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Morphogenesis of pancreatic cancer: role of pancreatic intraepithelial neoplasia (PanINs)

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Abstract

Introduction—Pancreatic ductal adenocarcinoma (i.e., pancreatic cancer) is an almost universally lethal disease. The identification of precursor lesions of pancreatic cancer provides an opportunity for early detection and potential therapeutic intervention before the development of invasive cancer.

Discussion—It is now established that pancreatic cancers do not arise de novo but rather exhibit a sequential histological and genetic progression of precursor lesions culminating in frank, invasive neoplasia. Pancreatic intraepithelial neoplasia (PanIN) is the most common non-invasive precursor lesion of pancreatic cancer. The development of a consensus nomenclature scheme for PanINs has facilitated research into pancreatic cancer precursors and enabled standardization of results across institutions.

Conclusion—PanINs harbor many of the molecular alterations observed in invasive pancreatic cancer, confirming their status as true non-invasive precursor lesions. Recently developed genetically engineered mouse models of pancreatic cancer also demonstrate the stepwise PanIN progression model, underscoring the commonalities in pancreatic neoplasia between mouse and man.

Keywords

Pancreatic cancer; Pancreatic intraepithelial neoplasia; PanIN; Genetics

Introduction

Pancreatic cancer is a disease with a dismal prognosis. In the United States, approximately 33,000 patients are diagnosed with pancreatic cancer annually, and nearly an equal number will die from their malignancy. Worldwide pancreatic cancer causes an estimated 213,000 deaths each year [1]. For all stages combined, the 1-year survival rate is around 20%, and the overall 5-year survival rate is only 4% despite the availability of improved surgical and medical avenues [2,3].

The high mortality rate for pancreatic cancer is primarily because of the advanced stage at which the neoplasm is diagnosed and because there are no sensitive and specific tools to detect the disease at an earlier stage. More than 80% of the patients with pancreatic cancer have locally advanced or distant metastatic disease at the time of diagnosis, rendering their malignancies surgically inoperable. Currently, 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

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patients undergoing surgical resection [4,5]. Even if pancreatic cancer is diagnosed early and surgical resection with curative intent is performed, nearly all patients develop local recurrence and/or distant metastases after surgery and eventually succumb to the debilitating effects of metastatic growth [6]. Unfortunately, conventional therapeutic modalities like chemo-radiation have had minimal impact, and the long-term survival of patients with pancreatic cancer has not improved in the last five decades [7,8].

Improved patient survival has been achieved in a variety of epithelial neoplasms (e.g., colorectal, lung, breast, cervix, and prostate cancer), largely because of identification of cancers at their primary anatomic sites at an early, often pre-invasive stage [9,10]. At this moment, however, there is no equivalent of a “Pap smear” or a “PSA test” for pancreatic cancer, which can conveniently detect early neoplasia. Nevertheless, it is now recognized that, analogous to other epithelial cancers, pancreatic cancers do not arise de novo but rather undergo a stepwise progression through histologically well-defined non-invasive precursor lesions, culminating in frank, invasive neoplasia. Although putative precursor lesions of pancreatic cancer were first documented over a century ago [11], it was only in the latter half of the last century that multiple lines of evidence began to coalesce, associating invasive pancreatic cancer with these lesions. For example, meticulous autopsy studies confirmed that the prevalence of what are now recognized as precursor lesions increased with age, thus paralleling the frequency of invasive pancreatic cancer. Similarly, most surgically resected pancreata harboring invasive cancer also tend to demonstrate non-invasive intra-ductular lesions in the surrounding parenchyma, suggesting an etiologic association [12–14]. Most importantly, careful molecular analyses over the last 10 years have unequivocally demonstrated that these precursor lesions share many of the underlying genetic alterations observed in the infiltrating cancer, underscoring their precursor status [15–17].

