Precursors to invasive pancreatic cancer

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease, with a 5-year survival rate of less than 5%. To better understand PDAC and to improve its dismal prognosis, we must understand its origins. PDAC has three distinct noninvasive precursor lesions including intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, and pancreatic intraepithelial neoplasia. Each of these precursor lesions has its own unique compendium of clinical findings, morphological features, and genetic aberrations. This review focuses on the clinical significance of precursor lesions of pancreatic cancer and how better understanding of these lesions can aid in early detection and treatment.

Keywords: pancreatic ductal adenocarcinoma, precursor lesions, intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, pancreatic intraepithelial neoplasia, PanIN

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a nearly uniformly lethal cancer that remains the 4th leading cause of cancer death in the United States, with a 5-year survival rate of less than 5%. The dismal prognosis associated with PDAC is most likely attributed to the late clinical presentation, at which time the disease is usually at an advanced, and surgically inoperable, stage. Only a minority (~15%–20%) of patients with PDAC present with resectable disease, and the overwhelming majority are candidates for chemoradiation therapy only. These therapies can prolong survival, but rarely lead to a cure. Thus, one of the best opportunities for increasing the survival in PDAC is to detect the disease at an early and potentially noninvasive stage, the stage of precursor lesions. Enhancing our knowledge of the morphology, biological behavior, and molecular aberrations of these precursor lesions gives us more insight into the pathogenesis and natural history of this fatal disease.

PDAC is associated with multiple precancerous lesions: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN). These are noninvasive epithelial neoplasms with an increased risk of transformation into PDAC. Each of these three entities exhibits a unique multistep morphologic and genetic progression to invasive adenocarcinoma. Herein, we review the clinicopathologic features, molecular aberrations, natural history, recommended treatment, and prognosis of these precursor lesions.
PanIN

Clinical features

PanIN is the most common precursor lesion of PDAC. The term PanIN was adopted in 1999 by a Pancreas Cancer Think Tank sponsored by the National Cancer Institute to classify a spectrum of noninvasive intraductal lesions of the pancreas. PanINs are microscopic clonal lesions, located in the smaller pancreatic ducts, and measure less than 5 mm in diameter. The incidence of PanIN tends to increase with age, and both men and women are affected equally. PanINs are usually asymptomatic and isolated PanIN lesions are not detectable upon noninvasive abdominal imaging with rare exceptions. Andea et al found that PanINs were identified in 82% of pancreata with invasive carcinoma, in 60% of pancreata with chronic pancreatitis, and in 16% of otherwise normal pancreata resected secondary to trauma or benign pancreatic neoplasms. Of note, higher grade PanINs are almost exclusively found in association with invasive PDAC.

Pathologic features

Microscopically, PanIN lesions are characterized by cuboidal-to-columnar cells with varying amounts of cytoplasmic mucin and degrees of cytologic and architectural atypia (Figure 1A–D). The degree of dysplasia is used as a universal grading tool, and represents the progression from low-grade lesions to carcinoma in-situ/high-grade lesions. PanIN-1 lesions show minimal nuclear atypia, inconspicuous nucleoli, and absent mitotic figures (Figure 1A and B). PanIN-1 lesions are further classified based on architectural subtypes, flat (PanIN-1A) and papillary or micropapillary (PanIN-1B). Lesions with moderate nuclear atypia, pseudostratification, loss of polarity, and hyperchromasia are classified as PanIN-2 lesions (Figure 1C). Mitotic figures are rarely identified in PanIN-2 lesions. Marked atypia is characteristic of PanIN-3 lesions (Figure 1D). PanIN-3 lesions have a papillary, micropapillary, or occasional flat architecture. Cribriform structures may be present as well as necrosis and “budding” epithelial cells in the gland lumen. Unlike PanIN-1 and PanIN-2, mitotic figures, including atypical mitotic figures, are present. Yet PanIN-3 is confined to the basement membrane and no invasive growth is present. Immunohistochemical stains for PanINs show positive staining for MUC1, MUC5AC, and MUC6, particularly in the higher grade lesions.

