The Molecular Pathology of Precursor Lesions of Pancreatic Cancer

Aatur D. Singhi and Anirban Maitra

Contents

A. D. Singhi
Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
e-mail: singhiad@upmc.edu

A. Maitra (✉)
Departments of Pathology and Translational Molecular Pathology, Sheikh Ahmed Pancreatic Cancer Research Center, University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: amaitra@mdanderson.org

J. P. Neoptolemos et al. (eds.), *Pancreatic Cancer*,
https://doi.org/10.1007/978-1-4939-7193-0_5

Abstract

It has become evident over the past decade that pancreatic ductal adenocarcinoma (PDAC) does not originate de novo, but rather, through a multistep progression that involves histologically defined precursor lesions. Three major subtypes of precursor lesions of PDAC have been identified to date, including pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN). PanINs constitute by far the most common precursor lesion, and are, by definition, microscopic in nature, while IPMNs and MCNs occur less frequently and are macroscopic (e.g., radiographically detectable) precursor lesions. In addition to the development of consensus histopathological criteria for the identification and classification of PDAC precursors, there has also been considerable progress made in characterizing the genetic alterations underlying these lesions. Elucidating the molecular pathology of precursor lesions has enabled a better understanding of the pathogenesis of early pancreatic neoplasia, and provided a seedbed for developing tools for early detection and chemoprevention of PDAC. The histopathology, molecular genetics as well as clinical implications and possible directions for future research of PanINs, IPMNs, and MCNs will be discussed in this chapter.

Keywords

Pancreatic ductal adenocarcinoma · Precursor neoplasms · Molecular genetics · Early detection · Pathogenesis

Introduction

The first example linking the progression from a noninvasive precursor lesion to invasive cancer with a cumulative sequence of genetic aberrations was established for the adenoma-carcinoma sequence in colon cancer [1]. This concept has since been extrapolated to many solid cancers, including pancreatic cancer or pancreatic ductal adenocarcinoma (PDAC). In fact, there is now increasing evidence to suggest, that almost all of the major epithelial malignancies may be associated with discrete noninvasive precursor lesions, and that histological progression of such lesions is paralleled by an underlying genetic progression. The general concept that PDAC does not arise de novo, but rather originates from tangible noninvasive precursor lesions, was first proposed over a century ago [2]. However, only over the past few decades have the identity of these precursor lesions been solidified through

Table 1 Clinicopathologic features of PanINs, IPMNs, and MCNs

	PanIN	IPMN	MCN
Predominant age	Prevalence increases with age	60–70 years	40–50 years
Female: male ratio	1:1	2:3	20:1
Preferential location	Head > body/tail	Head (80%) > body/tail	Body/tail (90%) > head
Ductal communication	N/A	Yes	No
Cyst contents	N/A	Viscous	Viscous
Stroma	Collagen-rich	Collagen rich	Ovarian type
Multifocal disease	Often	In ~20–30%	Extremely rare
EUS findings	Normal	Ampullary mucin extrusion, dilated pancreatic duct, and filling defects	None
Key genes involved in pathogenesis and progression	*KRAS*, *CDKN2A*, *TP53*, and *SMAD4*	*KRAS*, *GNAS*, *RNF43*, *CDKN2A*, *TP53*, *PIK3CA, PTEN*, and *SMAD4*	*KRAS*, *RNF43*, *CDKN2A*, *TP53*, *PIK3CA, PTEN*, and *SMAD4*

Abbreviations: *IPMN*, intraductal papillary mucinous neoplasm; *MCN*, mucinous cystic neoplasm; *N/A*, not applicable; *PanIN*, pancreatic intraepithelial neoplasia

meticulous histopathological and molecular biological analysis, and through introduction of a consensus nomenclature [3, 4]. Three different types of precursor lesions to PDAC are recognized: pancreatic intraepithelial neoplasia (PanIN), by far the most common, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs). The key features of these three precursors are listed in Table 1, and each will be discussed independently within the text.

Pancreatic Intraepithelial Neoplasia (PanIN)

Clinical and Histopathological Features of PanINs

PanIN lesions are microscopic noninvasive precursor lesions with varying degrees of architectural and cytologic atypia, and are located in the interlobular ducts of <5 mm in diameter [3]. Based on the degree of both architectural and cytologic atypia, PanINs are divided into two grades: low-grade and high-grade. Low-grade PanINs consist of flat-to-papillary ductal epithelium with abundant supranuclear mucin. The nuclei may be round or elongated and basally oriented or show some loss of polarity, crowding, enlargement, pseudostratification, and hyperchromasia (Fig. 1a). Mitoses are only rarely seen, and when present, are basal and morphologically normal. In contrast, high-grade PanINs are characterized by significant architectural and cytologic atypia. These lesions are usually papillary and, in some instances, demonstrate

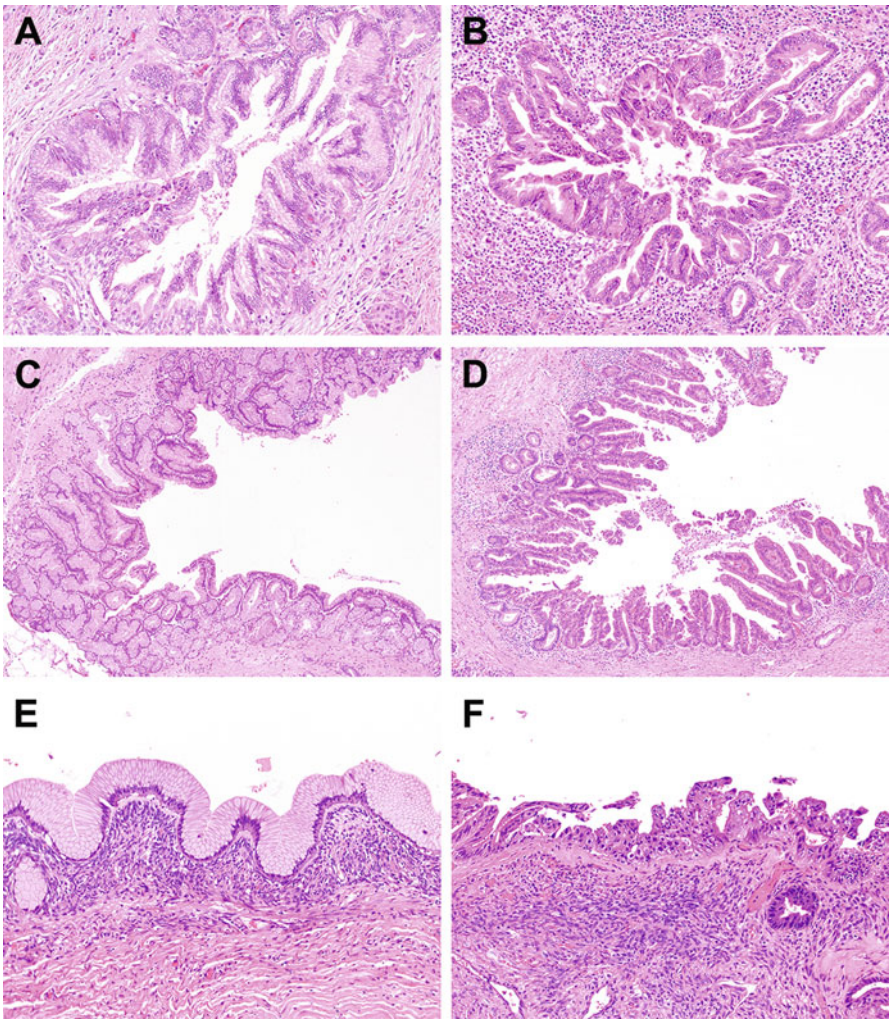

Fig. 1 Representative histologic sections of low-grade (**a**) and high-grade (**b**) PanINs, low-grade (**c**) and high-grade (**d**) IPMNs, and low-grade (**e**) and high-grade (**f**) MCNs. Note the presence of ovarian-type stroma underlying the mucinous epithelium of MCNs (**e**, **f**)

cribriform architecture and luminal necrosis. The nuclei are enlarged, hyperchromatic and show loss of orientation, such that they are no longer perpendicular to the basement membrane (Fig. 1b). Further, the nuclear-to-cytoplasmic ratio is significantly increased. Nucleoli may be prominent, and mitoses, some of which are luminal and atypical, may be present.

The overall prevalence of PanINs increases with age, and low-grade PanINs are found in over half of the population above the age of 65 years [5]. An increased

prevalence of PanINs in not only observed in PDAC, but also in the setting of chronic pancreatitis [6]. In one series, Andea and colleagues found PanIN lesions in 67 of 82 (82%) pancreata from patients with PDAC and in 54 of 86 (63%) of patients with chronic pancreatitis, but only in 10 of 36 (28%) patients with otherwise normal pancreata. Interestingly, PanINs are also frequently found adjacent to other periampullary neoplasms, including ampullary adenomas and adenocarcinomas, acinar cell carcinomas, well-differentiated pancreatic neuroendocrine tumors, serous cystadenomas, and solid-pseudopapillary neoplasms [7, 8].

Molecular Genetics of PanINs

The histological progression of PanIN lesions has been linked to progressive accumulation of genetic aberrations that are shared with PDAC. These aberrations do not occur in a random manner, but rather in a well-described sequence of early and later events (Fig. 2), as depicted in the PanIN progression model ("PanINgram").

Oncogene Mutations in PanIN Lesions

A growing number of oncogenes have been identified that contribute to pancreatic carcinogenesis upon activation, usually through intragenic mutations or copy number alterations. The most commonly observed activating point mutations in PDAC, as well as in PanINs, are found in the *KRAS* oncogene on chromosome 12p. These mutations, which are also among the earliest genetic alterations observed during pancreatic carcinogenesis, can be detected in up to 90% of PDACs and most often occur on codons 12, 13, or 61 [10, 11]. Utilizing pyrosequencing, a highly sensitive DNA sequencing technique, Kanda et al. showed more than 90% of low-grade PanINs harbor *KRAS* mutations, suggesting that this oncogene plays a critical role in PDAC initiation [12]. The importance of constitutively activated *KRAS* in PDAC initiation is further underscored by the development of genetically engineered mouse models of PDAC, wherein a mutant *Kras* allele is sufficient for the development of murine PanIN (mPanIN) lesions [13, 14]. Activating mutations impair the intrinsic GTPase activity of the *KRAS* gene product, leading to constitutive activation of downstream intracellular signaling cascades [15]. Three major downstream Ras effector cascades have been identified that are involved in mediating the oncogenic properties conferred by constitutively active *KRAS*, namely the RAF/MEK/ERK, the PI3K/AKT, and the RalGDS/Ral pathways. Of note, oncogenic Ras signaling seems to be involved in not only PDAC initiation, but also required for tumor maintenance in established cancers [16, 17]. Interestingly, in a proportion of PDAC more than one distinct mutation within the *KRAS* gene can be detected, suggesting that within the same organ, multifocal precursor lesions can develop independently from the one that eventually culminates in PDAC [18].