By the late 1990s, over 70 different terminologies were in use to describe these non-invasive ductal lesions, leading to considerable difficulties in comparing inter-institutional studies. Therefore, there was a dire need for the establishment of an international nomenclature scheme for precursor lesions of pancreatic adenocarcinomas. In 1999, the National Cancer Institute hosted a Pancreatic Cancer Think Tank at Park City, Utah, from which meeting emerged a consensus nomenclature scheme for precursor lesions of pancreatic cancer. The “*Pan*creatic *I*ntraepithelial *N*eoplasia” (PanIN) scheme for classifying these lesions, first proposed by Klimstra and Longnecker, has since become a gold standard at academic centers worldwide [18,19].

Histology

The detailed histopathological grading of PanIN lesions and their distinction from other neoplastic and non-neoplastic conditions in the pancreas have been described elsewhere [18, 19]. The reader is also directed to a freely accessible “teaching site” on the World Wide Web for this purpose, located at http://pathology.jhu.edu/pancreas_panin. Briefly, PanINs are microscopic lesions in the smaller (less than 5 mm) pancreatic ducts. PanINs can be papillary or flat, and they are composed of columnar to cuboidal cells with varying amounts of mucin. PanINs are classified into a fourtier classification, including PanIN-1A, PanIN-1B (low-grade PanINs), PanIN-2 (intermediate grade PanINs), PanIN-3 (high-grade PanIN), reflecting a progressive increase in histologic grade culminating in invasive neoplasia. The lowest grade PanIN lesions can be flat (1A) or papillary (1B) but are characterized by absence of nuclear atypia and retained nuclear polarity. PanIN-2 lesions are architecturally slightly more complex than PanIN-1 lesions, and they have more nuclear changes including loss of nuclear polarity, nuclear crowding, variation in nuclear size (pleomorphism), nuclear hyperchromasia, and nuclear pseudostratification. Mitoses are rarely seen. In contrast, PanIN-3 lesions, also referred to as “carcinoma-in-situ”, demonstrate widespread loss of polarity, nuclear atypia, and frequent

mitoses. However, as a pre-invasive lesion, PanIN-3 is still contained within the basement membrane [18,19]. As discussed above, PanINs are often present in the pancreatic parenchyma adjacent to infiltrating adenocarcinomas, and several case reports have documented patients with high-grade PanINs in the remnant pancreas who later developed an infiltrating pancreatic cancer [15]. In summary, just as there is a progression in the colorectum from adenoma, to adenoma with dysplasia, to invasive cancer, so too is there histologic and genetic progressions from PanIN-1, to PanIN-2, to PanIN-3, to invasive ductal adenocarcinoma in the pancreas [20].

It is important to note that PanINs are the most common, albeit not the only, recognized precursor lesions for pancreatic cancer. Two “macroscopic” precursor lesions (so called because they present typically as radiologically detectable cysts in the pancreas [21]) are intraductal papillary mucinous neoplasm and mucinous cystic neoplasms (MCNs). Intraductal papillary mucinous neoplasms (IPMNs) are mucin-producing epithelial neoplasms, which arise within the main pancreatic duct or one of its branches, and that often, although not always, have a papillary architecture [19,22]. By definition, IPMNs involve the larger pancreatic ducts. Those that involve the main pancreatic ducts are designated “main duct type”, while those that involve the secondary branches of the main pancreatic duct are designated “branch duct type” [18,19,23]. Two features characterize MCNs at the light microscopic level. First, the cysts are lined by columnar, mucin-containing epithelium. Second, the underlying stroma has the appearance of ovarian stroma, and in fact, expresses hormonal receptors like estrogen and progesterone [24,25]. Similar to PanINs, the cystic precursor lesions also demonstrate a multi-step histological and genetic progression to invasive neoplasia but will not be discussed within the scope of the current review.

As discussed above, the strongest evidence establishing the precursor lesional status for PanINs has been derived from comparative molecular analyses with invasive pancreatic cancer. Herein, we discuss some of the most common seminal alterations that are seen in PanIN lesions and likely contribute to the stepwise genetic progression model of pancreatic cancer.

