REVIEW

Pancreatic Ductal Adenocarcinoma and Its Precursor Lesions

Histopathology, Cytopathology, and Molecular Pathology

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Pancreatic ductal adenocarcinoma is one of the most aggressive malignant neoplasms with poor outcomes. At the time of diagnosis, the disease is usually at an advanced stage and only a minority is eligible for surgical resection. To improve the prognosis, it is essential to diagnose and treat the disease in an early stage before its progression into an invasive disease. This article reviews clinical features, histopathology, cytopathology, and molecular alterations of pancreatic ductal adenocarcinoma and its precursors. Moreover, we review a recently updated two-tier classification system for precursor lesions, new findings in premalignant cystic neoplasms, and recently updated staging criteria for invasive carcinoma based on the Cancer Staging Manual, eighth edition, from the American Joint Committee on Cancer. Finally, we discuss the potential clinical applications of the rapidly growing molecular and genetic information of pancreatic cancer and its precursors. (*Am J Pathol* 2019, 189: 9–21; <https://doi.org/10.1016/j.ajpath.2018.10.004>)

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, comprising 90% of all malignant pancreatic neoplasms, with a dismal 5-year survival rate of 8%.^{1,2} In the United States, it is the fourth leading cause of cancer death in both males and females. In 2018, the estimated number of new pancreatic cancer cases in the United States is 55,440, and the estimated death caused by pancreatic cancer is 44,330.¹ Its frequency has been increasing in the past several years, and it is predicted to be the second leading cause of cancer death by 2030.² Studies have suggested that it takes at least 10 years for a mutated pancreatic cell to develop into an invasive disease, which provides hope for early detection.

In the past decade, our knowledge on identification and characterization of pancreatic carcinoma and its precursor lesions has dramatically improved because of large-scale genome sequencing. Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) has become the adopted

method in procuring tissue for PDAC diagnosis. In this article, we present a review of clinical and pathologic features, molecular alterations, prognosis, and management of PDAC and its precursor lesions. We discuss the recently introduced two-tier classification system of precursor lesions and updates in staging criteria based on the recently published eighth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual.³ Finally, we discuss the potential clinical applications based on molecular and genetic information for early detection.

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This article is a part of a review series on benign and neoplastic pancreatic lesions from their pathologic to molecular profiles and diagnoses.

Precursor Lesions of Pancreatic Ductal Adenocarcinoma

PDAC arises from noninvasive precursor lesions, which include a noncystic lesion [pancreatic intraepithelial neoplasia (PanIN)] and cystic lesions [intraductal papillary mucinous neoplasm (IPMN), intraductal tubulopapillary neoplasm (ITPN), and mucinous cystic neoplasm (MCN)]. PanINs are diagnosed microscopically in pancreatic resection or biopsy specimens, whereas cystic lesions are diagnosed clinically by radiological examinations.

Based on the 2010 World Health Organization classification,⁴ precursor lesions of PDAC (PanIN, IPMN, and MCN) are divided into three grades of dysplasia—low, intermediate, and high grade. Clinical evidence shows that lesions with low- and intermediate-grade dysplasia have a low risk of malignant progression and are amenable to clinical observation, whereas lesions with high-grade dysplasia have a high risk of progression into invasive carcinoma and require surgical management. Therefore, a two-tier classification system—low and high grade—was proposed at an international consensus meeting to improve diagnostic concordance and to align histopathology with clinical practice. The former PanIN-2 and intermediate-grade dysplasia are classified into low grade in the two-tier system.⁵

Pancreatic Intraepithelial Neoplasia

PanINs are histologically identified as microscopic mucinous pancreatic ductal lesions (<0.5 cm). Low-grade PanIN encompasses flat, papillary to micropapillary columnar mucinous epithelium with bland to atypical nuclei, nuclear stratification, crowding, and hyperchromasia (Figure 1A). High-grade PanIN encompasses flat to papillary, micropapillary, or cribriform formation with severe nuclear atypia, loss of polarity, macronucleoli, and abnormal mitotic figures (Figure 1B).