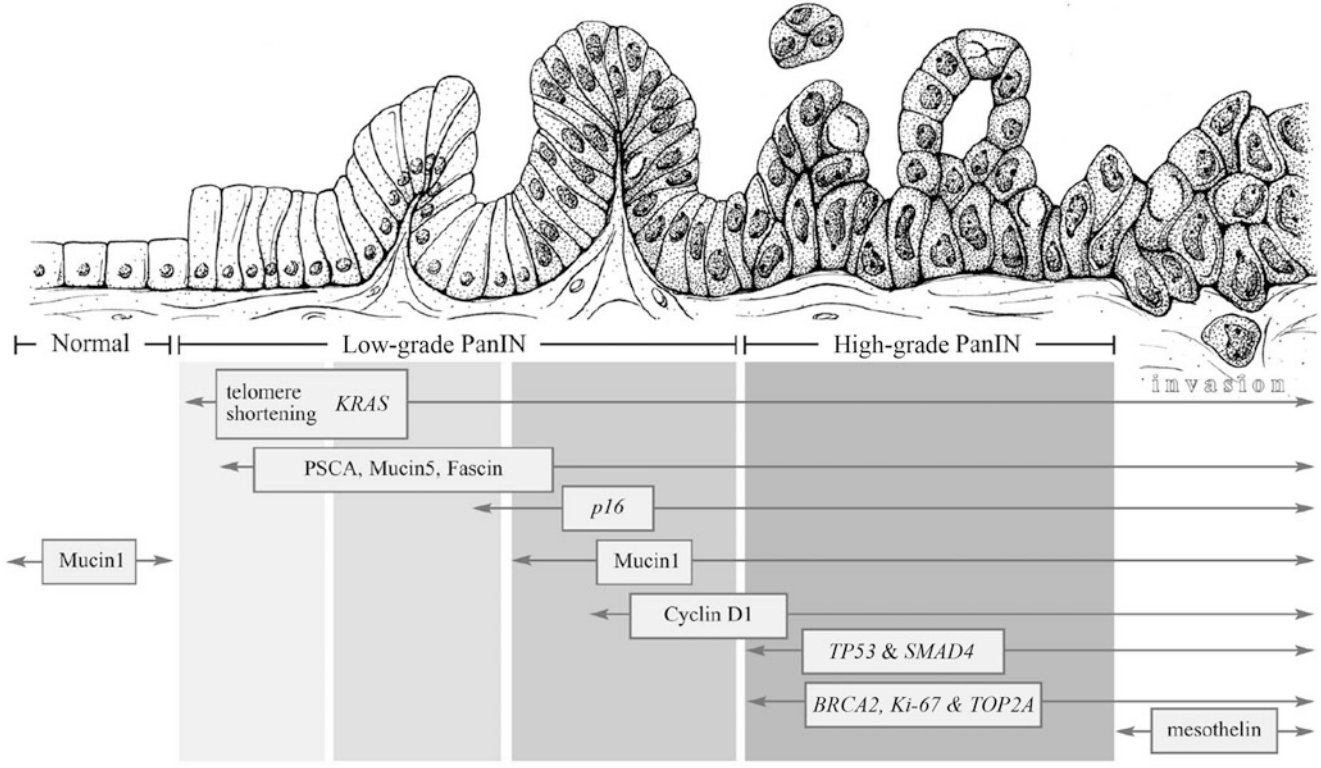

Fig. 2 Schematic illustration of some of the key genetic alterations observed during the multistep progression from the normal duct to PanIN and eventually PDAC in the form of a "PanIN-gram." The alterations shown are not comprehensive and are discussed in detail within the text (Reproduced from Maitra et al. [9] with permission)

Tumor Suppressor Gene Mutations in PanIN Lesions

Three tumor suppressor genes frequently inactivated in PanIN lesions, mirroring their common loss of function in PDAC, are *CDKN2A*, *TP53*, and *SMAD4/DPC4*. The *CDKN2A* gene on chromosome 9p21 encodes for the cell-cycle checkpoint protein p16, which binds to the cyclin-dependent kinases CDK4 and CDK6, and thereby inhibiting cyclin D1-binding and causing cell-cycle arrest in G1-S [19]. The *CDKN2A* gene is inactivated in virtually all PDACs: in approximately 40% of cases, this is due to homozygous deletion; another 40% carry intragenic mutations and show loss of the second allele; and 15% demonstrate epigenetic inactivation [20, 21]. Loss of p16 expression, which can be exploited as surrogate marker of the *CDKN2A* gene status, correlates with PanIN progression and is observed in 30–55% of low-grade PanINs and 71% of high-grade PanINs [22]. Interestingly, the frequencies of *CDKN2A* inactivation appear to be lower in PanIN lesions associated with chronic pancreatitis [23]. In a subset of cases, homozygous deletions of *CDKN2A* at 9p21 can also include homozygous deletion of the *methylthioadenosine phosphorylase* (*MTAP*) gene, whose product is required for the salvage pathway of purine synthesis. Codeletion of *MTAP* and *CDKN2A* is observed in approximately one-third of PDACs, and 10% of high-grade PanINs [24, 25].

The tumor suppressor gene *TP53* on the short arm of chromosome 17 encodes the protein p53, which plays a key role in mediating several important physiological functions, including regulation of the G1/S cell-cycle checkpoint, maintenance of G2/M arrest, and induction of apoptosis. Therefore, the inactivation of p53 in the majority of PDACs affects two major mechanisms controlling cell number: cell proliferation and apoptosis. Moreover, p53 abrogation contributes to genomic instability observed in PDACs [26]. Loss of *TP53* function is observed in 50–75% of PDAC and almost exclusively through intragenic mutations and loss of the second allele [27]. Nuclear accumulation of p53 using immunohistochemistry largely correlates with the mutational status of *TP53* and can therefore be used as a surrogate marker of *TP53* mutations in PanIN lesions. Immunohistochemistry reveals intranuclear p53 accumulation mostly in high-grade PanINs, and, thus, suggesting that *TP53* mutations constitute rather late events in the multistep pancreatic cancer progression cascade [9].

SMAD4 on chromosome 18q is inactivated in approximately 55% of PDACs by homozygous deletion in 30% of cases, or through intragenic mutation and loss of the second allele in another 25% [28]. *SMAD4* encodes the protein Smad4, which is involved in transforming growth factor (TGF)-beta signaling. The activation of the TGF-beta signaling pathway leads to binding of Smad4 to a phosphorylated Smad2/3 protein complex and its translocation to the nucleus, where it binds to specific promoter regions and induces expression of respective target genes [29]. Therefore, the loss of Smad4 function interferes with the intracellular signaling cascade downstream of TGF-beta and leads to reduced growth inhibition through loss of proapoptotic stimuli and inappropriate G1/S transition [30]. A potential alternative mechanism was recently unmasked in an elegant study showing that selective loss of Smad4-dependent signaling in T-cells leads to development of

epithelial cancers of the gastrointestinal tract in mice, while no tumor development was observed in mice with epithelial-specific deletion of *SMAD4*. These observations suggest that in addition to the abovementioned cell functions, Smad4 might also be crucially involved in interactions between cancer cells and the microenvironment and/or modulation of immune surveillance [31]. As described above for p53, immunohistochemical labeling for Smad4 can be used as a surrogate marker of the *SMAD4* mutational status [32]. Loss of Smad4 nuclear expression is observed in about one-third of high-grade PanINs, while it is preserved in normal ducts and low-grade PanINs [9, 33]. Therefore, *SMAD4* mutations, like mutations of *TP53*, represent a relatively late genetic event in the progression model for pancreatic cancer.

Caretaker Gene Mutations in PanIN Lesions

Caretaker genes comprise a third class of cancer-related genes, which are not directly involved in controlling cell growth or apoptosis, but rather help to maintain DNA integrity, e.g., by means of mismatch repair, nucleotide-excision repair, and base-excision repair [34]. By repairing subtle changes in the genomic DNA sequence that occurs due to polymerase errors or as a result of exposure to mutagens, as well as gross chromosomal aberrations, caretaker genes prevent accumulation of mutations within a cell that might provide a selective advantage leading toward a malignant phenotype.

The Fanconi anemia gene family is a group of caretaker genes known to be involved in pancreatic carcinogenesis [35, 36]. The Fanconi anemia gene family is involved in homologous recombination repair in response to DNA damage, e.g., by crosslinking agents or radiation [37]. One member of this family, the breast and ovarian cancer susceptibility gene *BRCA2* on chromosome 13q, is of particular interest in the setting of familial pancreatic cancer, since germline *BRCA2* mutations are found in 5–10% of familial cases, especially in individuals of Ashkenazi Jewish heritage [38, 39]. In addition, PDACs harboring Fanconi anemia mutations are exquisitely sensitive to DNA crosslinking agents, presenting an avenue for synthetic lethal therapy [36]. In patients with germline *BRCA2* mutations, loss of the second allele is observed in high-grade PanINs, suggesting that akin to p53 and Smad4, inactivation of BRCA2 function also constitutes a late genetic event [40].

Genomic Instability and Telomere Length Alterations in PanIN Lesions

Telomeres consist of hexameric TTAGGG repeats at the ends of chromosomal DNA strands, which confer chromosomal stability during cell division by preventing the ends from becoming sticky. Telomere attrition is among the earliest and most common alterations observed in PanIN lesions. Interestingly, significant telomere shortening is observed in over 90% of low-grade PanINs [41]. It has been speculated

that telomeres conduct a similar function to those of caretaker genes in pancreatic carcinogenesis, such that telomere dysfunction facilitates progressive accumulation of additional chromosomal abnormalities that culminates in the development of PDAC.

Reflecting their inherent genomic instability, structural and numerical chromosomal aberrations can be found in almost all cases of PDAC and often involve loss of significant proportions or the entirety of chromosomal arms. Chromosomal regions frequently involved in loss of one allele (designated loss of heterozygosity [LOH]) in PanINs include 1q, 6q, 7p, 9p, 10q, 14, 16q, 17p, and 18q [42]. Of note, the frequency of LOH observed at a given locus commonly increases from low- to high-grade PanINs. It has been proposed that LOH might in many cases be the first event in the "two-hit" cascade leading to inactivation of tumor suppressor genes. This concept is in line with the hypothesis of genomic instability beginning early in the PanIN progression model.