Oncogene mutations in PanIN lesions

Oncogenes can be activated through a variety of mechanisms including point mutations within the gene and amplification of the gene itself. A growing numbers of oncogenes have been identified that are targeted in pancreatic cancer. The most common activating point mutation involves the *KRAS* oncogene, on chromosome 12p, in over 90% of pancreatic ductal adenocarcinomas [26,27]. This is the highest fraction of 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 [28]. The *KRAS* family proteins encode small GTP-binding cytoplasmic proteins and regulate cell-cycle progression via the mitogen-activated protein kinase and AKT cascades [29]. Activating mutations impair the intrinsic GTPase activity of the *KRAS* gene product, resulting in a protein that is constitutively active in intracellular signal transduction [30]. Mutations of the *KRAS* gene are one of the earliest genetic abnormalities observed in the progression model of pancreatic cancer, demonstrable in approximately 36%, 44%, and 87% of cancer-associated PanIN-1A, PanIN-1B, and PanIN-2/3 lesions, respectively [31]. The frequency of *KRAS* gene mutations is somewhat lower (~10%) in PanIN lesions arising in the backdrop of chronic pancreatitis [32]. Of note is given that PanIN lesions and an adenocarcinoma within the same pancreas may harbor different *KRAS* gene mutations, suggesting that some precursors evolve as independent clones from the one that eventually progress to the invasive cancer [33]. The high frequency of *KRAS* gene mutations in human PanINs supports its role as an initiating event for pancreatic cancer formation. This fact has been reiterated in several recent animal models (see discussion below) where expression of mutant Kras is a prerequisite for the development of ductal pre-neoplasia

and cancer [34,35]. In addition to its role in pancreatic cancer initiation, constitutive RAS signaling appears to be required for pancreatic cancer maintenance as well [36].

Tumor-suppressor gene mutations in PanIN lesions

Tumor-suppressor genes are genes that promote tumor growth when inactivated. Tumor-suppressor genes are recessive, which means that 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, by deletion of both alleles (homozygous deletion); and third, by hypermethylation of the promoter of the gene, thus 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. Three tumor-suppressor genes, *p16INK4A/CDKN2A*, *TP53*, and *DPC4/SMAD4/MADH4*, are inactivated in a significant proportion of PanINs, mirroring their relative frequencies of loss of function in invasive adenocarcinomas.

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 [37]. 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 [37,38]. The *p16INK4A/CDKN2A* gene encodes the cell-cycle checkpoint protein p16, which binds to the cyclin-dependent kinases Cdk4 and Cdk6, thereby inhibiting binding of cyclin D1, resulting in G1-S cell-cycle arrest [39]. Loss of *p16INK4A/CDKN2A* results in inappropriate phosphorylation of retinoblastoma (Rb)-1, thereby facilitating progression of the cell cycle through the G1/S transition [40]. 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. Loss of p16 expression is also seen in cancer-associated PanINs, with 30% of PanIN-1A and PanIN-1B, 55% of PanIN-2, and 71% of PanIN-3 lesions, demonstrating loss of nuclear p16 protein expression [41]. In contrast, loss of p16 expression is less frequently observed in PanIN lesions arising in the backdrop of chronic pancreatitis (respectively, 0%, 11%, 16%, and 40% for PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3) [42].

The *TP53* tumor-suppressor gene on chromosome 17p encodes for the p53 protein [43,44]. The p53 protein has a number of important functions in the cell, including regulation of the G1/S cell-cycle checkpoint, maintenance of G2/M arrest, and the induction of apoptosis. The *TP53* gene is inactivated in 55–75% of pancreatic cancers, almost always by an intragenic mutation in one allele coupled with loss of the second allele [44]. The loss of *TP53* means that two critical controls of cell number (cell division and cell death) are deregulated in the majority of pancreatic cancers. By immunohistochemistry, p53 accumulation is usually seen in the advanced PanIN-3 lesions, which is consistent with *TP53* gene mutations being a late genetic event in pancreatic cancer progression [45,46].

