

# Pancreatic Carcinogenesis

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## Abstract

Pancreatic cancer is an almost universally lethal disease. Research over the last two decades has shown that pancreatic cancer is fundamentally a genetic disease, caused by inherited germline and acquired somatic mutations in cancer-associated genes. Multiple alterations in genes that are important in pancreatic cancer progression have been identified, including tumor suppressor genes, oncogenes, and genome maintenance genes. Furthermore, the identification of non-invasive precursor lesions of pancreatic adenocarcinoma has led to the formulation of a multi-step progression model of pancreatic cancer and the subsequent identification of early and late genetic alterations culminating in invasive cancer. In addition, an increased understanding of the molecular basis of the disease has facilitated the identification of new drug targets enabling rational drug design. The elucidation of genetic alterations in combination with the development of high-throughput sensitive techniques should lead to the discovery of effective biomarkers for early detection of this malignancy. This review focuses mainly on the current knowledge about the molecular insights of the pathogenesis of pancreatic ductal adenocarcinoma.

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## Epidemiology

Pancreatic cancer is a disease with a dismal outlook. In the United States approximately 33,000 patients are diagnosed with pancreatic cancer annually, and nearly an equal number will die from the disease, representing the fourth most common cause of cancer-related mortality. Men and women have an approximately equal risk [1]. Worldwide, pancreatic cancer causes an estimated 213,000 deaths each year [2]. For all stages combined, the 1-year survival rate is around 20%, and the overall 5-year survival rate is less than 5%, despite even the most aggressive therapies currently available [1].

A number of risk factors have been identified [3]. 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. Cigarette smoking is by far the leading preventable cause of pancreatic cancer [4]. Cigarette smoking doubles the risk of pancreatic cancer (relative risk = 2) [3]. Other risk factors include diets high in meats and fat, low serum folate levels, obesity, long-standing diabetes mellitus, and chronic pancreatitis [3, 5–7]. Approximately 10% of patients demonstrate

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a familial predisposition for pancreatic cancer, and a subset of these patients harbor germline mutations in *BRCA2*, *p16/CDKN2A*, *PRSS1*, *STK11/LKB1*, or the DNA mismatch repair genes (see further discussion below). In the vast majority of patients with familial risk, however, the underlying genetic predisposition remains unknown.

Complete surgical resection remains the only curative treatment. Studies from high-volume centers with optimal staging report up to a 15–20% 5-year survival rate in patients undergoing surgical resection [8, 9]. The mortality rate is so high because pancreatic cancer usually only produces symptoms when it has already metastasized, and because there are no sensitive and specific tools to detect the disease at an earlier stage. Although multiple histological subtypes of pancreatic cancer have been described, the most common and deadliest form is pancreatic ductal adenocarcinoma [10]. Novel approaches to the management of patients with this aggressive disease are urgently needed.

Research over the last two decades has shown that pancreatic cancer is fundamentally a genetic disease, caused by inherited germline and acquired somatic mutations in cancer-associated genes. A compendium of alterations in tumor suppressor genes, oncogenes, and genome-maintenance genes that are important in pancreatic cancer progression have now been identified (fig. 1). This review focuses mainly on the molecular insights on pancreatic ductal adenocarcinoma and its precursor lesions, including insights gained through experimental models of pancreatic carcinogenesis.

### Precursor Lesions of Pancreatic Cancer

Prior to a discussion on molecular genetics of pancreatic cancer, we will briefly discuss the current state of knowledge on precursor lesions of the pancreas. This is essential from the context of separating ‘early’ genetic changes (i.e. those associated with tumor initiation) from ‘late’ abnormalities (i.e. those associated with tumor progression). A recent review in *Pancreatology* has extensively discussed the histology and genetics of pancreatic cancer precursors [11]; therefore, we will only discuss these in fleeting detail. Briefly, pancreatic intraepithelial neoplasias (PanINs) are classified into a four tier classification, including PanIN-1A, -1B, -2, -3, reflecting a progressive increase in histologic grade culminating in invasive neoplasia (fig. 2). The lowest grade PanIN lesions can be flat (1A) or papillary (1B), but are characterized by ab-

sence of nuclear atypia and retained nuclear polarity. PanIN-2 lesions have micropapillary features with evidence of nuclear atypia and infrequent mitoses, while PanIN-3 lesions (a.k.a. carcinoma in situ) demonstrate widespread loss of polarity, nuclear atypia, and frequent mitoses. In addition to microscopic PanIN lesions, there are now recognized macroscopic (cystic) precursor lesions of pancreatic adenocarcinoma – including intra-ductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms. Akin to PanINs, the cystic precursor lesions also demonstrate a multistep histological and genetic progression to invasive neoplasia. Since IPMNs and mucinous cystic neoplasms can be detected by radiologic scans, they represent an opportunity to diagnose invasive pancreatic cancer before it can develop [11].

### Tumor Suppressor Genes

Tumor suppressor genes are genes that promote tumor growth when inactivated. Tumor suppressor genes are recessive, i.e. the two copies need to be mutated for loss of function, and they can be inactivated by a variety of mechanisms. First, by an intragenic mutation in one allele (copy of a gene) coupled with loss of the second allele; second, through a deletion of both alleles (homozygous deletion), and third, by hypermethylation of the promoter of the gene-silencing gene expression. In sporadic cancers these alterations are both somatic mutations acquired during life, while patients with inherited forms of cancer inherit one mutant allele in the germline while the second allele is somatically mutated in the cancer cells.

