonucleotide microarrays, and serial analysis of gene expression have helped elucidate large numbers of differentially expressed transcripts in pancreatic cancer and their precursor lesions (29,85–89). Multiple insights have been gained into the biology of pancreatic cancer and translational applications thereof from these global expression studies.

First, expression profiling of noninvasive lesions has helped elucidate the role of signaling pathways, including Hedgehog (Hh) and Notch, that play a role in pancreatic cancer initiation (see below) (86,90). Second, comparison of expression profiles between noninvasive precursor lesions and the accompanying adenocarcinoma has identified transcripts such as *S100A4*, *claudin4*, *CXCR4*, and *mesothelin* that likely play a role in the invasion process (88). Third, these studies have identified an important role for host stromatumor cell interactions in the pathogenesis of pancreatic cancer. Specifically, these studies have helped define that on the basis of distinct expression profiles, “bulk” tumors can be divided into four architectural compartments: the neoplastic epithelium itself, juxtatumoral stroma (i.e., stroma immediately adjacent to the neoplastic cells), panstroma (i.e., the rest of the stroma beyond the juxtatumoral location), and angioendothelium (i.e., the tumor vasculature) (91). Finally, global expression profiling has identified numerous promising diagnostic, imaging, and therapeutic candidates with immediate translation relevance in pancreatic cancer. The example of mesothelin highlights the versatile applications that can be generated from discovery of a single over-expressed gene. *Mesothelin*, which encodes for a GPI-anchored cell surface protein, is upregulated almost ubiquitously in pancreatic adenocarcinomas, is expressed only infrequently in noninvasive precursor lesions, and is essentially absent in non-neoplastic pancreatic tissues (32,92). Immunolabeling for mesothelin is useful as an ancillary marker for the diagnosis of pancreatic adenocarcinoma in equivocal biopsy or cytology specimens (93). Because mesothelin is also secreted, detection of elevated serum levels has been proposed as a potential biomarker for pancreatic cancer (94). Mesothelin also appears to be a potent immunogen, and development of a T cell immune response to mesothelin epitopes is a reliable indicator of response to a pancreatic cancer whole cell vaccine in patients (95). Efforts are under way to develop a recombinant mesothelin vaccine and thus eliminate the need for a whole cell vaccine with its attendant nonspecific immunogenic effects. Lastly, humanized antimesothelin monoclonal antibodies and antimesothelin-conjugated *Pseudomonas* immunotoxins have been developed as a therapeutic strategy in pancreatic cancer, and early phase clinical studies are under way with these agents (96). Thus, global expression studies have identified promising candidates that have the potential to impact all aspects of pancreatic cancer care in the clinic.

MicroRNAs (miRNAs) are a highly conserved family of 18–24-nucleotide RNA molecules that regulate the stability or translational efficiency of complementary target mRNAs (97). In the past five years, ~400 mammalian miRNAs have been identified, and recent evidence suggests the existence of many more that have yet to be characterized. In *Caenorhabditis elegans* and *Drosophila*, miRNAs have been demonstrated to regulate cellular differentiation, proliferation, and apoptosis. These functions have led to speculation that miRNAs may participate in tumorigenesis in humans. Indeed, multiple reports of altered miRNA expression in human cancers have been published (98,99). Furthermore, miRNAs are enriched at sites in the human genome that are often targets of amplification, loss of heterozygosity, or chromosomal breakpoints in neoplastic cells. These initial studies suggest that abnormal expression of miRNAs may be a fundamental feature of cancer cells. There is also evidence that suggests that miRNA deregulation plays a causative role in certain neoplasms. For

example, in one study, *C. elegans let-7* and its human homolog were shown to regulate the oncogene *RAS* (100). Human let-7 shows diminished expression in lung cancers, whereas *RAS* is overexpressed. Moreover, overexpression of let-7 in lung cancer cell lines decreases growth rates. In another study, a group of miRNAs located on human chromosome 13, known as the *mir-17* cluster, was shown to accelerate Myc-mediated lymphomagenesis in a mouse model; this is the first established oncogenic miRNA cluster and has been denoted as OncomiR-1 (101). Not unexpectedly, several recent studies showed that miRNA expression is deregulated in pancreatic cancers. A miRNA signature of pancreatic cancer has been elucidated, and it includes the upregulation of miR-21, miR-155, miR-221, and miR-222 (102–104). It is unclear which of these miRNAs plays a pathogenic role in pancreatic cancer and at what stage of tumorigenesis. In addition to enhancing our understanding of the biology of pancreatic cancer, these aberrantly expressed miRNAs might be useful as potential therapeutic targets, with the recent availability of in vivo miRNA knockdown strategies (antagomirs) (105).

Developmental Signaling Pathways: Hedgehog and Notch

The mammalian Hh signaling pathway comprises three secreted ligands—Sonic, Desert, and Indian hedgehog (Shh, Dhh, Ihh)—which bind to and inactivate the 12-transmembrane Patched (PTCH) receptor (106). This, in turn, releases the 7-transmembrane Smoothened (SMO) receptor from its inhibition by PTCH and allows SMO to inhibit Suppressor of Fused (SUFU). In the absence of signaling within this pathway, SUFU enables cytoplasmic sequestration of the Hh transcription factor glioma-associated oncogene homolog 1 (GLI), and inhibition of SUFU results in nuclear translocation of GLI, with transcriptional activation of multiple Hh target genes. Of note, PTCH is itself an Hh target, thus creating a negative feedback loop and regulating physiologic Hh levels. The Hh signaling pathway not only plays critical and diverse roles in developmental patterning, but is also being increasingly recognized as a key component of mature tissue homeostasis. Specifically, sustained Hh signaling within the stem cell niche of various tissues appears to be a prerequisite for maintaining stem cell number and function (107). In a wide range of epithelial sites, injury leads to transient expansion of an Hh-dependent stem cell population that is critical in accomplishing tissue regeneration. Given these significant roles for Hh signaling in the tissue-specific stem cell niche, it is not surprising that deregulation of this pathway has been reported in multiple human cancers, including pancreatic cancer.