Another commonly inactivated tumor-suppressor gene in pancreatic cancer is *DPC4*, also known as *SMAD4/MADH4. DPC4* 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% [47–49]. The *DPC4* gene codes for the protein Smad4, and Smad4 plays a critical role in signaling through the transforming growth factor type β (TGF-β) pathway. The TGF-β pathway is activated when the TGF-β proteins bind to specific cell surface

receptors. This triggers an intracellular cascade that results in the nuclear localization of Smad4. Once in the nucleus, Smad4 has growth controlling effects by regulating the expression of specific target genes [29,50]. Therefore, loss of *DPC4* and, thus loss of Smad4 protein, interferes with the intracellular signaling cascades downstream from TGF-β and activin, resulting in decreased growth inhibition via loss of pro-apoptotic signaling or inappropriate G1/S transition [51,52]. Immunohistochemical labeling for Smad4 protein expression mirrors *DPC4/SMAD4/MADH4* gene status with rare exceptions, and like *TP53*, loss of Smad4 expression is a late genetic event in pancreatic carcinoma progression. Smad4 expression is intact in PanIN-1 and PanIN-2 lesions, but loss of Smad4 expression is observed in 31–41% of PanIN-3 lesions [48].

Genome-maintenance genes mutations in PanIN lesions

Genome-maintenance genes are those that function to identify and repair damage to DNA. They do not directly influence cell growth and proliferation but rather prevent the accumulation of DNA damage and maintain genomic fidelity. 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 [53]. Although gross chromosomal abnormalities are frequent in pancreatic ductal adenocarcinomas, genetic instability also occurs through DNA mismatch repair defects [54]. The DNA mismatch repair genes *hMLH1* and *hMSH2* are examples of genome-maintenance genes targeted in pancreatic cancer [49]. Their encoded proteins work together to repair small insertions, deletions, and other sequence mismatches in newly replicated DNA. Either by mutation or promoter hypermethylation, one of these genes can be inactivated. As a result, DNA repair is compromised, and mutations accumulate in repetitive tracts, producing alterations known as “microsatellite instability” (MSI). Approximately 4% of pancreatic cancers have MSI, and these cancers have a specific microscopic appearance called “medullary histology”. Medullary histology is characterized by pushing borders, syncytial growth pattern, and lymphocytic infiltrate. Furthermore, MSI is associated with poor differentiation and lack of *KRAS* and *TP53* mutations, and germline mutations of this gene are associated with the human non-polyposis colorectal cancer syndrome [55–57].

Another class of genome-maintenance genes includes the Fanconi anemia family of genes. 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 [58]. The genes that mutated in pancreatic cancer include the *BRCA2*, the *FANCC* gene, and the *FANCG* gene [58,59]. These genes are targeted in a small percentage of pancreatic cancers, namely less than 10%. Of these, *BRCA2* appears to be particularly significant, because germline *BRCA2* mutations, including a founder germline mutation prevalent in the Ashkenazi Jewish population, result in a predisposition to pancreatic cancer in the affected kindred [60]. In ductal pancreatic cancers 7% to 10% harbor an inactivating intragenic inherited mutation of one copy of the *BRCA2* gene accompanied by loss of heterozygosity [61,62]. Among the three cases of pancreatic cancer with germline mutation of *BRCA2*, loss of remaining wild-type allele was present in a single PanIN-3 lesion but none in 13 low-grade PanINs, confirming that bi-allelic inactivation of the *BRCA2* gene, like the *TP53* gene, is a late event in pancreatic cancer [63].

