

Pancreatic adenocarcinoma pathology: changing “landscape”

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Abstract: Pancreatic cancer is a devastating disease. At time of diagnosis the disease is usually advanced and only a minority of patients are eligible for surgical resection. The overall 5-year survival is 6%. However, survival of patients with early stage pancreatic cancer is significantly better. To improve the prognosis of patients with pancreatic cancer, it is essential to diagnose and treat pancreatic cancer in the earliest stage. Prevention of pancreatic cancer by treating noninvasive precursor lesions just before they invade tissues can potentially lead to even better outcomes. Pancreatic carcinogenesis results from a stepwise progression in which accumulating genetic alterations drive neoplastic progression in well-defined precursor lesions, ultimately giving rise to an invasive adenocarcinoma. A thorough understanding of the genetic changes that drive pancreatic carcinogenesis can lead to identification of biomarkers for early detection and targets for therapy. Recent next-generation sequencing (NGS) studies have shed new light on our understanding of the natural history of pancreatic cancer and the precursor lesions that give rise to these cancers. Importantly, there is a significant window of opportunity for early detection and treatment between the first genetic alteration in a cell in the pancreas and development of full-blown pancreatic cancer. The current views on the pathology and genetics of pancreatic carcinogenesis that evolved from studies of pancreatic cancer and its precursor lesions are discussed in this review.

Keywords: Pancreatic cancer; genetics; precursor lesions; pancreatic intraepithelial neoplasia (PanIN); intraductal papillary mucinous neoplasm (IPMN)

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an extremely poor prognosis. The overall 5-year survival rate is 6%. The median survival varies from almost 2 years for patients with local and resectable disease, to only a few months for patients with advanced metastatic disease. Unfortunately, the vast majority of patients present at an advanced inoperable stage, whereas only about 20% of patients have localized disease that is amenable for surgery (1). To improve prognosis for patients with PDAC, it is essential to diagnose and treat the disease in the earliest stages, ideally even before a full blown invasive PDAC is established, by treating precursor lesions (2).

A growing body of evidence has helped establish that

invasive PDAC develops from well-defined noninvasive precursor lesions (3). The most common precursor to invasive PDAC, pancreatic intraepithelial neoplasia (PanIN), is microscopic (3). In addition to this microscopic lesion, there are two macroscopically discernible cystic precursor lesions in the pancreas (4). These cystic precursor lesions are intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) (4).

In addition to morphologic characterization of pancreatic carcinogenesis, our understanding of the genetic alterations that drive carcinogenesis has increased dramatically over the last decades. In particular, recent advancements in sequencing technologies have immensely deepened our understanding of the genetics of PDAC (5-9). Whereas earlier studies have focused on the major driver genes

involved in invasive PDAC, more recent studies using next-generation sequencing (NGS) have produced a more complete understanding of the genetics of PDAC, its variants, and its precursor lesions. Mathematical modeling of genetic data suggests that the genetic evolution of PDAC takes almost 12 years from the earliest genetic alteration in a precursor lesion to the development of a full-blown invasive cancer (10). Thus there is an almost 12-year window of opportunity to prevent PDAC from even developing if we can identify and treat noninvasive precursor lesions. In addition, the genes targeted in pancreatic neoplasms may serve as future biomarkers in the genetic diagnosis of PDAC and its precursors (7,8). This review article discusses the pathology and the current knowledge of genetics of PDAC and its precursor lesions PanIN, IPMN and MCN.

Genetics of invasive PDAC and its precursor lesions

Genetics of invasive PDAC

Invasive PDAC is one of the best understood tumors at the genetic level (5-7,10,11). Invasive PDACs are genetically very complex, with wide-spread chromosome abnormalities, numerous losses and gains of large segments of DNA, and on average more than 60 exomic alterations in each cancer (12,13). The genes most commonly targeted in PDAC are *KRAS*, *CDKN2A*, *TP53* and *SMAD4*. In addition, several less commonly mutated genes, including *MLL3*, *SMAD3*, *FBXW7* and *ARID1A* have been identified. Germline mutations in *BRCA2* and *CDKN2A*, and less frequently in *BRCA1*, *PALB2* and *ATM* have been identified in a small subset of patients with familial PDAC (14-16). In addition, patients with Lynch syndrome (caused by germline mutation in one of the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2*) and Peutz-Jeghers syndrome (PJS) (caused by germline mutation of the *STK11* gene) are at increased risk of PDAC (17,18).

Importantly, despite the relatively large number of genes targeted in PDAC, genetic alterations in PDAC have been shown to involve several core cellular signaling pathways and processes (Table 1). These include chromatin modification (*EPC1* and *ARID2*), DNA damage repair (*TP53*, *ATM*, *PALB2* and *BRCA2*) and other mechanisms (*ZIM2*, *MAP2K4*, *NALCN*, *SLC16A4* and *MAGEA6*) (6). In addition, a recent study has also suggested that genes described traditionally as embryonic regulators of axon guidance, particularly signaling through slit ligands and

roundabout receptors (SLIT/ROBO), may also be targeted in pancreatic cancer (5). Most PDACs harbor a mutation in a gene in each core pathway, but the specific gene mutated in a given pathway can differ among different PDACs. Therapeutic targeting of one or more of these pathways may thus be more effective than targeting of a specific genetic alteration.

With the advances in sequencing technologies, the genetic alterations in PDAC can now be studied at unprecedented levels, providing insights into the disease in ways that simply were not possible a decade ago. For example, comparisons of the genetic alterations in metastases to the primary tumors from which they arose provided insight into the length of time it takes for metastases to develop. Yachida *et al.* found that the genetic alterations in metastatic PDACs are surprisingly similar to those in matched primary tumors (7). By investigating whether mutations identified in the index metastasis were present or absent in multiple additional samples from the primary tumor they identified two categories of mutations. First, mutations present in all samples from a given patient were considered “founder mutations”, which were likely established in the noninvasive precursor lesion that gave rise to the invasive PDAC. Founder mutations included mutations in the major genes known to be involved in pancreatic carcinogenesis (i.e., *KRAS*, *CDKN2A*, *TP53*, and *SMAD4*). Mutations that were only present in a subset of the samples from each patient were considered “progressor mutations”. Progressor mutations occurred later than founder mutations and represent subclonal evolution beyond the parental clone. Of interest, Yachida *et al.* found that clonal populations that give rise to distant metastases were represented within the primary carcinoma, but these clones were genetically evolved from the original parental, nonmetastatic clone. Thus, genetic heterogeneity of metastases reflects the heterogeneity within the primary carcinoma. Extending this observation further using quantitative analyses of the timing of the genetic evolution of PDAC, Yachida and colleagues calculated that almost 12 years pass between the initiating mutation and the birth of the nonmetastatic invasive PDAC. Five more years are required for the acquisition of metastatic ability and the average patient dies 2 years thereafter (7). Compared to the traditional view on PDAC as a very rapidly progressing disease that is almost instantaneously metastatic, these studies revealed that genetic evolution and growth of PDAC resembles that of other tumor types and that there is a wide window of opportunity for early detection and treatment (10).

