Pancreatic Cancer: Translating Lessons from Mouse Models and Hereditary Syndromes

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Abstract
Pancreatic ductal adenocarcinoma is the overwhelmingly predominant form of pancreatic cancer and the second most common type of gastrointestinal cancer (behind colorectal cancer) in the United States. Recent exciting advances in two areas of pancreatic ductal adenocarcinoma (i.e., the development and characterization of genetically engineered mouse models and the dissection of the genetic basis of hereditary forms in families) have been illuminating. These preclinical models and clinical syndromes provide the first tangible basis for progress in screening and prevention in high-risk populations and in the development of molecular diagnostics and experimental therapeutics.

Pancreatic cancers comprise a variety of malignancies, including the preponderant form, pancreatic ductal adenocarcinoma (PDAC), and islet cell tumors (part of a class of neuroendocrine tumors), rare acinar cell tumors, mucinous neoplasms (divided further into mucinous cystic neoplasms and intraductal papillary mucinous neoplasms), lymphoma, and sarcoma. There are approximately 37,000 new cases of PDAC each year in the United States, with an increasing annual incidence in African-Americans. Median survival for PDAC is 6 months, and 5-year survival is <5%. When PDAC is identified in its early stage, 5-year survival approaches 20%. Yet, most PDAC cases present at late stages, when therapies are limited. These staggering data spawned attention on PDAC, and as a result, the past 5 or 6 years have witnessed robust and rewarding efforts to model PDAC (1) largely in mouse models, and these models (dealt in a later section) are advancing our understanding of molecular diagnostics and therapeutics. Additionally, compelling insights have been gained from hereditary PDAC. It is estimated that 5% to 10% of PDAC has a hereditary basis with penetrance (the percentage of individuals with a specific genotype that develop PDAC) approaching 80% with the remaining cases of PDAC sporadic in nature arising in average-risk patients. This substantial frequency of hereditary PDAC has implications for genetic counseling, genetic testing, and preventive and screening measures for at-risk family members. Evaluation of familial PDAC kindreds in the Surveillance, Epidemiology and End Results database revealed that the risk of pancreatic cancer was elevated in individuals with three (32.0-fold increased risk), two (6.4-fold increase), or one (4.6-fold increase) first-degree relative(s) with pancreatic cancer (2); risk was more elevated in smokers than nonsmokers (2). A comparison of a Mayo Clinic population with the Surveillance, Epidemiology and End Results database showed a nearly 2-fold increased risk of pancreatic cancer (and also liver cancer) in the first-degree relatives of a PDAC proband (the first identified family member with PDAC), which increased to about 3-fold when the probands were less than 60 years old (3).
A helpful distinction in hereditary pancreatic cancer would be well-defined familial PDAC syndromes where the underlying genetic basis has been elucidated and genetic testing and counseling can be offered. The more prevalent current situation is increased pancreatic cancer risk in families, without an identified genetic basis. In the following sections, we outline the known hereditary PDAC syndromes that have an identified genetic basis, and also discuss some scenarios for familial PDAC in which germ-line mutations have yet to be identified.

Family X and Human Chromosome 4q32-34

Family X with early-onset and highly penetrant, autosomal-dominant pancreatic cancer was subjected to genome-wide analysis with about 370 microsatellite markers, which revealed a significant linkage on chromosome 4q32-34 (4). A candidate gene in this locus is *palladin*, a cytoskeletal gene whose protein product regulates cell shape and motility. A *palladin* P239S gene mutation was detected in affected family members but not in nonaffected members (5). Furthermore, palladin RNA expression is increased in pancreatic cancer tissues from familial and sporadic PDAC. The expression pattern of palladin may be restricted to mesenchymal stromal fibroblasts, especially those activated in response to invading tumor cells, and perhaps triggers the desmoplasia (dense stromal response in the extracellular matrix) observed in human PDAC (6). The frequency of germ-line *palladin* mutations in PDAC kindreds is not clear. In a study by the Pancreatic Cancer Genetic Epidemiology Consortium (PACGENE), 21 microsatellite markers on chromosome 4 did not reveal linkage to 4q32-34 in affected individuals within 42 familial pancreatic cancer kindreds (7).

Hereditary and Chronic Pancreatitis

Hereditary pancreatitis, an autosomal-dominant disorder, involves symptoms and signs of recurrent acute pancreatitis culminating in chronic pancreatitis with evidence of both endocrine and exocrine insufficiency. These symptoms and signs begin early in life, even before age 10 but inevitably before age 30, and confer an estimated 40-fold risk of pancreatic adenocarcinoma by age 70 (8). The underlying genetic defect is germ-line “gain of function” missense mutations in the cationic trypsinogen (*PRSS1*) gene, especially at R122H or N29I, leading to less cleavage of the trypsinogen molecule by trypsin and resulting in autolysis of pancreatic acinar cells (9). Other *PRSS1* mutations have been reported in this disorder, including A16V, R122C, and R116C. Triplication of a 605-kb segment containing *PRSS1* on chromosome 7 in five families with hereditary pancreatitis also has been reported, suggesting a gain of trypsin gene copy number (10). At-risk relatives of affected individuals should be offered genetic testing (11) and, when confirmed to have a germ-line *PRSS1* mutation, should receive genetic counseling and be advised to eat mainly low-fat foods and avoid cigarettes because high-fat diet and cigarette smoking have been shown to accelerate PDAC in the background of hereditary pancreatitis.