Telomere length abnormalities in PanIN lesions

Telomeres are structures present at the ends of linear chromosomes, comprising hexameric DNA repeat sequences (TTAGGG) in association with telomere-binding proteins. These telomeric repeat sequences prevent fusion between ends of chromosomes, and so we can assume that telomeres serve as sort of protective “caps”. It appears that telomeres become

abnormally short very early in the development of pancreatic neoplasia [64]. 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 [53]. Such a chromosome fusion leads to so-called anaphase bridges during mitosis [65]. During cellular replication, these anaphase bridges frequently break, generating unstable chromosome ends that are subject to abnormal fusion events and subsequent chromosomal rearrangements [66]. Telomere length abnormalities are one of the earliest event in the pancreatic progression model, with more than 90% of even the lowest grade PanIN lesions demonstrating marked shortening of telomeres as compared with normal ductal epithelium [64]. It is believed that this loss of telomere integrity in PanIN lesions is one of the major causes for the loss of tumor-suppressor genes and the gain of oncogenes described earlier.

Epigenetic abnormalities in PanIN lesions

In addition to genetic changes, we now know that epigenetic abnormalities are a common hallmark of cancers. Epigenetic abnormalities in cancer occur predominantly trough methylation of CG dinucleotides ("CpG islands") in the promoter region of genes, leading to silencing of transcription [67]. In cancers, there is preferential methylation of the gene promoter in the neoplastic cells but not in the corresponding normal cells within the tissue of origin. Numerous studies have showed promoter hypermethylation of several genes, which have a function in tumor suppression and/or critical homeostatic pathways, to be an important mechanism for gene inactivation in many types of cancer [68,69]. A recent study of a large number of microdissected PanIN lesions has found that as many as 70% of the earliest PanIN-1A lesions demonstrate evidence of aberrant promoter methylation [70]. In addition to previously documented genes –*p16* and *proenkephalin*, this study found evidence of progressive hypermethylation in *NPTX2*, *SARP2*, *Reprimo*, and *LHX1* [70–73]. These results suggest that aberrant CpG island hypermethylation begins in early stages of PanINs, and its prevalence progressively increases during neoplastic progression. The aberrantly methylated genes in PanIN lesions can be detected with methylation-specific PCR, making them potentially attractive for early detection. For that reason, the detection of aberrantly methylated genes in the pancreatic juice of patients with pancreatic carcinoma might be a promising diagnostic strategy [74].

Alterations in apomucin expression in PanIN lesions

The apomucins MUC1, MUC2, and MUC5 are frequently overexpressed in epithelial cancers, particularly those arising in the gastrointestinal tract and pancreas [75]. MUC1 is expressed in the normal pancreatic ducts and acini and is responsible for the maintenance of lumen formation. MUC1 expression is also often encountered in invasive pancreatic ductal adenocarcinomas [76,77]. Maitra et al. showed that MUC1 expression was present 43% in PanIN-2 and 85% in PanIN-3 but in only 6% and 5% in PanIN-1A/B. Thus, in the multi-step progression of pancreatic adenocarcinomas, MUC1 expression within normal intra- and interlobular ducts appears to be decreased in the low-grade PanINs (PanIN-1A and 1B). However, MUC1 appears to be subsequently re-expressed in the advanced PanIN lesions, and this expression persists into invasive adenocarcinoma. Of interest, unlike MUC1, the expression of the apomucin MUC2 is uncommon in both normal pancreas and in invasive ductal adenocarcinomas [45,76]. In contrast, MUC2 expression is commonly seen in IPMNs and their associated invasive colloid carcinomas [78]. These mucins can be used to distinguish PanINs from IPMNs, because PanINs, in contrast to IPMNs with intestinal differentiation, do not express MUC2. Furthermore, MUC5 is similar to MUC1 in that it is also expressed in the majority of invasive ductal adenocarcinomas. In contrast to MUC1, however, MUC5 is not expressed in normal ducts, but its expression is up-regulated even in the earliest PanIN lesions and persists thereafter in the majority of lesions of all histologic grades [45,79]. These mucins

are also potentially detectable by imaging [80], and they may be useful for screening and as therapeutic targets for the treatment of precursor lesions [81,82] (Fig. 1).