Table 1 Core signaling pathways in pancreatic ductal adenocarcinoma

Regulatory process or pathway	Representative altered genes
Invasion	<i>ADAM11, ADAM12, ADAM19, ADAM5220, ADAMTS15, DPP6, MEP1A, PCSK6, APG4A, PRSS23</i>
TGF β signaling	<i>TGFBR2, BMPR2, SMAD4, SMAD3</i>
KRAS signaling	<i>KRAS, MAP2K4, RASGRP3</i>
JNK signaling	<i>MAP4K3, TNF, ATF2, NFATC3</i>
Integrin signaling	<i>ITGA4, ITGA9, ITGA11, LAMA1, LAMA4, LAMA5, FN1, ILK</i>
Wnt signaling	<i>MYC, PPP2R3A, WNT9A, MAP2, TSC2, GATA6, TCF4, RNF43*</i>
Hedgehog signaling	<i>TBX5, SOX3, LRP2, GLI1, GLI3, BOC, BMPR2, CREBBP</i>
Control of G1/S phase transition	<i>CDKN2A, FBXW7, CHD1, APC2</i>
Apoptosis	<i>CASP10, VCP, CAD, HIP1</i>
DNA damage control	<i>ERCC4, ERCC6, EP300, RANBP2, TP53, ATM, PALB2, BRCA1, BRCA2[#]</i>
Small GTPase signaling	<i>AGHGEF7, ARHGEF9, CDC42BPA, DEPDC2, PLCB3, PLCB4, RP1, PLXNB1, PRKCG</i>
Homophilic cell adhesion	<i>CDH1, CDH10, CDH2, CDH7, FAT, PCDH15, PCDH17, PCDH18, PCDH9, PCDHB16, PCDHB2, PCDHGA1, PCDHGA11, PCDHGC4</i>
Chromatin regulation	<i>ARID1A, EPC1, ARID2</i>
Axon guidance	<i>ROBO1, ROBO2, SLIT2, SEMA3A, SEMA3E, SEMA5A, EPHA5, EPHA7</i>

*, *RNF43* is mutated in a subset of MCNs and IPMNs (8). See text; [#], *ATM, PALB2, BRCA1* and *BRCA2* are mutated in hereditary pancreatic cancer and less frequently in sporadic PDAC (14-16). See text. Adapted from Jones *et al.* (6) and Biankin *et al.* (5).

Pancreatic intraepithelial neoplasia (PanIN)

The vast majority of PDACs are believed to arise from PanIN (3,19). PanINs are small microscopic lesions that are <5 mm. They are composed of a flat or papillary neoplastic epithelium. Three grades of dysplasia can distinguished in PanIN lesions (*Figure 1*). PanIN-1A and PanIN-1B have low-grade dysplasia. They are characterized by tall columnar cells with basally located small round-to-oval nuclei and abundant supranuclear mucin. PanIN-1A has flat epithelium, whereas PanIN-1B is characterized by papillary or micropapillary architecture. PanIN-2 is considered intermediate-grade dysplasia and shows mostly papillary epithelium with mild to moderate cytological atypia. PanIN-3 is considered high-grade dysplasia (carcinoma *in situ*) and characterized by usually papillary or micropapillary proliferations of cells with significant cytological atypia (19). Of note, PanINs are often surrounded by lobular parenchymal atrophy which, when multifocal, can be detected by endoscopic ultrasound and may serve as a biomarker in patients at high-risk for PDAC (20).

PanIN lesions are common in the pancreas. For example, Konstantinidis and colleagues found PanINs in 153 (26%) of 584 pancreata surgically resected for a reason other than PDAC. Most of these lesions were PanIN-1 (50% of

pancreata with PanIN) and PanIN-2 (41% of pancreata with PanIN), whereas PanIN-3 was only present in 13 cases (8% of pancreata with PanIN) (21). By contrast PanIN-3 has been reported to be present in 30-50% of pancreata with an invasive PDAC (19). Moreover, the number of PanINs, in particular those with high-grade dysplasia, is higher in patients with a strong family history of PDAC compared to patients with a PDAC but no family history of the disease (22).

Genetic studies support the hypothesis that PanINs can be a precursor to invasive pancreatic cancer, and have shown that the increasing morphologic grades of dysplasia in PanIN are accompanied by the accumulation of genetic alterations (*Figure 1*) (3). Telomere shortening and activating mutations in the *KRAS* oncogene are the most common alterations in low-grade PanIN lesions (23-25). Studies in genetically modified mouse models have shown that *KRAS* mutations can initiate PanIN development (26), and deep sequencing using NGS techniques have shown that *KRAS* mutations are present in >90% of all PanIN lesions, even those with low-grade dysplasia. These deep sequencing studies suggest a gradual expansion of the *KRAS*-mutant clone during PanIN progression (24). It appears that *KRAS* mutation alone provides only a modest selective advantage