In the vast majority of epithelial malignancies with aberrant Hh activation, constitutive signaling is a consequence of Hh ligand overexpression, rather than a mutational event in one of the pathway components (108). Several lines of evidence implicate the Hh signaling pathway as one of the initiating events in pancreatic cancer. First, Hh ligand is overexpressed in two precursor lesions of pancreatic adenocarcinoma, PanINs and IPMNs, as well as in chronic pancreatitis, a known risk factor for pancreatic cancer (109). Second, the expression profile of immortalized human pancreatic ductal cells selected for stable GLI overexpression closely resembles that of microdissected human PanIN lesions, consistent with the Hh pathway regulating a distinct transcriptional program in these precursor lesions (86). Finally, in recently developed animal models, Hh signaling cooperates with oncogenic Kras in the development of extensive PanIN lesions in mice (see below) (110). Sustained Hh signaling also appears to be critical in the maintenance of pancreatic cancers, as blockade of the Hh pathway in vivo with the small molecule inhibitor cyclopamine results in modest growth inhibition of subcutaneous pancreatic cancer xenografts (109); a more profound effect is observed in spontaneously metastasizing orthotopic xenograft models, where cyclopamine abrogates pancreatic cancer metastases disproportionate to its effects on the primary cancer (111). These findings are consistent with the role of the Hh pathway in regulating metastases, and they have important therapeutic implications for the clinical translation of this class of agents (see below).

The Notch pathway is another pathway critical in developmental patterning and in cell fate determination (112). The Notch pathway in mammals comprises membrane-bound Notch ligands (Jagged and Delta-like) that interact with Notch receptors 1–4 on neighboring cells. The resulting intramembrane proteolysis of the Notch receptor by tumor necrosis factor α-converting enzyme and γ-secretase enzyme allows nuclear translocation of the Notch intracellular domain (NICD). In the nucleus, NICD displaces a transcriptional corepressor complex bound to the CBF1/RBP-Jκ protein, and the NICD-CBF1 association activates transcription of a family of basic helix-loop-helix (bHLH) genes, including Hes1, Hey1, and HeyL. The translated bHLH proteins mediate Notch pathway effects, principal among which is the ability to restrain differentiation and maintain cells in an undifferentiated, precursor state. For further discussion on the Notch signaling pathway, the reader is referred to the review by Aster et al. (112a) in this volume of the *Annual Review of Pathology: Mechanisms of Disease*.

Notch signaling plays a seminal role in pancreatic development by maintaining an appropriate pool of precursor cells for exocrine differentiation and restricting premature differentiation along the endocrine lineage (113). In the mature pancreas, Notch signaling is active principally in the centroacinar cells (CACs), located at the junction of acini and ducts (see Cellular Origins of Pancreatic Cancer) (90). Expression of Notch receptor, ligands, and gene targets is elevated in PanIN lesions as well as in invasive cancer (90). Moreover, expression of the Notch gene target Hes1 is seen in precursor lesions arising in mouse models of pancreatic cancer (44), suggesting a role for this pathway in pancreatic cancer initiation (see Animal Models of Pancreatic Cancer). Blockade of Notch signaling in pancreatic cancer cell lines, with RNA interference or pharmacologic agents, leads to growth inhibition and apoptosis, underscoring the importance of this pathway as a new therapeutic target in established cancer (114).

ANIMAL MODELS OF PANCREATIC CANCER

Although the pancreas was one of the first organs wherein tissue-specific transgene expression was achieved (115,116), almost two decades passed before the development of an animal model that faithfully recapitulated the cognate human disease. These early genetically engineered mice (GEM) utilized acinarspecific promoters such as *elastase* to drive oncogene expression in the mouse pancreas, which, not surprisingly, yielded neoplasms of predominantly acinar histogenesis (116,117). A major breakthrough was achieved in 2003 with the development of a GEM that expressed an oncogenic *Kras*^{G12D} allele from its endogenous promoter (and thus, at physiologic levels) in the developing pancreas, through *Cre*-mediated recombination driven by *Pdx1* (or *Ptf1/p48*) regulatory elements (44).

Pdx1 is a homeodomain protein critical for early pancreatic development, and both differentiated exocrine and endocrine cell types in the mature pancreas arise from a *Pdx1*-expressing progenitor population (118). *Ptf1/p48* is a marker of early pancreatic specification, and its expression persists in the exocrine acinar compartment postnatally (119). The *Pdx1*-Cre; LSL-*Kras*^{G12D} mice develop the entire compendium of precursor ductal lesions (murine PanIN or mPanIN) observed in the human pancreatic cancer progression model, with a minor proportion (~10%) developing invasive, metastatic adenocarcinomas after a long latency of several months (44); a near identical phenotype is observed in the Ptf1/p48-Cre; LSL-*Kras*^{G12D} mice. The low frequency of progression to invasive adenocarcinoma in these GEM is likely a consequence of *Kras*-induced senescence in the preinvasive lesions, underscoring the need for cooperating mutations in the *Ink4a-Rb* or *Arf-p53* checkpoints to develop invasive neoplasia (120). This, in fact, is the case because engineering mice that misexpress oncogenic *Kras* in the pancreas in combination with a conditional *Ink4a/Arf* or *p53*-null allele (45,47), or a conditional knock-in mutant *p53*^{R172H} (121), develop lethal, metastatic pancreatic cancers with complete penetrance and shorter latency (weeks to few months, based on the genotype).