Aberrant expression of proteins in PanIN lesions

The protein cyclin D1 is a co-factor in the phosphorylation and inactivation of the Rb protein, which plays a central role in cell-cycle regulation [39]. Over-expression of the cyclin D1 protein has been documented in 60–85% of invasive pancreatic adenocarcinoma in imunohistochemistry studies [83,84]. Cyclin D1 overexpression in pancreatic cancer has been associated with a poor prognosis and decrease in survival [85]. In the development of pancreatic cancer, cyclin D1 overexpression appears to be an intermediate step with nuclear overexpression in 29% of PanIN-2 lesion and 57% of PanIN-3 lesions but no expression in normal pancreatic ducts, PanIN-1A, or PanIN-1B lesions [45].

Cyclooxygenase-2 (COX-2) regulates the metabolism of arachidonic acid into prostaglandins and other pro-inflammatory products. COX-2 has been implicated in tumorigenesis in which metabolites of COX-2 activate a range of signaling pathways, leading to cancer cell proliferation, survival, invasion, and angiogenesis [86]. These processes may be secondary to activation of the MAP kinase signaling pathway and nuclear factor kappa B (NF κB)-mediated signaling [87]. In pancreatic cancer, COX-2 levels are up-regulated, and also in PanIN lesions, COX-2 is expressed. In general, COX-2 follows the trend of expressions, which increases from normal pancreatic ducts to PanIN to adenocarcinoma, with significantly higher expression in PanIN-2/3 compared with PanIN-1A/1B [88]. The appearances of COX-2 in PanIN lesions suggest the possibility of a potential target for chemoprevention using selective COX-2 inhibitors [89].

Certain proteins were first identified as overexpressed in pancreatic cancer based on global expression analyses and subsequent validation in tissue sections. Many of these proteins, not surprisingly, are also overexpressed in precursor lesions. For example, protein prostate stem cell antigen (PSCA) is overexpressed in 30% of PanIN-1 lesions, and respectively 40%, 60%, and 60% in PanIN-2, PanIN-3, and invasive cancer, mandating the classification of PSCA as an early event in the progression model [45]. The patterns of protein expression in PanIN lesions are important, because the proteins expressed in low-grade PanINs may be reasonable chemoprevention targets, while those expressed late (in PanIN-3 lesions) are potential markers for the early detection of pancreatic neoplasia.

Signaling pathways and PanIN lesions

It is known that several embryonic signaling pathways (Notch, Hedgehog, and Wnt pathways) play an important role in multiple tissues during development in utero, and these pathways are for the most part turned off in adult somatic cells, including the exocrine pancreas. Recently, abnormal transcriptional activation of these pathways has been reported in both human and mouse models of pancreatic neoplasia [90–93]. The Notch signaling plays a critical role in maintaining the balance among cell proliferation, differentiation, and apoptosis. Over-expression of Notch pathway receptors (Notch 1–4), ligands (Jagged 1–2), and transcriptional targets (Hes 1) are up-regulated in PanIN lesions and in invasive adenocarcinoma. Notch activation in PanIN lesions appears to be ligand dependent, with Jagged-1 identified by micro-array analysis as one of the significantly overexpressed genes in early PanIN lesions [90,94].

Aberrant activation of the Hedgehog signaling pathway has been reported in PanINs and pancreatic cancer, as well as in genetically engineered murine models (see discussion below) of PanIN [91,92]. Global transcriptional profiling of human PanINs revealed up-regulation of extra-pancreatic foregut markers including pepsinogen C, MUC6, Sox-2, KLF4, and TFF1 as a consequence of overexpression of Gli1, a downstream mediator of Hedgehog signaling.

Furthermore, activation of the Hedgehog pathway in a human pancreatic ductal epithelial cell line resulted in a similar up-regulation of foregut markers seen in the early PanIN lesions [95]. It is interesting to note that the aberrantly expressed markers of foregut are not present in normal ductal epithelium.