Figure 1 PanIN. (A) PanIN-1A is composed of flat columnar epithelium with abundant mucin and basally oriented, small, uniform nuclei. (B) PanIN-1B is identical to PanIN-1A, except it has either papillary or micropapillary architecture. (C) PanIN-2 often demonstrates papillary architecture and, by definition, has nuclear abnormalities that include moderate nuclear atypia, pseudostratification, loss of polarity, and hyperchromasia. Mitoses may be present, although rare, and are basally located and morphologically normal. (D) PanIN-3 is characterized by papillary or micropapillary architecture and marked nuclear atypia that is indistinguishable from invasive pancreatic ductal adenocarcinoma.

Note: However, it is confined to the basement membrane.

Abbreviation: PanIN, pancreatic intraepithelial neoplasia.

Molecular features

Molecular analysis of PanINs show similar genetic alterations to PDAC and can be divided into early and late events. Telomeric shortening is one of the earliest molecular aberrations detected in low-grade PanIN that likely facilitates genetic instability. Another early event is activating point mutations in the KRAS2 oncogene. Ultra-sensitive sequencing technologies applied to microdissected PanIN lesions have found near ubiquitous frequency of KRAS2 mutations in all histological grades, although the lower grade PanINs tend to be admixtures of cells with wild-type and mutant alleles. Later genetic events include inactivating mutations in p16/CDKN2A. Loss of p16 expression by immunohistochemistry can be found in 30%, 50%, and 70% of PanIN-1, PanIN-2, and PanIN-3 lesions, respectively. Mutations in the tumor suppressor genes TP53, DPC4/SMAD4, and BRCA2 are late events and generally observed in PanIN-3 lesions. Loss of heterozygosity (LOH) is observed, particularly in higher-grade PanIN lesions, and at chromosomal loci harboring genes frequently mutated in PDAC. Of note, while most of the LOH events are concordant between the noninvasive precursor and adjacent PDAC, a minority of events can be discordant, reiterating that PanIN lesions arise as independent clones. In addition to gnomic aberrations, PanINs also accrue epigenetic abnormalities such as promoter hypermethylation.
and aberrant micro-RNA (miRNA) expression during the multistep progression to invasive PDAC.\textsuperscript{18,19}

**Diagnosis, natural history, and treatment**

Due to their microscopic nature, PanINs are typically found incidentally in resection or in biopsy specimens, and are not associated with clinical signs or symptoms. Most PanINs are undetectable with modern imaging modalities, making early detection challenging. However, a recent study done by Brune et al and Detlefsen et al, showed that multifocal PanIN lesions, such as those arising in patients with familial pancreatic cancer, can be associated with parenchymal atrophy, which can be detected using endoscopic ultrasound (EUS).\textsuperscript{20,21} Anecdotal evidence suggests that higher-grade PanIN lesions that are left in situ upon partial pancreatectomy for PDAC can progress to adenocarcinoma within a timespan of months to a few years, underscoring the precursor nature of such lesions.\textsuperscript{22} Nonetheless, a recent study has demonstrated that the presence of PanIN lesions at the surgical margin of pancreata with otherwise invasive PDAC does not influence prognosis for the patient, and additional surgery is not required.\textsuperscript{23}

**IPMN**

**Clinical features**

IPMNs are mucin-producing epithelial neoplasms that are characterized by intraductal proliferation of cells with varying degrees of atypia, which usually form papillae and lead to cystic dilation of the pancreatic ducts.\textsuperscript{24} By definition, IPMNs involve either the main pancreatic duct, or one of its branches, or both. These lesions arise within ducts that are greater than 1 cm in diameter, most frequently are located in the head of the pancreas, can be multifocal, and lack the ovarian-type stroma that is seen in MCNs.\textsuperscript{25} An IPMN that is less than 1 cm in size is called an “incipient” IPMN.\textsuperscript{26} These lesions are histologically identical to classical IPMNs, but fail to meet the size criteria. Men are more affected than women (3:2 ratio), and IPMNs tend to affect elderly patients with a mean age of diagnosis at 65 years (ranging in age from 25 to 95 years). Due to obstruction of the pancreatic ducts by mucin or the tumor itself, patients may present with intermittent episodes of pancreatitis, abdominal pain, nausea, and vomiting; other symptoms include weight loss, jaundice, and back pain.