In a resection specimen, high-grade PanIN is sometimes difficult to distinguish from dilated neoplastic glands of PDAC. Furthermore, high-grade PanIN may represent intraductal spread of invasive carcinoma or cancerization of the ducts. Therefore, when high-grade PanIN is identified, adequate sampling and cautious histologic examination are crucial in excluding the presence of an invasive component.⁵

The progression of PanIN, along with IPMN and MCN, to PDAC is considered similar to stepwise carcinogenesis of colorectal carcinoma. In this process, the early events include telomere shortening, *KRAS* mutation, and *CDKN2A* (*p16*) loss, whereas *p53* and *SMAD4* loss occurs in the late stage. Studies have shown that *KRAS* mutations, telomere shortening, and Her2 overexpression were detected in low-grade PanIN, whereas *p16* and *SMAD4* loss and *TP53* and *BRCA2* mutations were found in high-grade PanIN.^{4,6} A recent study of isolated high-grade PanIN (not associated

with invasive PDAC) showed that *KRAS* and *CDKN2A* mutations were detected in the low-grade PanINs, whereas the *TP53* mutation only occurred in a minor portion of high-grade PanINs and *SMAD4* mutation was completely absent.⁷ A possible explanation of this finding is that many high-grade PanIN lesions in the earlier studies were intraductal spread of invasive cancer, or isolated high-grade PanIN might be biologically different from high-grade PanIN with associated PDAC.⁷

Intraductal Papillary Mucinous Neoplasm

IPMN is defined as radiologically or grossly recognizable cystic neoplasms (>1 cm) arising in and communicating with the pancreatic ductal system. IPMNs are equally prevalent in men and women, and they are usually diagnosed at 60 to 70 years of age.⁸ The characteristic radiologic features of IPMNs are cystic lesions, involvement of pancreatic duct system, dilation of main and/or branch ducts, and atrophy of surrounding pancreatic parenchyma.⁹ The endoscopic finding of mucin extrusion from dilated ampulla of Vater is pathognomonic for IPMN.¹⁰ Based on the involvement of pancreatic ducts, IPMNs can be divided into three types: main duct, branch duct, and mixed types. Main-duct IPMN usually occurs in the head of the pancreas with a dilated main pancreatic duct. Branch-duct IPMN involves side branches of pancreatic duct. Mixed-type IPMN involves both main and branch ducts.

According to the morphology of mucinous epithelium, IPMNs are classified into four histologic subtypes: gastric, intestinal, pancreatobiliary, and oncocytic types (Figure 1, C–F). Gastric-type IPMN resembles gastric foveolar epithelium. Intestinal-type IPMN is similar to a colonic villous adenoma with goblet cells. Pancreatobiliary-type IPMN has cuboidal neoplastic lining cells with minimal mucin, enlarged nucleoli, and complex architecture. Oncocytic-type IPMN [alias intraductal oncocytic papillary neoplasm (IOPN)] typically presents with eosinophilic neoplastic cells and complex architecture, including arborizing papillae, solid nests, cribriform growth pattern, and intraepithelial lumina. Multiple histologic types can be found within an IPMN, and the dominant component determines its subtype. Approximately 25% of IPMNs do not have one dominant type of epithelium; instead, they are lined by a mixed type of epithelium.¹¹

Different types of IPMNs have distinct mucin expression, which is helpful in determining their subtypes. Gastric-type IPMN expresses MUC5AC; the intestinal type expresses MUC2 (and CDX2); the pancreatobiliary type expresses MUC1 and sometimes focal MUC6; IOPN expresses MUC1 (in some cases), MUC6, and Heppar1 (and expression of Heppar1 is uncommon in other types of IPMNs).¹²

Based on the degree of dysplasia, IPMNs are classified into low and high grade. The morphologic changes of the spectrum of dysplasia in IPMN parallel those seen in

Figure 1 A and B: Pancreatic intraepithelial neoplasia: low grade (A) and high grade (B). C–F: Subtypes of intraductal papillary mucinous neoplasms (IPMNs): gastric (C), intestinal (D), pancreatobiliary (E), and oncocytic (F) types. G: Invasive tubular carcinoma arising in IPMN with infiltrating growth pattern and desmoplastic stroma. H: Invasive colloid carcinoma arising in IPMN with mucinous neoplastic epithelium floating in the extracellular mucin pools. I and J: Mucinous cystic neoplasm, low grade (I) and high grade (J), with ovarian-type stroma underlying the mucinous neoplastic epithelium. K: Intraductal tubulopapillary neoplasm with cribriform architecture, minimal mucin production, and high-grade dysplasia. Hematoxylin and eosin stain was used for all images. Original magnification, $\times 200$ (A–K).