Activation of the Wnt signaling pathways usually occurs via activating mutations of β-catenin or loss-of-function mutations of the *APC* tumor-suppressor gene; either event leads to stabilization and nuclear translocation of β-catenin and transcription of Wnt target genes [96]. Several studies demonstrated that Wnt pathway mutations are rare in pancreatic ductal adenocarcinoma, although they are frequently observed in non-ductal tumors (e.g., solid pseudopapillary tumors, pancreatoblastomas, and acinar cell carcinomas) [97,98]. In PanIN lesions, nuclear β-catenin expression is a rare event, and this reiterates the existence of two distinct, genetically divergent pathways of neoplasia in the pancreas: one resulting in the more common, conventional ductal adenocarcinoma and the other resulting in the less common non-ductal neoplasms [45].

Mouse models

Since the development of genetically engineered mouse models with pancreatic cancer, our understanding of the genetics of human PanINs and invasive pancreatic cancer has improved a lot. A major breakthrough was achieved in 2003, when Hingorani and colleagues developed a mouse model with pancreatic neoplasia that expressed an oncogenic $KRAS^{G12D}$ allele from its endogenous promoter through Cre-mediated recombinant driven by *Pdx1* regulatory elements [35]. *Pdx1* is involved in early pancreatic cell fate determination. *Pdx1* expression is critical in pancreatic development, and homozygous deletion of *Pdx1* causes pancreatic agenesis [99]. The Pdx1-Cre, LSL-$Kras^{G12D}$ mice develop the entire histologic compendium of murine PanIN (mPanIN) lesions observed in the cognate human disease, and in a subset of mice, develop invasive pancreatic carcinomas as well. Although expression of mutant *Kras* itself is not enough for developing invasive cancer, it is sufficient to initiate PanINs. The fact that these animals developed PanIN lesions before they developed invasive cancer has helped to validate the hypothesis that PanINs can progress to invasive cancer. However, when engineering mice that mis-express oncogenic *Kras* in the pancreas were combined with bi-allelic *INK4a*/*Arf* deletion or an oncogenic $Trp53^{R172H}$ allele, these mice developed aggressive, metastatic pancreatic cancers, with complete penetrance and shorter latency. On the other hand, abrogation of either *INK4a/Arf* or *TP53* signaling alone in the absence of oncogenic *Kras* does not lead to the development of pancreatic carcinomas or associated precursor lesions, underscoring the crucial importance of *Kras* signaling in initiating the cascade of events, which result in pancreatic carcinogenesis [34,100,101]. Of interest, the mPanIN lesions in the various LSL-$Kras^{G12D}$ mice not only demonstrate the morphological spectrum of human PanIN lesions but they also carry many of the alterations described above, such as overexpression of Notch, Hedgehog, and COX-2 [35,101]. These mouse models have significantly facilitated defining the role of these genes in the progression of pancreatic neoplasia.

Mouse models can also be used to examine the role of other medical conditions and environmental factors in the development of pancreatic cancer [102,103]. For example, Guerra et al. reported that when *Kras* mutations are created in adult mice, these genetically engineered mice do not develop lesions or pancreatic cancer. However, if these mice are challenged with a mild form of pancreatitis, they will develop the full spectrum of PanINs and invasive pancreatic carcinoma. This study provides an excellent example of how genetics and environmental factors interplay in the development of pancreatic cancer, especially when we translate these studies into human observations [103,104].

At last, mouse models are potentially useful tools to explore pre-clinical diagnostic and therapeutic strategies for pancreatic neoplasia. As already mentioned, these mouse models recapitulate not only the morphology of the cognate human disease but also many of the signaling pathways like Notch, Hedgehog, and COX-2 [35,101]. Thus, there is a unique opportunity to explore chemoprevention and treatment strategies in a biologically relevant pre-clinical model.

