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REVIEW ARTICLE

Genetics and pathology of pancreatic cancer

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States [1,2]. Approximately 32 000 individuals in the USA and over 200 000 individuals worldwide die from the disease each year [1–3]. The incidence approximates the mortality rate, which reflects the poor prognosis of pancreatic cancer. Although there have been many advances in pancreatic cancer research, the 5-year survival rate for affected patients remains under 5% [2].

The aggressiveness that characterizes pancreatic cancer arises from multiple heterogeneous genetic changes that occur before the onset of clinical symptoms. Studies performed over the past decade have shed some light on the molecular and histological events that are associated with pancreatic carcinogenesis. This chapter will focus on the genetics and pathology of pancreatic ductal adenocarcinoma. Future progress in this area will hopefully lead to improved diagnostic tests, early detection, and new treatments for patients who suffer from this devastating disease.

Genetics

The accumulation of multiple nonrandom genetic changes over time is a hallmark of pancreatic cancer. Genetic abnormalities include alterations in chromosome or gene copy number, microsatellite instability, epigenetic silencing, intragenic point mutations, and gene overexpression secondary to increased transcription (Table I) [4].

Chromosomal alterations

The most common techniques used to study chromosome losses and gains are karyotyping (G-banding), comparative genomic hybridization (CGH), and allelotyping [4]. Metaphase spread karyotyping (G-banding) is a cytogenetic technique, developed in the 1960s, where chromosomes are stained and their banding patterns are examined during metaphase. Karyotyping can reveal large deletions, insertions, translocations, inversions, and other rearrangements, but suffers from a lack of submicroscopic resolution. Conventional CGH has an improved resolution (5–10 mB) [5], but in the post-genomic era, even this technology has become arcane. Array-based CGH, a molecular cytogenetic technique developed in the early 1990s, is an application of CGH using microarray technology. In microarray analysis, thousands of molecules may be immobilized on an insoluble solid support. This strategy enables the simultaneous detection of a large number of analytes [6]. A second application of microarray technology, gene expression analysis, will be discussed in greater detail below. The general principles of microarray analysis [7], applicable to both CGH and global gene expression studies, are illustrated in Figure 1.

As demonstrated in Figure 1, fragments of tumor DNA and non-tumor DNA are differentially labeled with fluorescent dye and allowed to compete for hybridization sites in either a metaphase chromosome spread (conventional CGH) or in an array of genomic probes (array CGH). The color and intensity of the

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Table I. Overview of the genetic abnormalities described in this paper.

Abnormalities observed in pancreatic ductal adenocarcinoma	Example
Chromosome alterations	Losses: 9p, 17p, 18q, 3p, 8p, 6q Gains: 3q, 5p, 7p, 8q, 20q
Microsatellite instability	Medullary carcinoma with mutations in *hMLH1*
Epigenetic silencing	Hypermethylation of promoter CpG islands in *ppENK*
Mutations	*p16, p53, DPC4*
Gene overexpression	Mesothelin

fluorescence emitted by the hybridized fragments of labeled DNA reveal areas of the genome that may be deleted or amplified in the test sample relative to the control sample [5,8]. In allelotyping analysis, microsatellite markers (short tandem repeats of DNA that are interspersed throughout the genome) in normal and cancer DNA from a single patient are PCR amplified and sequenced. Since individuals are often heterozygous at microsatellite loci, genomic deletions can be detected by evaluating microsatellite markers in tumor DNA for losses of heterozygosity (LOH). Chromosome positions that have frequent LOH in pancreatic cancer often harbor important tumor suppressor genes [9–11].

Chromosome losses are more common than chromosome gains in pancreatic cancer [4]. According to allelotyping data in pancreatic cancer xenografts, DNA loss in xenografts ranges from 1.6% to 32% of the genome [10]. Despite this variability, certain reproducible patterns have been observed. The most common regions of genomic loss in primary pancreatic cancers and pancreatic cancer xenografts include chromosome arms 9p, 17p, 18q, 3p, 8p, and 6q [10,12,13]. Some of these loci contain known tumor suppressor genes such as *CDKN2A/P16/MTS1* (9p21), *p53* (17p13), and *MADH4/SMAD4/DPC4* (18q). Further investigations may reveal additional tumor suppressor genes in the other LOH 'hot spots' [10].

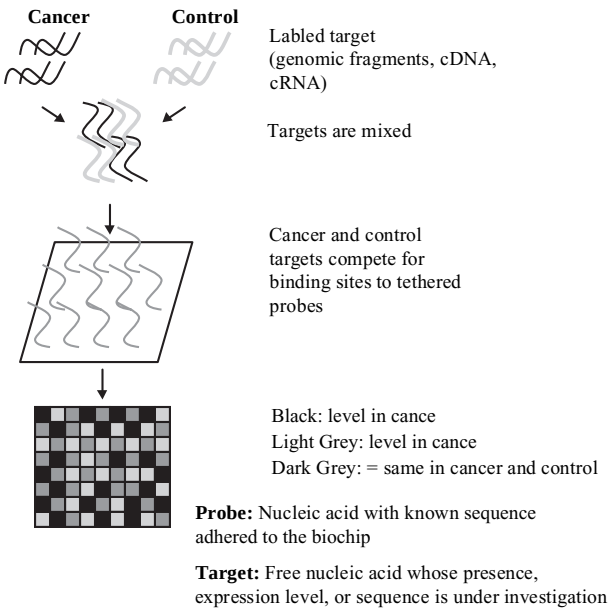

Figure 1. Microarray technology is based on high throughput competitive hybridization of fluorescently labeled sample DNA and control DNA (target sequences) with an organized array of sequences deposited on a biochip (probe sequences). Molecular cytogenetics (e.g. CGH) and gene expression analysis (e.g. cDNA microarray) are two widely used applications of microarray technology. The cartoon is based on a schematic from Coe and Antler [7].