The *p16INK4A/CDKN2A* gene, located on the short arm of chromosome 9 (9p), is one of the most frequently inactivated tumor suppressor genes in pancreatic cancer [12] (table 1). Remarkably, virtually all pancreatic carcinomas have loss of *p16INK4A/CDKN2A* function, in 40% of pancreatic cancer through homozygous deletion, in 40% by an intragenic mutation coupled with loss of the second allele, and in 15% by hypermethylation of the *p16INK4A/CDKN2A* gene promoter [12, 13]. The protein p16 belongs to the cyclin-dependent kinase (CDK) inhibitor family and functions to prevent the phosphorylation of Rb-1 by CDKs, and cyclin D-Cdk4 and cyclin D-Cdk6 complexes, which act as cell-cycle regulators [14, 15]. Loss of *p16INK4A/CDKN2A* results in inappropriate phosphorylation of Rb-1, thereby facilitating progression of the cell cycle through the G1/S transition [16].

Thus, the p16/Rb pathway is inactivated in virtually all pancreatic cancers, leading to an inappropriate progression through the G1 phase of the cell cycle. Of note, in a small group of patients, inherited mutations of the *p16INK4A/CDKN2A* gene cause the familial atypical multiple mole melanoma (FAMM) syndrome, which is associated with an increased risk of developing melanoma and an increased risk of developing pancreatic cancer [17, 18]. Particularly, the *p16* Leiden deletion, a 19-bp deletion, is associated with an increased pancreatic cancer risk [19].

In addition, the homozygous deletions, which inactivate *p16*, can encompass adjacent genes, including the *MTAP*, *IFNA1* and *IFNB1* genes [20, 21]. The *MTAP* gene is located approximately 100 kilobases telomeric to the *p16INK4A/CDKN2A* gene on chromosome 9p21, and is frequently contained in the *p16INK4A/CDKN2A* homozygous deletions. As a result, *MTAP* function is completely lost in approximately 30% of pancreatic adenocarcinomas. This is a potentially promising finding, because it may have therapeutic implications [22]. The product of the *MTAP* gene, the enzyme methylthioadenosine phosphorylase plays an important role in the synthesis of adenosine [23]. Chemotherapeutic agents, such as L-alanosine, a purine biosynthesis inhibitor, have been developed, to specifically target the selective loss of *MTAP* function in cancers, implicating that it might be effective against one third of the adenocarcinomas of the pancreas [22, 23].

Mutation of the *p53* gene on chromosome 17p is the most common somatic alteration in human cancer. The *p53* protein plays a central role in modulating cellular responses to cytotoxic stress by contributing to both cell-cycle arrest and programmed cell death. Loss of *p53* function during carcinogenesis can lead to inappropriate cell growth, increased cell survival, and genetic instability [24]. In pancreatic cancer, the *p53* tumor suppressor gene is inactivated in 50–75% of the cases and occurs predominantly through single allelic loss coupled with an intragenic mutation of the second allele [25]. The loss of *p53* means that two critical controls of cell number (cell division and cell death) are deregulated in the majority of pancreatic cancers. Of interest, *14-3-3 $\sigma$* , a *p53*-regulated gene, plays a role in signal transduction, apoptosis, stress response and cytoskeletal organization [26]. *14-3-3 $\sigma$*  is transcribed in response to DNA damage and in a number of cancers it is an important mediator of *p53*-induced G2 arrest [27].

In addition, *p53*-induced growth arrest is also achieved by transactivation of *p21*. *p53* binding to DNA

stimulates production of the protein *p21*, which negatively regulates the complex consisting of cyclin D and the cell division-stimulating protein cyclin-dependent-kinase-2 [28], thereby preventing the cell from progressing from G1-S phase. This mechanism allows time for repair to damaged DNA. If *p53* mutated, it is not able to bind DNA, so *p21* is not made available and abnormal growth can occur. Cell lines which lack wild-type *p53* show a reduced or complete absence of *p21* [29]. Loss of *p21* activity has been observed in approximately 30–60% of pancreatic tumor specimens [30–32]. Pancreatic cell lines and pancreatic tumors show a correlation between active *p53* and *p21* [33].

As stated, *p53* loss is a ‘double threat’, because it results in both loss of cell cycle checkpoints, as well as deregulation of programmed cell death (i.e. apoptosis). It is now known that *p53*-induced apoptosis is mediated by activation of genes involved in the apoptotic pathway, for example genes such as *PUMA* (*p53*-upregulated modulator of apoptosis) and *Noxa*. *PUMA* and *Noxa* are activated in a *p53*-dependent manner following DNA damage. Once activated, they bind to Bcl-2, localize to the mitochondria to induce cytochrome c release, and activate the induction of programmed cell death [34–36].

Finally, the micro-RNA miR-34a deserves mention (miRNAs in general are discussed later): miR-34a is a direct transcriptional target of *p53*. MiR-34a activation can recapitulate elements of *p53* activity, including induction of cell-cycle arrest and promotion of apoptosis, and loss of miR-34a can impair *p53*-mediated cell death [37, 38]. Chang et al. [39] showed that reduced expression of miR-34a is a very frequent feature of pancreatic cancer cells.

*DPC4* (*Smad4*) is a tumor suppressor gene on chromosome 18q and is one of the most commonly inactivated genes in pancreatic ductal adenocarcinoma, detected in approximately 55% of the cases. Inactivation occurs either through homozygous deletion, in approximately 30%, or loss of one allele coupled with an intragenic mutation in the second allele in approximately 25% [40–42]. The transcription factor SMAD4 is an important regulator of the transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling pathway [43]. Upon receptor activation, SMAD proteins become phosphorylated and heterodimerize with Smad4 to transmit upstream signals to the nucleus and transactivate transcription of specific target genes [44]. Loss of *SMAD4/DPC4* interferes with the intracellular signaling cascades downstream from TGF- $\beta$  and activin, resulting in decreased growth inhibition via loss of pro-apoptotic signaling or inappropriate G1/S transition [43,

45]. The *SMAD4* gene is remarkable for two reasons. First, inactivation of the *DPC4* gene is relatively specific to pancreatic cancer, although it occurs with low incidence in other cancers, such as colon, breast, and ovarian or biliary tract carcinomas [46, 47]. Secondly, immunohistochemical labeling for Smad4 protein expression mirrors *DPC4/SMAD4* gene status in pancreatic cancers with rare exceptions [42]. Inactivation of *DPC4/SMAD4* is uncommon in nonductal neoplasms of the pancreas [10], and is rare in most extrapancreatic malignancies [10, 46]. Therefore, immunolabeling for loss of Smad4 is a convenient ancillary diagnostic marker in clinical specimens, including suspected metastases from an occult pancreatic primary.