PanINs. The cytologic and architecture atypia increases as IPMN progresses from low to high grade. Compared with low-grade IPMN, high-grade IPMN shows complex architecture, irregular and hyperchromatic nuclei with loss of polarity, and stratification. Gastric-type IPMNs are usually low grade, intestinal type may be low or high grade, and pancreatobiliary IPMN and IOPN are frequently high grade.⁵

Whole-genome sequencing of IPMNs revealed frequent *KRAS*, *GNAS*, and *RNF43* mutations. A *KRAS* mutation was detected in most IPMNs (47% to 81%), even in small

incipient IPMNs (defined as 0.5 to 1 cm), which supports the hypothesis that *KRAS* is one of the most important driver genes during IPMN development.^{13,14} *GNAS* is the second most commonly mutated gene (41% to 66%), and almost all intestinal-type IPMNs harbor a *GNAS* mutation. *RNF43* is a potential tumor suppressor gene, a negative regulator of the Wnt signaling pathway, and frequently inactivated in IPMNs.¹⁵ Genetic alterations of *TP53*, *CDKN2A*, *STK11*, *BRAF*, *SHH*, and *BRG1* have also been reported in IPMNs.¹⁶ Inactivation of both *TP53* and *CDKN2A* is associated with transformation of IPMN to invasive carcinoma.

There is a correlation between the *STK11* mutation and Peutz-Jegher syndrome. Gradual loss of *BRG1* expression correlates with increasing degree of dysplasia. Recently, telomere fusion was detected in high-grade IPMN or IPMN with associated invasive carcinoma, but not in normal-duct or low-grade IPMNs.¹⁷

Although IOPN was regarded as an oncocytic variant of IPMN in the World Health Organization 2010 classification, there is growing evidence to suggest that it should be distinguished from other variants of IPMNs because of its distinct morphologic appearance, mucin and molecular profiles. In contrast to other IPMNs, IOPN lacks mutations in *KRAS* and *GNAS*, but instead exhibits mutations in *ARHGAP26*, *ASXL1*, *EPHA8*, and *ERBB4* genes.^{12,18}

Invasive carcinoma was found in approximately 30% of surgically resected IPMNs.^{19,20} The risk factors of an IPMN harboring invasive carcinoma are main-duct type, distal common bile duct dilation, size >3.0 cm, multicystic lesion, and solid component. Small, branch-duct IPMNs have a low risk of progression and are not recommended for surgical removal.²¹ Among subtypes, the pancreatobiliary type has the most aggressive behavior, whereas the gastric type has the lowest risk of harboring an invasive carcinoma.¹¹ Invasive carcinoma arising in IPMNs can have tubular (commonly associated with pancreatobiliary-type IPMN) or colloid morphology (commonly associated with intestinal-type IPMN) (Figure 1, G and H).²²

The estimated 5-year survival rate of IPMNs with associated invasive carcinoma is approximately 30% to 50%, which is higher than that of invasive PDACs without IPMNs, but much lower than that of noninvasive IPMNs.⁸ Therefore, radiologic correlation, careful gross examination, and adequate sampling of IPMNs are important to confirm the presence of an invasive component in resected specimens. In some institutions, mucinous cystic lesions are entirely submitted for histologic examination to exclude invasive carcinoma.

The rate of recurrence or new disease in the remnant pancreas is <20% after partial pancreatectomy.^{20,23} The rate increases to approximately 50% in patients who had IPMNs with associated invasive carcinoma, and usually as metastatic disease.²⁴ Currently, the postoperative recommendation for patients with partial pancreatectomy is surveillance for at least 5 years.²⁵

Mucinous Cystic Neoplasm

MCN is a radiologically and grossly identified mucinous cystic lesion. MCN is different from IPMN in several aspects. First, MCN does not communicate with the pancreatic ductal system. Second, MCN is lined by neoplastic mucinous columnar epithelium and pathognomonic ovarian-type spindle cell stroma. MCNs commonly occur in the tail of the pancreas and exclusively in

women with a mean age of 50 years.²⁶ Some MCNs are incidental findings, whereas others cause abdominal pain and fullness. Because they do not communicate with pancreatic duct, patients with MCN are less likely to have pancreatitis, jaundice, or new-onset diabetes mellitus compared with those with IPMN. Radiologic features favoring MCN are solitary cyst with thick wall, internal septations, and peripheral calcifications.²⁷