Epigenetic Alterations in PanIN Lesions

The most common form of epigenetic alterations found in PDAC, and also in PanIN lesions, consists of methylation of CpG islands within promoter regions, leading to transcriptional silencing of the regulated gene. Over recent years, epigenetic gene silencing – in addition to genetic alterations such as deletions and intragenic mutations – has increasingly been recognized as one of the most ubiquitous mechanisms exploited by cancer cells to alter their inherent transcriptomic programs in favor of more rapid cell growth, invasiveness, and resistance to apoptosis [43].

Current evidence supports the notion that aberrant DNA methylation occurs early during the progression of pancreatic cancer. Using a gene candidate approach, Rosty et al. demonstrated that PanIN lesions in patients with chronic pancreatitis show the loss of p16 expression, suggesting that this alteration may contribute to the predisposition of patients with chronic pancreatitis to develop PDAC [23]. In a large-scale methylation analysis with subsequent validation via methylation-specific PCR, Sato et al. analyzed DNA samples from 65 PanINs for methylation status of eight genes (*ST14, CDH3, CLDN5, LHX1, NPTX2, SARP2, SPARC*, and *Reprimo*) that were identified previously through a microarray approach as aberrantly hypermethylated in PDAC [44]. Among PanINs examined in this study, methylation of any of these eight genes was identified in 68% of cases with methylation prevalence increasing from low-grade to high-grade PanIN for *SARP2*, *Reprimo* and *LHX1*. Peng et al. had examined promoter methylation patterns of 12 cancer-related genes (*p14, p15, p16, p73, APC, hMLH1, MGMT, BRCA1, GSTP1, TIMP-3, CDH1*, and *DAPK-1*) in 40 microdissected PanIN lesions and 147 discrete areas sampled from PDACs [45]. The frequency of at least one methylated gene locus increased significantly from normal ductal epithelium lacking signs of inflammation to PanINs, and from PanINs to PDAC, respectively, further underscoring that epigenetic progression is also a feature of the traditional "PanIN-gram" model. Determination of aberrantly methylated gene promoters in pancreatic juice samples

has emerged as a potential diagnostic tool for PDAC and its precursor lesions, with a suggestion that it might be more specific than detection of mutated or differentially expressed genes [46]. In particular, certain promoter sequences like that of the *TSLC1* gene are methylated only in higher-grade PanIN lesions, and therefore, might identify those lesions that pose a greater relative risk of progression to invasive adenocarcinoma.

Transcriptomic Abnormalities in PanIN Lesions

With the advent and increasingly widespread deployment of global gene expression profiling techniques, including RNA sequencing, serial analysis of gene expression (SAGE), and various forms of oligonucleotide and cDNA/miRNA microarrays, there has been a dramatic increase in our knowledge of differential gene expression patterns in PDAC [47–51]. A few compelling examples of differentially expressed genes with translational potential will be discussed here. Although initially discovered in the context of invasive cancer, the differential expression of these genes has since been validated in varying grades of PanIN lesions as well.

Prostate stem cell antigen (PSCA) is overexpressed in 30–40% of low-grade IPMNs and 60% of high-grade IPMNs, in line with PSCA upregulation being an early event in the PanIN progression model [9]. Of note, the recent pilot studies showed that PSCA overexpression might be a suitable target for the development of novel diagnostic tools for PDAC [52]. Another example is mesothelin, a membrane-bound GPI-anchored protein known to play a role in cell adhesion. Unlike PSCA, mesothelin expression was detected only in 11% of PanIN lesions, but close to 100% of PDACs, suggesting that mesothelin overexpression is a late event [9, 53]. Recent studies have examined mesothelin as an antigen for cancer cell-specific drug delivery and for cancer immunotherapy [54]. A study by Sutherland et al. using oligonucleotide microarrays described the upregulation of several components of the retinoic acid signaling pathway, including RAR-alpha, HOXB6 and HOXB2 in PDAC, as compared to the normal pancreas [55]. In particular, HOXB2 expression was identified as prognostic marker in PDAC that correlated with survival, surgical resection, and tumor stage at the time of diagnosis. Nuclear immunostaining for HOXB2 was observed in 8% of normal pancreatic ducts, 14% of PanIN lesions, and 38% of PDACs. This suggests HOXB2 overexpression increases during pancreatic carcinogenesis.

Changes in microRNA (miRNA) expression are also important in the development of PDAC. miRNAs are small endogenous noncoding RNAs of 14–24 nucleotides that negatively regulate protein expression at the posttranscriptional level by inhibiting translation and/or by targeting mRNAs for degradation. Furthermore, because miRNAs are stable and detectable in human plasma, they are being investigated for their use as diagnostic serum markers. PDACs overexpress several miRNAs including miR-21, miR-34, miR-146a, miR-155, miR-196b, and miR-200a/b [56–58]. In a large comprehensive miRNA study by Yu et al., the authors identified 107 aberrantly expressed miRNAs based on the PanIN grades

and compared with normal pancreatic duct samples [59]. Further, 35 aberrantly expressed miRNAs in high-grade PanINs compared with normal pancreatic duct samples. These differentially expressed miRNAs included those that have been previously identified in PDACs as well as miRNAs not previously described as differentially expressed in these lesions (e.g., miR-125b, miR-296-5p, miR-183*, miR-603, miR-625/*, and miR-708). Interestingly, miR-196b was the most differentially expressed miRNA in high-grade PanINs.

Cell Cycle and Proliferation Abnormalities in PanIN Lesions

Much like PDAC, PanIN lesions also demonstrate aberrations in cell cycle checkpoint control and proliferation. While low-grade PanINs are minimally proliferative, this index significantly increases in high-grade PanINs, as assessed by nuclear expression of the proliferation antigen Ki-67/MIB-1. Klein et al. described mean nuclear Ki-67/MIB-1 labeling indices as 0.41% for normal ducts, 5.7% for low-grade PanIN, and 22.0% for high-grade PanIN [60]. The average labeling index for PDACs was 37.0%, reflecting the progressive increase in proliferative potential during the progression from normal ducts to PDAC [60]. Cyclin D1 is involved in regulating cell cycle progression by acting as a cofactor in phosphorylating and inactivating the retinoblastoma (Rb) protein, and its expression has been linked to poor prognosis and decreased survival in PDAC. Overexpression of cyclin D1 is observed in 14% of low-grade PanINs, 57% of high-grade PanINs, and up to 60–85% of PDACs [9]. $p21^{WAF/CIP1}$ acts as cyclin-dependent kinase inhibitor that inhibits cyclin E/CDK2 complexes and prevents phosphorylation of Rb. Overexpression of $p21^{WAF/CIP1}$ is an early event and is observed in 33% of low-grade PanINs, 80% of high-grade PanINs, and 85% of PDACs [61].

Aberrantly Activated Growth Factor Signaling Pathways in PanIN Lesions

Cyclooxygenase-2 (COX-2) is upregulated in PDAC, possibly secondary to activation of nuclear factor kappa B signaling, and is postulated to be involved in cell proliferation and tumor angiogenesis [62]. In PanINs, COX-2 is generally found to be overexpressed in high-grade PanINs as compared to low-grade PanINs and normal ducts [63]. COX-2 inhibitors have been suggested as potential chemopreventive agents against PDAC [64], but initial clinical efficacy data have been equivocal thus far. Members of the matrix metalloproteinase (MMP) family of zinc-dependent extracellular proteinases are involved in enabling cell invasion and metastasis [65]. Overexpression of MMP-7 is observed in the majority of PDACs, as well as in greater than half of low-grade PanINs [66]. Urinary plasminogen activator (uPA) converts plasminogen into plasmin, which in turn activates MMP precursors. In addition, uPA induces the upregulation of various downstream signaling molecules, including fibroblast growth factor 2 (FGF2) and angiostatin [67, 68]. In one study, uPA

immunolabeling was observed not only in the majority of PDACs but also in 19 of 27 (70%) low-grade PanINs and 12 of 27 (44%) high-grade PanINs [69].

Aberrantly Activated Embryonic Signaling Pathways in PanIN Lesions

Embryonic signaling pathways, including Hedgehog, Notch, and Wnt, which are usually inactive in differentiated tissues of the adult pancreas, have been found to be aberrantly reactivated in PDACs as well as in a variety of other epithelial human cancers [70–72]. This finding is of particular interest, since these signaling networks might contribute to maintain specific subpopulations of cancer cells with enhanced tumor-initiating properties, often referred to as "cancer stem cells." This concept has direct translational implications, since all of the three abovementioned embryonic signaling pathways represent candidate drug targets. The phenotype of the putative cancer stem cell compartment in PDAC has recently been elucidated by multiple groups. For example, Simeone et al. have demonstrated that a subpopulation of CD44+/CD24+/ESA+ cells, which represent less than 1% of cancer cells within a "bulk" isolate, harbor more than 100-fold increased tumorigenic potential in immunodeficient mice, as compared to nontumorigenic cells. Of note, in this population they also observed a ~10-fold overexpression of the Hedgehog ligand sonic hedgehog (Shh) as compared to bulk tumor tissues [73]. Similarly, Feldmann et al. found that inhibition of Hedgehog signaling by means of small molecule inhibitors diminished tumor initiation and metastasis in orthotopic xenograft models of PDAC, mirrored by significant reduction of a subpopulation of cancer cells with high aldehyde dehydrogenase (ALDH) activity in vivo and in vitro [74]. The concept that Hedgehog signaling is involved in maintaining a "cancer stem cell niche" would imply, that Hedgehog pathway reactivation occurs very early during the carcinogenic cascade, and indeed overexpression of Shh has been observed by immunohistochemistry in low-grade PanINs, but not in normal pancreatic ductal epithelium [75]. Further evidence came from another study by Leach et al. demonstrating that low-grade PanINs express a cluster of "foregut-specific" markers, including pepsinogen C, MUC6, KLF4, GATA6, Sox-2, Forkhead-6, and TFF1, which is very similar to differential gene expression patterns observed in immortalized pancreatic ductal epithelial cells upon transfection with the Hedgehog transcription factor Gli1 [76].