Several caveats have emerged from the generation of these genetically engineered mouse models:

1. GEM that harbor either pancreas-specific deletion of *Ink4a/Arf* or *p53*, in the absence of conditional *Kras*^{G12D} expression, do not demonstrate an obvious pancreatic phenotype (44,45,47). Thus, mutant *Kras* appears to be the initiator genetic lesion, and the additional genetic hits serve as accelerators of disease progression (122). As a corollary, *Ink4a*, *Arf*, and *p53* appear to be dispensable for normal pancreatic development because *Cre*-mediated deletion under *Pdx1* or *Ptf1/p48* regulatory elements abrogate the corresponding transcripts in all differentiated exocrine and endocrine cell types.
2. Expectedly, there is an increasing latency for the development of invasive neoplasia, if the second conditional allele with *Kras*^{G12D} is heterozygous for *Ink4a/Arf* or *p53*, versus homozygous null (47); in the former instances, the invasive cancer usually demonstrates loss of the retained wild-type allele. Furthermore, murine pancreatic cancers that arise in the backdrop of conditional homozygous alleles (i.e., with a rapidly progressive course) have locally advanced lethal tumor burden accompanied by micrometastatic disease, whereas cancers arising with longer latencies in the compound heterozygous mice typically develop gross systemic metastases (44,45, 47). It is postulated that the longer latency permits emergence of subclones with more aggressive metastatic potential (123).
3. The choice of cooperating mutation with *Kras*^{G12D} has a bearing on the histologic spectrum of the invasive cancer; thus, GEM with conditional *Ink4a* deletions are biased toward developing poorly differentiated neoplasms, including those with sarcomatous or anaplastic histology (something uncommonly observed in human pancreatic cancer), whereas a mutant *p53* backdrop usually lends itself to well- or moderately differentiated adenocarcinomas (44,45,47). The presence or absence of an intact TGF-β signaling pathway also has an impact on tumor histology, as discussed subsequently.

Recently, three models of pancreatic neoplasia have been described wherein the integrity of the TGF-β signaling pathway is compromised by either conditional deletion of the *Smad4* allele (46,124) or the type II *TGFβ receptor* (*Tgfbr2*) (48). These new models have provided fascinating insights into the role of TGF-β signaling in pancreatic neoplasia. First, it appears that conditional deletion of *Tgfbr2* or *Smad4* is not sufficient to induce either mPanIN lesions or invasive cancer, underscoring the importance of multiple genetic hits (46,48,124). Second, deletion of either *Tgfbr2* or *Smad4* cooperates with mutant *Kras* in inducing pancreatic neoplasia, with shorter latencies than observed in *Pdx1*-Cre; *Kras*^{G12D} GEM alone. There is, however, one crucial distinction: Although GEM with pancreas-specific loss of *Tgfbr2* and mutant *Kras* expression develop well-differentiated adenocarcinomas with 100% penetrance and lethality (48), the combination of *Smad4* loss with mutant *Kras* yields pancreata that harbor cystic neoplasms, either IPMNs (46) or MCNs (124), respectively. The basis for this attenuated phenotype in the setting of *Smad4* loss is unclear, but it might reflect a more pervasive abrogation of signal transduction downstream of the deleted *Tgfbr2* receptor. Third, consistent with the role of TGF-β signaling in promoting epithelial-to-mesenchymal transition (125), loss of *Smad4* is associated with decreased evidence of epithelial-to-mesenchymal transition in the murine pancreatic cancers. Thus, pancreatic cancers that arise in *Smad4* wild-type, *Ink4a/Arf*-null, *Kras*^{G12D} mice demonstrate a high proportion of poorly differentiated carcinomas, whereas *Ink4a/Arf*-null, *Kras*^{G12D} mice that also harbor conditional *Smad4* deletions typically develop well- to moderately differentiated adenocarcinomas expressing epithelial markers, such as Cytokeratin 19 and E-cadherin (46).

In addition to expanding our understanding of the basic biology of pancreatic cancer, the recently developed compendium of GEM is a treasure trove for performing studies on early detection, molecular imaging, tumor immunology, and experimental therapeutics (126). Many of the oncogenic pathways and genes upregulated in human pancreatic cancers (EGFR, Hh, Notch, cyclooxygenase-2, matrix metalloproteinases, etc.) are overexpressed in the preinvasive lesions and/or the associated invasive cancer (44,46,47,121). Thus, there is an opportunity to explore chemoprevention and treatment strategies in a biologically relevant preclinical model. How these mouse hospitals will compare to the more traditional human tumor xenograft models remains to be seen, but there is little doubt that their emergence has garnered tremendous interest in the scientific community to study pancreatic cancer.

CELLULAR ORIGINS OF PANCREATIC CANCER

The cell of origin of human pancreatic cancer is not defined; however, significant progress has been made in defining specific populations within the cancer that are selectively able to initiate new tumors when transplanted into mice. Although there are significant differences between normal stem cells and these tumor-initiating cells, the tumor-initiating cells are often termed cancer stem cells.

Emerging evidence from multiple solid cancers supports the existence of cancer stem cells (127), which were first identified within hematopoeitic malignancies (128). Normal stem cells undergo asymmetric cell division, with one daughter cell retaining self-renewal capacity and the other differentiating into a transit-amplifying cell, with limited proliferative potential; it is postulated that cancer stem cells divide in an identical manner. On the basis of tumor initiation in heterotransplantation experiments, cancer stem cells typically comprise 0.1%–1% of cells in a given cancer (127). A series of elegant studies in solid cancers (breast cancer, gliomas, colon cancer, etc.) has confirmed the existence of self-renewing tumor-initiating subpopulations, which can be enriched on the basis of a variable expression of a panel of cell surface markers, such as CD133, CD24, CD44 (129–131). Li and colleagues (132) have recently isolated a tumor-initiating population from human pancreatic cancer, which is characterized by a surface $CD44^+$, $CD24^+$ ESA^+ immunophenotype. These cells, comprising <1% of the bulk population, had a 100-fold increased tumorigenic potential compared with nontumorigenic cancer cells, with 50% of animals injected with as few as 100 $CD44^+CD24^+ESA^+$ cells, forming tumors that were histologically indistinguishable from the human tumors from which they originated (132).