Many other tumor suppressor genes that are targeted at low frequency in pancreatic cancer (<10%) deserve mentioning. Mutations in the *LKB1/STK11* gene are the cause of the autosomal-dominant inherited Peutz-Jeghers syndrome. Patients with Peutz-Jeghers syndrome have an increased risk of pancreatic cancer and it is conceivable that *LKB1* acts as tumor suppressor gene in pancreatic cancer as well [48, 49]. Intragenic mutations and homozygous deletions of the *MKK4* gene occur in a small percentage of pancreatic cancers [50]. The *MKK4* gene encodes for a component of a stress-activated protein kinase cascade and has a function in apoptosis and growth control. Furthermore, *MKK4* is preferentially inactivated in subsets of pancreatic cancer metastases, suggesting that the protein product may function as a metastasis suppressor [51]. Other less frequently affected tumor suppressor genes include the *TGF- $\beta$ /activin* signaling pathway receptors such as the TGF- $\beta$  type I receptor (*TGF- $\beta$ R1*; *ALK5*; chromosome 9q), *TGF- $\beta$ R2* (chromosome 3p), *ACVR1 $\beta$*  (*ALK4*; chromosome 12q) [52] and *ACVR2* (chromosome 2q) [53, 54]. The *TGF- $\beta$ R1* *ALK5* forms a heterodimer with the TGF- $\beta$  type II receptor (*TGF- $\beta$ R2*) to mediate signaling of TGF- $\beta$  ligands. A downstream component of this pathway includes *DPC4* (*SMAD4*). Signaling initiated after binding of TGF- $\beta$ -related ligands to their cognate receptors leads to heteromerization and nuclear translocation of the Smad proteins and the transcriptional activation of target genes [55, 56]. TGF- $\beta$  is a pleiotropic factor that regulates cell proliferation, angiogenesis, metastasis, and immune suppression. The involvement of the TGF- $\beta$  pathway has been established in cancers of many organs including the breast, lung, colon and pancreas. TGF- $\beta$  signaling is frequently attenuated in pancreatic cancer because of alterations in the components of the pathway [57, 58].

## Oncogenes

Oncogenes are genes that contribute to oncogenesis when mutationally activated. In contrast to tumor suppressor genes they act in a dominant fashion, i.e. mutation of one copy of the gene suffices for activation. Oncogenes can be activated through a variety of mechanisms, including point mutations within the gene and amplification of the gene itself. A growing number of oncogenes have been identified that are targeted in pancreatic cancer.

The most common activating point mutation involves the *KRAS2* oncogene, on chromosome 12p, in over 90% of pancreatic ductal adenocarcinomas [59, 60] (table 1). This is the highest fraction of K-ras alteration found in any human tumor type. Frequent mutation sites involve codons 12, 13 and 61, but in pancreatic ductal cancers the majority occur in codon 12. The *KRAS* gene product mediates signals from growth factor receptors and other signal inputs. Mutation of *KRAS* results in a constitutive gain of function, because the RAS protein remains trapped in the activated state even in the absence of growth factor signals, which leads to proliferation, suppressed apoptosis and cell survival.

The RAS family proteins encode small GTP-binding cytoplasmic proteins [44]. The constitutively active RAS intrinsically binds to GTP and confers uncontrolled stimulatory signals to downstream cascades including Ras effectors. Activated *KRAS* engages multiple effector pathways, notably the RAF-mitogen-activated protein kinase, phosphoinositide-3-kinase (PI3K) and RalGDS pathways.

Mutant *KRAS* has been extensively investigated as a marker of pancreatic cancer because mutations are basically entirely limited to one codon, can be readily detected using molecular assays and are present in approximately 90% of pancreatic ductal adenocarcinomas. Unfortunately, *KRAS* mutations are not specific for invasive pancreatic cancer and they occur in patients with chronic pancreatitis, in individuals who smoke, and in situ neoplasias from patients without pancreatic cancer [61, 62].

The *BRAF* gene on chromosome 7q is a member of the RAS-RAF-MEK-ERK-MAP kinase pathway, and is mutated in one-third of the pancreatic cancers with wild-type (normal) *KRAS* [63]. *BRAF*, a serine/threonine kinase located immediately downstream in RAS signaling, is a frequent mutational target in several cell lines and nonpancreatic primary cancers including 66% of melanomas and 10% of colorectal carcinomas [64, 65]. Interestingly, *KRAS* and *BRAF* mutations are mutually exclu-

sive and tumors with mutant forms of one of these 2 genes invariably retain wild-type copies of the other. The requirement of oncogenic *KRAS* or *BRAF* pathway-related signal transduction appears to be critically important for most instances of pancreatic ductal carcinogenesis.

The PI3K-kinase-AKT pathway is a key effector of RAS-dependent transformation of many cell types and plays a role in cell survival, cell proliferation and other growth-related processes [66]. Activated PI3K results in phosphorylated phosphatidylinositides (PIP3), a step inhibited by product of the tumor suppressor gene, *PTEN*. PIP3 in turn phosphorylates and activates AKT [29]. Recently activating mutations of *PIK3CA*, the gene encoding PI3K, have been reported in a subset of pancreatic cancer precursors, specifically in IPMNs [67]. Even in the absence of mutations, the PI3K/AKT pathway is constitutively active in the majority of pancreatic cancers [68]. This might be due to the aberrant expression of their natural antagonist *PTEN* [69]. Although *PTEN* is not mutated in pancreatic cancers, the reduction of its expression may give pancreatic cancer cells an additional growth advantage [70]. Furthermore, amplification or activation of *AKT2* kinase, a major target of the PI3K complex, occurs in up to 60% of pancreatic cancers [71–74], supporting the participation of an activated PI3K-AKT axis in this disease.