Frequent gains of DNA, secondary to unbalanced chromosome rearrangements, have been observed in primary pancreatic cancers on chromosome arms 3q, 5p, 7p, 8q, and 20q [12,14]. Fluorescent *in situ* hybridization (FISH) performed on pancreatic cancer cell lines demonstrates amplified regions of DNA at 19q, 12p, 12q, 17q, and 20q. These amplified sites correspond to the locations of the following oncogenes: *AKT2, KRAS2, MDM2, ERBB2*, and *AIB1*, respectively [13,15].

Microsatellite instability

Medullary carcinoma, a subtype of pancreatic adenocarcinoma seen in approximately 5% of operative cases, often contains a defective DNA mismatch repair (MMR) mechanism [16]. Altered MMR gives rise to microsatellite instability (MSI), characterized by hypermutability at so-called minisatellite repeat sequences. Unlike most ductal adenocarcinomas of the pancreas, MSI tumors show minimal LOH and aneuploidy [17]. They also frequently lack mutations in KRAS2 and P53. Histologically, medullary cancers are poorly differentiated. They grow in a syncytial pattern, exhibit pushing borders, and demonstrate extensive necrosis. Interestingly, these tumors appear to have a better prognosis than the more common ductal adenocarcinoma [16].

Epigenetic silencing

Epigenetic phenomena are heritable DNA modifications that do not involve alterations in DNA sequence [18]. Examples include structural changes to chromatin, such as post-translation histone modifications and nucleosome rearrangements, and deregulation of cytosine methylation at promoter CpG islands. The expression of many tumor suppressor genes and oncogenes appears to be influenced through this mechanism. Genes that are silenced in pancreatic cancer by epigenetic changes include *ppENK* (90%), *RARB* (20%), *CDKN2A/P16* (18%), *CACNA1G* (16%), *TIMP3* (11%), *CDH1* (7%), *THBBS1* (7%), and *hMLH1* (4%) [19,20]. Approximately

60% of pancreatic cancers have multiple genes that are silenced by hypermethylation of promoter cytosines [20].

Mutations and specific gene alterations

Specific genes that play an important role in carcinogenesis can be classified as tumor suppressor genes, oncogenes, or genes involved in genomic maintenance. Many tumor suppressors regulate cell proliferation. Therefore, biallelic inactivation of a tumor suppressor gene can provide a growth advantage for tumors. Gene loss may result from allelic deletion, intragenic mutation, chromosomal recombination, epigenetic phenomena, or a combination thereof. Conversely, oncogenes promote cell growth. Genetic alterations that activate oncogenes include gain-of-function mutations, translocations, amplifications, and gene overexpression. Table II provides a list of genes that affect pancreatic carcinogenesis through intragenic mutations [21].

CDKN2A/p16/MTS1, located on chromosome arm 9p, is the most frequently inactivated tumor suppressor gene in pancreatic cancer (95%). The gene can be inactivated through homozygous deletion (40%), single allele loss combined with an intragenic mutation in the second allele (40%), or promoter hypermethylation (15%) [22,23]. p16 prevents Rb-1 inhibition by CDK4-phosphorylation during G_1 of the cell cycle (Figure 2). When p16 is inactivated, phosphorylation of Rb-1 by G_1-CDKs increases, facilitating progression through the G1/S-cell cycle checkpoint [24].

p53, located on chromsome arm 17p, is the second most commonly inactivated tumor suppressor gene in pancreatic cancer (50–75%) [25,26]. Usually, one p53 allele is deleted while the other allele acquires an intragenic mutation [25]. Following DNA damage to normal cells, activated p53 causes the cell to arrest in G1 or G2 of the cell cycle (Figure 2) [27]. p53 also responds to genetic injury by activating apoptotic pathways. Thus, p53 inactivation allows the tumor to proliferate despite the continued accumulation of other genetic defects.

MADH4/SMAD4/DPC4, located on chromosome arm 18q, is the third most frequently inactivated tumor suppressor gene in pancreatic cancer (55%). Approximately 30% of pancreatic cancers have homozygous deletions at the *SMAD4* locus, and 25% have allelic loss with an intragenic mutation in the second allele [28]. *SMAD4* is a downstream component of the transforming growth factor (TGF)-β and activin signaling pathways (discussed below, Figure 3). Like p16, loss of *SMAD4* impairs the cell cycle check mechanism during the G1/S transition. *SMAD4* loss is also believed to affect pro-apoptotic signaling [29].

Mutations have been reported in additional components of the TGF-β and activin signaling pathways, such as *TGFβR1* (1% homozygous deletion rate), *TGFβR2* (4%), *ACVR1B* (2%), and *ACVR2* (4%) [30,31]. Rare mutations have also been identified in genes outside of the TGF-β and activin pathways. *MKK4*, a component of the mitogen activated protein kinase pathway involved in stress and cytokine-induced signaling, has a 4% mutation rate in pancreatic cancer [32]. *STK11/LKB1,* a serine/threonine kinase mutated in Peutz-Jeghers syndrome, is also mutated in 4% of pancreatic cancers [33]. Recent studies demonstrate that STK11 is important to the maintenance of intracellular polarity and intracellular energy metabolism [34]. *BRCA2*, a genomic maintenance gene in the Fanconi anemia (FA) pathway, is altered in 7% of pancreatic cancers [35], but germline mutations can be seen in a larger proportion of patients (17%) that cluster in pancreatic cancer families (see below) [36]. *FANCC* and *FANCG* are two other components in the FA pathway that have been shown to be mutated in pancreatic cancer (~5%) [37]. FA signaling directs homologous recombination after double-strand DNA breaks.