**Pathologic features**

Noninvasive IPMNs are subdivided grossly into two groups based on the predominant site of involvement: main duct type (Figure 2A) and branch duct type (Figure 2B);\textsuperscript{27} although examples of mixed main and branch ducts also exist. Main duct IPMNs tend to have higher degrees of dysplasia, and are more often associated with invasive carcinoma (40% to 50% of cases).\textsuperscript{28,29} Microscopically, the epithelium lining the ducts is typically characterized by tall papillary structures with fibrovascular cores lined by a layer of mucin-secreting columnar epithelium with varying degrees of atypia. However, some subtypes, more often in branch duct IPMNs, may have flat epithelium. High-grade IPMNs show both increased cytologic atypia (nuclear pseudostratification and loss of polarity) and complex architectural patterns (branching papillae, pseudopapillary structures, and cribriform glands with neoplastic cells filling the lumen). High-grade IPMNs with complex papillary excrescences may fill the duct lumen leading to duct obstruction and proximal duct dilatation. Noninvasive IPMNs are graded on a histological spectrum, based on the degree of architectural and cytologic atypia, as low- (aka IPMN adenoma), intermediate-, and high-grade dysplasia (carcinoma in situ), respectively.\textsuperscript{30}

IPMNs are generally classified into four different histologic subtypes based on their cytomorphological features: gastric foveolar type, intestinal type, pancreatobiliary type, and oncocytic type.\textsuperscript{24,31} Immunohistomchemical staining for mucin antibodies enables differentiation between the four types of IPMNs. Intestinal-type IPMNs are the most common and resemble villous adenomas found in the large intestine (Figure 3A). They form long finger-like projections that are lined by columnar mucin-rich neoplastic cells. By immunohistochemistry, intestinal-type IPMNs stain positive for both MUC2 and CDX2. The gastric-type IPMNs have an eosinophilic cytoplasm with abundant mucin and basally oriented nuclei (Figure 3B). Branch duct-type IPMNs often show gastric differentiation. Gastric-type IPMNs stain negative for both MUC1 and MUC2. The pancreatobiliary-type IPMNs

![Figure 2 IPMN gross features. Grossly, IPMNs are classified based on their level of pancreatic duct involvement. (A) Those involving the main pancreatic duct are designated as main duct type and characterized by a dilated, tortuous main pancreatic duct. (B) IPMNs involving the secondary branches of the main pancreatic duct are designated as branch duct type.](image-url)
oncocytic IPMNs are typically oncocytic rather than ductal adenocarcinoma. Invasive adenocarcinoma arising from pancreatobiliary-type IPMNs more often develop ductal

described: colloid, oncocytic, and ductal adenocarcinoma; ent histological subtypes of adenocarcinoma have been

The nuclei are large and have prominent nucleoli, and the cytoplasm often stains positive for MUC1. These subtypes of IPMNs have clinical and prognostic relevance, for example the gastric subtype is associated with the better prognosis, followed by oncocytic and intestinal subtypes, while the pancreatobiliary subtype is associated with a poor prognosis and adverse clinical behavior. It is important to note that IPMNs can consist of a mixture of subtypes.

One-third of IPMNs, usually main duct, are associated with invasive pancreatic adenocarcinoma. Three different histological subtypes of adenocarcinoma have been described: colloid, oncocytic, and ductal adenocarcinoma; with colloid being the most common of the three. Intestinal-type IPMNs often progress to colloid carcinoma, while pancreatobiliary-type IPMNs more often develop ductal adenocarcinoma. Invasive adenocarcinoma arising from oncocytic IPMNs are typically oncocytic rather than ductal or colloid. IPMNs can also be associated with extrapancreatic malignancies including gastric and colorectal carcinoma.