The identification of pancreatic cancer tumor-initiating cells is an important milestone for two reasons: First, it is well documented that tumor-initiating cells in other malignancies demonstrate significantly greater resistance to conventional cytotoxic agents and to radiation than the differentiated bulk population (133,134). This has enormous implications for therapy of pancreatic cancer, as most strategies are currently based on reducing tumor volume rather than eradicating the cells responsible for tumor initiation and maintenance (135). Using combined therapeutic approaches that target both the tumor-initiating cells and the differentiated bulk population is most likely to eradicate disease recurrence and achieve a lasting cure (111). Second, identification of pancreatic tumor-initiating cells provides an opportunity to elucidate the putative cell of origin for pancreatic cancer, on the premise that pancreatic tumor-initiating cells are the most proximate derivative of this elusive cell type. Compelling evidence suggests that tumor-initiating cells arise from either resident normal tissue stem cells or a progenitor cell that acquires capacity for self-renewal through dysregulation of critical self-renewal pathways (136,137); the subsequent acquisition of oncogenic mutations permits niche-independent, cell-autonomous expansion of these deregulated stem/progenitor cells, culminating in neoplasia (138).

In parallel with these advances using human pancreatic cancer samples, experimental animal models continue to provide insights into the cellular origins of experimentally induced pancreatic cancer. Of note, the expression of a mutant *Kras* gene driven by CK19 regulatory elements in GEM, which targets transgene expression to the mature ductal epithelium, yields a minimal phenotype, characterized by periductal lymphocytic infiltration and an absence of mPanINs or neoplasia (139). This suggests that differentiated ductal cells are unlikely to be the proximate cell of origin for pancreatic adenocarcinoma, and supports a stem/progenitor cell origin, as implicated in the discussion above. By contrast, targeting mutant *Kras* to the entire pancreatic anlage by *Pdx1* regulatory elements results exclusively in ductal neoplasms, without acinar or endocrine lesions (44,121). Thus, even if differentiated ductal cells can be excluded as the source of mPanINs and adenocarcinoma, the putative cell of origin is such that it responds to mutant *Kras* expression by differentiating along a ductal lineage. Unlike the marrow and neural tissues, a discrete pancreatic stem cell with multilineage differentiation potential has not been isolated (132).

If tumor-initiating cells represent the neoplastic transformation of resident tissue stem or progenitor cells, where do the latter reside in the pancreas? Two potential candidates with at least limited epithelial plasticity have emerged in the exocrine pancreas, which fulfill some of the criteria for stemness (see also Figure 2) (140). The first is the CAC, located at the junction of acini and ducts. CACs are the only cell type with retained Notch activation (as assessed by nuclear Hes1 expression) in the mature exocrine pancreas (90). As stated above, Notch signaling in the developing pancreas represses differentiation, and sustained activation of this pathway in CACs suggests persistence of a precursor-like transcriptional program (90,113). Further evidence is derived from studies in mice with conditional deletion of the *PTEN* protein phosphatase gene in the pancreas; these mice develop prominent ductal metaplasia resulting from expansion of Hes1-expressing CACs (141). A minority of *Pten*-deficient mice develop pancreatic adenocarcinoma, bolstering a role for CACs in exocrine neoplasia (141).

The second contender for the cell of origin for pancreatic neoplasia is more provocative, in that differentiated acinar cells are themselves considered to harbor facultative progenitor properties. Unlike dedicated stem cells, facultative progenitors acquire a precursor phenotype under specific circumstances, such as organ injury and regeneration (113). For example, in caerulein-induced chemical pancreatitis, surviving acinar cells can repress the terminal exocrine transcriptional program and undergo a process of dedifferentiation, expressing markers of undifferentiated pancreatic progenitor cells such as Pdx1 and Hes1 (142). Under conditions of growth factor stimulation (e.g., activated EGFR signaling), acinar cells undergo a process of acinar-to-ductal metaplasia (ADM), characterized by the abnormal formation of tubular structures (i.e., tubular complexes) with both ductal and acinar differentiation (143). Rigorous lineage-tracing experiments in reporter mice have confirmed ADM to be a true transdifferentiation process; in other words, ductal elements within foci of ADM are tagged, confirming their acinar histogenesis (144). ADM occurs through the generation of nestin-positive intermediates (113). Nestin is an intermediate filament that marks precursor cells at many tissue sites, including the pancreas (145), and its expression in cells of acinar lineage reiterates the ability of differentiated acinar cells to acquire a progenitor phenotype. One of the strongest lines of evidence supporting the role of the acinar compartment in pancreatic neoplasia is derived from a recent study by Guerra and colleagues (146), wherein targeting an oncogenic $Kras^{G12D}$ allele to elastase-expressing acinar cells during development results in the formation of mPanINs and adenocarcinomas. The mPanINs arise in the backdrop of ADM and demonstrate focal expression of acinar markers. In this model of conditional $Kras^{G12D}$ expression, activating the oncogenic allele in adult pancreata did not yield mPanINs, unless coexistent chronic caerulein-induced injury was superimposed (146). Thus, injury-induced expansion of acinar facultative progenitors that respond to an oncogenic $Kras^{G12D}$ allele might be a prerequisite for the development of mPanINs and subsequent adenocarcinoma in the adult

pancreas; this study also provides a rationale for the established association between longstanding chronic pancreatitis (or some rare forms of hereditary pancreatitis) and the risk for pancreatic adenocarcinoma in humans (2).