Grossly, MCN is a single multilocular cyst with a thick and fibrotic capsule. Solid areas within the cyst or at the capsule should be sampled extensively for the presence of an invasive component. The lining epithelium consists of columnar cells with abundant apical mucin. Intestinal-type epithelium with goblet cells can sometimes be seen in MCN. The underlying ovarian-type stroma should be present for the diagnosis of MCN (Figure 1, I and J). Stromal hyalinization resembling ovarian corpora albicans can be seen in the stroma.²⁸

Epithelial cells of MCN express cytokeratin and glycoprotein markers, such as CEA and CA19-9. MUC5AC is expressed diffusely, whereas MUC2 expression is seen in intestinal-type epithelium. MUC1 is usually detected in invasive carcinomas arising from MCNs. In addition to estrogen and progesterone receptors, the stromal cells also frequently express inhibin.²⁸

The criteria for evaluating dysplasia in IPMN also apply to MCN. MCN is classified into low and high grade (Figure 1, I and J).

Frequent genetic alterations identified in other precursor lesions are also identified in MCN, including *KRAS*, *TP53*, *SMAD4*, and *CDKN2A*. Approximately half of mucinous cystic neoplasms harbor the *RNF43* mutation. In contrast to IPMN, *GNAS* mutation is not found in MCN. There is no specific genetic mutation in MCN; therefore, the preoperative diagnosis requires exclusion of other pancreatic cystic lesions.

Invasive pancreatic carcinoma is found in approximately 15% to 30% of MCN cases, and they have a better prognosis than conventional PDAC.^{20,26,27} The risk of invasive carcinoma arising from MCNs increases with age. Radiologic features suggestive of an associated invasive carcinoma include large cyst size, thickening of septae, and the presence of an intracystic solid mass.^{26,27} In contrast to IPMN, patients with noninvasive MCN have a 5-year survival rate of nearly 100%; therefore, postoperative follow-up is usually not necessary. MCN with associated minimally invasive adenocarcinoma, defined as invasion into the ovarian-type stroma but not into the pancreatic parenchyma, demonstrates good prognosis with rare recurrence.²⁹ The 5-year survival rate for patients with MCN with invasive carcinoma is 25% to 35%.^{20,26} The single most important factor for pathologic evaluation of MCN is to confirm or exclude the presence of an invasive component, similar to the assessment of IPMNs. Therefore, extensive sampling is essential in the evaluation of MCN.²⁶

Intraductal Tubulopapillary Neoplasm

ITPN is a rare intraductal epithelial neoplasm and is recognized as a distinct entity in World Health Organization classification. ITPN can be detected radiologically and grossly. ITPN occurs almost equally in both sexes and is commonly diagnosed in patients between 35 and 78 years of age (mean age, 61 years).³⁰ It is difficult to differentiate ITPN from IPMN by imaging alone because of overlapping features, but a more solid growth pattern favors ITPN over IPMN. A cork-of-wine-bottle sign is a characteristic feature observed on magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography, which indicates intraductal growth.³¹ Approximately 95% of ITPNs arise in the main pancreatic duct, whereas a small number arises in branch ducts.³²

The size of ITPN ranges from 1 to 15 cm (average size, 3 cm). Approximately 50% of ITPNs are located in the head of pancreas, 33% in the body and/or tail, and >10% in the pancreatic duct with diffuse involvement.³⁰ ITPN is defined as a macroscopic (≥ 1 cm), intraductal, tubule-forming epithelial mass lesion with high-grade dysplasia. Histologically, ITPN consists of closely packed tubules with focal areas of papillary and cribriform structures and with none to minimal mucin (Figure 1K). The neoplastic cells are cuboidal to low columnar with a moderate amount of cytoplasm and with moderately to severely atypical nuclei. Apical snouts, intraluminal secretion, or comedo-like necrosis can be observed in some cases. Clear cell changes, stromal osseous, and cartilaginous metaplasia have been reported in ITPN.³³

The neoplastic cells of ITPN are positive for cytokeratin 7, cytokeratin 19, MUC1, and MUC6; and they are negative for MUC2, MUC5AC, trypsin, and β -catenin.³⁰ MUC6 and MUC5AC are useful in differentiating ITPNs from IPMNs because most IPMNs are MUC5A⁺/MUC6⁻, whereas ITPNs are MUC6⁺/MUC5A⁻. An exception is IOPN, which has a similar mucin profile to ITPN (MUC6⁺MUC1⁺/MUC5A⁻MUC2⁻³³), and this may imply that ITPN is more closely related to IOPN than other subtypes of IPMN.