### Molecular features

Unlike PanINs, MCNs, and PDAC, DPC4/SMAD4 gene mutations are an uncommon finding in IPMNs. KRAS2 mutations increase with increasing degrees of dysplasia, and TP53 and p16/CDKN2A gene inactivation, both commonly found in PDAC, can vary among IPMN cases. Interestingly, expression of the protein product of STK11/LKB1, a gene associated with Peutz–Jeghers syndrome, is observed in 25% of IPMNs, and the PIK3CA gene is mutated in 10% of cases. In a recent study, Wu et al. identified recurrent point mutations of GNAS at codon 201 in 60% of IPMNs. GNAS encodes for the alpha subunit of a stimulatory G-protein, which in turn, activates cyclic AMP pathways within cells. Interestingly, GNAS mutations were more often seen in intestinal-type IPMNs. Furukawa et al. evaluated the rate of mutations involving codon 201 of GNAS in 118 IPMNs and 32 conventional pancreatic adenocarcinoma cases, and found that ~40% of the IPMNs, and none of the pancreatic adenocarcinomas, harbored GNAS mutations. The G-protein alpha unit and downstream effectors, including phosphorylated substrates of protein kinase A (PKA), were expressed in the IPMN tissues bearing mutant GNAS. The frequency of GNAS mutations did not vary with grades of IPMN dysplasia however the expression of PKA was associated with neoplastic grade. PKA expression was greater in high-grade and invasive IPMNs versus low-grade IPMNs. These findings suggest that GNAS mutations are unique to IPMNs and play a role in their pathogenesis and that activation of G-protein alpha and downstream components may contribute to the progression of IPMNs.

### Diagnosis, natural history, and treatment

A finding, that is strongly consistent with IPMNs, is mucin extruding from a patulous ampulla of Vater on upper endoscopy. Although atypical cells and mucin may be found on endoscopic ultrasound-guided fine-needle aspiration, the sensitivity of aspiration cytology in cystic lesions of the pancreas is suboptimal. Imaging modalities may be rather helpful in the diagnosis of IPMNs. CT and endoscopic retrograde cholangiopancreatography usually reveal dilated main and branched pancreatic ducts, due to obstruction of the lumen by mucous plugs and papillary projections of the tumor. Plugging of the ductal lumina leads to the formation of grape-like cystic lesions that are diagnostic of IPMN lesions. Magnetic resonance cholangiopancreatography may also be necessary to evaluate the extent of pancreatic duct involvement.
a useful tool, and usually demonstrates ductal dilation and occasionally mural nodules.\textsuperscript{45}

The natural history of noninvasive IPMNs and the precise timeline for their progression to invasive cancer is not well known, because the majority of large and symptomatic lesions are removed before invasion. Nonetheless, given that many patients report a long history of symptoms before being diagnosed with an invasive component associated with an IPMN suggests that the natural history is at least 5 to 10 years for such a progression.\textsuperscript{29,30} Features suggestive of malignant IPMNs include: main duct IPMN, clinical symptoms, cysts greater than 3 cm, mural nodules, lymphadenopathy, distension of the pancreatic main duct, positive cytology on EUS, and a CEA level greater than 200 in the cystic contents.\textsuperscript{24,28}

Main-duct IPMNs have a greater risk of malignancy, approaching 40\textendash{}50\%, while patients with branch duct IPMNs are at a much lower risk of malignant transformation with a risk \textendash{}10\% (the range is somewhat variable based on the specific series).\textsuperscript{29,47\textendash}50 Surgery remains the standard of care in the management of main duct IPMNs given their malignant potential.\textsuperscript{51} Treatment of IPMNs involving the branch ducts is somewhat controversial, in terms of definitive surgery versus conservative follow up and management. High risk features and indications for surgery include: size > 3 cm, symptoms, and suspicious radiological findings such as solid or mural nodules and distension of the main pancreatic duct (“Sendai criteria”).\textsuperscript{28} However, preoperative radiological assessments in asymptomatic branch duct IPMNs are not precise in up to one-third of cases, underscoring the need for ancillary diagnostic parameters like mutational analysis in cyst fluid.\textsuperscript{52} It is important to note that surgical resection does not always result in a definite cure due to the fact that noninvasive IPMNs can be multifocal and recur.\textsuperscript{53} A small percentage of patients develop a second pancreatic neoplasm, which can be fatal. Therefore, careful follow up and clinical management is needed after resection of IPMNs to evaluate for metachronous disease.