Analogous to the aberrant expression of Hedgehog pathway components, murine and human PanINs and PDACs also express multiple Notch components [72]. As observed for Hedgehog signaling, Notch pathway activation during pancreatic carcinogenesis is most likely to be due to endogenous ligand overexpression, rather than mutational events. For example, the activating Notch ligand, Jagged-1, is overexpressed in low-grade PanINs [76]. The activation of Wnt signaling in cancer tissues usually occurs due to intragenic mutations, i.e., either activating *CTNNB1/beta-catenin* mutations or loss-of-function mutations within the *APC* gene, resulting in nuclear translocation of beta-catenin and subsequent transcription

of Wnt target genes [77]. In PDAC, however, canonical pathway activation is more often ligand-dependent, than through mutational events [78]. Immunohistochemical detection of nuclear beta-catenin can be used as a surrogate marker of Wnt pathway activation. Al-Aynati et al. reported nuclear overexpression of beta-catenin in a small proportion of high-grade PanINs [79], but observations regarding PDACs have been conflicting [71].

Genetically Engineered Mouse Models and Murine PanINs (mPanINs)

A remarkable advance achieved in the last decade for pancreatic cancer research has been the development of genetically engineered mouse models, which resemble cognate properties of the human disease, such as a multistep progression involving noninvasive precursor lesions culminating in lethal disseminated malignancy [13, 14, 26]. In order to distinguish precursor lesions in mice from those arising in human pancreata, the former have been designated as murine PanIN (mPanIN) [80]. Interestingly, mPanIN lesions observed in these models also harbor many of the molecular alterations found in humans, including activation of the Notch and Hedgehog signaling pathways [13, 81]. These mouse models represent a unique platform for discovery of early pancreatic neoplasia-associated biomarkers in serum, as recently demonstrated by Hanash and colleagues [82]. In this study, the investigators identified a large panel of abnormally expressed protein the sera of mice from both early and late stage disease. Of note, when five of these proteins were examined in human sera obtained from PDAC patients, they were able to predict the diagnosis of malignancy as much as 7–13 months prior to onset of clinical symptoms, underscoring the commonalities between mouse and human disease models. Genetically engineered mouse models of mPanINs and PDAC have also begun to be utilized as in vivo platforms for assessment of novel chemoprevention and treatment modalities. For example, it has been demonstrated that the COX-2 inhibitor nimesulide can downregulate mPanIN formation in genetically predisposed mice [83], an expected finding, given that mPanINs (as well as their human counterparts) overexpress COX-2 [13].

Therapeutic Implications of Isolated PanIN Lesions

Currently, the detection of PanIN lesions is hampered by the lack of sensitive noninvasive diagnostic tools. Due to their microscopic size, PanIN lesions are usually not diagnosed by standard clinical imaging techniques. Recent data from the Johns Hopkins Hospital suggest that a combinatorial approach of collecting secretin-stimulated pancreatic juice, endoscopic ultrasound (EUS), and computer tomography might enable the detection of morphological and genetic changes associated with PanIN lesions in the adjacent pancreatic parenchyma [84]. In particular, Brune et al. showed that PanINs can be associated with a lobulocentric form of atrophy in the adjacent parenchyma, and a diffuse distribution of this atrophy

observed in patients with multifocal PanIN lesions confers a diagnostic pattern on EUS [85]. Even if further improvements in imaging techniques and other diagnostic tools will provide the means to reliably and noninvasively screen for the presence of PanIN lesions, the therapeutic implications that of such findings are largely unknown. While the pathophysiological concept of a multistep progression of PanINs culminating in PDAC has become acceptable, the appropriate clinical management of noninvasively diagnosed PanIN lesions in an individual patient still needs to be defined. In an effort to estimate the approximate probability of a single PanIN to progress to cancer, Terhune et al. applied a mathematical model, assuming that PanIN lesions can be found in 37.5% of cases in a normal population with an average of five foci per affected pancreas, and that 0.8% of pancreata develop PDAC [86]. The authors argued that based on these assumptions only about 1% on PanIN lesions progress to PDAC. These considerations underscore the caution mandated in drawing therapeutic conclusions based on the identification of PanIN lesions alone, in the absence of a discernible malignancy.

Interestingly, collection of secretin-stimulated pancreatic juice has emerged as a promising adjunct to the evaluation of precursor neoplasms. Digital next-generation sequencing ("digital NGS") to detect low-abundance mutations in secretin-stimulated pancreatic juice samples collected from the duodenum in subjects with a family history of PDAC has identified low abundance of *KRAS* mutations that are thought to arise from small PanIN lesions [87]. However, further studies are needed to assess whether the genetic alterations associated with high-grade PanINs can be reliably detected by digital NGS with a high sensitivity and high specificity.

Intraductal Papillary Pucinous Neoplasms (IPMN)

Clinical Features of IPMNs

Intraductal papillary mucinous neoplasms (IPMNs) are mucin-producing epithelial neoplasms that arise from the main pancreatic duct (main duct IPMN), branches (side branch IPMN), or both (mixed main and branch duct IPMN). A population-based study estimated the age and sex-adjusted cumulative incidence of an IPMN to be 2.04 per 100,000 individuals per year [88]. In comparison, the incidence of PDAC is 0.8 per 100,000 individuals per year. These neoplasms occur more frequently in men than women with a mean age at presentation of approximately 65 years. The majority of IPMNs are identified incidentally on abdominal computed tomography (CT) or magnetic resonance imaging (MRI), but a subset can be associated with epigastric/abdominal pain, pancreatitis, weight loss, and jaundice [89, 90]. IPMNs are usually greater than 1 cm in size, commonly arise in the head of the pancreas and can be multifocal. Similar to PanINs, the neoplastic cells may show varying degrees of dysplasia that can progress from low-grade dysplasia to high-grade dysplasia and PDAC [91]. In addition, the risk of high-grade dysplasia and PDAC is higher in patients with main duct IPMN and mixed main and branch duct IPMN than branch duct IPMN (60% vs. 25%, respectively) [89, 92]. Although the

rate of progression to advanced neoplasia in an IPMN has yet to be defined, patients with a PDAC arising in an IPMN are generally 3–5 years older than those with a non-invasive IPMN [93]. Thus, it is hypothesized that there is a substantial window of opportunity to detect and treat noninvasive IPMNs before they progress to PDAC.

Histopathological Features of IPMNs

As mentioned previously, IPMNs can be subdivided into three groups based on their location with respect to the pancreatic ductal system: main duct, branch duct, and mixed main and branch duct. Interestingly, main and branch duct IPMNs differ in their clinicopathologic features (Table 2). Based on the degree of architectural and cytologic atypia, IPMNs are graded as either low-grade or high-grade. Representative histologic images of these lesions are shown in Fig. 1c, d. Main duct IPMNs have an increased frequency of harboring high-grade dysplasia and more often associated with a PDAC than branch duct IPMNs [96, 97]. The neoplastic epithelium lining the papillae can demonstrate a variety of directions of differentiation, but the biologic and clinical significance of patterns of differentiation remain controversial. Most IPMNs adopt an intestinal differentiation and resemble intestinal adenomas with well-formed, long villous projections, lined by columnar mucinous epithelium with cigar-shaped nuclei. Most of the neoplastic cells contain abundant apical mucin and, in some cases, have scattered goblet cells. Gastric foveolar differentiation is characterized by eosinophilic cytoplasm, basally oriented nuclei, and abundant apical cytoplasm mucin. Gastric foveolar type IPMNs can be papillary or flat in appearance. The pancreatobiliary type IPMN is less common and the neoplastic cells form more complex papillae with bridging and cribriform structures. The nuclei are rounder than the intestinal type and the chromatin pattern is open, often with prominent nucleoli. This type contains less apical mucin and tends to harbor at least high-grade dysplasia. The intestinal and pancreatobiliary types of IPMN more commonly arise in the main duct, while the gastric type of IPMN is usually a branch duct lesion. The histological subtypes also demonstrate different patterns of apomucin labeling, with the intestinal-type IPMNs expressing MUC2, the pancreato-biliary type expressing MUC1, and the gastric type IPMN expressing MUC5AC, but usually lacking MUC1 and MUC2. In addition to intestinal, gastric

Table 2 Clinical and pathologic features associated with main duct and branch duct IPMNs [94, 95]

	Main duct IPMN	Branch duct IPMN
Age peak	55 years	65 years
Location in pancreas	57% in head	93% in head
Dysplasia/malignancy		
Low-grade dysplasia	43%	85%
High-grade dysplasia	20%	15%
Invasive adenocarcinoma	37%	0%

and pancreatobiliary type IPMNs, there is another histologic variant that is referred to as intraductal oncocytic papillary neoplasm (IOPN). The neoplastic cells found within IOPNs show abundant eosinophilic cytoplasm, due to the high number of mitochondria in these cells. However, whether IOPNs should be classified as a subtype of IPMNs or a distinct entity remains controversial.

IPMNs can be associated with two predominant subtypes of PDAC that include colloid (mucinous noncystic) carcinoma and conventional ductal adenocarcinoma [98]. Distinguishing the subtypes of PDAC is clinically important, since colloid carcinomas carry a significantly better prognosis [99]. Great care should be taken not to overlook an associated focal carcinoma, particularly because the neoplastic epithelium in an IPMN can extend intraductally for several centimeters beyond the grossly dilated duct. Of note, patients with IPMNs show an increased risk for extrapancreatic malignancies. In particular, higher rates of colorectal, gastric, esophageal, and lung malignancies have been reported [100].

Molecular Features of IPMNs

Studies have identified a variety of genetic alterations in IPMNs. The most frequent genetic alteration is an oncogenic *KRAS* mutation, which has a prevalence of >80%. *KRAS* encodes for a G-protein, or a guanosine-nucleotide-binding protein, that functions as a small GTPase and mediates downstream MAPK/ERK signaling from growth factor receptors [101, 102]. Missense mutations result in constitutive activation of *KRAS* and occur primarily in codon 12 and, to a lesser extent, codons 13 and 61 [101]. *KRAS* mutations are detected in all histologic subtypes of IPMNs, but are more likely present in the gastric and pancreatobiliary types. Further, Nikiforova et al. found *KRAS* mutations in IPMNs are associated with a branch duct location [101]. In addition to *KRAS*, 65% of IPMNs harbor somatic mutations in the oncogene *GNAS*, which encodes for the G-protein stimulating α subunit (Gsα) [102]. Mutations in *GNAS* at either codon 201 or 227 result in constitutive activation of Gsα and its effector adenylate cyclase, leading to autonomous synthesis of cAMP and uncontrolled growth signaling [102, 103]. *GNAS* mutations are more often present in IPMNs involving the main pancreatic duct than branch duct, and of an intestinal histologic subtype. Collectively, activating mutations in *KRAS* and/or *GNAS* are present in >96% IPMNs and considered early genetic events in the progression to PDAC.