A third downstream pathway activated through *RAS* is the RalGDS pathway. RalGDS is one of several known Ras-regulated guanine-nucleotide exchange factors, or *GEFs*, that function by activating Ral A and Ral B GTPases [75]. Recently, RAL A was shown to be activated in a variety of pancreatic cancers, and knockdown of *RAL A* suppressed tumorigenicity of RAS-transformed human cells [76]. In the same studies, knockdown of *RAL B* had no effect on tumor initiation, but suppressed tumor progression (i.e. metastases), suggesting divergent roles for the two RAL proteins in the context of pancreatic neoplasia. Whether or not these signaling moieties can be utilized as therapeutic targets remains to be determined.

The mammalian Hedgehog family of secreted signaling proteins – comprised of Sonic, Indian, and Desert Hedgehog (Shh, Ihh, and Dhh) – regulates the growth and patterning of many organs, including the pancreas, during embryogenesis [77]. The Hedgehog pathway is under negative regulation by the Patched (PTC) tumor suppressor protein that inactivates the Smoothed (SMO) protein. The Hedgehog ligands engage the PTC transmembrane protein, disrupting the inhibition of SMO and thereby enabling signaling transduction to the *GLI* fam-

ily of transcriptional regulators [78]. Loss of *PTC*, activating mutations in *SMO* and overexpression of *GLI* and Hedgehog proteins are associated with a variety of cancers [79]. Activation of the Hedgehog pathway has been implicated in both the initiation of pancreatic ductal neoplasia and in the maintenance of advanced cancers [80]. The expression of the Hedgehog ligands, the transcriptional target gene *PTC*, and the essential pathway component *SMO* is undetectable in normal human pancreatic ducts. In contrast, a relative increase in the expression of these proteins is observed during pancreatic ductal tumorigenesis [78, 81, 82]. Moreover, it has been confirmed that the Hedgehog pathway plays a role in metastases. Inhibition of Hedgehog signaling has been shown to reduce the incidence of systemic metastasis in pancreatic adenocarcinoma xenografts [83]. Recently, Ji et al. [84] showed that there is a cross-talk between oncogenic *KRAS* and the Hedgehog signaling pathway in pancreatic cancer cell lines. Their studies suggest that oncogenic *KRAS* through the *RAF/MEK/MAPK* pathway suppresses *GLI1* protein degradation and consequently plays an important role in activating Hedgehog signaling pathway in the absence of additional Hedgehog ligand during pancreatic tumorigenesis.

The Notch signaling pathway is another pathway which is important in directing cell fate and cell proliferation during embryonic development. Later in life, the Notch signaling pathway plays a critical role in maintaining the balance among cell proliferation, differentiation, and apoptosis [85]. In mammals, this signaling pathway involves interaction of the membrane-bound Notch receptors (Notch 1–4) and Notch ligands (Delta-like, and Jagged) on adjacent cells [85, 86]. The function of Notch signaling in tumorigenesis can be either oncogenic or antiproliferative, and the function is context dependent. In a limited number of tumor types, including human hepatocellular carcinoma and small cell lung cancer, Notch signaling is antiproliferative rather than oncogenic. However, most of the studies show an opposite effect of Notch in many human cancers including pancreatic cancer [87]. In the normal adult pancreas, Notch and its ligands are expressed at low levels. Interestingly, aberrant expression of its ligands, expression of mutant Notch1 oncoprotein, and abnormal expression of transcription targets of Notch signaling can be observed in early stages of pancreatic tumorigenesis as well as in invasive pancreatic cancer [88].

Several other oncogenes that are targeted in pancreatic cancer by amplifications deserve mentioning. First, the *AKT2* gene on chromosome 19q is a downstream effector of the PI3K/AKT pathway, and is amplified in 10–

**Table 1.** Frequency of selected tumor suppressor genes, oncogenes and genome maintenance genes

Gene mutations	Incidence in pancreatic adenocarcinoma, %
<i>p16</i>	80–95
<i>p53</i>	50–75
<i>DPC4</i>	45–55
<i>K-RAS</i>	75–90
<i>BRAF</i>	5–10 (estimated)
<i>hMLH1, hMSH2</i>	4
<i>BRCA2</i>	7–10

15% of pancreatic cancers [73, 89]. *AKT2* can be activated by stimuli such as platelet-derived growth factor, basic fibroblast growth factor, and insulin through the *PI3K/AKT* pathway, suggesting this pathway's importance in this tumor type [72]. Secondly, the *AIB1* gene on chromosome 20q is amplified in approximately 60% of pancreatic cancers [90]. The nuclear receptor coactivator amplified in breast cancer 1 (*AIB1/SRC-3*) belongs to the p160/steroid receptor coactivator family (*SRC*) [91]. *AIB1* amplification and/or overexpression is not only detected in hormone-sensitive tumors, such as breast, prostate and ovarian, but it is also found in nonsteroid-targeted tumors such as pancreatic cancer, colorectal carcinoma and hepatocellular carcinoma [92]. Thirdly, the *MYB* gene on chromosome 6q is amplified in 10% of pancreatic carcinomas [93]. Abnormalities in the locus of the human *MYB* gene have been observed in several human cancers. In a majority of these tumors, these abnormalities seem to be accompanied by an amplification of the *MYB* gene followed by enhanced transcription [94].