KRAS2, located on chromosome arm 12p, is the most commonly mutated oncogene in pancreatic cancer (90%) [38–40]. In the majority of cases, aspartic acid is substituted for glycine at codon 12. Additional mutational 'hot spots' occur in codons 13 and 61 [41]. *KRAS2* functions as a GTPase involved in intracellular signaling. Gain-of-function mutations that impair *KRAS2* GTPase activity augment downstream signaling and increase cell proliferation. Pancreatic cancers with wild-type *KRAS2* may have mutations in *BRAF* (3%) [42]. *BRAF* is a serine/threonine kinase and a key downstream effector of *KRAS2*.

Table II. Genes that may be mutated in pancreatic cancer.

Gene	Chromosome location	Percentage of pancreatic cancers with mutations
Oncogenes		
KRAS2	12p	95
BRAF	7q	3
Tumor suppressors		
p16	9p	95
p53	17p	75
DPC4	18q	55
LKB1/STK11	19p	5
MKK4	17p	4
TGFBR2	3p	4
ACVR2	2q	4
ACVR1B	12q	2
TGFBR1	9q	1
Genome maintenance		
BRCA2	13q	7–10
FANCC	9q	6
FANCG	9p	3
MLH1	3p	3

Table modified from Sohn [21].

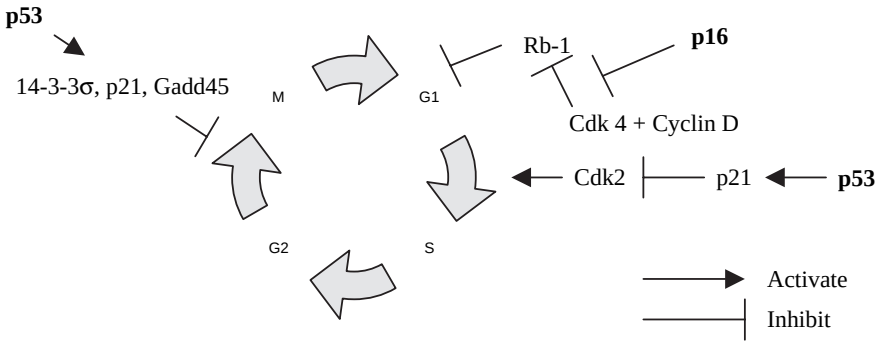

Figure 2. p16 and p53 regulation of the cell cycle. In normal cells, cyclin D complexes with Cdk4 during G_1 of the cell cycle. This complex inhibits Rb by phosphorylating it, allowing the cell to progress into S-phase. p16 prevents cell proliferation during stress by interfering with the cyclin D-Cdk complex [24]. p53 responds to DNA damage by inducing p21. Activated p21 binds and inhibits G_1 and G_1/S-Cdks (Cdk2), causing the cell to arrest in G_1. p53 prevents entry into mitosis through the induction of 14-3-3σ, p21, and Gadd45. These proteins inactivate M-Cdks (Cdk1, Cdc2) [27]. Inactivation of p16 or p53 in pancreatic cancer promotes cell tumorigenesis.

Gene overexpression

Altered gene expression in tumors can result from chromosomal rearrangements, epigenetic silencing, or mutations in genes that are upstream of a target gene. Gene expression may be examined one gene at a time, using techniques like Northern blot analysis, real-time PCR, and mRNA *in situ* hybridization [43]. However, recently developed techniques such as serial analysis of gene expression (SAGE) and microarray analysis permit simultaneous evaluation of virtually the entire human transcriptome [43]. In SAGE analysis, mRNA is reverse transcribed into cDNA, and then cleaved close to the poly A tail (3' end). These steps produce 10–14 base pair fragments, or tags, that correspond to the transcripts in the original sample RNA pool. The tags are linked together, amplified, and sequenced. The prevalence of each tag in the amplified product corresponds to the expression level of a unique transcript in the tumor RNA library [43]. Data from the target pool can be compared to data from a control RNA library to identify changes in gene expression. An advantage of the SAGE technique is that genomes do not have to be characterized before analysis [43].

Gene expression microarrays, like genomic microarray analysis (i.e. CGH), involve hybridization reactions between labeled targets and a library of probes (either cDNA or oligonucleotides) that are adhered to a biochip. However, in the case of gene expression analysis, the labeled targets are made from cDNA instead of genomic fragments. Therefore, the readout provides expression data rather than cytogenetic data. cDNA microarray analysis follows the general scheme for microarray experiments illustrated in Figure 1. Specifically, the RNA from tumor and non-tumor cells is differentially labeled with fluorescent dye during cDNA synthesis. The target cDNAs are allowed to hybridize to the cDNA probes on the biochip in a competitive fashion. The colors and intensities of the hybridization products are analyzed to determine the level of gene expression in the tumor compared to the reference sample [7,44]. In oligonucleotide microarray analysis (trademarked as GeneChip by Affymetrix), probes that are 25 nucleotides long are synthesized on a biochip, one nucleotide at a time. Labeled tumor cRNA, synthesized through sequential reverse transcription and *in vitro* transcription steps, is allowed to hybridize to the chip in a non-competitive fashion. The intensity of the signals on the GeneChip corresponds to the level of transcript present in the tumor RNA sample. These data can be compared to the signal produced by the hybridization of reference cRNA with a separate, but identical, gene chip [7]. Gene expression microarray analysis is perhaps better suited than SAGE analysis for high-throughput investigations, but the technique requires prior characterization of a group of genes in order to synthesize a library of probes.