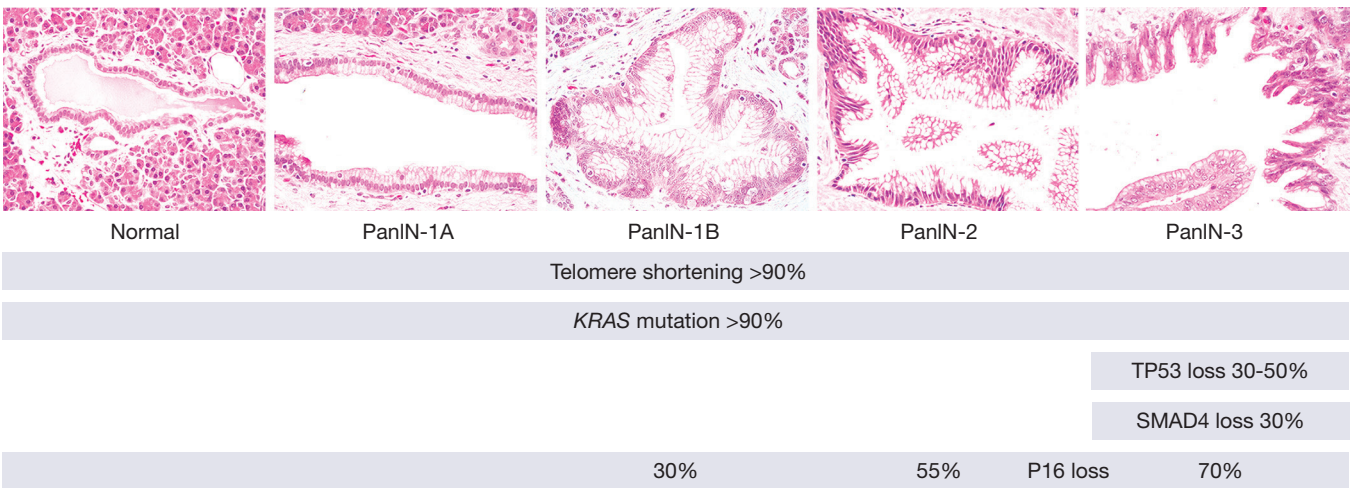


Figure 1 PanIN progression model of pancreatic cancer. Each step in the progression from normal epithelium to low-grade PanIN, and on to high-grade PanIN is accompanied by accumulating genetic alterations. From left to right: a normal pancreatic duct is lined by cuboidal to low-columnar epithelium with amphophilic cytoplasm. PanIN-1A shows flat epithelial lining with tall columnar cells with basally located nuclei and abundant supranuclear mucin. PanIN-1B identical to PanIN-1A except for a papillary, micropapillary, or basally pseudostratified architecture in PanIN-1B. PanIN-2 demonstrates full-thickness pseudostratification of nuclei with mild-to-moderate cytologic abnormalities. PanIN-3 is characterized by complete loss of polarity, budding of cellular tufts into the duct lumen, and significant nuclear pleomorphism. PanIN, pancreatic intraepithelial neoplasia.

over neighboring cells and that additional genetic or epigenetic events are needed for neoplastic progression (24).

A subset of PanINs (10%) harbors a *GNAS* mutation, a recently discovered oncogene mutated in about 60% of IPMNs (9,24). Interestingly, in some PanINs a *GNAS* mutation is the only mutation and in other PanINs the *GNAS* mutation seems to have occurred earlier than the *KRAS* mutation. Some of these PanIN lesions with a *GNAS* mutation may progress to IPMNs, as Matthaei and colleagues found that 33% of lesions with a size between PanINs and IPMNs (the so called incipient IPMNs) harbor *GNAS* mutations (27). Together these data suggest that *GNAS* mutations in PanIN may drive the lesion towards the IPMN pathway, although specificity of *GNAS* mutations for the IPMN pathway needs further confirmation.

The other genes targeted in invasive PDAC, including *CDKN2A/P16*, *TP53* and *SMAD4*, are also altered in PanIN lesions, supporting the hypothesis that PanINs are a precursor to invasive PDAC (3,28-30). These genetic alterations appear to occur after telomere shortening and *KRAS* gene mutations, as they are usually not found in low-grade PanINs, but instead are found in higher-grade PanIN lesions.

Some of the genetic changes in PanINs appear to be associated with progression (24). For example, loss of *P16*

protein expression, a marker for genetic inactivation of *CDKN2A/P16*, correlates with increasing PanIN grade (30% of PanIN-1A/B, 55% of PanIN-2, and 70% of PanIN-3 lost *P16* expression) (28,31). This finding suggests that loss of *P16* may be more important for progression of PanIN than for initiation (3,24). Late genetic events that almost exclusively occur in PanIN-3 are inactivation of *TP53* and *SMAD4*, which are found in 30-50% of PanIN-3 lesions (29,30).

In addition to genetic changes, epigenetic alterations also play a role in PanIN progression. Hypermethylation of the promoters of tumor suppressor genes can be seen in low-grade PanIN lesions and they increase with grade of dysplasia (32). Promoter hypermethylation of *CDKN2A/P16* is responsible for a third of *P16* silencing, whereas homozygous deletions and intragenic mutation coupled with loss of heterozygosity (LOH) account for the remaining two-thirds (31). Many microRNAs are aberrantly expressed in PanINs and some of these are likely to be important in pancreatic carcinogenesis. Expression of some microRNAs, such as miR-196b, appears specific for high-grade lesions (PanIN-3 and PDAC) (24).

The genetic alterations, if any, that are crucial for transition from high-grade PanIN (*in situ* carcinoma) to an invasive carcinoma are still largely unknown. Direct

comparative sequencing of a precursor lesion and the associated invasive carcinoma can greatly increase our knowledge of the genetic changes that drive this transition. However, it is almost impossible to identify the exact PanIN that gave rise to the PDAC since much of the pancreas is usually overgrown by the PDAC once the tumor is resected. Also distinction between PanIN-3 adjacent to PDAC and the process of “cancerization of a pancreatic duct” by a PDAC can be difficult (19). Despite these difficulties, Murphy and colleagues tried to address the mechanisms that control progression to invasion by exome sequencing of 10 PDACs and 15 adjacent PanIN-2 and PanIN-3 lesions (33). PanINs and invasive carcinomas appeared to harbor similar numbers of mutations. There was a trend towards fewer mutations in PanIN-2 (average of 30 mutations) compared to the invasive carcinomas (average 50 mutations), but, surprisingly, PanIN-3 showed on average more mutations (63 mutations). In total, 66% of mutations were common to the invasive carcinoma and the adjacent PanIN, 10% of mutations were only present in the invasive carcinoma, and 25% of the mutations were only present in the PanIN lesions. When individual PanIN lesions were analyzed, genetic overlap between PanIN and adjacent invasive carcinoma ranged from 34% to 96%, but >50% commonality of mutations was present in 10 of the 15 PanIN lesions (33). The very high commonality between PanIN and invasive carcinoma in a few cases may represent very recent genetic divergence, but also raises the concern that a lesion is actually ductal spread of the adjacent invasive cancer instead of a true PanIN-3 lesion.

A number of clinical studies of PanIN lesions have been performed in parallel to the previously mentioned genetic studies, and the clinical significance of PanIN lesions in different settings is now being understood. PanIN at a resection margin does not affect survival in patients who have a resection for invasive PDAC. This is likely because the patients with invasive cancer and a PanIN at a margin are likely to die from their invasive PDAC long before the residual PanIN has time to progress to an invasive cancer (34). Although the data are not so strong, Konstantinidis and colleagues investigated the significance of incidentally discovered PanIN in pancreatic resections for reasons other than PDAC (21). They found that presence of PanIN-1 or 2 in the resection margin or PanIN of any grade anywhere in the pancreas did not result in an appreciable cancer risk in the pancreatic remnant after resection (21). Follow-up of patients in this study was relatively short [median 3 years (range, 0.5-11 years)]



Figure 2 Endoscopic picture of a bulging ampulla of Vater with extruding thick mucin in a patient with an IPMN, sometimes referred to as “fish-eye” ampulla, and virtually pathognomonic of IPMN. IPMN, intraductal papillary mucinous neoplasm.

compared to the time needed for PDAC development (11,21).