### Genome Maintenance Genes

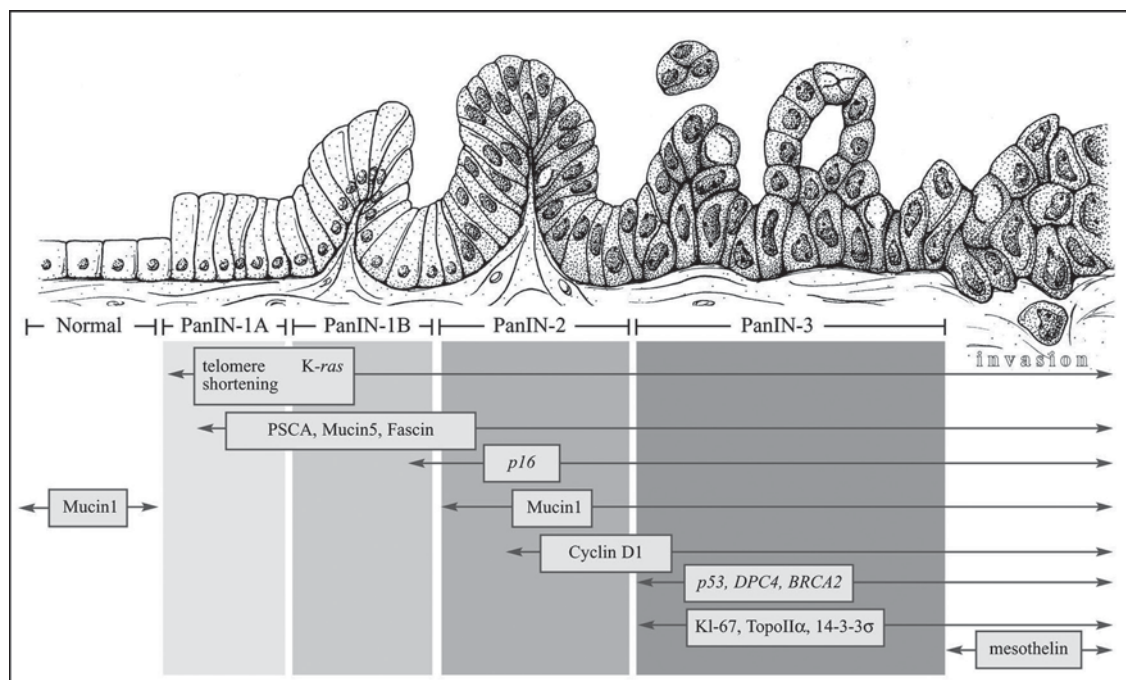
Genome maintenance genes are those that function to identify and repair damage to DNA. When a genome maintenance gene is inactivated, DNA damage is not repaired efficiently and DNA mutations accumulate. If these mutations occur in cancer-associated genes they can contribute to tumorigenesis [90]. Although gross chromosomal abnormalities are frequent in pancreatic ductal adenocarcinomas, genetic instability also occurs through DNA mismatch repair defects [95]. The DNA mismatch repair genes *hMLH1* and *hMSH2* are examples of genome maintenance genes targeted in pancreatic cancer [96]. When one of these genes is inactivated, DNA

changes occur leading to 'microsatellite instability' (MSI). MSI is associated with poor differentiation, lack of *KRAS2* and *p53* mutations, and germline mutations of this gene are associated with the human nonpolyposis colorectal cancer syndrome (HNPCC) [96–98]. Approximately 4% of pancreatic cancers have MSI and these cancers have a specific microscopic appearance called 'medullary type', which includes a syncytial growth pattern, pushing borders and lymphocytic infiltrate [96].

The causative genes of Fanconi anemia, *FANCC* and *FANCG*, also play a role in pancreatic tumorigenesis [99]. Fanconi anemia is a hereditary cancer susceptibility disorder, with the occurrence of hematologic abnormalities or acute myelogenous leukemia at an early stage, usually leading to death before the age of 20. Patients who survive into adulthood often develop solid tumors [99]. The *BRCA2* gene represents Fanconi complementation group D1 and is thought to aid DNA strand and interstrand crosslinking repair. *BRCA2* has therefore been categorized as genome maintenance gene rather than a standard tumor suppressor. In ductal pancreatic cancers 7–10% harbor an inactivating intragenic inherited mutation of one copy of the *BRCA2* gene, accompanied by loss of heterozygosity [100, 101]. Of interest, it has been shown that the presence of *BRCA2*/Fanconi anemia gene mutations in pancreatic cancer may make them particularly sensitive to chemotherapeutic agents that cause DNA crosslinks such as Mitomycin C, because these cancers are unable to repair DNA interstrand crosslinks [102].

### Growth Factors

Several of the genes known to be overexpressed in pancreatic cancer include growth factors and their receptors. Growth factors are the proteins that control cell differentiation and proliferation. Disturbances in growth inhibition and an abundance of growth-promoting factors give cancer cells a distinct growth advantage, which clinically results in rapid tumor progression. The epidermal growth factor receptor (EGFR) is overexpressed and plays a distinct role in pancreatic cancer. The four receptors of the EGF family are membrane-spanning glycoproteins composed of an amino terminal extracellular ligand-binding domain, a hydrophobic transmembrane region and a cytoplasmic domain that contains both the tyrosine kinase domain as well as the receptor [103]. The classical EGF receptor is also known as HER1 or ErbB-1. The remaining three receptors are designated HER-2/Neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4).