Global gene expression technologies have identified hundreds of overexpressed genes in pancreatic ductal adenocarcinoma [45–53]. Our group at Johns Hopkins identified 422 overexpressed genes using SAGE, cDNA microarrays, and U133 oligonucleotide arrays [45]. Forty genes were identified by more than one method, and six genes were detected by all three methods. The six genes detected by each technique are keratin 19, transglutaminase 2, stratifin retinoic acid-induced 3, secretory leukocyte protease inhibitor, and tetraspan1. Increased expression of the first three genes listed was observed in earlier studies using antibody detection or mass spectrometry [54–56].

Familial pancreatic cancer

Focused investigations on high-risk families have yielded new insights into the genetics of pancreatic cancer. Registries, such as the National Familial Pancreas Tumor Registry (NFPTR) at Johns Hopkins, track patients and family members at risk for pancreatic cancer [57,58]. Inherited mutations may account for 2–10% of all pancreatic cancers [58–61]. Individuals with one, two, or three first degree relatives (parent, child, or sibling) with a history of

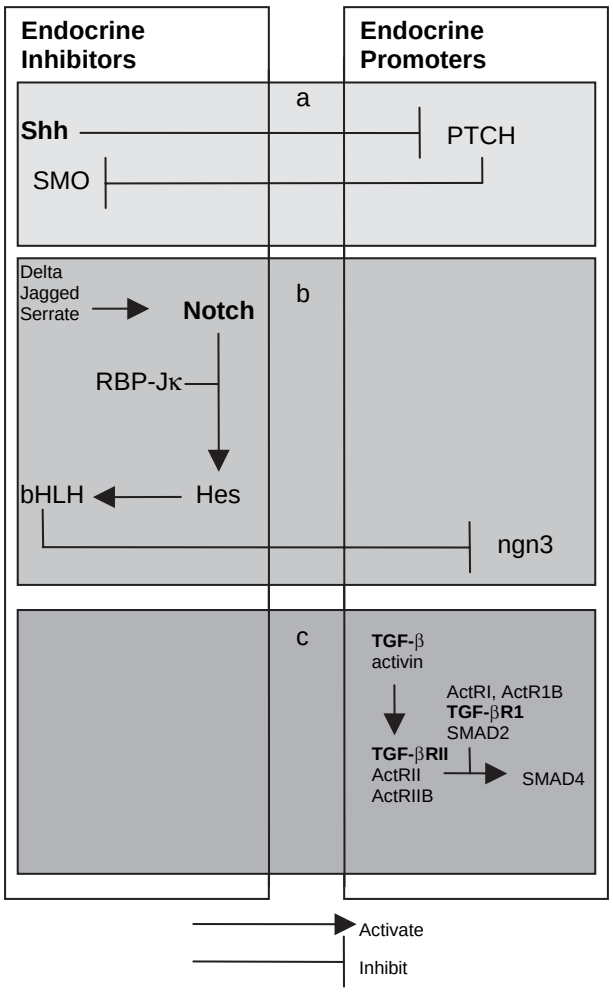

Figure 3. Signaling pathways in pancreas development. Genes that maintain epithelial progenitor cells, and have oncogenic properties in pancreatic cancer, appear on the left of the figure. Genes that stimulate endocrine differentiation, and have tumor suppressor properties in pancreatic cancer, appear on the right of the figure. The role of three specific signaling pathways in pancreatic development are summarized. (a) Shh signaling inhibits endocrine differentiation. (b) Notch signaling inhibits endocrine differentiation. (c) TGF-β and activin signaling promote endocrine differentiation. Modified from Kim et al [85].

pancreatic cancer demonstrate risk increases for pancreatic cancer that are approximately 6-, 18-, and 57-fold above baseline, respectively [58]. Pancreatic cancer surveillance protocols in high-risk families using endoscopic ultrasound and/or endoscopic retrograde cholangiopancreatography demonstrate that routine screening, combined with directed pancreatectomy for suspicious lesions, can effectively identify and treat patients with pancreatic dysplasia prior to the onset of invasive pancreatic cancer [62,63]. EUS-based surveillance at Johns Hopkins identified one high-risk individual with an asymptomatic invasive ductal adenocarcinoma. The patient had a disease-free survival of >5 years when Canto et al. published the study [63].

Five hereditary tumor predisposition syndromes associated with pancreatic cancer have been identified thus far (Table III) [64], although these known syndromes account for only a minority (<20%) of familial cases. Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disorder responsible for roughly 3% of colorectal cancers, and has an estimated population prevalence of 1/740 [65]. At least six distinct DNA mismatch repair (MMR) genes have been linked to HNPCC. Mutations in MMR genes result in the MSI phenotype with enhanced tumorigenesis. Individuals with HNPCC have a 1–5% lifetime risk of developing pancreatic cancer [60,66,67]. Other extra-colonic cancers in HNPCC patients include endometrial cancer (60%), stomach cancer (10%), ovarian cancer (12%), genitourinary cancer (4%), biliary cancer (2%), nervous system (4%), and small bowel cancer (1–4%) [68].

Peutz-Jeghers syndrome (PJS) is an autosomal dominant disorder with an incidence of 1:25 000. The disease is characterized by mucocutaneous pigmentations and hamartomatous intestinal polyps, due to mutations in the serine/threonine kinase 11 (*STK11/LKB1*) gene. Patients with PJS have a 132-fold increased risk of developing pancreatic cancer; which translates into a lifetime risk of >30% [60,67,69].