Although ITPN has overlapping macroscopic and histologic features with IPMN, molecular studies support the concept of ITPN and IPMN as two separate entities given their distinct molecular genetic profiles. In contrast to IPMNs, in which the *KRAS* mutations are more common, the *PIK3CA* mutation is more common in ITPNs (up to 27%), which suggests that activation of the phosphatidylinositol 3-kinase–AKT signaling pathway plays a role in the pathogenesis of ITPNs. *KRAS* mutation occurs at a relatively low frequency in ITPNs (0% to 10%). A next-generation sequencing study of 11 cases of ITPN revealed mutations in histone H3 methyltransferase genes, *MLL2* and *MLL3*, and *MCL1* (a member of the Bcl-2 family) amplification.¹⁸

Approximately 50% of the ITPNs are associated with invasive carcinoma. Therefore, adequate sampling and

careful microscopic examination are essential for the identification of an invasive component.^{30,34} Male, large tumor size, and high Ki-67 proliferation index are risk factors associated with invasive carcinoma.³⁰ Postoperative recurrence and liver metastasis of ITPN have been reported.³³ The 5-year survival rate of patients with ITPN-associated invasive carcinoma is >30%, which is better than conventional PDAC.²¹

ITPN shares overlapping morphologic features with pancreatobiliary IPMN and IOPN. Papillary architecture and abundant mucin production support the diagnosis of IPMN, whereas solid intraductal mass, tubular architecture, minimal mucin, and luminal comedo-like necrosis favor ITPN. Another less common differential diagnosis is intraductal acinar cell carcinoma, which consists of sheets of back-to-back acinar structures with neoplastic cells demonstrating acinar differentiation (positive for trypsin, chymotrypsin, and BCL-10 on immunohistochemical stains).^{35,36}

Cytopathology of Pancreatic NMCs, Including IPMNs and MCNs

Pancreatic cysts are detected in 2.4% to 13% of abdominal imaging,³⁷ and they can be categorized as nonneoplastic cysts, neoplastic cysts, and solid tumors with cystic change. Malignant risk of neoplastic cysts is variable. A non-mucinous neoplastic cyst, such as serous cystadenoma, is commonly benign, whereas neoplastic mucinous cysts (NMCs), including IPMN and MCN, are considered premalignant neoplasms. A multimodal approach, which combines clinical and radiologic information, cytologic evaluation, and ancillary tests, is required for accurate classification of pancreatic cysts.

Imaging modalities, such as computed tomography and magnetic resonance, yield 40% to 60% accuracy in classifying pancreatic cysts.³⁸ EUS-FNA cytology has added significant value to imaging studies in assessing the risk of malignancy of pancreatic cysts.³⁹

The Papanicolaou Society of Cytopathology guidelines classify neoplastic mucinous cysts in category IV.^{40,41} According to the guidelines, diagnostic criteria for NMC include one of the followings: thick colloid-like extracellular mucin, elevated CEA (>192 ng/mL), *KRAS*/*GNAS* mutation, or presence of neoplastic mucinous epithelial cells.⁴⁰

EUS-FNA is performed through the duodenal wall if the lesion is located at the pancreatic head. A transgastric method will be used if the lesion is at body or tail. Knowing the relevant endoscopic approach, the pathologist will recognize normal gastric or duodenal epithelium as procedure-associated contaminants and not as lesional material. Thick colloid-like mucin with or without lining epithelium of the cyst is diagnostic of a neoplastic mucinous cyst. Mucin with cellular or inflammatory debris is also associated with mucinous cysts.