In addition to *KRAS* and *GNAS*, inactivating mutations in the tumor suppressor gene *RNF43* occur in 14–38% of IPMNs with frequent loss of heterozygosity [104, 105]. *RNF43* encodes for an E3 ubiquitin ligase that regulates the Wnt signaling pathway. Similarly, activating mutations in *CTNNB1* also occur in small subset of IPMNs [106]. Other potential genes mutated in IPMNs include *TP53*, *PIK3CA*, *PTEN*, *CDKN2A*, and *SMAD4*. *TP53* mutations occur late in the neoplastic progression of IPMNs and are frequently seen in advanced neoplasia [107]. Similarly, Garcia-Carracedo et al. found *PIK3CA* mutations and deletions in *PTEN* are strongly associated with high-grade IPMNs and PDAC [108, 109]. Losses in *CDKN2A* are an

uncommon finding, but more prevalent in IPMNs with high-grade dysplasia than low-grade dysplasia [110, 111]. *SMAD4* is also rarely inactivated in low-grade IPMNs, but mutations with corresponding loss of heterozygosity are typically seen in the setting of advanced neoplasia. More recently, Hata et al. demonstrated an elevated telomerase activity, presumably due to *TERT* promoter mutations, in IPMNs is more often seen in IPMNs with high-grade dysplasia and/or invasive adenocarcinoma [112].

Epigenetic silencing by aberrant promoter methylation has been described for a number of candidate tumor suppressor genes in IPMNs, including *SOCS1, ppENK, CDKN1C*, and *CDKN2A* [113, 114]. In recent years, several studies have uncovered a plethora of differentially expressed genes in IPMNs. Transcripts found to be overexpressed in IPMNs that represent candidate biomarkers, and which might also potentially be involved in IPMN progression, include *lipocalin-2, galactin-3, cathepsin-E, claudin-4, TFF-1, TFF-2, TFF-3, CXCR-4, S100A4, matrix metalloproteinase 7 (MMP-7),* and *sonic hedgehog (SHH)* [115–117]. The recent availability of technologies that can enable mass spectrometric based approaches on microdissected tissues has enabled one of the first global proteomic analysis of a noninvasive IPMN [118]. This study, using microdissected material from an archival IPMN, identified tissue transgluaminase-2 (TTG-2) and deleted in malignant brain tumor 1 (DMBT1) as candidate biomarkers in these precursor lesions.

Genetically Engineered Mouse Model of IPMNs

In an elegant study, Schmidt and coworkers described that concomitant pancreas-specific expression of an oncogenic *Kras* allele and transforming growth factor-alpha (TGF-alpha) led to formation of acinar-ductal metaplasia, accelerated progression of *Kras*-induced mPanINs, as compared to *Kras* expression alone, and to the development of cystic lesions resembling key features observed in human IPMNs starting at 2–3 months after birth [119]. Histologically, these cystic lesions were characterized by papillary proliferations which had formed in branches of the main pancreatic duct. In line with findings in humans, the observed murine IPMNs were shown to express CK19, MUC1 and MUC5AC.

Studying the potential role of *GNAS* in pancreatic carcinogenesis, Taki et al. generated transgenic mice that included activated *GNAS* [120]. These mice showed elevated cAMP levels, small dilated tubular complex formation, loss of acinar cells, and fibrosis in the pancreas; but, no macroscopic tumorigenesis was apparent by 2 months of age. However, the combination of *KRAS* and *GNAS* resulted in mice developing cystic tumors consisting of markedly dilated ducts lined by papillary dysplasia epithelium in the pancreas that closely mimicked human IPMNs.

Interestingly, mutations in *Brg1* and other members of the SWI/SNF complex have been observed in over 30% of PDACs, and decreased Brg1 protein expression has been identified in a subset of IPMNs [121]. Inactivation of *Brg1* in combination with mutant *Kras* in mice promoted the development of cystic neoplastic lesions that resemble

IPMNs and over time progress to PDAC [122]. These findings suggest that chromatin remodeling may underlie the development of IPMNs and the formation of PDAC.

Therapeutic Considerations Regarding IPMNs

The major clinical challenge with IPMNs is differentiating IPMNs with high-grade dysplasia and PDAC from IPMNs with low-grade dysplasia. Moreover, another clinical conundrum is predicting whether an IPMN will follow an indolent or malignant disease course. As a consequence, a number of consensus- and evidence-based management and treatment guidelines have been developed for IPMNs and heavily rely on cross-sectional abdominal imaging, endoscopic ultrasound, and pancreatic cyst fluid ancillary studies, such as carcinoembryonic antigen (CEA) and cytopathology [89, 123, 124]. However, these diagnostic modalities have clear limitations in predicting malignancy with a high sensitivity and high specificity. Thus, there has been a growing interest in identifying molecular markers to guide management for IPMNs.

In a pilot study, Khalid et al. prospectively evaluated the presence of mutations in *KRAS* and allelic imbalance in seven tumor suppressor genes by Sanger sequencing in preoperative pancreatic cyst fluid [125]. The authors found the combination of *KRAS* mutations and allelic loss to be predictive of advanced neoplasia within an IPMN with a sensitivity and specificity of 91% and 93%, respectively. These results were later expanded into a multicenter prospective study (Pancreatic Cyst DNA Analysis Study or PANDA study) of 113 patients [126]. Pancreatic cyst fluid was collected preoperatively by EUS-fine needle aspiration (FNA) and assessed for *KRAS* mutations and the overall fraction of alleles lost compared to germline DNA (mean allelic loss amplitude or MALA).

In the PANDA study, the presence of mutant *KRAS* alone had a sensitivity and specificity of 45% and 96%, respectively, for a mucinous cyst, but was not predictive of advanced neoplasia. In contrast, a high MALA (>82%) had 90% sensitivity and 67% specificity for advanced neoplasia. But there were a number of weaknesses in the study design that diminished the overall significance of these results. Notably, it was unclear if DNA analysis would add value to established pancreatic cyst management guidelines. Furthermore, there was concern that MALA may be confounded by DNA degradation, gastrointestinal contamination during EUS-FNA, and other variables. Indeed, follow-up studies demonstrated broad variability in agreement between molecular and clinical diagnoses. Shen et al. reported an 89% concordance between molecular and clinical consensus diagnoses, while Panarelli et al. and Toll et al. reported a concordance rate of 39% and 56%, respectively [127–129].

Regardless of the issues with MALA, *KRAS* testing proved to be a cost-effective strategy to identify patients with IPMNs and MCNs. In a cohort of 618 patients, Nikiforova et al. found mutant *KRAS* had 54% sensitivity and 100% specificity for a mucinous cyst [101]. This assay was superior to CEA testing and utilized significantly less pancreatic cyst fluid for analysis. Moreover, the combination of *KRAS*

point mutations and elevated CEA improved the sensitivity to 83% and maintained a high specificity of 85%. The sensitivity of molecular analysis for mucinous cysts was further increased by the addition of *GNAS* testing. Singhi et al. showed the detection of mutant *KRAS* and/or *GNAS* had a sensitivity and specificity of 65% and 100%, respectively [103]. However, there was significant discordance in the rates of detection of *KRAS* and *GNAS* mutations between preoperative EUS-FNA and studies using postoperative pancreatic cyst fluid. The authors underscored the limitations of their assay may be due to the inherent sensitivity and specimen requirements of conventional Sanger sequencing.

The limit of detection of Sanger sequencing is approximately 15–20% of mutant alleles. In comparison, next-generation sequencing (NGS) has a limit of detection of approximately 3–5% of mutant alleles. Recent studies have shown the application of NGS to pancreatic cyst fluid ranges from 86% to 90% in sensitivity and 75% to 100% in specificity for mucinous differentiation [130, 131]. Other advantages of NGS are the small amounts of DNA required for analysis and the ability to assay multiple genes simultaneously. Using a broad panel of genes to include *KRAS*, *GNAS*, *VHL*, *TP53*, *CDKN2A*, and *SMAD4*, among others, Jones et al. identified a high concordance rate between molecular and clinical diagnoses [131]. Similarly, Singhi et al. found mutations in *TP53*, *PIK3CA*, and/or *PTEN* to have 83% sensitivity and 97% specificity in detecting advanced neoplasia within an IPMN [130]. However, as diagnostic DNA testing of pancreatic cyst fluid continues to evolve, questions remain as to how these alterations will influence patient management.

Mucinous Cystic Neoplasms (MCN)

Clinical Features of MCNs

Mucinous cystic neoplasms (MCNs) are also mucin-producing epithelial neoplasms that arise outside of the large ducts of the pancreas. The exact incidence and prevalence of MCNs is difficult to assess, but within a large surgical series represented, MCNs comprise a quarter of all resected cystic neoplasms of the pancreas. Over 90% of MCNs are diagnosed in females, and the mean age at diagnosis is between 40 and 50 years, with a wide range described in the literature (14–95 years) [132, 133]. Not surprisingly, patients presenting with noninvasive MCNs tend to be 5–10 years younger on average as compared to those carrying MCNs with associated invasive carcinoma, in line with the concept of MCN being a precursor lesion eventually progressing to PDAC. Clinical symptoms are often unspecific and include epigastric pain, a sense of abdominal fullness and abdominal mass. Carcinoembryonic antigen 19-9 (CA19-9) blood concentrations are usually normal in noninvasive MCN patients and elevated only in cases that are associated with a PDAC [134]. Of note, MCNs, like IPMNs, can be discovered as incidental cystic lesions of the pancreas. Computed tomography typically reveals a relatively large (up to 10 cm) intrapancreatic cystic mass. Intramural nodules are more common in MCNs with an associated invasive adenocarcinoma. The cysts

themselves are usually 1–3 cm in diameter and divided by fibrous septa, cyst contents vary from mucoid to hemorrhagic fluid. MCNs do not communicate with the pancreatic duct, and this feature is often exploited to differentiate MCNs from IPMNs in the clinical setting.