Intraductal papillary mucinous neoplasm (IPMN)

IPMNs are epithelial mucin-producing tumors that arise within the larger pancreatic ducts. At endoscopy a so-called “fish-eye” ampulla of Vater, i.e., a bulging ampulla with extruding mucin, can be seen and is almost diagnostic for IPMN (*Figure 2*). IPMNs are by definition >5 mm in diameter, and they typically are characterized by papillary proliferations that dilate the existing duct infrastructure. IPMNs are usually found in the head of the pancreas, but they can involve any portion of the pancreas and some involve the entire length of the gland (35,36). IPMNs are very common, and studies of asymptomatic individuals who undergo a CT scan have revealed that close to 3% of asymptomatic individuals have pancreatic cysts, approximately 25% of which is consistent with an IPMN (35,37,38). The prevalence of IPMN is equal in men and women; the majority of patients are diagnosed around 60 years of age (4).

IPMNs can macroscopically be categorized in three groups: 10-35% arises in the main pancreatic duct (MD), 40-65% in a branch duct (BD), and 15-40% involves both the main and BDs (mixed type) (39-44). These numbers vary greatly from study to study, but the pattern of duct involvement does guide therapy. For example, examination

Table 2 International consensus guidelines for surgical resection of pancreatic cysts according to Tanaka *et al.* (36)

Main duct IPMN	Surgical resection is strongly recommended for all surgically fit patients. If the margin is positive for high-grade dysplasia, additional resection should be attempted to obtain at least moderate-grade dysplasia. However, MPD dilation of 5-9 mm should be considered as one of the “worrisome features”, similar to the case for BD-IPMN, with a recommendation of evaluation but no immediate resection. To date, there have been no consistent predictive factors for malignancy in MD-IPMN, including the degree of MPD dilation, presence of symptoms, or mural nodules
BD IPMN	Any of the following high-risk stigmata of malignancy present? • Obstructive jaundice in a patient with cystic lesion of the head of the pancreas • Enhancing solid component within cyst • Main pancreatic duct >10 mm in size ❖ Consider resection if clinically appropriate Are any of the following worrisome features present? • Pancreatitis^a • Cyst >3 cm • Thickened/enhancing cyst walls • Main duct size 5-9 mm • Nonenhancing mural nodule • Abrupt change in caliber of pancreatic duct with distal pancreatic atrophy ❖ Perform endoscopic ultrasound, and if any of these features are present: • Definite mural nodule^b • Main duct features suspicious for involvement^c • Cytology: suspicious or positive for malignancy ❖ Consider resection if clinically appropriate
MCN	Surgical resection is recommended for all surgically fit patients. Observation may be considered in elderly frail patients. In patients with MCNs of <4 cm without mural nodules, parenchyma-sparing resections (i.e., middle pancreatectomy) and distal pancreatectomy with spleen preservation as well as laparoscopic procedures should be considered

^a, pancreatitis may be an indication for surgery for relief of symptoms; ^b, differential diagnosis includes mucin. Mucin can move with change in patient position, may be dislodged on cyst lavage and does not have Doppler flow. Features of true tumor nodule include lack of mobility, presence of Doppler flow and FNA of nodule showing tumor tissue; ^c, presence of any one of thickened walls, intraductal mucin or mural nodules is suggestive of main duct involvement. In their absence main duct involvement is inconclusive; BD, branch duct; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm.

of resected IPMNs has shown that 62% of MD and 58% of mixed type IPMNs have high-grade dysplasia, and that 44% of MD and 45% of mixed type IPMNs have an associated invasive carcinoma (36). In contrast, only 24% of BD-IPMNs have high-grade dysplasia, and 17% an associated invasive carcinoma (36). Risk assessment and decision-making on which IPMNs to resect and which IPMNs can be safely followed is based on these percentages. However, relying solely on MD *vs.* BD in decision making can be treacherous as a recent study of 512 IPMNs found that 30% of suspected BD-IPMNs (67/233) had histological involvement of the main pancreatic duct not evident in

preoperative imaging (44). Importantly, the misdiagnosed BD-IPMNs had significantly more high-grade dysplasia and were more likely to harbor an associated carcinoma than histologically pure BD-IPMNs (44).

In order to address the complexities of managing patients with an IPMN, consensus guidelines for the management of IPMN and MCN were established in 2012. These guidelines advise that most IPMNs that involve the MD should be surgically resected because of their high rate of malignancy, whereas surgical indications for BD-IPMNs include the presence of “high-risk stigmata” such as mural nodules and symptomatology (*Table 2*) (36). If there are

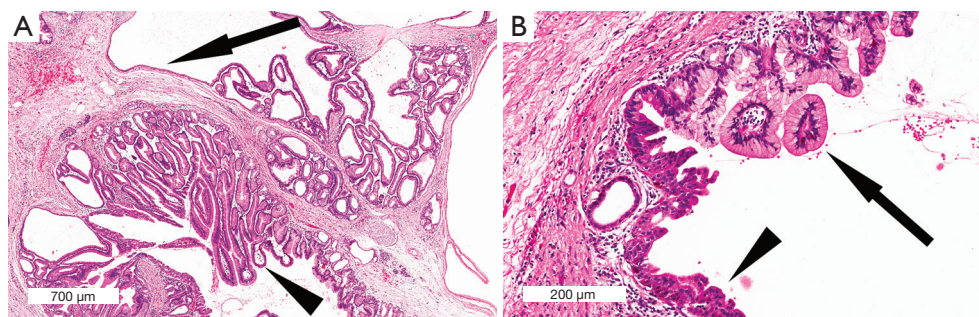


Figure 3 (A) Gastric-foveolar type IPMN with areas with low-grade (arrow) and intermediate-grade dysplasia (arrowhead); (B) gastric-foveolar type IPMN with transition from intermediate-grade dysplasia (arrow) to high-grade dysplasia (arrowhead). IPMN, intraductal papillary mucinous neoplasm.

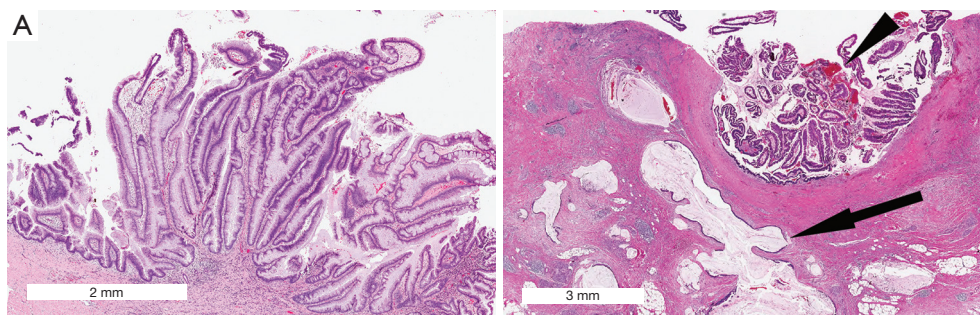


Figure 4 (A) Intestinal type IPMN with intermediate-grade dysplasia; (B) mucinous adenocarcinoma (arrow) arising from an intestinal type IPMN (arrowhead). IPMN, intraductal papillary mucinous neoplasm.

only so-called “worrisome features” (defined in *Table 2*), further diagnostic workup is advised. A recent study showed that “high-risk stigmata” had a good correlation with malignancy, but “worrisome features” did not (45). Clearly, development of additional biomarkers that can be used to predict presence of high-grade dysplasia or invasive growth has great potential to improve clinical decision-making (4).