TRANSLATIONAL STUDIES IN PANCREATIC CANCER

The past two decades have seen advances in our fundamental understanding of the nature of pancreatic cancer, from the characterization of precursor lesions to the identification of an ever increasing compendium of molecular abnormalities, and the development of relevant animal models. Unfortunately, despite these advances, pancreatic cancer remains a disease of near uniform lethality. Therefore, much attention has now focused on translational studies in pancreatic cancer, that is, those that can directly impact patient care and outcome. Although a comprehensive cataloging of these efforts would be beyond the scope of this review, we highlight selected examples of bench-to-bedside translation, particularly those in the areas of early detection and therapy.

Early Detection of Pancreatic Cancer

Pancreatic cancer, once invasive, is for the most part incurable. Therefore, the best chance for a cure currently rests in early detection of this neoplasm before it invades (20). The most widely utilized tumor marker for pancreatic cancer in the clinic is the sialylated Lewis^a blood group antigen CA19–9. Although the sensitivity of CA19–9 as a tumor marker in patients presenting with symptoms suspected to be due to pancreatic cancer is ~80%, this value is considerably diminished (~55%) in small, resectable cancers (<3 cm) (147). Furthermore, in high-risk, asymptomatic individuals whose pancreata harbor preinvasive lesions (IPMNs, high-grade PanINs), serum CA19–9 is often normal, thus largely precluding its role as a screening tool on the lines of a prostate-specific antigen test for the pancreas (22,40). The development of tumor markers that can supplant serum CA19–9 in the diagnosis of asymptomatic pancreatic neoplasms, and particularly high-grade noninvasive lesions, is thus a priority.

Global gene expression studies of pancreatic cancers have yielded a plethora of differentially expressed genes, whose secreted gene products have shown considerable potential as new serum markers for pancreatic cancer. A promising candidate to emerge from these studies has been *macrophage inhibitory cytokine 1* (*MIC1*) (148), identified by serial analysis of gene expression and oligonucleotide microarrays as differentially overexpressed in invasive pancreatic cancers (149) as well as in IPMNs (150). Elevated serum MIC1 antigen levels significantly outperformed CA19–9 and other tumor markers in distinguishing patients with resectable pancreatic cancers from healthy controls (150,151). Other tumor markers whose discovery has been facilitated by global gene expression profiling strategies include the gene products of *osteopontin* (152), *tissue inhibitor of metalloproteinase-1* (153), and *mesothelin* genes (32), among others; preliminary studies in cohorts of pancreatic cancer and control patients have confirmed the potential utility of some of these markers in the diagnosis of early-stage (i.e., resectable) pancreatic cancers (151–153). In addition to gene expression profiling, ongoing efforts at large-scale proteomic analyses of pancreatic cancers have also facilitated the discovery of candidate biomarkers (154,155). Along these lines, mass spectrometric analysis of the pancreatic cancer secretome is a novel strategy for biomarker discovery because secreted proteins, rather than the whole cell proteome, is the subset most likely to yield promising circulating tumor markers (156). Quantitative secretome analysis has identified several secreted protein candidates (e.g., perlecan, CD9, and the fibronectin receptor), which await validation in clinical material (156). One important caveat that has emerged from these studies has been the realization that there is unlikely to be a single magic bullet in the serum that will provide the sensitivity and specificity needed to screen asymptomatic individuals, and therefore, any screening assay for pancreatic cancer will likely incorporate two or more tumor markers.

In addition to serum, pancreatic juice is also a bona fide clinical source for developing biomarkers of pancreatic cancer (157,158). Pancreatic juice, being a more proximate surrogate of the ductal epithelium than serum, would expectedly have an enriched fraction of tumor markers, unadulterated by serum components (147). As an example, mutant *KRAS2* is readily detected in pancreatic juice but can be identified in the peripheral circulation only at the stage of advanced, inoperable pancreatic cancers (159). Unlike serum, however, pancreatic juice is obtained during the course of an invasive endoscopic procedure, and therefore, juice-based biomarkers cannot be utilized as a screening modality in the general population. The assays developed in the context of pancreatic juice are best applicable to high-risk individuals suspected of harboring early-stage pancreatic cancers or high-grade noninvasive lesions (22, 40).

Aberrantly methylated genes may also serve as markers of pancreatic cancer. For example, quantitative methylation-specific polymerase chain reaction for assessment of promoter methylation of a panel of genes in pancreatic juice better predicts for pancreatic cancer than conventional methylation-specific polymerase chain reaction, with 100% specificity if >1% methylation at two or more gene promoters is utilized as an inclusion criterion (82). Along the same lines, recent advances in quantitative assays allow for the measurement of *KRAS2* gene mutation load in pancreatic juice using an ultrasensitive ligation-based amplification strategy (160) and for the identification of differentially expressed transcripts by microarrays and real-time polymerase chain reaction (161). All of these quantitative techniques are currently in the experimental realm, and their performance in larger-scale clinical cohorts needs to be elucidated, either independently or as an adjunct to radiology and cytologic examination.

Developing Improved Pancreatic Cancer Therapies

The clinical reality is that the majority of patients present with locally advanced or distant metastatic, surgically inoperable disease. Therefore, the development of more potent therapies for advanced pancreatic cancer mandates great urgency. Gemcitabine is the first line of therapy for advanced pancreatic cancer (162) and is increasingly being used as adjuvant treatment in patients with resectable disease (163). The pivotal Phase III trial, which demonstrated the superior efficacy of gemcitabine over the then standard of care 5-fluorouracil, reported an enhancement of median survival from 4.4 to only 5.6 months in patients with advanced pancreatic cancer (164). The EGFR tyrosine kinase inhibitor erlotinib (Tarceva®) is the first targeted agent approved by the Food and Drug Administration for pancreatic cancer (165); unfortunately, the combination of erlotinib and gemcitabine improves survival by less than a month compared with gemcitabine alone in patients with advanced disease. Clearly, there is considerable room for improvements in pancreatic cancer therapy. A large number of investigational agents are being developed for application to pancreatic cancer, targeting growth factor, angiogenic, and other signaling pathways upregulated in this malignancy (166); the discussion of these is beyond the scope of this review. We discuss two specific paradigms illustrating how the knowledge gleaned from pancreatic cancer genetics is serving as a road map for identifying new therapeutic agents against this malignancy.