Histopathology of MCNs

The cysts of MCNs are lined by a columnar mucin-producing epithelium, associated with a spectrum of architectural and cytologic atypia, akin to what is observed in IPMNs. MCNs with low-grade dysplasia consist of uniform columnar cells with abundant supranuclear mucin. The nuclei are typically uniform, small, and basally located basally (Fig. 1e). In contrast, MCNs with high-grade dysplasia demonstrate significant degree of architectural and cytologic atypia, similar to what is seen in high-grade PanINs and high-grade IPMNs [4, 132] (Fig. 1f). In addition to neoplastic epithelium, MCNs comprise a distinct "ovarian-type" stroma [133, 135]. This ovarian-type stroma consists of densely packed spindle-shaped cells, which can in some cases even show luteinization, and that form a band directly underneath the neoplastic epithelium. Per the current consensus definition, the ovarian-type stroma is an essential prerequisite for the diagnosis of an MCN. Therefore, a proportion of lesions previously referred to as MCNs are now categorized as IPMNs, and the ratio of MCNs relative to IPMNs tends to decrease in newer reports. Diagnostically, ovarian-type stroma can be particularly useful for MCN samples where the neoplastic epithelium is focally denuded. Around one-third of resected MCNs are found to be associated with PDAC [132]. These carcinomas may arise focally in an MCN, and the extent of invasion has been shown to be one of the most important prognostic factors [133].

Molecular Genetics of MCNs

The genetic alterations found in MCNs are similar to those in IPMNs. Analogous to IPMNs, activating *KRAS* mutations are the most common finding, but their prevalence increases with the degree of dysplasia. Jimenez et al. detected KRAS mutations in 26% of low-grade MCNs, while in 89% of MCNs with advanced neoplasia [136]. *RNF43* alterations are also present in MCNs and range from 8% to 35% [104, 105]. In addition, mutations and/or deletions in *TP53*, *PIK3CA*, *PTEN*, *CDKN2A*, and *SMAD4* are detected in MCNs with advanced neoplasia. However, in contrast to IPMNs, *GNAS* mutations are distinctly absent in MCNs [102, 104]. Moreover, recent studies on global expression profiling of MCNs have uncovered tissue specific overexpression of a variety of proteins. Among others, c-met, S100P, prostate stem cell antigen (PSCA), jagged-1, c-myc, cathepsin E, and pepsinogen C were found to be overexpressed by neoplastic epithelial cells, and steroidogenic acute regulatory protein (STAR) and estrogen receptor-1 (ESR-1) by ovarian-type stroma cells, respectively [137, 138].

Genetically Engineered Mouse Models of MCN

Within the past decade, at least two genetically engineered mouse models have been described, closely resembling key features of human MCNs. Mao et al. reported that the activation of the Hedgehog signaling pathway through overexpression of a mutationally activated smoothened allele (R26-Smo-M2) in mice led to the rapid development of rhabdomyosarcomas, basal cell carcinomas, and medulloblastomas [139]. Of interest, they also observed the development of a novel form of pancreatic lesions resembling low-grade MCNs in approximately half of tamoxifen-induced mice. These lesions were characterized by cyst formation of varying size, lined by cuboidal epithelium with foci of columnar metaplasia and by a supporting proliferative ovarian-like stroma. Moreover, PAS and Alcian blue stains indicated mucin expression by the epithelial cells within these lesions. Izeradjene et al. described that pancreas-specific expression of oncogenic *Kras* in combination with *Smad4* haploinsufficiency led to the formation of macroscopically visible cystic lesions in the body and tail of murine pancreata [140]. Histopathological examination revealed formation of low-grade mPanINs as well as cystic lesions resembling histological features of human MCNs, including lining by a neoplastic epithelium consisting of columnar, mucin-filled, CK19 positive epithelial cells displaying focal areas of low to high-grade dysplasia, as well as a surrounding stroma that was frequently very cellular and contained spindle-shaped cells with distinctive "wavy" nuclei. Interestingly, the cysts did not seem to communicate with the duct system.

Therapeutic Implications of MCNs

The prognosis of MCNs depends largely on whether or not there is an associated adenocarcinoma and the extent of adenocarcinoma invasion. If a PDAC is not diagnosed after thorough histopathological evaluation of a surgically completely resected MCN, the patient has an excellent prognosis and can be considered as cured [133]. If, on the other hand, a surgically resected MCN is found to be associated with an invasive carcinoma, patients show a worse 5-year overall survival of only about 60%, which is, nevertheless, still considerably better than survival rates observed for PDAC that are not associated with an MCN [133]. However, the extent of invasion of the adenocarcinoma largely dictates prognosis. Both Crippa et al. and Lewis et al. found intracapsular PDAC as defined by invasion that did not go beyond the wall of the MCN to be associated with an excellent prognosis [141, 142]. Between both studies, only 4 of 30 (13%) adenocarcinomas with intracapsular invasion recurred. Similarly, Zamboni et al. reported three patients with intracapsular invasion and five cases with extracapsular invasion. All three patients with intracapsular invasion were alive and well after a mean follow-up of 22 months [133]. In contrast, two of three patients with extracapsular invasion died of disease.

At least two clinically relevant conclusions can be drawn from these observations: First, the striking difference in prognosis between MCN with and without accompanying PDAC underscores the importance and potential of early detection

and resection of these precursor lesions. Unlike noninvasive IPMNs, MCNs are typically unifocal and represent surgically curable lesions even if they are associated with high-grade dysplasia and minimally invasive adenocarcinoma at the time of diagnosis. The observed age difference of patients with and without associated adenocarcinoma further indicates that there is probably a sufficient time window of probably several years in a given patient, before an existing MCN develops an invasive adenocarcinoma, and during which early detection and resection with curative intent are possible. Secondly, pathologists need to carefully and entirely sample MCNs for histopathologic review to adequately assess for the presence of a PDAC and document the extent of invasion.

Conclusion

In summary, PanINs, IPMNs, and MCNs represent three well-defined precursor lesions of PDAC. In the last decade, significant progress has been made in understanding their molecular genetics, development of animal models, and improvements in early detection of these lesions in asymptomatic individuals. Further advances in early detection and possibly chemopreventive clinical trials are expected to occur within the next decade and are essential in the fight against pancreatic cancer.

Box 1 Key Research Points

Three types of precursor lesions are recognized that can progress to invasive adenocarcinoma of the pancreas – pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN). Over the past decade, consensus histopathological criteria have been established that facilitate the accurate diagnosis and classification of these precursors, and permit comparable data to be generated between different institutions. The multistep progression from early to later stages of these precursor lesions is mirrored by a series of accumulating genetic alterations.

Box 2 Future Scientific Directions

While potent therapeutic options for established PDAC are lacking accounting for its overall dismal prognosis, the precursor lesions of PDAC (e.g., PanINs, IPMNs, and MCNs) represent a unique therapeutic opportunity for curative intervention. Future research should be aimed at developing diagnostic and imaging tools which allow for reliable early detection of these precursor lesions in a clinical setting. This is particularly desirable for PanINs, which are by far the most frequently observed precursor lesions and are difficult or close to impossible to detect with current clinically available imaging

(continued)

Box 2 Future Scientific Directions (continued)
techniques. Moreover, prospective studies should address individual risk estimation of diagnosed precursor lesions to enable evidence-based guidelines for the appropriate clinical management in individual cases.

Box 3 Clinical Implications
Early detection of precursor lesions of PDAC has the potential to identify high-risk patients and treat a pancreatic lesion before it progresses into a frank malignancy. The clinical implications for some precursor lesions are more obvious than others. MCNs should always be resected and thoroughly evaluated histopathologically for the presence of an associated PDAC. The same holds true for main duct type IPMNs. However, there are currently opposing opinions as to the management and treatment of branch duct type IPMNs. PanINs are a common finding in the elderly population, but to date appropriate tools to reliably diagnose isolated PanINs in a clinical setting are lacking. Recently, endoscopic ultrasound has enabled the diagnosis of multifocal PanIN lesions in patients at risk for developing PDAC (e.g., individuals with a familial pancreatic cancer). Improvements in imaging strategy and the incorporation of molecular techniques in the diagnosis and workup of precursor lesions should facilitate improved therapeutic decision making.

Cross-References

- ▶ Animal modeling of Pancreatitis-to-Cancer Progression
- ▶ Developmental Molecular Biology of the Pancreas
- ▶ Epigenetics and Its Applications to the Progression Model of Pancreatic Cancer
- ▶ Molecular Pathology of Carcinomas of the Ampullary/Periampullary Region
- ▶ Molecular Pathology of Pancreatic Endocrine Tumors

References

1. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319:525–32.
2. Hulst SLP. Zur Kenntnis der Genese des Adenokarzinoms und Karzinoms des Pankreas. Virchows Archiv. 1905;180:288–316.
3. Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol. 2004;28:977–87.

4. Hruban RH, Maitra A, Kern SE, et al. Precursors to pancreatic cancer. Gastroenterol Clin North Am. 2007;36:831–49.
5. Cubilla AL, Fitzgerald PJ. Morphological lesions associated with human primary invasive nonendocrine pancreas cancer. Cancer Res. 1976;36:2690–8.
6. Andea A, Sarkar F, Adsay VN. Clinicopathological correlates of pancreatic intraepithelial neoplasia: a comparative analysis of 82 cases with and 152 cases without pancreatic ductal adenocarcinoma. Mod Pathol. 2003;16:996–1006.
7. Agoff SN, Crispin DA, Bronner MP, et al. Neoplasms of the ampulla of vater with concurrent pancreatic intraductal neoplasia: a histological and molecular study. Mod Pathol. 2001;14:139–46.
8. Stelow EB, Adams RB, Moskaluk CA. The prevalence of pancreatic intraepithelial neoplasia in pancreata with uncommon types of primary neoplasms. Am J Surg Pathol. 2006;30: 36–41.
9. Maitra A, Adsay NV, Argani P, et al. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. Mod Pathol. 2003;16:902–12.
10. Almoguera C, Shibata D, Forrester K, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 1988;53:549–54.
11. Hruban RH, van Mansfeld AD, Offerhaus GJ, et al. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. Am J Pathol. 1993;143:545–54.
12. Kanda M, Matthaei H, Wu J, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology. 2012;142:730–733.e9.
13. Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 2003;4:437–50.
14. Aguirre AJ, Bardeesy N, Sinha M, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev. 2003;17:3112–26.
15. Hingorani SR, Tuveson DA. Ras redux: rethinking how and where Ras acts. Curr Opin Genet Dev. 2003;13:6–13.
16. Baines AT, Lim KH, Shields JM, et al. Use of retrovirus expression of interfering RNA to determine the contribution of activated K-Ras and ras effector expression to human tumor cell growth. Methods Enzymol. 2006;407:556–74.
17. Brummelkamp TR, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell. 2002;2:243–7.
18. Laghi L, Orbetegli O, Bianchi P, et al. Common occurrence of multiple K-RAS mutations in pancreatic cancers with associated precursor lesions and in biliary cancers. Oncogene. 2002;21:4301–6.
19. Sherr CJ. Cell cycle control and cancer. Harvey Lect. 2000;96:73–92.
20. Caldas C, Hahn SA, da Costa LT, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat Genet. 1994;8:27–32.
21. Schutte M, Hruban RH, Geradts J, et al. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res. 1997;57:3126–30.
22. Wilentz RE, Geradts J, Maynard R, et al. Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. Cancer Res. 1998;58:4740–4.
23. Rosty C, Geradts J, Sato N, et al. p16 Inactivation in pancreatic intraepithelial neoplasias (PanINs) arising in patients with chronic pancreatitis. Am J Surg Pathol. 2003;27:1495–501.
24. Hustinx SR, Hruban RH, Leoni LM, et al. Homozygous deletion of the MTAP gene in invasive adenocarcinoma of the pancreas and in periampullary cancer: a potential new target for therapy. Cancer Biol Ther. 2005;4:83–6.
25. Hustinx SR, Leoni LM, Yeo CJ, et al. Concordant loss of MTAP and p16/CDKN2A expression in pancreatic intraepithelial neoplasia: evidence of homozygous deletion in a noninvasive precursor lesion. Mod Pathol. 2005;18:959–63.