Table 1 Cytologic Features of Low- and High-Grade Atypia in NMC

Cytologic features of low-grade atypia in NMC	Cytologic features of high-grade atypia in NMC
<ul style="list-style-type: none"> • Low cellularity • Single cells, small groups or flat sheets of bland-appearing glandular epithelial cells ($\geq 12\text{-}\mu\text{m}$ duodenal enterocyte) • Nuclei round and regular with even chromatin and inconspicuous to occasional discernible nucleoli • Low N/C ratio • Apical cytoplasmic mucin and basally located nuclei; the cells may be indistinguishable from gastric contamination • Background muciphages, no necrosis 	<ul style="list-style-type: none"> • Low to high cellularity • Single cells, small to large three-dimensional crowded cluster ($< 12\text{-}\mu\text{m}$ duodenal enterocyte); papillary architecture (support IPMN) • Nuclear hypochromasia or hyperchromasia, with or without prominent nucleoli, and nuclear membrane irregularity • High N/C ratio • Variable amount of cytoplasm with or without visible mucin or vacuoles; variable cellular necrosis in the background

IPMN, intraductal papillary mucinous neoplasm; N/C, nuclear/cytoplasmic; NMC, neoplastic mucinous cyst.

CEA level analysis of the cystic fluid can add diagnostic value. Brugge et al⁴² showed that an elevated CEA > 192 ng/mL is the optimal threshold level for distinguishing mucinous cysts from nonmucinous cysts with 75% sensitivity and 84% specificity. Van der Waaij et al⁴³ showed that a higher cutoff level of > 800 ng/mL is associated with higher specificity (98%), but low sensitivity (48%). Nevertheless, an increased level of CEA cannot absolutely distinguish a benign from a neoplastic mucinous cyst, and a low level of CEA cannot absolutely exclude a mucinous cyst.⁴⁰

There are many overlapping features between IPMN and MCN in EUS-FNA cytology specimens. Distinguishing these two entities solely based on cytologic evaluation alone is almost impossible; it often requires clinical and radiologic correlation. The subepithelial ovarian stroma, a diagnostic feature of MCN, is often not sampled in cytology aspirates. Studies have shown that molecular analysis for *KRAS* and *NRAS* mutations can add values in diagnosing NMC.^{44–46} The presence of *KRAS* mutations supports a mucinous cyst, but a *KRAS* mutation alone cannot separate premalignant cysts (NMCs with low-grade atypia) from malignant cysts (NMCs with high-grade atypia or associated invasive carcinoma).⁴⁷ A combined analysis of *KRAS* mutation, loss of heterozygosity, and DNA quantity/quality correlates well with malignant cysts.⁴⁴ Both IPMN and MCN commonly harbored mutations in *KRAS* and *RNF43*, whereas a *GNAS* mutation was only found in IPMN, but not in MCN.^{45,46} Therefore, the presence of a *GNAS* mutation, in addition to a *KRAS* mutation, supports the diagnosis of IPMN.^{45,46,48} Similar to a *KRAS* mutation, alterations in *RNF43* and *GNAS* cannot distinguish high-grade or invasive from low-grade IPMNs.^{45,46} However, Singhi et al⁴⁹ recently reported that combination of *KRAS* and/or *GNAS* mutation with *TP53*, *PIK3CA*, and/or *PTEN* alterations detected in cystic aspirate can predict NMC with advanced neoplasia (high-grade dysplasia or invasive adenocarcinoma) with 79% sensitivity and 96% specificity.

Once the presence of NMC is determined, cytologic grading of neoplastic mucinous epithelium becomes

necessary. A two-tier system for grading epithelial atypia has been recommended by the Papanicolaou Society of Cytopathology, which includes low-grade atypia (low- and intermediate-grade dysplasia) and high-grade atypia (high-grade dysplasia or worse).^{40,50}

Low-grade NMC aspirate often shows low cellularity. Cytologic features of low-grade neoplastic epithelium are single cells, small groups, or flat sheets of bland-appearing glandular epithelial cells with apical cytoplasmic mucin and basally located nuclei, which are often difficult to distinguish from gastrointestinal epithelium contaminant (Table 1).^{40,51}

High-grade NMC aspirate tends to be more cellular. The neoplastic cells are often smaller than duodenal enterocytes with an increased nuclear/cytoplasmic ratio, nuclear hypochromasia or hyperchromasia, prominent nucleoli, and nuclear membrane irregularity. They are arranged in single cells or in small to large crowded clusters. Variable cellular necrosis is often present in the background (Figure 2).^{39,50,52} Identification of high-grade dysplastic cells is important because surgical management is recommended for high-grade lesions.²⁰