Histologically, IPMNs can be categorized as gastric-foveolar, intestinal, pancreatobiliary, or oncocytic type based on the direction of differentiation of the neoplastic epithelium as defined by histology and immunolabeling (46,47). In addition, intraductal tubulopapillary neoplasms (ITPN) are recognized as an intraductal neoplasm distinct from IPMNs; however, these lesions are rare, and their precise relationship to the other IPMN subtypes remains to be defined. Most BD-IPMNs have gastric-foveolar histology, whereas intestinal, pancreatobiliary, and oncocytic histologies are seen more often in the main duct type IPMNs. The histologic directions of differentiation in IPMNs have clinical implications and therefore deserve a

more detailed discussion.

Gastric-foveolar IPMNs are lined by epithelium resembling foveolar epithelium of the gastric mucosa (*Figure 3*). The neoplastic epithelial cells have apical mucin with small basally oriented nuclei. The epithelium is usually flat and composed of a single layer of cells, although the neoplastic epithelium can form papillae. Mitoses are rare and most lesions have low-grade dysplasia, although intermediate-/high-grade dysplasia is present in 10% (48). Immunohistochemically the neoplastic epithelium expresses MUC5AC and MUC6, but does not express MUC1 and MUC2 (47). MUC4 is expressed in lesions with higher grades of dysplasia (49). Gastric-foveolar IPMNs can be mixed with pancreatobiliary and intestinal type epithelium. Associated invasive carcinomas are rare, but when present tend to be ductal adenocarcinomas.

Intestinal IPMNs resemble villous adenomas of the gastrointestinal tract (*Figure 4*). Long papillae, lined by mucin-secreting neoplastic epithelial cells, protrude from the cyst wall. The neoplastic cells have elongated

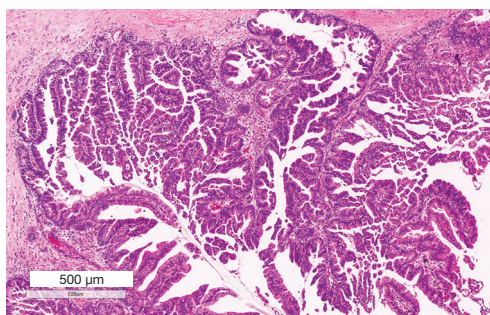


Figure 5 Pancreatobiliary type IPMN with high-grade dysplasia. IPMN, intraductal papillary mucinous neoplasm.

nuclei and can be pseudostratified. Intestinal IPMNs usually have moderate- to high-grade dysplasia (46). Immunohistochemically the neoplastic cells strongly express MUC2 and MUC5AC, but do not express MUC1. MUC6 is focally expressed in some cases (50). Some also express MUC4 (49). CDX2, a marker of intestinal differentiation, is also expressed in this subtype (47). Associated invasive carcinomas arising from intestinal IPMNs are typically colloid carcinomas (mucinous noncystic adenocarcinomas) with a similar mucin profile (51), but can also be ductal adenocarcinomas or mixed ductal/colloid carcinomas (52).

Pancreatobiliary IPMNs are usually high-grade lesions with complex architecture with cribriform papillae and bridging (*Figure 5*). The neoplastic cells are cuboidal and have atypical round nuclei with clearly visible nucleoli. Lower-grade dysplasia is rare but when present is characterized by mild atypia with hyperchromasia and enlarged nuclei (46). The neoplastic cells express MUC1, MUC5AC and some also express MUC6. MUC2 is not expressed. Associated invasive carcinomas usually are ductal adenocarcinomas, with the same mucin expression pattern (47,51,53).

Oncocytic IPMNs, also known as intraductal oncocytic papillary neoplasms (IOPNs), are morphologically the most complex lesions, and have intricate branched papillae with cribriform formations and solid cell nests. They almost always harbor high-grade dysplasia (*Figure 6*). The cells have abundant eosinophilic cytoplasm, but can have intracellular mucin and intraepithelial mucin pools. MUC1 and MUC6 are expressed by the neoplastic cells. Incidentally goblet cells may be seen expressing MUC2 and MUC5AC. When present, an associated invasive carcinoma is usually the rare oncocytic carcinoma. Although only a small number of cases have been reported this may represent a true subtype based on distinct histology and genetics (54).

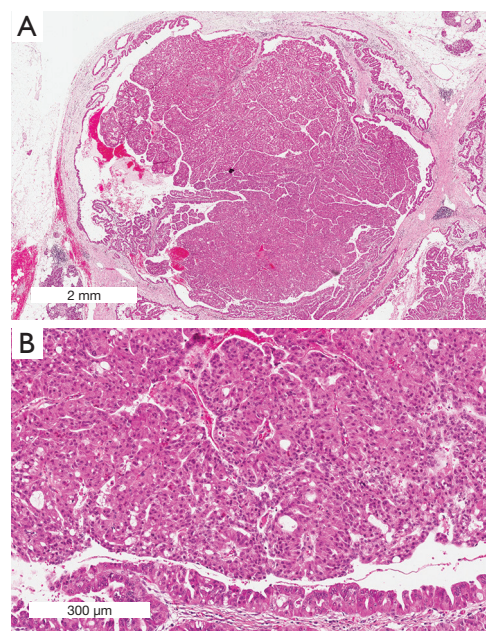


Figure 6 Oncocytic type IPMN with high-grade dysplasia. (A) Overview; (B) detail. IPMN, intraductal papillary mucinous neoplasm.

ITPN are the most recently recognized pancreatic intraductal neoplasm and, as mentioned above, may represent a separate entity from IPMN. A predominant tubulopapillary growth of cuboidal neoplastic cells in the affected duct combined with a more solid architecture with minimal cytoplasmic mucin and frequent necrotic foci define this neoplasm (*Figure 7*). ITPNs often have an overall cribriform appearance. The lesions are always high-grade. MUC6 is expressed in all cells and MUC1 is expressed focally. MUC2 and MUC5AC are negative (55).

IPMN subtypes have been categorized based on their histologic and morphologic features. Although there are clear differences, many IPMNs show mixed histologic features suggesting that these phenotypes do not represent completely distinct underlying pathways. For instance, intestinal and pancreatobiliary IPMNs can both harbor areas with gastric differentiation, and it has been suggested that the low-grade gastric-foveolar type is a common precursor to other types of IPMNs (47).