26. Hingorani SR, Wang L, Multani AS, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell. 2005;7:469–83.
27. Redston MS, Caldas C, Seymour AB, et al. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. Cancer Res. 1994;54:3025–33.
28. Hahn SA, Hoque AT, Moskaluk CA, et al. Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res. 1996;56:490–4.
29. Gordon KJ, Blobe GC. Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim Biophys Acta. 2008;1782:197–228.
30. Massague J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell. 2000;103:295–309.
31. Kim BG, Li C, Qiao W, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. Nature. 2006;441:1015–9.
32. Wilentz RE, Su GH, Dai JL, et al. Immunohistochemical labeling for dpc4 mirrors genetic status in pancreatic adenocarcinomas : a new marker of DPC4 inactivation. Am J Pathol. 2000;156:37–43.
33. Wilentz RE, Iacobuzio-Donahue CA, Argani P, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res. 2000;60:2002–6.
34. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004;10:789–99.
35. van der Heijden MS, Yeo CJ, Hruban RH, et al. Fanconi anemia gene mutations in young-onset pancreatic cancer. Cancer Res. 2003;63:2585–8.
36. van der Heijden MS, Brody JR, Gallmeier E, et al. Functional defects in the Fanconi anemia pathway in pancreatic cancer cells. Am J Pathol. 2004;165:651–7.
37. D'Andrea AD, Grompe M. The Fanconi anemia/BRCA pathway. Nat Rev Cancer. 2003;3:23–34.
38. Couch FJ, Johnson MR, Rabe KG, et al. The prevalence of BRCA2 mutations in familial pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007;16:342–6.
39. Klein AP, Hruban RH, Brune KA, et al. Familial pancreatic cancer. Cancer J. 2001;7:266–73.
40. Goggins M, Hruban RH, Kern SE. BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: evidence and implications. Am J Pathol. 2000;156:1767–71.
41. van Heek NT, Meeker AK, Kern SE, et al. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. Am J Pathol. 2002;161:1541–7.
42. Yamano M, Fujii H, Takagaki T, et al. Genetic progression and divergence in pancreatic carcinoma. Am J Pathol. 2000;156:2123–33.
43. Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet. 2000;16:168–74.
44. Sato N, Fukushima N, Hruban RH, et al. CpG island methylation profile of pancreatic intraepithelial neoplasia. Mod Pathol. 2008;21:238–44.
45. Peng DF, Kanai Y, Sawada M, et al. DNA methylation of multiple tumor-related genes in association with overexpression of DNA methyltransferase 1 (DNMT1) during multistage carcinogenesis of the pancreas. Carcinogenesis. 2006;27:1160–8.
46. Goggins M. Identifying molecular markers for the early detection of pancreatic neoplasia. Semin Oncol. 2007;34:303–10.
47. Iacobuzio-Donahue CA, Ashfaq R, Maitra A, et al. Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res. 2003;63:8614–22.
48. Han H, Bearss DJ, Browne LW, et al. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res. 2002;62:2890–6.

49. Logsdon CD, Simeone DM, Binkley C, et al. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res. 2003;63:2649–57.
50. Buchholz M, Braun M, Heidenblut A, et al. Transcriptome analysis of microdissected pancreatic intraepithelial neoplastic lesions. Oncogene. 2005;24:6626–36.
51. Nakamura T, Furukawa Y, Nakagawa H, et al. Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. Oncogene. 2004;23:2385–400.
52. Tanaka M, Komatsu N, Terakawa N, et al. Increased levels of IgG antibodies against peptides of the prostate stem cell antigen in the plasma of pancreatic cancer patients. Oncol Rep. 2007;18:161–6.
53. Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res. 2001;7:3862–8.
54. Li M, Bharadwaj U, Zhang R, et al. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. Mol Cancer Ther. 2008;7:286–96.
55. Segara D, Biankin AV, Kench JG, et al. Expression of HOXB2, a retinoic acid signaling target in pancreatic cancer and pancreatic intraepithelial neoplasia. Clin Cancer Res. 2005;11:3587–96.
56. Kent OA, Mullendore M, Wentzel EA, et al. A resource for analysis of microRNA expression and function in pancreatic ductal adenocarcinoma cells. Cancer Biol Ther. 2009;8: 2013–24.
57. Lee EJ, Gusev Y, Jiang J, et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer. 2007;120:1046–54.
58. Zhang Y, Li M, Wang H, et al. Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. World J Surg. 2009;33:698–709.
59. Yu J, Li A, Hong SM, et al. MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin Cancer Res. 2012;18:981–92.
60. Klein WM, Hruban RH, Klein-Szanto AJ, et al. Direct correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN): additional evidence for a recently proposed model of progression. Mod Pathol. 2002;15:441–7.
61. Biankin AV, Kench JG, Morey AL, et al. Overexpression of p21(WAF1/CIP1) is an early event in the development of pancreatic intraepithelial neoplasia. Cancer Res. 2001;61: 8830–7.
62. Tucker ON, Dannenberg AJ, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res. 1999;59:987–90.
63. Maitra A, Ashfaq R, Gunn CR, et al. Cyclooxygenase 2 expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia: an immunohistochemical analysis with automated cellular imaging. Am J Clin Pathol. 2002;118:194–201.
64. Sclabas GM, Uwagawa T, Schmidt C, et al. Nuclear factor kappa B activation is a potential target for preventing pancreatic carcinoma by aspirin. Cancer. 2005;103:2485–90.
65. Bloomston M, Zervos EE, Rosemurgy AS 2nd. Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. Ann Surg Oncol. 2002;9:668–74.
66. Crawford HC, Scoggins CR, Washington MK, et al. Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. J Clin Invest. 2002;109:1437–44.
67. O'Mahony CA, Seidel A, Albo D, et al. Angiostatin generation by human pancreatic cancer. J Surg Res. 1998;77:55–8.
68. Ribatti D, Leali D, Vacca A, et al. In vivo angiogenic activity of urokinase: role of endogenous fibroblast growth factor-2. J Cell Sci. 1999;112(Pt 23):4213–21.

69. Harvey SR, Hurd TC, Markus G, et al. Evaluation of urinary plasminogen activator, its receptor, matrix metalloproteinase-9, and von Willebrand factor in pancreatic cancer. Clin Cancer Res. 2003;9:4935–43.
70. Berman DM, Karhadkar SS, Maitra A, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature. 2003;425:846–51.
71. Doucas H, Garcea G, Neal CP, et al. Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance. Eur J Cancer. 2005;41:365–79.
72. Miyamoto Y, Maitra A, Ghosh B, et al. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell. 2003;3:565–76.
73. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007;67:1030–7.
74. Feldmann G, Dhara S, Fendrich V, et al. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res. 2007;67:2187–96.
75. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature. 2003;425:851–6.
76. Prasad NB, Biankin AV, Fukushima N, et al. Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. Cancer Res. 2005;65:1619–26.
77. Taipale J, Beachy PA. The Hedgehog and Wnt signalling pathways in cancer. Nature. 2001;411:349–54.
78. Pasca di Magliano M, Biankin AV, Heiser PW, et al. Common activation of canonical Wnt signaling in pancreatic adenocarcinoma. PLoS One. 2007;2:e1155.
79. Al-Aynati MM, Radulovich N, Riddell RH, et al. Epithelial-cadherin and beta-catenin expression changes in pancreatic intraepithelial neoplasia. Clin Cancer Res. 2004;10:1235–40.
80. Hruban RH, Adsay NV, Albores-Saavedra J, et al. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. Cancer Res. 2006;66:95–106.
81. Feldmann G, Habbe N, Dhara S, et al. Hedgehog inhibition prolongs survival in a genetically engineered mouse model of pancreatic cancer. Gut. 2008;57:1420.
82. Faca VM, Song KS, Wang H, et al. A mouse to human search for plasma proteome changes associated with pancreatic tumor development. PLoS Med. 2008;5:e123.
83. Funahashi H, Satake M, Dawson D, et al. Delayed progression of pancreatic intraepithelial neoplasia in a conditional Kras(G12D) mouse model by a selective cyclooxygenase-2 inhibitor. Cancer Res. 2007;67:7068–71.
84. Canto MI, Goggins M, Hruban RH, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol. 2006;4:766–81; quiz 665.
85. Brune K, Abe T, Canto M, et al. Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. Am J Surg Pathol. 2006;30:1067–76.
86. Terhune PG, Phifer DM, Tosteson TD, et al. K-ras mutation in focal proliferative lesions of human pancreas. Cancer Epidemiol Biomarkers Prev. 1998;7:515–21.
87. Yu J, Sadakari Y, Shindo K, et al. Digital next-generation sequencing identifies low-abundance mutations in pancreatic juice samples collected from the duodenum of patients with pancreatic cancer and intraductal papillary mucinous neoplasms. Gut. 2017;66:1677–87.
88. Reid-Lombardo KM, St Sauver J, Li Z, et al. Incidence, prevalence, and management of intraductal papillary mucinous neoplasm in Olmsted County, Minnesota, 1984–2005: a population study. Pancreas. 2008;37:139–44.
89. Tanaka M, Fernandez-del Castillo C, Adsay V, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. Pancreatology. 2012;12:183–97.