Synthetic lethal screening was developed in the context of mapping genetic interactions in *Drosophila* and in yeast, wherein the mutation of two functionally related genes in parallel is lethal to the organism, whereas mutation of any one by itself does not affect the organism's viability. Hartwell and colleagues (167) were the first to suggest that this paradigm be extrapolated to human cancer therapy, such that an intrinsic genetic or biochemical defect specific to the neoplastic cells is capable of inducing cell death when combined with an extrinsic insult (i.e., therapy) that is synthetic lethal to the former. Non-neoplastic cells, by virtue of lacking this specific genetic or biochemical defect, would bypass the effects of synthetic lethality, providing a broad therapeutic window for cancer-specific cytotoxicity (168). An

example of synthetic lethality in the context of pancreatic cancers encompasses the ~5%–7% of cases that harbor loss-of-function mutations in the *BRCA2* gene or other members of the Fanconi anemia complex (e.g., *FANCC* and *FANCG*), which play a critical role in homologous recombination repair (26). Neoplastic cells lacking *BRCA2* (or *FANC* family) gene function are defective in homologous recombination repair and are rendered selectively sensitive to the effects of DNA cross-linking agents such as Mitomycin C, and to γ-irradiation. In the minority of pancreatic cancers harboring defects in homologous recombination repair, Mitomycin C provides a potential therapeutic window not accorded by standard therapies such as gemcitabine (27,169).

A second example of synthetic lethality involves the *methylthioadenosine phosphorylase* (*MTAP*) gene, whose gene product is the critical enzyme in the salvage pathway of purine synthesis. The *MTAP* gene is located on chromosome 9p21, approximately 100 kb telomeric to the *p16/CDKN2A* gene, which undergoes frequent homozygous deletion in pancreatic cancer and, less commonly, in high-grade noninvasive lesions (170,171). In approximately half of these cases with *p16/CDKN2A* homozygous deletion, the *MTAP* gene is codeleted, thereby blocking the salvage pathway of purine synthesis in the affected pancreatic cancer cells (170, 171). In these instances, purine synthesis in the neoplastic cells is essentially entirely dependent upon the de novo pathway, and therefore, blockade of de novo purine synthesis by systemic inhibitors (e.g., L-alanosine, an analog of L-aspartic acid) can effectively terminate ongoing purine synthesis in the *MTAP*-null cancer cells (172). In contrast, non-neoplastic cells, which retain *MTAP* gene function, will escape synthetic lethality and continue to synthesize purines via the intact salvage pathway. A convenient immunohistochemical assay for Mtap protein can help identify which cancers harbor *MTAP* gene deletions and thus enable appropriate patient selection (170,171).

Another loss-of-function mutation in pancreatic cancer that has recently been utilized as a substrate for synthetic lethal screening is *DPC4/SMAD4*, which is inactivated in ~55% of pancreatic cancers (62). A large-scale screening of ~20,000 compounds identified a single agent that is selectively toxic to cells lacking *DPC4/SMAD4* function, with minimal impact at comparable dosages on cells with retained gene function (173); this compound currently awaits further evaluation in pancreatic cancer.

Although synthetic lethal targeting generally involves a loss-of-function mutation specific to the cancer cells, a second related paradigm that is increasingly being used to develop targeted therapies involves aberrant gene activation and has been termed oncogene addiction. This term, first coined by Bernard Weinstein, describes a phenomenon of acquired dependence by the cancer cells on the proliferation and survival signals exerted by mutated, constitutively activated oncogenes (174). Oncogene addiction predicts that acute inhibition of an aberrantly activated oncogene will profoundly impact growth and survival in neoplastic cells bearing the mutant gene, but that these effects will be mitigated in cells with the wild-type allele (168). The best known examples of oncogene addiction result from mutations in genes encoding receptor and nonreceptor protein kinases, such as *EGFR*, *ERBB2*, *BRAF*, and *KIT* (CD117), among others (175–178). Neoplasms that are relatively resistant to conventional cytotoxic agents, yet harbor kinase gene mutations, often respond dramatically to small molecule inhibitors or monoclonal antibodies directed against the mutant oncoprotein (179). The ability to selectively target critical oncogenic mutations in subsets of cancers has given new impetus to the concept of individualized therapy (177,180).

In the context of pancreatic cancers, the obvious oncogenic target would be mutant *KRAS2* or its gene product, which is constitutively activated in 90% of cases and is considered a critical initiating event for this malignancy (122). Unfortunately, farnesyltransferase inhibitors, which interfere with critical posttranslational modification of RAS proteins, have been largely

ineffective in clinical trials (181). Although the reason for this is not entirely clear, it is believed to be a result of compensatory increase in the activity of geranylgeranyltransferase enzymes, which bypass the farnesyltransferase-inhibitor-induced blockade of farnesyltransferase (182).

As our knowledge of the pancreatic cancer genome has expanded, additional mutant oncogenes have emerged as candidates for targeted therapies. For example, *EGFR* is mutated in a small subset (~3%) of pancreatic cancers, and patients bearing one of these tumors might benefit from tyrosine kinase inhibitors or antibodies that antagonize the mutant receptor (183). Recently, activating *PI3KCA* (*PI3K catalytic, alpha*) gene mutations were reported in ~10% of pancreatic neoplasms, particularly IPMNs and associated colloid cancers, and these cancers are likely susceptible to small molecule inhibitors of the protein kinase (54).