IPMNs can be a precursor to invasive PDAC. Although IPMNs show many of the genetic alterations involved in PanIN and classic invasive PDAC, such as *KRAS*, *TP53*, *SMAD4*, *CDKN2A/P16* (3,6), some genetic alterations, such as activating *GNAS* mutations and inactivating

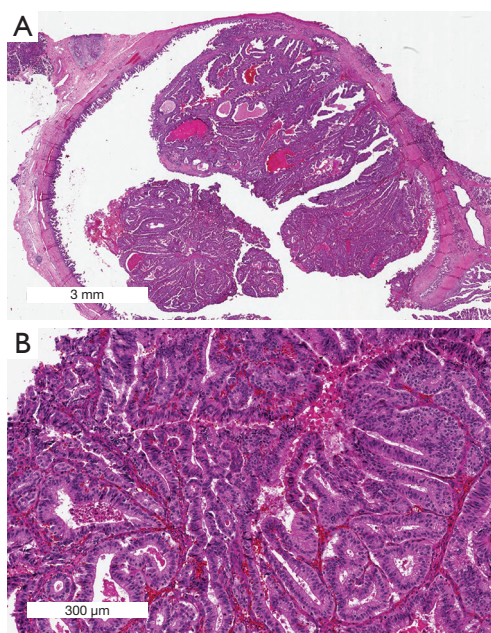


Figure 7 Intraductal tubulopapillary neoplasm with high-grade dysplasia. (A) Overview; (B) detail.

RNF43 mutations, seem to be more specific for the IPMN precursor pathway (8,9).

Wu *et al.* sequenced the exomes of eight IPMNs and found that IPMNs contain an average of 26 ± 12 somatic mutations (8). The genes most frequently targeted in IPMNs appear to be *KRAS*, *GNAS*, *CDKN2A/P16*, *RNF43*, *TP53*, and *SMAD4*. In a large follow-up study in which 51 cancer genes were sequenced in 48 IPMNs, Amato and colleagues found that virtually all IPMNs (>90%) harbor a *KRAS* and/or *GNAS* mutation, and that *CDKN2A/P16*, *RNF43*, *TP53*, *BRAF*, and *SMAD4* are less commonly targeted (56). *KRAS* mutations appear to be early events, as close to 90% of low-grade and intermediate-grade IPMNs harbor a *KRAS* mutation (9). Both intestinal and pancreatobiliary type IPMNs harbor *KRAS* mutations, while *GNAS* mutations appear to be more common in intestinal type IPMNs (9,56,57). Of interest, *KRAS* and *BRAF* mutations have not been reported in ITPN (58). Interestingly, *in vitro* research in pancreatic ductal cells found that mutated *GNAS* may extensively alter gene expression, including expression of mucin genes through the interaction with MAPK and PI3K pathways. Extensively altered expression of MUC2 and MUC5AC in different cell lines suggested a role in morphologic and histologic presentation (59).

As >95% of IPMNs show either a *KRAS* or *GNAS* mutation, it is possible that all IPMNs are initiated by a mutation in either one of these genes. Recently an IPMN with associated carcinoma was reported in a patient with McCune-Albright syndrome (post-zygotic noninherited activating *GNAS* mutations), further establishing the causal role of *GNAS* in pancreatic tumorigenesis (60).

The targeting of *RNF43* tumor suppressor gene in IPMNs is of interest because the protein product of this gene plays an important role in the Wnt/ β -catenin pathway (8,56). *RNF43* is a transmembrane E3 ligase that down-regulates the Wnt pathway by removing Wnt receptors from the cell surface in intestinal stem cells (61). While further research on the role of *RNF43* in IPMN is needed, newer therapies targeting the Wnt/ β -catenin pathway may be applicable to IPMN associated invasive PDACs with an *RNF43* mutation (62,63).

Another gene that is frequently inactivated in IPMNs is *CDKN2A/P16*. Homozygous deletions, intragenic mutations coupled with LOH and epigenetic alterations can inactivate *CDKN2A/P16* (31). LOH at 9p was seen in 10% of low-grade, 20% of intermediate-grade, and 33% of high-grade IPMNs, and 100% of invasive PDACs (64). Loss of *P16* is thus a marker for progression to high-grade dysplasia/invasive carcinoma. A recent study found *CDKN2A/P16* mutations in about 5% of IPMNs by NGS, but showed loss of expression in the same tissue in 0% of low-grade, 25% of intermediate-grade, 30% of high-grade, and 50% of invasive IPMNs, suggesting an important role for inactivation by epigenetic mechanisms coupled with LOH (56).

TP53 is mutated late in IPMN progression (56). Mutation of *TP53* leads to protein inactivation and typically to the abnormal accumulation of the protein product in the neoplastic cells, reflected by very strong immunostaining for the *TP53* protein (Figure 8). Alternatively, completely absent immunostaining indicates a stop codon mutation coupled with LOH (65). *TP53* expression is usually normal in low-grade IPMNs, but *TP53* expression is altered in a third of intermediate-grade IPMNs and close to half of high-grade IPMNs (66). The tumor suppressor gene *SMAD4* is also inactivated late in IPMN progression. Although inactivated in 55% of invasive PDACs, *SMAD4* is rarely inactivated in low- or intermediate-grade IPMNs. *SMAD4* can be inactivated by homozygous deletion or by intragenic mutations coupled with LOH. Wilentz *et al.* (29) reported loss of immunohistochemical expression of the *SMAD4* protein is a marker for inactivation of the *SMAD4*

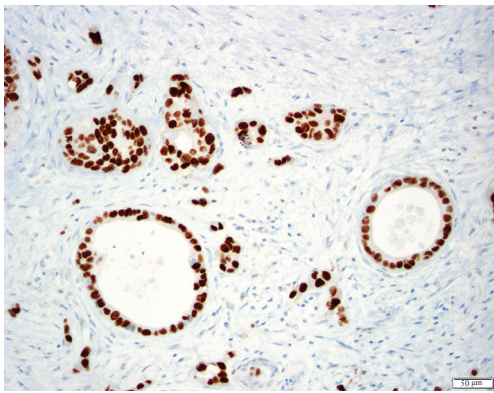


Figure 8 *TP53* immunohistochemistry showing strong aberrant expression of *TP53* consistent with somatic *TP53* mutation in a pancreatic ductal adenocarcinoma. A somatic mutation of *TP53* was confirmed in this case.

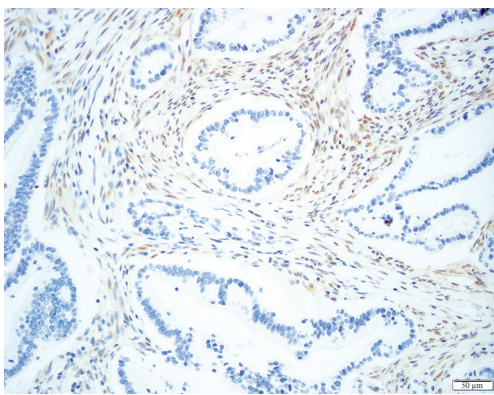


Figure 9 *SMAD4* immunohistochemistry showing loss of *SMAD4* expression in a pancreatic ductal adenocarcinoma. Note the normal expression in surrounding stromal cells compared to negativity in tumor cells.

gene (Figure 9). *SMAD4* expression was shown to be normal in IPMNs with low-, intermediate- and high-grade dysplasia, while 3 of 4 IPMN associated invasive carcinomas showed loss of *SMAD4* (67). Other studies showed similar results, with retained expression of *SMAD4* in non-invasive IPMNs but loss of *SMAD4* in 3-16% of IPMN associated invasive carcinomas. Iacobuzio-Donahue *et al.* reported that all 19 colloid carcinomas arising from an IPMN had normal expression of *SMAD4*, whereas weak staining was seen in 5 of 9 invasive ductal adenocarcinomas arising from an IPMN, suggesting a link of *SMAD4* loss with ductal differentiation (68).