Familial atypical multiple mole-melanoma syndrome (FAMMM) is associated with multiple nevi, melanomas, and extracutaneous tumors. The disease is caused by a mutation in *CDKN2A/p16* and accounts for approximately 12% of familial pancreatic cancers [60]. Affected individuals have a 20-fold increased risk for developing pancreatic cancer, and a lifetime risk that approaches 15% in some families [70].

Familial breast and ovarian cancer syndrome is due to mutations in the *BRCA1* or *BRCA2* genes. Individuals with germline *BRCA1* mutations have a twofold risk increase of pancreatic cancer; those with *BRCA2* mutations have between a 4- and 13-fold risk increase [71–73]. Inherited *BRCA2* mutations are responsible for roughly 17% of familial pancreatic cancer cases, which makes *BRCA2* the most common inherited defect contributing to pancreatic cancer identified to date [35]. Ashkenazi Jews have the highest incidence of *BRCA2*-deficient pancreatic cancer. Affected individuals in this subpopulation usually harbor the 6174delT *BRCA2* mutation, which occurs in 1% of all Ashkenazi Jews [72]. Familial breast cancer syndrome due to a mutated *BRCA2* gene carries a lifetime risk for pancreatic cancer which approximates 7% [74].

In addition to the above described familial cancer predisposition syndromes, familial pancreatic cancer can be associated with hereditary pancreatitis (HP). HP is an autosomal dominant disease caused in 70% of the cases by a mutation in cationic trypsinogen (protease serine 1; *PRSS1*) [75]. Mutations in the serine protease inhibitor Kazal type 1 gene (*SPINK1*) have also been linked to HP [76]. Mutated *PRSS1* is resistant to inactivation, leading to pancreatic auto-

Table III. Hereditary tumor predisposition syndromes associated with pancreatic cancer.

Syndrome	Features	Estimated lifetime risk of pancreatic cancer*	Gene	Locus
HNPCC	Colorectal cancers; less commonly cancers of the uterus, stomach, ovaries, genitourinary system, hepatobiliary system, brain, and small intestine	1–5%	Common	
			MSH2	2p15-16
			MLH1	3p21
			Rare	
			PMS1	2q31
			PMS2	7p22
			MSH6	2p15-16
Peutz-Jeghers syndrome	Mucocutaneous pigmentations, hamartomatous polyps of the GI tract, and increased risk of GI and non-GI malignancy	30%	STK11/ LKB1 kinase	19p13.3
FAMMM	Multiple nevi, melanomas, and increased risk of pancreatic cancer	15%	CDKN2A/p16	19p13.3
Hereditary breast and ovarian cancer (BRCA2)	Increased risk of breast, ovarian, and pancreatic cancer	5–10%	*BRCA2*	13q12.3
Hereditary pancreatitis (HP)	Recurrent pancreatitis and pancreatic cancer	40%	*PRSS1*	7q35
			SPINK1	5q32
Family X	Absence of extra-pancreatic cancer and pancreatitis; pancreatic insufficiency prior to the onset of cancer	80%	Unidentified	4q32-34

Table modified from Hansel et al. [4]. HNPCC, hereditary nonpolyposis colorectal cancer; FAMMM, familial atypical multiple mole-melanoma syndrome; GI, gastrointestinal.
*Lifetime risk for developing pancreatic cancer for all individuals in developing countries is 1% [64].

digestion. Affected individuals have a 50-fold increased risk of developing pancreatic cancer; their lifetime risk approaches 40% [77].

An unidentified pancreatic cancer gene has been mapped to chromosome arm 4q32-34 through linkage analysis in one family, referred to as 'family X' [61]. Affected family members have an 80% lifetime risk of pancreatic cancer and do not appear to be at increased risk for extra-pancreatic tumors. The cancers develop earlier (median age 43 years) than sporadic pancreatic cancer (median age 65 years) [78] and are often associated with a prodrome of pancreatic insufficiency [61].

In the overwhelming majority ($\sim$80%) of familial pancreatic cancer cases, the underlying genetic predisposition remains unknown. Some groups have aggressively proposed that unaffected family members undergo annual surveillance [61] to exclude development of pancreatic cancer prospectively (see EUS studies above). There is an urgent need to identify the gene or genes responsible for the vast majority of familial cases, so that a 'gene test' akin to other familial cancers like colon or breast cancer can be formulated for unaffected relatives of probands.

Pathology

Pancreatic cancer and development

The embryologic development of normal pancreatic tissue can be separated into three phases. First, dorsal and ventral buds arise from foregut endoderm. Next, cells differentiate into exocrine and endocrine cells. Finally, morphogenesis occurs through growth and branching. These events result in three distinct cell types derived from a common precursor: exocrine acinar cells synthesize and secrete digestive proenzymes into pancreatic ducts; ductal cells line the pancreatic ducts and secrete alkaline fluid; and endocrine cells, primarily involved in glucose homeostasis, are clustered into structures called islets of Langerhans [79].

The cell type of origin in pancreatic cancer has not been definitively characterized. Expression of acinar, ductal, and islet cell markers have each been identified in pancreatic ductal adenocarcinoma. In fact, markers for gastric, duodenal, and colonic epithelium have also been detected [80]. Experiments in transgenic mice have led some investigators to speculate that normal pancreata undergoes a metaplastic change into an embryonic-like epithelium early in cancer development. This theory is based on animal studies which demonstrate that metaplastic mouse pancreata develop histological and genetic changes that are similar to changes observed in pancreatic cancer [81–83].

The link between pancreatic development and pancreatic cancer, supported mainly by animal studies, has increased interest in certain developmental signaling pathways. The pathways that are best characterized include sonic hedgehog (Shh), Notch, and TGF-β signaling (Figure 3).