Efforts are under way to perform large-scale sequencing of pancreatic cancers to identify the entire gamut of germ-line and somatic coding sequence alterations. It is certain that these analyses will elucidate new oncogenic mutations, which in turn will be the substrate for targeted therapy in subsets of pancreatic cancers. In the future, individualized therapy of pancreatic cancers is likely to become the standard of care, with the choice of first-line agents dictated by the genetic alterations in an individual neoplasm. The availability of high-throughput mutation detection platforms that can garner information on hundreds of cancer-associated mutations in a single run will greatly facilitate the clinical applicability of genomic data to treatment protocol design (184).

SUMMARY POINTS

1. Pancreatic cancer runs in some families and a number of genes responsible for this aggregation (*BRCA2, BRCA1, CDKN2A/p16, STK11/LKB1,* DNA mismatch repair genes) have been discovered.
2. A number of histologically distinct variants of pancreatic cancer with distinct clinical features have been described, of which ductal adenocarcinoma is by far the most common and most lethal subtype.
3. Three noninvasive precursor lesions of invasive pancreatic cancer have been described, namely PanIN, IPMN, and MCN. These represent an opportunity to cure pancreatic neoplasia.
4. A genetic progression model of pancreatic cancer has been developed, which incorporates genomic, transcriptomic, and proteomic abnormalities implicated in the development of this malignancy. Specifically, alterations observed in the early (*KRAS2* mutations, telomere shortening), intermediate (*p16/CDKN2A* loss), and later (*DPC4, TP53,* and *BRCA2* mutations) stages of disease have been identified.
5. Genetically engineered mouse models of pancreatic cancer have been developed, which recapitulate the entire spectrum of histologic progression observed in the cognate human disease. In these models, mutant *Kras2* expression directed to the exocrine pancreas appears to be the prerequisite for developing ductal lesions.
6. Pancreatic cancer “stem cells” have been identified, raising the possibility of developing tumor-initiating cell-targeted therapies.
7. Understanding the genetic basis of pancreatic cancer has been instrumental in developing rational early-detection assays and improved therapeutic strategies. Sequencing of the pancreatic cancer genome is on the horizon and will enormously facilitate safer and more efficacious targeted therapies for this uniformly lethal disease.

RELATED RESOURCES

The Johns Hopkins Pancreatic Cancer Web site. http://pathology.jhu.edu/pancreas/

The National Familial Pancreas Tumor Registry. http://pathology2.jhu.edu/pancreas/nfptr.cfm

Pancreatic Cancer in the Ashkenazi Jewish Population. http://pathology2.jhu.edu/pancreas/ashkenazi jewish ancestry.cfm

Pancreatic Cancer in African Americans. http://pathology2.jhu.edu/pancreas/African Americans.cfm

Listing of Pancreatic Cancer Web sites. http://pathology2.jhu.edu/pancreas/othersites.cfm

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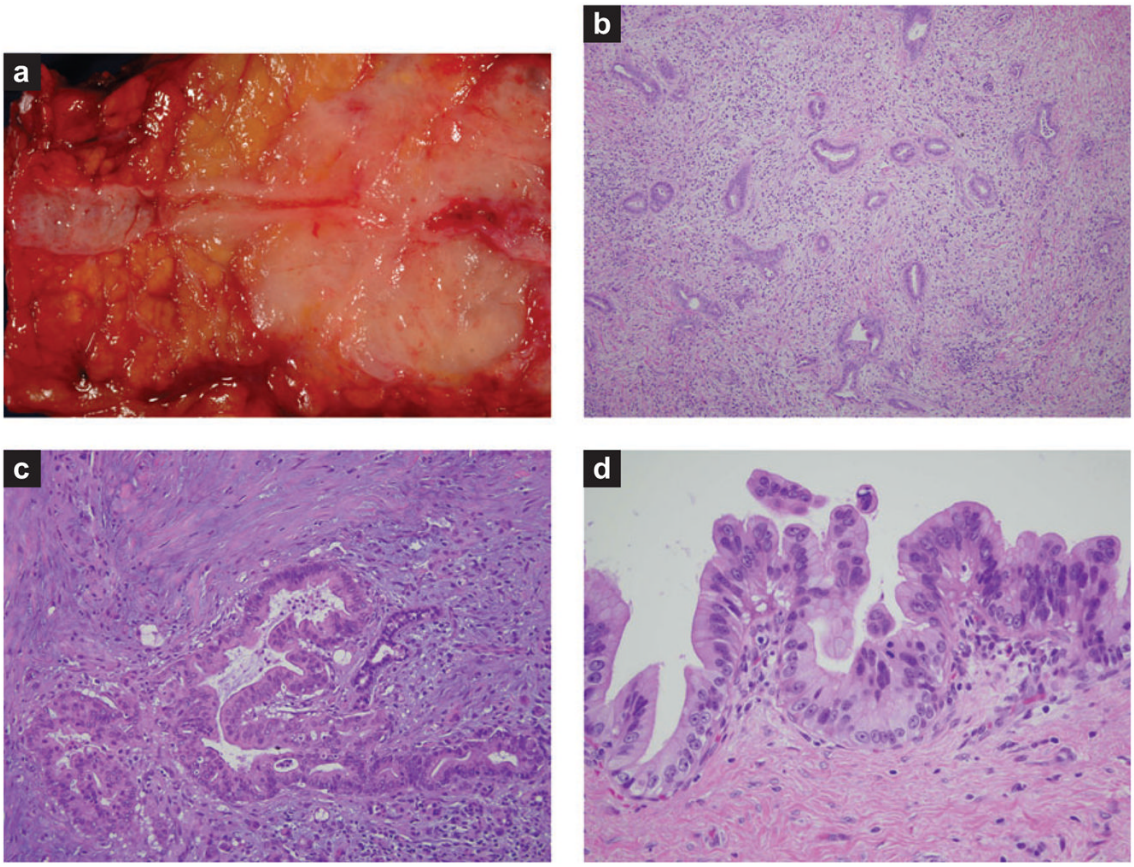

Figure 1.
Pathology of pancreatic adenocarcinoma and its precursor lesions. (*a*) Gross photograph of an infiltrating adenocarcinoma. Note the dramatic narrowing of the pancreatic duct associated with the poorly defined white neoplasm. (*b*) Low-power photomicrograph of an infiltrating adenocarcinoma. Note the haphazard arrangement of the glands and the associated non-neoplastic desmoplastic stroma. (*c*) High-power photomicrograph of an infiltrating adenocarcinoma. Note the desmoplastic stroma and the marked pleomorphism in the cancer relative to the trapped non-neoplastic duct. (*d*) High-grade pancreatic intraepithelial neoplasia.