Phosphatidylinositol-3 kinases (PI3K) are lipid-kinases that play a role in proliferation, differentiation, survival, and several other cellular functions. *PIK3CA* is an oncogene that activates the AKT pathway and is mutated in 10% of intermediate- and high-grade IPMNs, and this genetic targeting of *PIK3CA* seems to be another late event in the progression of IPMNs (69). *PIK3CA* was mutated 3 of 11 (37%) ITPNs, which also had overall significantly higher expression of phosphorylated AKT than the control group IPMNs, suggesting that this pathway may be a driver of ITPN development (58). LOH of *PTEN*, another tumor suppressor gene in the AKT pathway, has been reported in 0% low-grade, 30% intermediate-grade, and 40% high-grade IPMNs. *PTEN* expression in 30% of IPMNs was also significantly associated with higher nuclear grade, but further studies are needed to evaluate clinical value of *PTEN* in IPMNs (70). Intriguingly, alterations in the PI3K pathway do not occur commonly in PDACs, pointing to this pathway's unique importance in IPMNs (5).

STK11, a tumor suppressor gene encoding for the serine threonine protein LKB1, is mutated in the germline of patients with the PJS. PJS is known to cause a 132-fold increase in risk of invasive PDAC and some of these invasive cancers arise from IPMNs (71). Mutations in *STK11* are seen in 5% of nonPJS IPMNs (72).

The expression of human telomerase reverse transcriptase (hTERT) and of Sonic hedgehog (Shh) is increased in IPMNs with higher grade of dysplasia, most significantly in progression from intermediate- to high-grade IPMNs (73,74). The loss of expression of the tumor suppressor gene *BRG1* has also been associated with progression in IPMNs (75). Changes in the expression of some genes in IPMNs are driven by genetic alterations, while in other tumors gene expression changes are produced by epigenetic DNA modifications, microRNAs, post-translational protein modifications, and possible feedback mechanisms. For example, >90% of IPMNs show at least one aberrantly methylated tumor suppressor gene promoter site (76). Genes that have been reported to be methylated in IPMNs included *CDKN2A/P16*, *TP73*, *APC*, *bMLH1*, *MGMT*, and *E-Cadherin* (76). Significantly more genes are methylated in IPMNs with high-grade dysplasia than in IPMNs with low-grade dysplasia. Moreover, some genes may be selectively methylated in high-grade lesions, which may be useful in the clinical management of IPMNs (32,76,77). MicroRNAs are also aberrantly expressed in IPMNs (78). Both MiR-21 and miR-155 are up-regulated in invasive carcinomas associated with IPMNs compared to noninvasive IPMNs

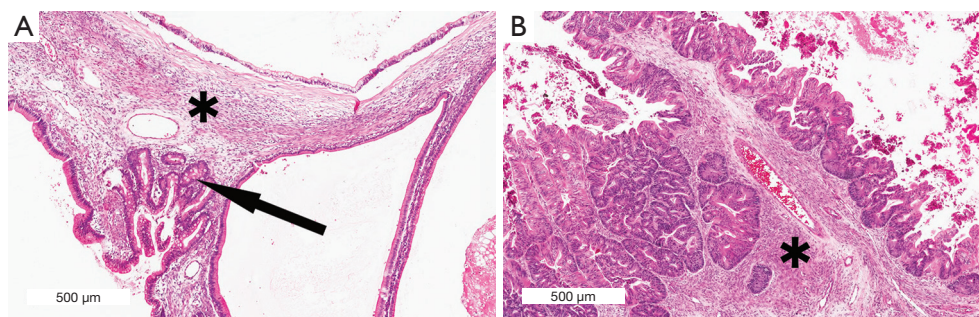


Figure 10 (A) Mucinous cystic neoplasm with low- to intermediate-grade dysplasia and focal goblet cells (arrow). Note the cellular ovarian-type stroma (asterisk); (B) mucinous cystic neoplasm with high-grade dysplasia and typical ovarian-type stroma (asterisk).

and normal tissue, suggesting a role for these microRNAs in carcinogenesis (79,80). These microRNAs regulate key tumor suppressor pathways: miR-21 represses several genes including *PTEN* (81), and miR-155 represses *TP53INP1* (80). Downregulation of microRNA MiR-101 has also been shown in progression of IPMNs. MiR-101 can silence *EZH2* expression in IPMN (82), and in IPMNs *EZH2* expression has an inverse correlation in expression with the tumor suppressor *CDKN1B/p27*. *EZH2* might transcriptionally silence *CDKN1B/p27* and is also known to methylate the protein histone 3 at lysine 27 (83).

It has also been suggested that the pattern of expression of certain microRNAs can be used as a marker of different IPMN subtypes. MiR-196a expression is associated with intestinal IPMN (84) and miR-200c, miR-141, miR-216 could be used to mark dysplastic progression in IPMN-tissue (85), and cyst fluid (86). MicroRNAs can also be detected in serum and can have discriminating diagnostic applications (87). Other diagnostic approaches for early diagnosis of high-grade/invasive IPMN may be detection of *TP53* and/or *SMAD4* mutations in pancreatic juice or cyst fluid (88), circulating tumor cells (89), mRNA binding proteins (90), ubiquitin and thymosin- β 4 in EUS FNA (91), and monoclonal antibodies (92).

Thus, a number of genetic, histological and clinical studies have defined the molecular basis for the development of IPMNs which in turn suggests novel molecular biomarkers and novel therapeutic approaches for these neoplasms.

Mucinous cystic neoplasm (MCN)

MCN is the least common of the precursor lesions that can give rise to invasive PDAC. MCNs occur almost exclusively women and usually in the tail of the pancreas. MCNs are cyst forming neoplasms, and characteristically the cysts

do not communicate with the pancreatic duct system. By definition, MCNs contain a characteristic ovarian-type stroma (Figure 10). One theory on the pathogenesis of MCNs argues that they are the result of ectopic gonadal mesenchyme that is incorporated in the pancreas during the fourth and fifth weeks of embryogenesis as a result of the close proximity of the left primordial gonad to the dorsal pancreatic anlage which gives rise to the pancreatic body and tail. This could also explain MCNs at the contralateral side in the hepatobiliary tract (93,94). However, because this cannot explain the rare occurrence of MCN in male patients, an alternative theory has been put forth which suggests that neoplastic epithelial cells of MCNs induce ovarian stromal differentiation in cells that are normally present in the pancreas (95).