The vast majority of chronic pancreatitis cases are not hereditary. Alcohol is the greatest risk factor, and other identifiable causes include cystic fibrosis (associated with mutations in *CFTR*, refs. 12, 13) and tropical calcific pancreatitis. Many cases are “idiopathic,” although they may involve mutations in serine peptidase inhibitor, Kazal type 1, also known as *SPINK1* or pancreatic secretory trypsin inhibitor (*PSTI*), detected in a small subset of hereditary pancreatitis patients, in whom the mutations have no discernible effect on disease phenotype, penetrance, or onset of diabetes mellitus (14, 15). *SPINK1* mutations occur thus, in patients with idiopathic chronic pancreatitis and tropical calcific pancreatitis, but the functional relevance is under debate. It is conceivable that *SPINK1* mutations and polymorphisms modify disease (15).

Therefore, it is unclear to what extent, if any, interrogation for CFTR or *SPINK1* mutations or other variants, such as in *chymotrypsin C* (16), should be pursued in patients with idiopathic chronic pancreatitis, where the lifetime risk of pancreatic cancer is 2-fold after 10 years and 4-fold after 20 years (17). Nevertheless, these patients should be advised to avoid alcohol and cigarette smoking because alcohol and cigarette smoking act in a synergistic fashion to foster chronic pancreatitis.

Genetic Syndromes with Increased Pancreatic Cancer Risk

Familial atypical mole and multiple melanoma syndrome

Familial atypical mole and multiple melanoma (FAMMM) syndrome is an inherited autosomal-dominant condition marked by the following characteristics: (a) one or more first- or second-degree relatives (parent, sibling, child, grandparent, grandchild, aunt, or uncle) with malignant melanoma; (b) many moles, some of which are atypical (asymmetrical, raised, and/or different shades of tan, brown, black, or red) and of different sizes; and (c) moles with specific features visible under a microscope. FAMMM syndrome increases the risk of melanoma and pancreatic cancer. Evaluation of kindreds has revealed that affected individuals harbor germ-line mutations in the tumor suppressor gene *p16INK4a* (*CDKN2A*; refs. 18, 19), which result in abrogation of a cell cycle regulatory checkpoint during G1 phase. Cancer preventive measures in these cases include careful skin examination on a regular basis, imaging of the pancreas (see later section), and genetic testing/counseling. A study of FAMMM families found that siblings of affected individuals would consider prophylactic pancreatectomy if biomarkers or imaging suggested a precancerous pancreatic condition (20).

Peutz-Jeghers syndrome

Peutz-Jeghers (PJ) syndrome is an inherited autosomal-dominant condition and a type of hamartomatous polyposis syndrome where PJ polyps develop in the small bowel and colon early in life, with common gastrointestinal bleeding and intussusception and a hallmark mucocutaneous pigmentation (21). Besides increased risks of colon and small bowel cancer, PJ patients may also have sex cord tumors and advancing age-related increased risks for cancers of the pancreas, lung, cervix, and breast (22, 23). The PJ syndrome is predominantly due to germ-line *LKB1* mutations. *LKB1* is a sensor of cellular energy and stress and a negative regulator of the mammalian target of rapamycin (mTOR) pathway (21).

Hereditary breast-ovarian cancer syndrome

Approximately 5% to 10% of all breast cancers are believed to have a hereditary basis with germ-line mutations of the tumor
suppressor gene \textit{BRCA1} or \textit{BRCA2}, as well as other yet to be identified genes, these mutations also confer an increased risk for pancreatic and other cancers. The relative risks of \textit{BRCA} mutation carriers (men and women) for pancreatic cancer are 3.51 (\textit{BRCA2}; ref. 24) and 2.26 (\textit{BRCA1}; ref. 25). In a similar vein, a subgroup of kindreds with familial PDAC has germline \textit{BRCA2} mutations or variants (19, 26, 27). Although \textit{BRCA1} and \textit{BRCA2} differ structurally and in chromosomal location, they share the ability to repair damaged DNA and maintain genomic stability.

As a related genetic consideration to hereditary PDAC, hereditary breast-ovarian cancer syndrome and Fanconi anemia may be related through biallelic \textit{BRCA2} mutations (28). Fanconi anemia is an autosomal recessive disorder that involves pancytopenia, congenital anomalies, and increased risk for cancers with chromosomal instability. The class of genes responsible for Fanconi anemia encodes complementation groups, designated \textit{FANC}, and is involved in homologous DNA recombination and DNA repair. Germ-line and somatic mutations in \textit{FANCC} and \textit{FANCG} may be present in early-onset PDAC (29, 30). Therefore, some of the Fanconi anemia genes and \textit{BRCA2} may be linked through a common pathway and impinge on hereditary PDAC.