In the Hedgehog (Hh) pathway, Shh binds and inhibits the transmembrane receptor, Patched (PTCH) (Figure 3a) [84]. This action allows Smoothened (SMO) to initiate downstream signals that stabilize Gli transcription factors, leading to the induction of target Hh genes [84,85]. The pathway is repressed during pancreatic development by TGF-β/

activin signaling [85]. This action is believed to maintain a precursor population and curb the progression of cells towards differentiated endocrine tissue [84,85]. Inactivated Shh during embryogenesis can result in developmental abnormalities such as ectopic pancreatic tissue or islet cell hyperplasia [85]. Conversely, increased Shh expression leads to the loss of pancreatic lineage markers and the transformation of pancreatic mesenchyme into gut mesoderm [85]. Shh pathway components are activated in pancreatic cancer [86,87]. Increased expression of Hh factors were observed in pancreatic cancer cell lines and xenografts. Developmental misexpression of Shh in murine pancreata under the control of a Pdx1 promoter resulted in genetic and histological abnormalities that resemble those seen in pancreatic cancer precursor lesions [86]. Furthermore, cyclopamine (a SMO antagonist) and a monoclonal antibody against Shh had a growth inhibitory effect on pancreatic cancer cells *in vitro*, suggesting a requirement for the pathway in growth sustenance.

Notch signaling also regulates epithelial progenitors and inhibits endocrine differentiation in the developing pancreas (Figure 3b) [84,85,88]. The Notch pathway is triggered when Delta, Serrate, and Jagged ligands bind Notch receptors. The intracellular component of the activated Notch receptor interacts with the DNA-binding protein RBP-Jκ, to activate the transcription of the hairy/enhancer-of-split genes (Hes). Hes activation directs basic helix-loop-helix factors (bHLH) to down-regulate differentiation-associated genes, like the endocrine activator ngn3 [85]. Oligonucleotide microarray studies detected overexpression of key Notch pathway components in pancreatic cancer [83]. Transfection of the Notch1 and Notch2 receptors into murine pancreatic epithelium induced acinar-to-ductal metaplasia [83]. This process can be disrupted *in vitro* by γ-secretase, an enzymatic inhibitor of Notch signaling [83].

Unlike the Notch and Hh pathways, TGF-β and activin signaling promote endocrine cell development (Figure 3c). The TGF-β and activin ligands bind their respective type II receptors. The type II receptors in turn bind TGF-β and activin type I receptors, and activate intracellular SMAD proteins [30,85]. Since the TGF-β and activin pathways counter Hh and Notch pathways during pancreatic development, it is not surprising that TGF-β and activin signaling affect tumor growth in a different manner than the Hh and Notch pathways. As described earlier, multiple factors in the TGF-β and activin pathways harbor inactivating mutations, suggesting that these genes are important tumor suppressors [29].

Pancreatic intraepithelial neoplasia

Dysregulated molecular signaling and the accumulation of genetic changes over time result in incremental changes in pancreatic epithelium that can be appreciated microscopically [89]. A unified nomenclature was developed in 1999 to classify intraductal precursor lesions of the pancreas, referred to as pancreatic intraepithelial neoplasia (PanINs) (Figure 4) [90,91]. PanIN-1A lesions are flat, tall columnar cells with basally located nuclei; PAN-1B lesions show papillary architecture; PanIN-2 lesions exhibit nuclear abnormalities, such as a loss of polarity or nuclear crowding; PanIN-3 lesions have marked nuclear and cytologic abnormalities and represent the lesions previously referred to as carcinoma *in situ* of the pancreas. Invasion through the basement membrane marks the transition from PanIN-3 to

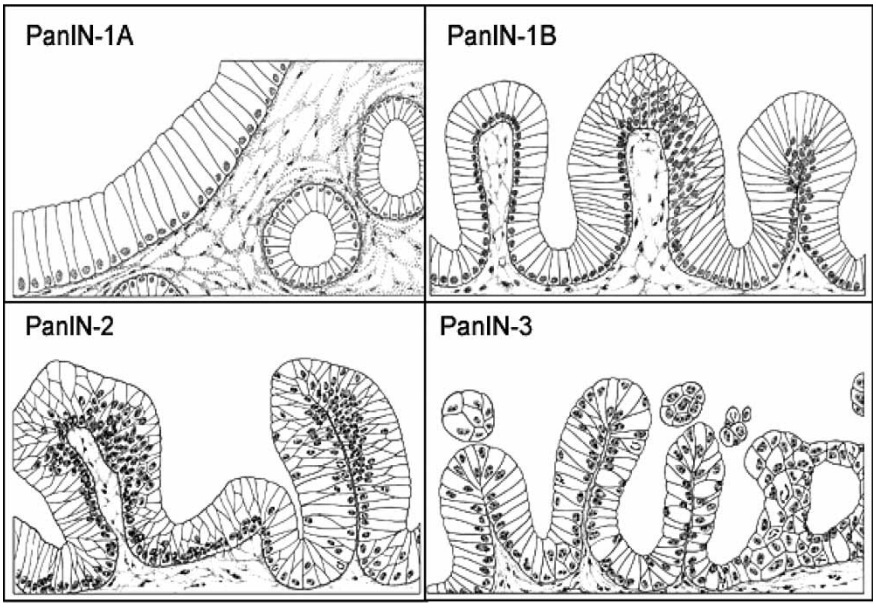

Figure 4. Pancreatic intraepithelial neoplasia: PanIN-1A (flat), PanIN-1B (papillary), PanIN-2 (papillary with nuclear changes), and PanIN-3 (severely atypical with mitoses, budding, and luminal necrosis). Images appear on the Johns Hopkins Pancreas Cancer Web [91].

invasive carcinoma. PanIN lesions demonstrate a progressive accumulation of genetic aberrations that mirror their histologic progression. For example, certain changes like telomere length abnormalities and KRAS2 mutations are observed as early as in PanIN-1 lesions [92]. *p16* inactivation has often occurred by the PanIN-2 stage [93], while certain changes such as loss of function of SMAD4 [94], BRCA2 [95], and p53 [96] tumor suppressor genes occur in advanced PanIN lesions.