MCNs account for approximately 8% of all resected cystic lesions of the pancreas (96,97). Small MCNs (<3 cm) are usually incidental findings, whereas larger MCNs may produce nonspecific complaints such as abdominal discomfort and the sensation of a mass in the epigastric region. Surgical resection is recommended for all surgically fit patients (Table 2). Up to one-third of resected MCNs have an associated invasive carcinoma, although more recent studies report lower percentages (5-15%), likely due to the fact that smaller low-grade MCNs are being detected incidentally in patients imaged for other reasons (98-100). Invasive adenocarcinomas arising in MCNs usually resemble a common PDAC but can also have a mucinous histology. Because invasive carcinoma can arise very focally in an MCN, when MCNs are resected they should be sampled extensively, if not completely, by the examining pathologist (99). Patients with a surgically resected noninvasive MCN are cured after the resection. The 5-year survival rate for patients with an MCN with an associated invasive carcinoma is about 50-60%, depending

on the extent of invasion (96).

Grossly, in contrast to IPMNs, MCNs do not communicate with the pancreatic ductal system. Most MCNs form large (average size 10 cm) multilocular lesions containing thick mucin, or sometimes mucin tinged by hemorrhage. Lesions with low-grade dysplasia usually have a smooth and glistening internal surface, whereas lesions with high-grade dysplasia are lined by epithelium with papillary projections. MCNs with an associated invasive carcinoma are often large and multilocular and contain papillary projections or mural nodules (96,101).

Microscopically, the cysts of MCNs are lined by a columnar mucin-producing neoplastic epithelium. By definition, they also have an ovarian-type stroma consisting of densely packed spindle cells with round to elongated nuclei and a small amount of cytoplasm (Figure 10). The stromal cells express inhibin, estrogen and progesterone receptors, as well as vimentin, smooth-muscle actin, and desmin. In some lesions the stroma may become fibrotic and hypocellular and be more difficult to recognize. The epithelial lining of the cysts consists of mucin-producing tall columnar epithelial cells with pseudopyloric, gastric-foveolar, small-intestinal or large-intestinal differentiation. Squamous differentiation is only rarely seen (95). The epithelial cells express cytokeratins 7, 8, 18, and 19, the gastric type mucin MUC5A, and pancreatic type mucin DUPAN-2 and CA19-9, whereas scattered goblet-like cells express the intestinal MUC2. MUC1 expression is observed in most ductal adenocarcinoma arising from MCN, but is negative in the associated noninvasive components (102). The degree of dysplasia in MCN can vary greatly and change abruptly from minimal to severe or even focal invasive growth. The highest degree of dysplasia present in an MCN determines the classification of the lesions as MCN with low-grade, intermediate-grade, or high-grade dysplasia (95). The vast majority (70-80%) of MCNs are low-grade (98,100).

MCNs is less well-characterized at the genetic level than are PanINs and IPMNs. However, recent whole-exome sequencing of carefully microdissected MCNs has revealed that the neoplastic epithelium has an average of 16 ± 7.6 somatic mutations and relatively few allelic losses (8). *KRAS* is the most frequently mutated gene in MCN. Using Sanger sequencing *KRAS* mutations have been found in 25% (7/27) of MCNs with low-grade dysplasia, 40% (5/13) of MCNs with intermediate-grade dysplasia, and 90% (8/9) of MCNs with high-grade dysplasia or invasive carcinoma. Mutations in *TP53* are a relatively late event occurring only

in areas with high-grade dysplasia or an associated invasive carcinoma (103). Whole-exome sequencing identified *RNF43* mutations in 3 of the 8 MCNs examined (8). Loss of *SMAD4* is a late event in neoplastic progression of MCN and found in associated invasive adenocarcinomas but not typically in noninvasive components of MCNs (104). Rarely *PIK3CA* gene mutations are found in MCN, but these seem confined to those with high-grade dysplasia (105). Hypermethylation of *P14* and *P16* has been reported in about 15% of non-invasive MCNs (106).

Global gene expression profiling identified a number of genes that are up-regulated in the epithelium of MCNs, including *S100*, *PSCA*, *C-MYC*, *STK6/STK15*, *cathepsin E*, *TCF4*, and *pepsinogen C*. In addition, activation of the Notch pathway was shown in the epithelial component by the demonstration of overexpression of Jagged1 and the downstream Notch pathway member Hes1. Overexpression of steroidogenic acute regulatory (STAR) protein and estrogen receptor 1 (ESR1) occurs in the stroma (107).

Conclusions

PDAC is a deadly disease. The key to reducing deaths from PDAC is to detect pancreatic neoplasia at a very early and still curable stage or, even better, to detect and treat precursor lesions before they transform into incurable invasive cancers. PDAC develops from several histologically and genetically distinct precursor lesions providing an opportunity for early detection and prevention (2). Moreover, genetic studies have suggested that the window of opportunity to diagnose and treat a precursor lesions is almost 12 years (10).

While the genes that are recurrently mutated in PDAC and in precursor lesions (such as *KRAS*, *GNAS*, *TP53*, *CDKN2A/P16*, *SMAD4*) are prime targets for early detection efforts, some of the genes that are less commonly mutated (such as *ATM*, *BRCA2*, and *RNF43*) are potentially more therapeutically targetable. Moreover, therapeutic targeting of one or more core signaling pathways involved in PDAC instead of a specific genetic alteration may be important to circumvent genetic heterogeneity of PDAC.

Although progress in the therapy of patients with PDAC is invaluable, early detection and prevention of PDAC are likely to be more effective to decrease mortality (2). Today biomarkers can be assessed in cyst fluid aspirated by fine needle aspiration or in secreted pancreatic juice collected in the duodenum (9,88,108,109). Recent studies revealed genetic alterations in pancreatic cyst fluid that can

discriminate between a completely harmless cyst such as serous cyst adenoma and a premalignant cyst such as IPMN and MCN (8). However, the ultimate goal is to identify those patients with a high-grade precursor lesion and/or early invasive PDAC (88). Those patients would benefit from a surgical resection, whereas patients with lesions with only low-grade dysplasia could be safely followed without surgery. Such definitive biomarkers are not yet available, but further dissection of the genetic progression of PDAC precursor lesions will hopefully lead to the identification of biomarkers that indicate high-grade dysplasia or transition to invasive growth. Ultimately this will lead to better risk stratification of patients with pancreatic cancer precursor lesions and patients at increased risk of PDAC. Newer gene-based tests have the potential to greatly aid in clinical decision-making and the selection of patients who would benefit from surgical treatment, while on the other hand patients with low-risk lesions could be spared from an operation.

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