**MCNs**

**Clinical features**

MCNs are mucin-producing, cyst-forming epithelial neoplasms, most commonly arising in the body/tail of the pancreas, and they are supported by a distinctive ovarian-type stroma.\textsuperscript{30} These lesions are almost exclusively diagnosed in young women with a mean age of 40 to 50 years (ranging in age from 14 to 95 years). MCNs can be found incidentally in asymptomatic patients on a physical exam or by imaging and may also be detected in symptomatic patients.\textsuperscript{44} Patients most often present with epigastric pain, a sense of abdominal fullness, nausea and vomiting, diarrhea, or symptoms due to a mass effect. Approximately 5\% to 30\% of symptomatic patients might harbor a palpable mass upon physical examination. Patients with MCNs usually have normal CA 19-9 levels, unless the MCN is accompanied by an invasive adenocarcinoma.

**Pathologic features**

MCNs are predominately identified in the body/tail of the pancreas. MCNs can be substantially large in size, ranging in diameter from 1.5 cm to 30 cm in size. Grossly, less than 20\% of MCNs communicate with the pancreatic duct.\textsuperscript{55} The cyst surface is smooth and glistening, and covered with large dilated blood vessels and a thick fibrous capsule (Figure 4A). MCNs tend to be multilocular, are rarely multifocal, and contain abundant mucin and/or hemorrhagic fluid (Figure 4B to D). Microscopically, the cysts are lined by columnar mucin-producing epithelium arranged in a flat architecture (Figure 5A and B). However, low papillary projections, cribriform, and glandular formation may be present, especially in high-grade neoplasms. As mentioned above, these lesions have a distinctive ovarian type stroma, which forms a “Grenz” zone underneath the epithelium, and can be highlighted with immunohistochemical stains for PR, ER, and inhibin A (Figure 5B).

These neoplasms, like the other precursor lesions, are graded based on cytologic and architectural atypia. Low-grade MCNs are composed of a uniform single-layer of benign mucinous columnar cells; the nuclei are basally

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**Figure 4** MCN gross features. Grossly, MCNs are solitary lesions that do not communicate with the pancreatic ducts. They may be either (A) unilocular or (B) multilocular. (C and D) The locules have thick walls and are filled with thick tenacious mucin, although some may be filled with hemorrhagic fluid.

**Abbreviation:** MCN, mucinous cystic neoplasm.
located, small, and uniform in size. Intermediate- and high-grade MCNs consist of columnar epithelium with varying degrees of nuclear atypia with loss of nuclear polarity. Typically, the tumor epithelium can have a spectrum of different degrees of dysplasia, and the tumor should be graded as the highest grade present, even if only focal.

**Molecular features**

Early molecular events in the pathogenesis of MCNs include activating point mutations in *KRAS2* and late events include *TP53* and *DPC4/SMAD4* mutations.56 Also documented in a minority of MCNs is the aberrant methylation of *p16/CDKN2A*.57 However, a recent study identified that MCNs often harbor mutations of *RNF43* with accompanying LOH of the wild-type allele.58 RNF43 encodes for an E3 ubiquitin ligase, and recent data suggests that the encoded protein leads to accelerated turnover of the Wnt receptor and therefore antagonizes oncogenic Wnt signaling.58 Of note, overall, approximately 50% of pancreatic mucinous cysts – IPMNs and MCNs – harbor inactivate mutations of *RNF43*. It is unclear whether disruption of *RNF43* activity leads to oncogenic Wnt signaling in exocrine cystic neoplasms, or other targets of this ubiquitin ligase are aberrantly activated.