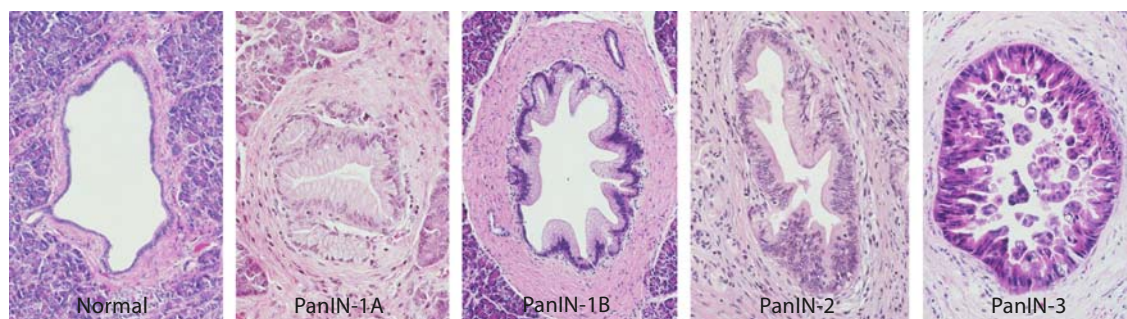
**Fig. 1.** Progression model of pancreatic ductal adenocarcinoma from normal (left) to carcinoma (right). The histological progression is associated with the accumulation of specific genetic alterations. Reprinted with permission from Maitra et al. [164].

HER-2/neu overexpression is most prominent in well-differentiated ductal adenocarcinoma, as well as in the early-stage precursor lesions, and appears to correlate with the grade of dysplasia in the precursor lesions [104, 105]. In pancreatic cancer, *HER-2/neu* amplification has been observed with a variable incidence of 10–60% [106, 107]. In addition, increased levels of fibroblast growth factor (FGF), FGF-receptor, insulin-like growth factor I (IGF-I), IGF-I receptor, nerve growth factor, and vascular endothelial growth factor (VEGF) are also reported in pancreatic cancer [108, 109].

Tumor growth requires accompanying expansion of the host vasculature with tumor progression, which is often correlated with vascular density. VEGF is the best-characterized inducer of tumor angiogenesis. Interestingly, Delta-like ligand 4 (Dll4), a Notch ligand, is dynamically regulated by VEGF [110]. Several studies demonstrated that Dll4 may act downstream of VEGF as a ‘brake’ on VEGF-mediated angiogenic sprouting [111]. Dll4, a transmembrane ligand for the Notch family of receptors, is induced by VEGF as a negative feedback regulator and acts to prevent overexuberant angiogenic sprouting [112].

### Telomere Shortening

Defective telomeres may be the major cause of the chromosomal instability observed in many cancers and in the vast majority of pancreatic cancers [113]. Telomeres are structures at the end of linear chromosomes that normally function to protect the terminal sequences and prevent the ends of chromosomes from joining aberrantly [114, 115]. Telomeres serve as protective ‘caps’ and are composed of short repeated DNA sequences and associated proteins. It appears that telomeres become abnormally short very early in the development of pancreatic neoplasia [114]. These shortened telomeres can presumably lead to the abnormal fusion of chromosome ends and in this fashion to chromosome instability, promoting further neoplastic progression in these cells [90]. Such a chromosome fusion leads to so-called anaphase bridges during mitosis [116]. These anaphase bridges frequently break during cellular replication, generating unstable chromosome ends that are subject to abnormal fusion events and subsequent chromosomal rearrangements [117]. This process, called breakage-fusion-bridge cycles, has been observed in pancreatic cancers and is



**Fig. 2.** Consecutive PanIN lesions with progressive histological changes from normal to PanIN-3. Reproduced with permission from [http://pathology.jhu.edu/pancreas\\_panin](http://pathology.jhu.edu/pancreas_panin).

believed to be one of the major causes underlying loss of function of tumor suppressor genes and the gain of function of oncogenes as described earlier [90]. In most instances, cells harboring this degree of genomic instability are eliminated through activation of *p53*. However, chromosomal rearrangements likely persist in cells with *p53* mutations, and these cells will then quickly accrue further genomic alterations [118]. Thus telomere dysfunction and *p53* loss cooperate to promote the development of carcinomas in multiple tissues [79]. Chromosomal instability provides a tumor with the genetic diversity to overcome certain barriers in carcinogenesis. However, ultimately, chromosomal instability might prove counterproductive to tumor growth, which may explain why neoplasms seem to acquire mechanisms to elongate their telomeres at later stages in the development of a malignancy, often through the reactivation of the enzyme telomerase, or through alternate lengthening of telomeres [119].

### Familial Pancreatic Cancer

In the majority of cases, cancer is a multifactorial disorder in which genetic and environmental factors interact to initiate carcinogenesis. However, in a minority, the disease follows a familial pattern of transmission, suggesting a hereditary cancer syndrome. Characterization of the genetic mutations segregating in such families has helped to elucidate the molecular events that underlie tumorigenesis in the more common multifactorial form of the disease. Elucidation of the mechanisms of hereditary colorectal cancer and breast/ovarian cancer syndromes represents some of the greatest triumphs of the last century in the field of cancer genetics.

It has been estimated that 10% of pancreatic cancers have a familial basis [120, 121]. Having a first-degree relative with pancreatic cancer doubles the risk of developing pancreatic cancer [122], and the risk increases with increasing numbers of affected relatives [123]. Segregation analyses have suggested that an autosomal dominant pattern of inheritance is the most parsimonious genetic model for this increased risk [124], but the gene responsible for the familial aggregation of pancreatic cancer in the majority of cases has not yet been identified [125]. In different countries familial pancreatic cancer registries have been established to investigate the epidemiology and genetic background of these families, and to organize the screening programs for high-risk relatives and for follow-up. The largest such registry, the National Familial Pancreas Tumor Registry, is located at the Johns Hopkins Medical Institutions, Baltimore, Md., USA (<http://pathology2.jhu.edu/pancreas/nfptr.cfm>) [125].