Conclusion

Putative precursor lesions of pancreatic cancer were documented over a century ago. However, it took many decades to define the various histological types of precursor lesions in the pancreas and to credential these lesions as true precursors to invasive adenocarcinoma. Nevertheless, the detailed mechanisms involved in the initiation and progression of these precursor lesions remain to be elucidated. An improved understanding of the pathogenesis of PanIN lesions will enable us to develop better tools for primary and secondary prevention of pancreatic cancer, as well as improve existing tools for early diagnosis.

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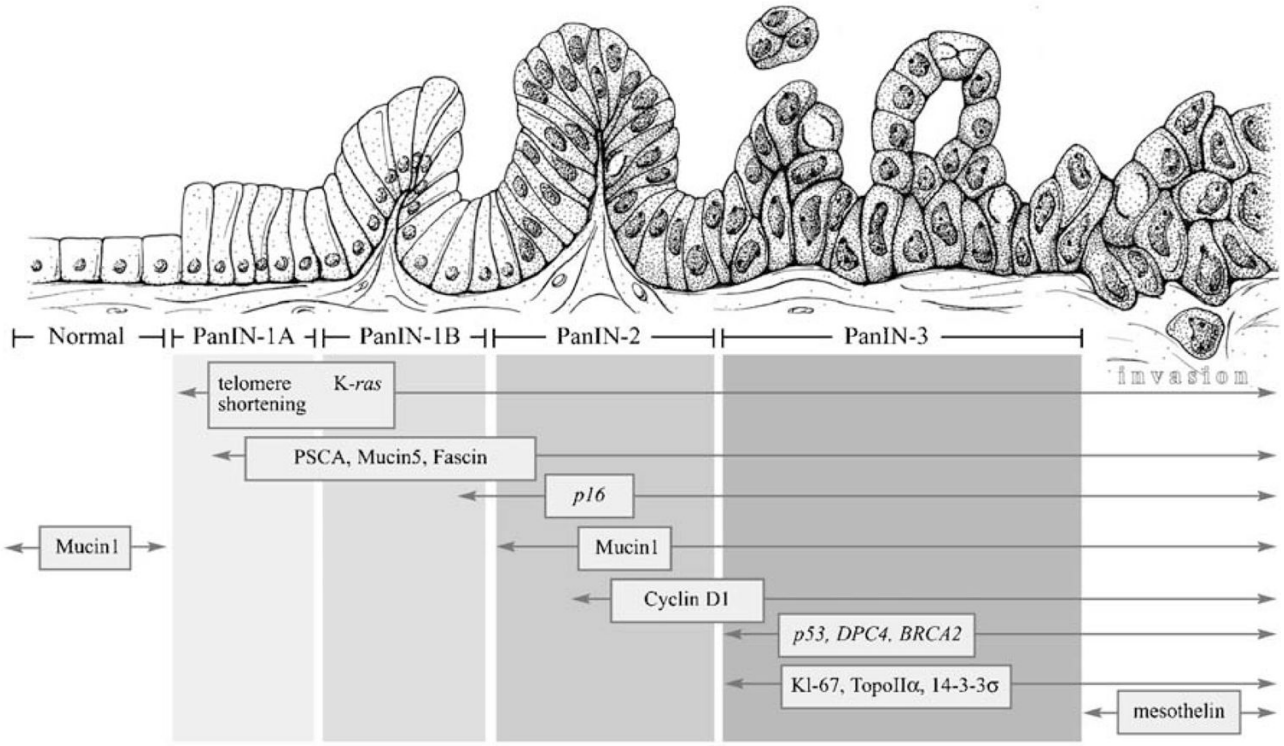

Fig. 1.

A "PanINgram" illustrating some of the molecular alterations that occur during the multi-step progression of pancreatic adenocarcinomas. The molecular abnormalities listed are not comprehensive, and additional alterations are discussed in the text at the appropriate juncture. Adapted from [45]