### Screening and Prevention of Hereditary PDAC

Genetic testing and counseling are valuable tools for controlling PDAC arising in high-risk individuals with hereditary syndromes (Table 1), and this has implications for sporadic PDAC in the average-risk patient. We would note that the many advances made in the diagnosis, screening, and therapy of colorectal cancer have emerged from a deep understanding of hereditary colorectal cancer syndromes and would advocate this type of approach to sporadic PDAC with an increasing appreciation of hereditary PDAC. Apart from understanding the genetic basis of PDAC, it is likely that gene-environment interactions are critical. Cigarette smoking is a major risk factor for hereditary and sporadic PDAC (31, 32). Vitamin B6 and methionine may be important risk factors as well (33).

A clinical practice program of screening and surveillance is gaining increasing acceptance for families with an increased risk of PDAC with or without a defined genetic basis (34, 35). Difficulties in entering an appropriate number of at-risk individuals and controls from familial pancreatic cancer kindreds into screening research programs have limited the robustness of the screening tools these programs develop, thus also limiting the screening recommendations based on them. Challenges include lack of long-term follow-up, variable economic reimbursements for screening, and implementing the appropriate endoscopic and radiological modalities for screening. One study concluded with the recommendation to screen with abdominal computed tomography scan and endoscopic ultrasound, the latter supplemented with endoscopic retrograde cholangiopancreatography as needed (36). This study was conducted in 78 high-risk patients (72 familial pancreatic cancer kindreds and 6 \textit{PJ} syndrome kindreds) and 149 control patients. Eight patients had pancreatic neoplasm, six had intraductal papillary mucinous neoplasms, one had an intraductal papillary mucinous neoplasm that progressed to invasive ductal adenocarcinoma, and one had pancreatic intraepithelial neoplasia (PanIN). Endoscopy showed that the at-risk kindreds seemed to have changes compatible with chronic pancreatitis. We would suggest identification of families predisposed to PDAC, and involving a multidisciplinary team (gastroenterologists, genetic counselors, surgeons, radiologists, oncologists, and pathologists), clinical screening with imaging and endoscopic modalities, genetic testing/counseling, and avoidance of cigarette smoking.

### Preclinical Models: Future Directions for Diagnosis and Therapy of PDAC

Preclinical models of PDAC have been burgeoning in recent years, including three-dimensional cell culture models that mimic the tumor microenvironment (37, 38), xenotransplantation models in immunodeficient mice that permit evaluation of the efficacy of new drugs, and innovative genetically engineered mouse models.

The first breakthrough in genetically engineered mouse models emerged from the targeting of \textit{Kras}\textsuperscript{G12D} to progenitor cells of the mouse pancreas (39). This resulted in human PanINs, which are precursor lesions to invasive PDAC (39, 40). At low frequency, these lesions progress to invasive and metastatic adenocarcinomas (39). The progression to PanIN and PDAC is accelerated through the cooperation of \textit{Kras}\textsuperscript{G12D} and \textit{Trp53R172H} or \textit{Kras}\textsuperscript{G12D} and \textit{p16INK4a/p19Arf} deficiency, or \textit{Kras}\textsuperscript{G12D} with impaired transforming growth factor signaling (41–43). The \textit{Kras}\textsuperscript{G12D} mouse has been used for proteomic methods to generate potential candidate biomarkers (39, 44), although it is too preliminary to be able to focus on one or a panel of several biomarkers. We would advocate “bridging” proteomic technologies for common biomarker patterns between genetically engineered mouse models and hereditary PDAC in patients. This would likely lead to the application of biomarkers to sporadic PDAC and possibly even PanIN lesions in these patients. Because PanIN lesions escape detection by current imaging modalities, linking biomarkers to PanIN lesions would represent a tremendous advance because curative surgical

Table 1. Hereditary forms of PDAC

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Defective gene(s) or chromosome</th>
<th>Genetic testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family X</td>
<td>Chromosome 4q32-34; palladin is candidate gene</td>
<td>No</td>
</tr>
<tr>
<td>Hereditary pancreatitis</td>
<td>\textit{PRSS1}</td>
<td>Yes</td>
</tr>
<tr>
<td>FAMMM</td>
<td>\textit{p16INK4a/CDKN2A}</td>
<td>Yes</td>
</tr>
<tr>
<td>PJ</td>
<td>\textit{LKB1}</td>
<td>Yes</td>
</tr>
<tr>
<td>Hereditary breast/ovarian cancer</td>
<td>\textit{BRCA1} or \textit{BRCA2}</td>
<td>Yes</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>\textit{BRCA} or \textit{FANCC, FANC}</td>
<td>Yes</td>
</tr>
</tbody>
</table>
resection for PDAC is only possible for very early stage disease. Furthermore, three-dimensional cell culture models, xenograft models, and genetically engineered mouse models are natural platforms for preclinical testing of chemopreventive and therapeutic agents, especially molecularly targeted therapeutics with inhibition of sonic hedgehog signaling or Notch signaling (45, 46).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

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