To date, at least five hereditary disorders that significantly increase the risk of pancreatic cancer have been described. These include familial breast/ovarian cancer syndrome (caused by inherited mutations in the *BRCA2* gene), the FAMM syndrome (caused by germline mutations in the *p16* gene), the Peutz-Jeghers syndrome (caused by inherited mutations in the *STK11/LKB1* gene), hereditary pancreatitis (caused by germline mutations in the *PRSS1* gene), and hereditary HNPCC caused by mutations in *hMLH1* or *hMSH2*.

Familial breast/ovarian cancer syndrome is associated with an increased risk of breast cancer in men and women, and a subset of these families also harbor an increased risk for pancreatic cancer [126]. Germline mutations of the *BRCA2* gene, residing on 13q12–13, are identified in 4–17% of familial pancreatic cancer, with a particular propensity for occurring in families of Ashkenazi Jewish

heritage [100, 127]. As mentioned earlier, the protein product of the *BRCA2* gene has been shown to interact with protein products of several of the Fanconi anemia genes and to function in the repair of double-strand DNA breaks [99].

The FAMM syndrome is an autosomal dominant disorder characterized by the familial occurrence of multiple melanocytic naevi, atypical naevi, and an increased risk of both melanoma and pancreatic cancer [128, 129]. FAMM can be caused by germline mutations in the *p16/CDKN2A* gene on chromosome 9p. The carriers of the germline *p16*-Leiden mutation have an estimated risk of 17% to develop pancreatic cancer by the age of 75 years [19, 130].

The Peutz-Jeghers syndrome is a rare, autosomal dominant condition characterized by the development of hamartomatous gastrointestinal polyps, mucocutaneous pigmentation and high lifetime risk of developing cancer, affecting both gastrointestinal and extragastrointestinal sites. The lifetime risk of developing pancreatic cancer is approximately 36% [131]. In 50% of families the pathogenesis is caused by germline mutations occurring in the *STK11/LKB1* gene [48, 132].

Hereditary pancreatitis is characterized by the familial occurrence of pancreatitis with an early age of onset [133]. Germline mutations in the *PRSS1* gene cause an autosomal dominant form of the disease, whereas germline mutations in *SPINK1* lead to an autosomal recessive pattern of inheritance. An estimated 40% of patients with familial pancreatitis will develop pancreatic cancer by the age of 70 years [134].

HNPCC has an autosomal dominant pattern of inheritance, it affects approximately 1 in 200 persons and is associated with multiple forms of cancer, most importantly colorectal, but also gastric, endometrial, and pancreatic cancer [135]. As discussed before, HNPCC is caused by mutations in one of the DNA mismatch repair genes. The group of individuals with a known predisposing familial syndrome, and with a history of familial pancreatic cancer would be among the first to benefit from screening tests for early detection of pancreatic cancer.

### Mouse Models of Pancreatic Cancer

Although the pancreas was the first organ where transgenesis was attempted over two decades ago [136], the development of a mouse model that faithfully recapitulates the multistep progression of human pancreatic adenocarcinoma has been elusive. In 2003, Hingorani et al.

[137] developed a mouse model of pancreatic neoplasia by conditional mis-expression of mutant *KRAS* in the pancreas from its endogenous promoter. The bitransgenic mice express a 'knock-in' *Kras*<sup>G12D</sup> upon Cre-mediated recombination and removal of a lox-STOP-lox allele within the *Pdx1* expression domain. *Pdx1* is a transcription factor that is expressed in the developing pancreas and foregut, restricting mutant *KRAS* expression to these organs. The *Pdx1*-Cre, lox-STOP-lox-*Kras*<sup>G12D</sup> mice develop the entire histologic compendium of murine PanIN (mPanIN) lesions observed in the cognate human disease, and a subset of mice develop invasive pancreatic carcinomas as well. Subsequent models have utilized additional cooperating mutations with *Kras* (for example, an oncogenic Trp53<sup>R172H</sup> allele or biallelic deletions of *INK4a/Arf*) – these compound transgenic mice develop metastatic pancreatic cancers with near-universal penetrance, and represent biologically relevant models of advanced pancreatic cancer in humans [138–140].

Several important lessons have been learnt from these newly developed mouse models of pancreatic cancer. First, these studies indicate the likely absolute requirement of mutant *Kras* in order to initiate pancreatic neoplasia along the mPanIN pathway, which might also explain the extremely high frequency of *KRAS* abnormalities in human PanIN lesions and pancreatic cancer [141]. Thus, misexpression of other oncogenes by themselves results in pancreatic 'cancer' in mice (for example, aberrant expression of the Hedgehog transcription factor *GLI2*) [82], but it is only upon coexpression with mutant *Kras* that these mice develop cancers preceded by mPanINs. Second, the expression of mutant *Kras* from its endogenous promoter appears to be a prerequisite as well, since earlier models of transgenic *Kras* expression have resulted in cancers of acinar histogenesis without mPanIN formation [142]. Third, these mouse models have helped elucidate some insights into the putative cell-of-origin of pancreatic cancer. For example, recent studies by Guerra and colleagues have demonstrated that mPanINs and adenocarcinomas can be reproduced in the pancreas of adult mice by conditional misexpression of mutant *Kras* to the elastase-expressing acinar/centroacinar compartment [143]; the one caveat is that the mature acinar/centroacinar compartment appears to be resistant to the oncogenic transformation unless accompanied by an ongoing injurious stimulus (i.e. chronic pancreatitis). These studies provide remarkable experimental reiteration to the longstanding epidemiological associations between chronic pancreatitis and an increased incidence of pancreatic cancer [3]. They also underscore the possibility that the mon-

iker of 'ductal' adenocarcinoma might not reflect the true histogenesis of these cancers, at least in the context of murine pancreatic neoplasia. Fourth, and not the least, the development of these models have provided an unprecedented opportunity to explore preclinical diagnostic and therapeutic strategies in autochthonous models not afforded by short-term xenograft studies. For example, the cancers developing in these mice recapitulate not only the morphology of the cognate human disease, but also many of the oncogenic signaling pathways like *EGFR*, *Notch* and *Hedgehog* [137, 140]. Small molecule inhibitors targeted against these pathways can now be tested in the transgenic models prior to clinical trials. There is little doubt that the development of these models has fulfilled a critical lacuna on the field of pancreatic cancer research.