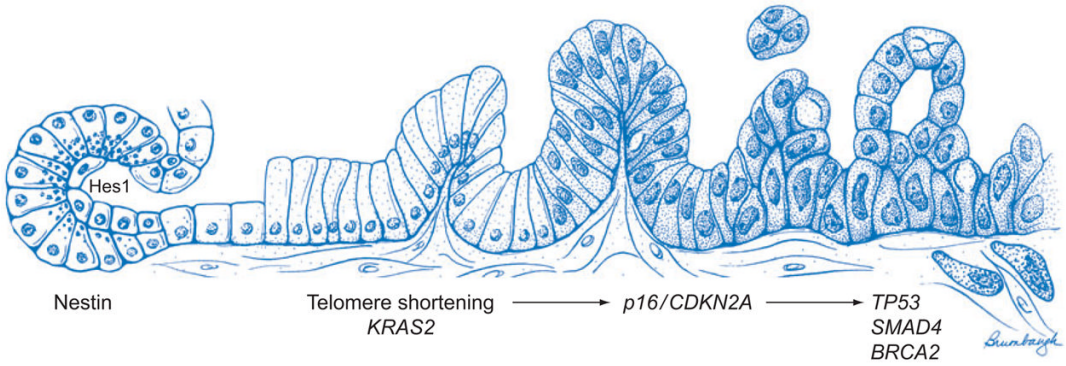

Figure 2.

Genetic progression model of pancreatic adenocarcinoma. The progression from histologically normal epithelium to low-grade pancreatic intraepithelial neoplasia (PanIN), to high-grade PanIN, to invasive carcinoma (*left to right*) is associated with the accumulation of specific genetic alterations. On the basis of their temporal appearance in this progression model, the molecular abnormalities can be classified as early (*KRAS2* mutation, telomere shortening), intermediate (*p16/CDKN2A* loss), or late (mutations of *DPC4/SMAD4, TP53, BRCA2*). These signature genomic alterations are accompanied by a multitude of expression abnormalities that are not illustrated (see text for details). Note that this progression model is specific for PanINs, and other recognized precursor lesions of adenocarcinoma (intraductal papillary mucinous neoplasm and mucinous cystic neoplasm) likely harbor a distinct compendium of genetic alterations in their path to invasive cancer. The state of the field does not definitively establish the cellular origin for PanIN and pancreatic cancer. On the basis of genetically engineered models, two candidates have emerged as the most likely cell of origin for ductal neoplasia in the pancreas: facultative progenitors within the acinar compartment and centroacinar cells. Both competing hypotheses are illustrated to the far left of the figure to underscore the current uncertainty in the literature. Rigorous lineage-tracing experiments have confirmed the ability of differentiated acinar cells to undergo acinar-to-ductal transdifferentiation, via generation of nestin-positive intermediates. This process, known as acinar-to-ductal metaplasia, is a frequent accompaniment of pancreatic neoplasia in experimental models, and is also observed in the setting of human pancreatic cancers. The second putative source of neoplastic ductal cells is centroacinar cells, which are found at the junction of acini and ducts. These are the only cell types in the mature exocrine pancreas with retained Notch activation, as determined via persistent nuclear Hes1 expression. Loss of regulatory signals (e.g., inactivation of *PTEN*) that lead to uncontrolled centroacinar cell expansion culminates in pancreatic adenocarcinoma. In addition to these putative candidates, this model does not exclude the possibility of a rare, as yet undefined, pancreatic stem cell as the source of ductal neoplasia. See Cellular Origins of Pancreatic Cancer for further details.

Table 1

Genes associated with an increased risk of pancreatic cancer

Individual	Gene	Relative risk	Risk by age 70	Cancer morphology	Other cancers	Reference(s)
No history	None	1	0.5%	NS	None	
Breast cancer	*BRCA2*	3.5–10X	5%	NS	Breast, ovary, and prostate	(10,11)
	BRCA1	2X	1%	Breast cancer with basaloid features	Breast, ovary, and prostate	(12)
FAMMM	*P16 (CDKN2A)*	20–34X	10%–17%	NS	Melanoma	(14,185)
Familial pancreatic cancer (3 FDR)	Unknown	32X	16%	NS	Unknown	(9)
Familial pancreatitis	*PRSS1*	50–80X	25%–40%	Pancreatic cancers in the background of severe diffuse chronic pancreatitis	None	(17)
Peutz-Jeghers	*STK11/LKB1*	132X	30%–60%	NS	Gastroesophageal, small bowel, colorectal, and breast	(13,16)
HNPCC	*hMLH1*, *hMSH2*, others	Unknown	< 5%	Medullary and colloid phenotypes	Colorectal, endometrial, stomach, ovarian, ureter and renal pelvis, biliary tract, and brain	(18,186)
Young-age-onset pancreatic cancer	*FANC-C* and *FANC-G*	Unknown	Unknown	NS	Unknown	(187,188)

3 FDR, 3 or more first-degree relatives with pancreatic cancer; FAMMM, familial atypical multiple mole melanoma syndrome; HNPCC, hereditary nonpolyposis colorectal cancer syndrome; NS, nonspecific.