Mucinous cystic neoplasm

Some noninvasive pancreatic lesions can mimic PanINs and, analogous to the latter, can be associated with invasive pancreatic cancers in subsets of cases [90]. Mucinous cystic neoplasms (MCN) of the pancreas are a heterogeneous group of cystic tumors that range from small, benign cysts to large cysts with infiltrating adenocarcinoma (Figure 5B) [97]. The cysts contain a cloudy fluid that stains positive for mucin, and they are lined by tall, mucin-producing columnar epithelium. MCNs can be large (mean tumor diameter is 10.5 cm) and do not communicate with the main pancreatic duct. They occur more often in women then men, and are usually diagnosed in the fifth decade of life. The presence of dense ovarian stroma underneath the cyst epithelium is a defining characteristic of MCNs [97].

MCNs may be subcategorized into four groups based on the degree of cellular atypia (e.g. high nuclear-cytoplasmic ratios, pleomorphism, and loss of cell polarity) and the presence or absence of invasion. Thus, mucinous cystadenomas lack significant atypia and are lined by a single layer of epithelium, borderline mucinous cystic neoplasms exhibit papillae and have moderate nuclear atypia, while mucinous carcinoma *in situ* lesions have severe nuclear atypia. All these categories of mucinous cystic lesions are noninvasive and complete surgical resection is nearly always curative [98]. In contrast, approximately a third of MCNs are associated with an invasive carcinoma (mucinous cystadenocarcinoma), which demonstrate invasion of tumor cells into the surrounding stroma, and are associated with a 33% 5-year survival post-resection [98].

IPMNs

Intraductal papillary-mucinous neoplasms (IPMNs) are similar in many aspects to MCNs (Figure 5C). For instance, IPMNs are a heterogeneous group of tumors that include both noninvasive (60%) and invasive (40%) neoplasms [99]. Microscopically, they are composed of columnar, mucin-secreting cells and can develop a papillary architecture [97]. Indeed, MCNs and IPMNs cannot be distinguished with an electron microscope. However, IPMNs are not associated with ovarian stroma, and they always communicate with the pancreatic duct system. The main pancreatic duct or major side branches are usually dilated and mucus-filled. Therefore, mucin may be seen oozing from the ampulla of Vater during endoscopic evaluation of an IPMN.

The genetic pattern in IPMNs is different from the genetics observed in PanIN lesions and ductal adenocarcinoma. Immunohistochemical studies reveal

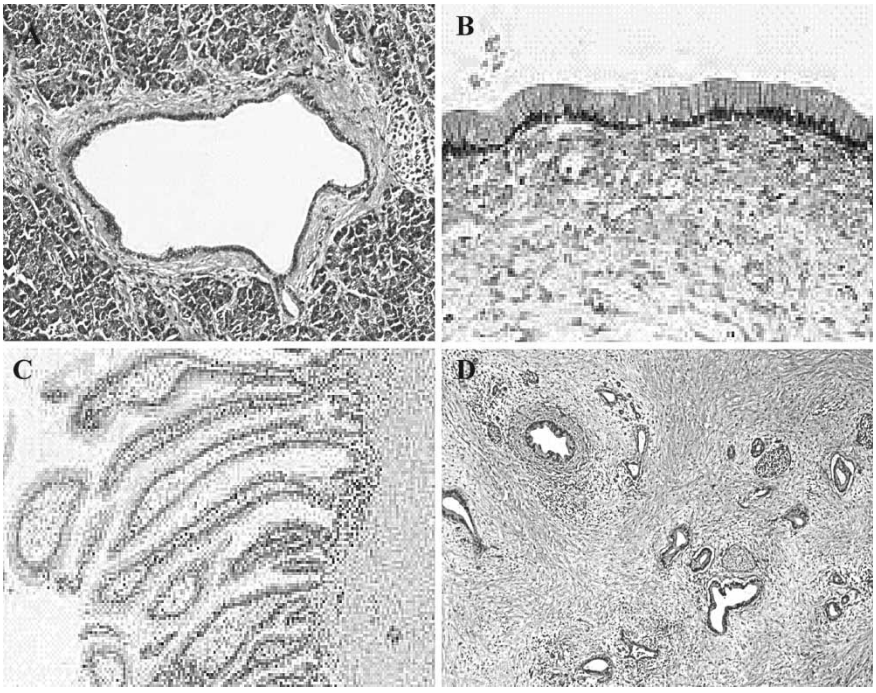

Figure 5. (A) Normal pancreatic tissue. (B) Mucinous cystic neoplasm. (C) Intraductal papillary-mucinous neoplasms (IPMNs). (D) Pancreatic ductal adenocarcinoma. Images appear on the Johns Hopkins Pancreas Cancer Web [91].

that 84% of IPMNs express the SMAD4 gene, compared with 45% of ductal adenocarcinomas of the pancreas [100]. Furthermore, IPMNs express a different mucin (MUC2) than PanIN lesions and ductal adenocarcinoma (MUC1) [101].