### Molecular Biomarkers and Therapy

The gene expression patterns in pancreatic cancer have been studied using multiple platforms. A decade ago, gene expression was studied through analysis of the product of one gene at a time. Currently, gene expression patterns can be studied using technologies that assay nearly the entire genome simultaneously. Examples of such technologies that have been applied to pancreatic cancer include serial analysis of gene expression, cDNA arrays and oligonucleotide arrays [144–147]. The protein products of differentially expressed genes have proven useful as diagnostic markers in tissue biopsies, as serum markers, and as therapeutic targets. For example, prostate stem cell antigen and mesothelin were identified to be overexpressed in the majority of pancreatic cancers by serial analysis of gene expression, and immunolabeling for these two proteins can be used to aid in the interpretation of challenging pancreatic biopsies [148, 149]. Similarly, osteopontin was identified as overexpressed in pancreatic carcinoma using oligonucleotide microarrays, and serum osteopontin levels have a sensitivity of 80% and a specificity of 97% for pancreatic cancer [150].

Recently, micro-RNAs (miRNAs), a novel class of 18–23 nucleotide noncoding RNAs, have gained attention as another family of molecules involved in cancer development. Current evidence has illustrated that miRNAs are misexpressed in various human cancers, and further indicates that miRNAs can function as tumor suppressors ('TSGmiRs') or oncogenes ('oncomiRs') [151, 152]. Upon binding to their target RNAs, miRNAs cause posttranscriptional gene silencing by either cleaving the target mRNA or by inhibiting the translation process [153].

As several studies have highlighted, miRNA expression is deregulated in pancreatic cancer. A miRNA signature of pancreatic cancer has been elucidated, and it includes the upregulation of miR-21, miR-155, miR-221 and miR-222 [154, 155]. Moreover, Chang et al. [39] found that miR-34a is frequently lost in pancreatic cancer cell lines. These studies demonstrate that miRNAs may become useful biomarkers for pancreatic cancer diagnostics. In addition, these aberrantly expressed miRNAs might be useful as potential therapeutic targets, with the recent availability of in vivo miRNA knockdown strategies ('antagomirs') [156].

The revolution in our understanding of the genetics of cancer and the exploration of gene expression on a large scale has brought with it the hope that novel therapies can be developed specifically exploiting the genetic deletions and resultant absolute biochemical deficiencies present in pancreatic cancer. Two promising examples of therapies using a specific biochemical difference, including mitomycin C for pancreatic cancers harboring *BRCA2* gene mutations and L-alanosine, a purine biosynthesis inhibitor, for pancreatic cancers with loss of *MTAP* function were already mentioned above.

The downregulation of Notch signaling could also be a novel therapeutic approach for pancreatic cancer. Numerous studies have proposed inhibition of Notch signaling as a strategy for cancer treatment, such as with the pharmacological block of  $\gamma$ -secretase enzyme with small molecule inhibitors, which has a striking antineoplastic effect in Notch expressing transformed cells in vitro and in xenograft models [157]. Inhibitors of  $\gamma$ -secretase prevent the second ligand-induced proteolytic cleavage of the Notch receptor, thereby blocking the Notch signaling pathway. Importantly, in pancreatic cancer cells it has been shown that downregulation of Notch1 inhibits cell growth and induces apoptosis [87]. In other compartments of the gastrointestinal tract, notably the colorectum and the esophagus, regression of tumorigenesis is observed after chemical inhibition of Notch [158, 159].

Furthermore, developmental signaling pathways, like the Hedgehog signaling pathway, have emerged as therapeutic targets in pancreatic cancers [160]. This pathway is aberrantly activated in the majority of pancreatic ductal adenocarcinomas [78]. Drugs such as cyclopamine which specifically inhibit the Hedgehog pathway have been shown to be effective in xenograft models of human pancreatic cancer in treated mice [81]. Interestingly, the realization of cross-talk between *RAS/MAPK* and Hedgehog signaling pathways in pancreatic carcinomas also suggest that targeting the *RAS* and Hedgehog pathways

synergistically may represent a new therapeutic strategy [84]. Additionally, there are a few promising agents on the therapeutic horizon, being tested in clinical trials, like bevacizumab, the monoclonal antibody against VEGF, which targets tumor vascularization and cetuximab, the monoclonal antibody against the EGFR [161]. Of note, trastuzumab (Herceptin®) is a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor, a member of the EGFR family, and shows profound beneficial results with breast cancer patients whose tumors overexpress this receptor [103]. Whether trastuzumab will be as effective a form of treatment in pancreatic cancer as it appears to be in breast cancer, is currently the focus of several studies [162, 163].

### Future Perspectives

Intensive research over the last two decades has shown that pancreatic cancer is fundamentally a genetic disease, caused by inherited germline and/or acquired somatic mutations in cancer-associated genes. It has uncovered multiple alterations in many genes that are important in

pancreatic cancer progression. In addition, an increased understanding of the molecular basis of the disease has provided the identification of new drug targets enabling rational drug design, and facilitated the production of animal models of the disease on which such therapies can be tested.

Pancreatic ductal adenocarcinoma is nevertheless still one of the most lethal cancers of all human malignancies. The poor prognosis and late presentation of pancreatic cancer patients emphasize the importance of early detection, which is the sine qua non for the fight against pancreatic cancer. It is hoped for the future that the understanding of genetic alterations in combination with the development of high-throughput sensitive techniques will lead to the rapid discovery of an effective biomarker.

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