**Diagnosis, natural history, and treatment**

Imaging studies are the best diagnostic tool for MCN lesions.55,59 CT often reveals well-circumscribed thick-walled multilocular cysts, ranging in size from 1 to 3 cm, typically located in the tail of the pancreas. Occasionally, noninvasive MCNs can be differentiated from invasive MCNs based on the finding of mural nodules within the cysts, which are more common in invasive lesions. Endoscopic retrograde cholangiopancreatography is another diagnostic method used to detect MCNs. The large cystic structures often displace or compress the pancreatic duct, and in the majority of cases the cysts do not communicate with the pancreatic ducts.55

MCNs progress to invasive carcinoma less often than IPMNs. Approximately 6% to 36% of MCNs are associated with an invasive component at the time of resection.60 Due to their malignant potential, all MCNs should be removed to prevent further progression. The disease-specific 5-year survival approaches 100% for patients with a noninvasive resected MCN. If an invasive component is present, the 5-year survival decreases to approximately 50% to 60%.61 Of special note, invasive adenocarcinomas usually arise focally in MCNs and complete resection of the MCN lesion is recommended. Partial resections of MCNs should be avoided to prevent missing an invasive component. If detected early and completely excised, MCNs are potentially curable neoplasms.

**Familial pancreatic cancer**

Approximately 10% of patients with pancreatic carcinoma have a known family history.62 Individuals with a first-degree relative with pancreatic cancer are thought to have a twofold increase in risk of developing pancreatic cancer, and this risk increases with each first-degree relative with a pancreatic cancer history.63 Understanding the genetic defects and precursor lesions associated with familial pancreatic carcinoma could therefore help explain the pathogenesis of this disease and aid in better screening and prophylactic methods. In a recent study done by Shi et al, noninvasive precursor lesions were prevalent in the resected pancreata from patients with familial pancreatic carcinoma, but they were found more often than those with sporadic disease, and were of higher grade.63 It is recommended that high-risk individuals with a family history of pancreatic cancer should be screened routinely for precursor lesions.64

**Future directions**

The vast majority of pancreatic cancer patients are diagnosed at an advanced stage or after metastatic dissemination. Detecting these tumors at the precursor level could help alleviate the poor prognosis associated with pancreatic cancer. Cystic pancreatic lesions (IPMNs and MCNs) can be detected using a wide variety of imaging techniques, either incidentally, or when they become symptomatic. PanIN lesions however, are, for the most part, too small to be identified using current imaging modalities. One of the challenges for the future is to be able to detect higher-grade PanIN lesions prior to acquisition of invasive properties.

A recent genetic study has demonstrated a large window of opportunity where pancreatic cancer can potentially be
detected prior to becoming invasive and terminally metastatic. In fact, one of the best opportunities for curing pancreatic cancer remains its early detection, given that 80% or more of patients are currently diagnosed at a surgically unresectable stage. One potential molecular target for screening is mutant KRAS2 DNA, which is shed by neoplastic cells into blood, pancreatic juice, or stool.65,66 The near ubiquitous nature of KRAS2 mutations renders this as an attractive marker for pancreatic cancer detection in peripheral biospecimens. However, KRAS2 mutations per se are not specific to invasive pancreatic cancer, and may occur in patients with low-grade precursor lesions as well as those with chronic pancreatitis;67 thus, any tractable assay cannot be a simple “yes/no” readout but must also quantify the amount of mutant DNA within the biospecimen of interest.65,66 Other common somatic mutational events, such as TP53 and CDKN2A/p16 mutations are also amenable to detection in biospecimens like pancreatic juice, as are epigenetic alterations such as aberrant methylation.68,69 For pancreatic cystic lesions, EUS and aspiration of cyst fluid contents provides an opportunity for molecular analysis, such as quantitative assessment of mutant DNA using next generation sequencing,37 or multiplex microRNA profiling for aberrant biomarker expression.70 Ultimately, there is unlikely to be one biomarker that proves to be the panacea for early detection, and most likely, we will utilize a multianalyte panel (encompassing DNA, RNA, proteins, or any combinations thereof) for the early diagnosis and therapeutic stratification of pancreatic neoplasia.

Conclusion
Our understanding and knowledge of precursor lesions of pancreatic cancer and their morphological appearance, biological behavior, molecular abnormalities, and clinical implications has significantly progressed over the years. We are now poised to apply the lessons learned over the past decade in precursor lesions of pancreatic cancer towards improving the diagnosis and survival of patients with this lethal malignancy. Ongoing advances in modern imaging techniques and molecular screening tools for high risk individuals provide an unprecedented opportunity for detecting and treating precursor lesions prior to their progression to invasive adenocarcinoma.

Disclosure
The authors report no conflicts of interest in this work.

References