90. Scheiman JM, Hwang JH, Moayyedi P. American Gastroenterological Association technical review on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. Gastroenterology. 2015;148:824–48.e22.
91. Adsay V, Mino-Kenudson M, Furukawa T, et al. Pathologic evaluation and reporting of intraductal papillary mucinous neoplasms of the pancreas and other tumoral intraepithelial neoplasms of pancreatobiliary tract: recommendations of Verona Consensus Meeting. Ann Surg. 2016;263:162–77.
92. Crippa S, Fernandez-Del Castillo C, Salvia R, et al. Mucin-producing neoplasms of the pancreas: an analysis of distinguishing clinical and epidemiologic characteristics. Clin Gastroenterol Hepatol. 2010;8:213–9.
93. Salvia R, Fernandez-del Castillo C, Bassi C, et al. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg. 2004;239:678–85; discussion 685–7.
94. Maitra A, Fukushima N, Takaori K, et al. Precursors to invasive pancreatic cancer. Adv Anat Pathol. 2005;12:81–91.
95. Terris B, Ponsot P, Paye F, et al. Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less aggressive pathologic features as compared with those involving the main pancreatic duct. Am J Surg Pathol. 2000;24:1372–7.
96. Seki M, Yanagisawa A, Ohta H, et al. Surgical treatment of intraductal papillary-mucinous tumor (IPMT) of the pancreas: operative indications based on surgico-pathologic study focusing on invasive carcinoma derived from IPMT. J Hepatobiliary Pancreat Surg. 2003;10:147–55.
97. Tanaka M. Intraductal papillary mucinous neoplasm of the pancreas: diagnosis and treatment. Pancreas. 2004;28:282–8.
98. Seidel G, Zahurak M, Iacobuzio-Donahue C, et al. Almost all infiltrating colloid carcinomas of the pancreas and periampullary region arise from in situ papillary neoplasms: a study of 39 cases. Am J Surg Pathol. 2002;26:56–63.
99. Adsay NV, Pierson C, Sarkar F, et al. Colloid (mucinous noncystic) carcinoma of the pancreas. Am J Surg Pathol. 2001;25:26–42.
100. Kamisawa T, Tu Y, Egawa N, et al. Malignancies associated with intraductal papillary mucinous neoplasm of the pancreas. World J Gastroenterol. 2005;11:5688–90.
101. Nikiforova MN, Khalid A, Fasanella KE, et al. Integration of KRAS testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts. Mod Pathol. 2013;26:1478–87.
102. Wu J, Matthaei H, Maitra A, et al. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. Sci Transl Med. 2011;3:92ra66.
103. Singhi AD, Nikiforova MN, Fasanella KE, et al. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. Clin Cancer Res. 2014;20:4381–9.
104. Springer S, Wang Y, Dal Molin M, et al. A combination of molecular markers and clinical features improve the classification of pancreatic cysts. Gastroenterology. 2015;149:1501–10.
105. Wu J, Jiao Y, Dal Molin M, et al. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. Proc Natl Acad Sci U S A. 2011;108:21188–93.
106. Amato E, Molin MD, Mafficini A, et al. Targeted next-generation sequencing of cancer genes dissects the molecular profiles of intraductal papillary neoplasms of the pancreas. J Pathol. 2014;233:217–27.
107. Kanda M, Sadakari Y, Borges M, et al. Mutant TP53 in duodenal samples of pancreatic juice from patients with pancreatic cancer or high-grade dysplasia. Clin Gastroenterol Hepatol. 2013;11:719–30.e5.
108. Garcia-Carracedo D, Chen ZM, Qiu W, et al. PIK3CA mutations in mucinous cystic neoplasms of the pancreas. Pancreas. 2014;43:245–9.
109. Garcia-Carracedo D, Turk AT, Fine SA, et al. Loss of PTEN expression is associated with poor prognosis in patients with intraductal papillary mucinous neoplasms of the pancreas. Clin Cancer Res. 2013;19:6830–41.

110. Sasaki S, Yamamoto H, Kaneto H, et al. Differential roles of alterations of p53, p16, and SMAD4 expression in the progression of intraductal papillary-mucinous tumors of the pancreas. Oncol Rep. 2003;10:21–5.
111. Biankin AV, Biankin SA, Kench JG, et al. Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut. 2002;50:861–8.
112. Hata T, Dal Molin M, Suenaga M, et al. Cyst fluid telomerase activity predicts the histologic grade of cystic neoplasms of the pancreas. Clin Cancer Res. 2016;22:5141–51.
113. House MG, Guo M, Iacobuzio-Donahue C, et al. Molecular progression of promoter methylation in intraductal papillary mucinous neoplasms (IPMN) of the pancreas. Carcinogenesis. 2003;24:193–8.
114. Sato N, Ueki T, Fukushima N, et al. Aberrant methylation of CpG islands in intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology. 2002;123:365–72.
115. Sato N, Fukushima N, Maitra A, et al. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. Am J Pathol. 2004;164:903–14.
116. Ohuchida K, Mizumoto K, Fujita H, et al. Sonic hedgehog is an early developmental marker of intraductal papillary mucinous neoplasms: clinical implications of mRNA levels in pancreatic juice. J Pathol. 2006;210:42–8.
117. Nishikawa N, Kimura Y, Okita K, et al. Intraductal papillary mucinous neoplasms of the pancreas: an analysis of protein expression and clinical features. J Hepatobiliary Pancreat Surg. 2006;13:327–35.
118. Cheung W, Darfler MM, Alvarez H, et al. Application of a global proteomic approach to archival precursor lesions: deleted in malignant brain tumors 1 and tissue transglutaminase 2 are upregulated in pancreatic cancer precursors. Pancreatology. 2008;8:608.
119. Siveke JT, Einwachter H, Sipos B, et al. Concomitant pancreatic activation of Kras(G12D) and Tgfa results in cystic papillary neoplasms reminiscent of human IPMN. Cancer Cell. 2007;12:266–79.
120. Taki K, Ohmuraya M, Tanji E, et al. GNAS(R201H) and Kras(G12D) cooperate to promote murine pancreatic tumorigenesis recapitulating human intraductal papillary mucinous neoplasm. Oncogene. 2016;35:2407–12.
121. Dal Molin M, Hong SM, Hebbar S, et al. Loss of expression of the SWI/SNF chromatin remodeling subunit BRG1/SMARCA4 is frequently observed in intraductal papillary mucinous neoplasms of the pancreas. Hum Pathol. 2012;43:585–91.
122. von Figura G, Fukuda A, Roy N, et al. The chromatin regulator Brg1 suppresses formation of intraductal papillary mucinous neoplasm and pancreatic ductal adenocarcinoma. Nat Cell Biol. 2014;16:255–67.
123. Vege SS, Ziring B, Jain R, et al. American Gastroenterological Association institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. Gastroenterology. 2015;148:819–22; quize12–3.
124. Tanaka M, Chari S, Adsay V, et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology. 2006;6:17–32.
125. Khalid A, McGrath KM, Zahid M, et al. The role of pancreatic cyst fluid molecular analysis in predicting cyst pathology. Clin Gastroenterol Hepatol. 2005;3:967–73.
126. Khalid A, Zahid M, Finkelstein SD, et al. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. Gastrointest Endosc. 2009;69:1095–102.
127. Shen J, Brugge WR, Dimaio CJ, et al. Molecular analysis of pancreatic cyst fluid: a comparative analysis with current practice of diagnosis. Cancer. 2009;117:217–27.
128. Panarelli NC, Sela R, Schreiner AM, et al. Commercial molecular panels are of limited utility in the classification of pancreatic cystic lesions. Am J Surg Pathol. 2012;36:1434–43.
129. Toll AD, Kowalski T, Loren D, et al. The added value of molecular testing in small pancreatic cysts. JOP. 2010;11:582–6.

130. Singhi AD, Zeh HJ, Brand RE, et al. American Gastroenterological Association guidelines are inaccurate in detecting pancreatic cysts with advanced neoplasia: a clinicopathologic study of 225 patients with supporting molecular data. Gastrointest Endosc. 2016;83:1107–1117.e2.
131. Jones M, Zheng Z, Wang J, et al. Impact of next-generation sequencing on the clinical diagnosis of pancreatic cysts. Gastrointest Endosc. 2016;83:140–8.
132. Hruban RH, Pitman MB, Klimstra DS. Tumors of the pancreas, Atlas of tumor pathology. Fourth series, Fascicle 6th edition. Washington, DC: American Registry of Pathology and Armed Forces Institute of Pathology; 2007.
133. Zamboni G, Scarpa A, Bogina G, et al. Mucinous cystic tumors of the pancreas: clinicopathological features, prognosis, and relationship to other mucinous cystic tumors. Am J Surg Pathol. 1999;23:410–22.
134. Bassi C, Salvia R, Gumbs AA, et al. The value of standard serum tumor markers in differentiating mucinous from serous cystic tumors of the pancreas: CEA, Ca 19-9, Ca 125, Ca 15-3. Langenbecks Arch Surg. 2002;387:281–5.
135. Zamboni G, Kloppel G, Hruban R, et al. Mucinous cystic neoplasms of the pancreas. In: Hamilton SR, Aaltonen LA, editors. World Health Organization classification of tumours. Pathology and genetics of tumours of the digestive system. Lyon: IARC Press; 2000. p. 234–6.
136. Jimenez RE, Warshaw AL, Z'Graggen K, et al. Sequential accumulation of K-ras mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg. 1999;230:501–9; discussion 509–11.
137. Fukushima N, Sato N, Prasad N, et al. Characterization of gene expression in mucinous cystic neoplasms of the pancreas using oligonucleotide microarrays. Oncogene. 2004;23:9042–51.
138. Lam MM, Swanson PE, Upton MP, et al. Ovarian-type stroma in hepatobiliary cystadenomas and pancreatic mucinous cystic neoplasms: an immunohistochemical study. Am J Clin Pathol. 2008;129:211–8.
139. Mao J, Ligon KL, Rakhlin EY, et al. A novel somatic mouse model to survey tumorigenic potential applied to the Hedgehog pathway. Cancer Res. 2006;66:10171–8.
140. Izeradjene K, Combs C, Best M, et al. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. Cancer Cell. 2007;11:229–43.
141. Crippa S, Salvia R, Warshaw AL, et al. Mucinous cystic neoplasm of the pancreas is not an aggressive entity: lessons from 163 resected patients. Ann Surg. 2008;247:571–9.
142. Lewis GH, Wang H, Bellizzi AM, et al. Prognosis of minimally invasive carcinoma arising in mucinous cystic neoplasms of the pancreas. Am J Surg Pathol. 2013;37:601–5.