The epithelium of IPMNs is subcategorized by a four-tiered histologic classification scheme, like the one described earlier for MCNs. Intraductal papillary-mucinous adenomas lack significant atypia; borderline IPMNs show an intermediate degree of atypia; and IPMN carcinoma *in situ* demonstrate severe atypia [102]. 'Colloid' (mucinous non-cystic) carcinoma is identified in the majority of IPMNs associated with invasive carcinoma; a tubular/ductal type occurs in most of the remaining cases [99].

IPMNs occur equally in men and women, and they generally arise during the seventh decade of life [99]. Demographic data suggest that the time interval between the development of an IPMN adenoma and progression to an IPMN with invasive cancer is over 5 years. Five-year survival rates for resected IPMNs without and with invasive cancer are 77% and 43%, respectively [99]. Approximately 8% of patients with a resected benign IPMN develop a recurrence or a second metachronous IPMN in the pancreatic remnant [99].

Ductal adenocarcinoma

Ductal adenocarcinoma accounts for >80% of pancreatic cancers. Grossly, they are white-yellow and firm masses. Sixty percent of the cases arise in the pancreatic head, 15% in the body or tail, and 20% involve the gland diffusely [97]. They are characterized microscopically by infiltrating small glands that are lined with low-columnar, mucin-containing cells (Figure 5D). Typically, a strong desmoplastic reaction occurs around the cancer [97,102]. The nuclei often demonstrate pleomorphism, hyperchromasia, loss of polarity, and prominent nucleoli [97].

Ductal adenocarcinomas are highly aggressive cancers with frequent invasion of vascular, lymphatic, and perineural tissue. Approximately 80% of surgical specimens show disease in regional lymph nodes. There is clinically evident disease at distant organ sites in up to 80% of all patients who are discovered to have pancreatic cancer. The most common sites for distant metastases are the liver (80%), peritoneum (60%), lung and pleura (50–70%), and adrenal glands (25%) [97]. The 5-year survival rate for all patients with pancreatic ductal adneocarcinoma is <5% [2]. The 5-year survival rate is 15–25% in patients who undergo pancreaticoduoedenectomy, and 30–40% in patients who undergo pancreaticoduoedenectomy with small tumors and node-negative disease [103,104].

Other rare primary non-endocrine tumors of the pancreas include adenosquamous carcinoma, acinar cell carcinoma, giant cell carcinoma, giant cell carcinoma with osteoclast-like giant cells, pancreatoblastoma, serous cystadenoma/cystadenocarcinoma, and solid-pseudopapillary (Hamoudi) neoplasm [97]. Table IV gives the AJCC TMN staging system for pancreatic cancer, last revised in 2002 [105].

Table IV. TNM classification of exocrine pancreatic cancer.

TNM definitions
Primary tumor (T)
TX: Primary tumor cannot be assessed
T0: No evidence of primary tumor
Tis: Carcinoma *in situ*
T1: Tumor limited to the pancreas, 2 cm or less in greatest dimension
T2: Tumor limited to the pancreas, more than 2 cm in greatest dimension
T3: Tumor extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery
T4: Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor)
Regional lymph nodes (N)
NX: Regional lymph nodes cannot be assessed
N0: No regional lymph node metastasis
N1: Regional lymph node metastasis
Distant metastasis (M)
MX: Distant metastasis cannot be assessed
M0: No distant metastasis
M1: Distant metastasis
AJCC stage groupings
Stage 0
Tis, N0, M0
Stage IA
T1, N0, M0
Stage IB
T2, N0, M0
Stage IIA
T3, N0, M0
Stage IIB
T1, N1, M0
T2, N1, M0
T3, N1, M0
Stage III
T4, any N, M0
Stage IV
Any T, any N, M1

From *AJCC Cancer Staging Manual* [105].

Conclusions

New insights into the molecular mechanisms that underlie pancreatic carcinogenesis have emerged during the past decade. There is a well supported model that outlines the genetic and histological progression of pancreatic neoplasia. Microarray analysis offers the opportunity to study genomic copy number and gene expression changes across the entire human genome and transcriptome, respectively. Consequently, the number of genes that are known, or suspected, to play a role in the development of

pancreatic cancer has increased exponentially in recent years.

However, there is still much to learn. Although there are five well characterized hereditary tumor predisposition syndromes associated with pancreatic cancer, the genetic abnormalities responsible for the majority of cases of familial pancreatic cancer have not been discovered. The exact roles of putative tumor suppressor genes and oncogenes implicated by microarray techniques remain unclear. In addition, the contribution of complex developmental signaling pathways (e.g. Shh, Notch, and TGF-β) to pancreatic carcinogenesis is just beginning to be understood.

Improved patient outcomes in pancreatic cancer will require continued progress in cancer genetics. The molecular analysis of serum and gastrointestinal excreta to detect the presence of pancreatic cancer precursor lesions is one promising area of investigation. Berthelemy et al. demonstrated the potential of this approach 10 years ago when they discovered KRAS2 mutations in pancreatic juice from two patients suspected of having benign pancreatic disease. Both patients presented with symptoms that led to the diagnosis of pancreatic cancer >1 year after the study [106]. KRAS2 mutations have also been identified in the stool of patients with pancreatic carcinoma and cholangiocarcinoma [107]. However, recent studies show that screening pancreatic juice and stool for KRAS2 mutations (76% and 50% specific, respectively) fails to achieve an appropriate specificity for a relatively low prevalence disease like pancreatic cancer [108], mandating the ongoing search for new markers such as HIP/PAP and PAP-2 [109]. While ERCP may be an inappropriate screening test due to its invasiveness, EUS is a promising strategy to identify subtle parenchymal changes before they can be visualized by CT or MR. Continued efforts to elucidate the molecular causes of pancreatic cancer will hopefully translate into better diagnostic tests and therapies in the near future.

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