Invasive Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma is the most common histologic subtype of pancreatic malignancy, comprising almost 90% of all malignant pancreatic neoplasms.⁵ The mean age of patients at diagnosis is 66 years.¹ At the time of diagnosis, most patients are inoperable because of locally advanced disease or distant metastasis. The median survival for patients with distant metastasis is < 1 year.⁵³ PDAC commonly presents as a hypoechoic solid mass with an irregular border. Most PDACs occur in the pancreatic head (60% to 70%), and the rest are in the body (5% to 15%) or tail (10% to 15%).⁴ Various imaging studies, including ultrasonography, computed tomography, magnetic resonance imaging, and EUS, are used to evaluate pancreatic solid

Figure 2 **A:** Thick colloid-like mucin (Diff-Quick stain). **B:** Low-grade neoplastic mucinous cysts (NMCs) show a small group of bland-appearing glandular epithelial cells with apical cytoplasmic mucin, basally located nuclei, and low nuclear/cytoplasmic (N/C) ratio (Papanicolaou stain). **C** and **D:** High-grade NMC shows neoplastic cells arranged in crowded clusters with high N/C ratio, nuclear hypochromasia or hyperchromasia, and nuclear membrane irregularity. Cellular necrosis and mucin are present in the background (Papanicolaou stain). **E** and **F:** Poorly differentiated ductal adenocarcinoma with marked nuclear pleomorphism, macronucleoli, and high N/C ratio [Diff-Quick stain (**E**) and Papanicolaou stain (**F**)]. **G:** Well-differentiated ductal adenocarcinoma with abundant mucin in the cytoplasm, low N/C ratio, and disorganized cell arrangement resembling a drunken honeycomb (Papanicolaou stain). Original magnification: $\times 20$ (**A**); $\times 400$ (**B–D**, **F**, and **G**); $\times 200$ (**E**).

lesions. Other benign pancreatic lesions (ie, chronic and autoimmune pancreatitis) can mimic pancreatic malignancy clinically and radiologically.^{54,55} It is difficult to distinguish benign mass-forming pancreatitis from malignant lesions solely based on radiographic appearance. Cytohistologic tissue evaluation remains the gold standard in diagnosing pancreatic malignancy.

Grossly, PDACs are firm, ill-defined white-yellow infiltrative lesions. The main pancreatic duct and distal common bile duct upstream of the lesion are usually dilated because of duct obstruction. Fibrosis and atrophy are common in PDAC, which sometimes obscure the demarcation between invasive carcinoma and chronic pancreatitis regions.

Microscopically, conventional PDAC is composed of haphazardly arranged infiltrating glandular and ductal structures, surrounded by desmoplastic stroma. Invasive glands are frequently identified well beyond the grossly identified border of the lesion. Poorly differentiated PDAC is composed of irregular and smaller glands with marked cellular pleomorphism. Well-differentiated invasive

carcinomas may have bland glands, which is difficult to distinguish from reactive glands in areas of chronic pancreatitis. Histologic criteria of invasive adenocarcinoma are haphazard arrangement of neoplastic glands, perineural invasion, vascular invasion, gland immediately adjacent to a muscular artery, luminal necrosis, incomplete lumina, nuclear variation ($>4:1$) in a single gland, isolated gland in peripancreatic adipose tissue, and atypical mitoses.⁵⁶ Neoplastic cells can replace endothelial cells of small vessels and reendothelialize these vessels, which can mimic PanIN. Identification of a well-defined smooth muscle layer can determine whether a neoplastic gland is in a vessel.⁵⁷

Most PDACs demonstrate immunoreactivities to antibodies of several cytokeratins (7, 8, 18, 19, and 20), several types of mucin (MUC1, MUC3, MUC4, and MUC5AC), and cancer marker antigens (CEA, B72.3, CA19-9, mesothelin, and S-100A4).^{28,58} There is no definite marker to distinguish PDAC from benign ductal structures. Aberrant p53 expression and loss of SMAD4 expression (in 55% of PDACs) support the diagnosis of PDAC, but absent p53

Table 2 Comparison of Staging Definitions of Pancreatic Carcinoma in the Seventh and Eighth Editions of the AJCC Cancer Staging Manuals

T category	T criteria for AJCC manuals	
	Eighth edition ³	Seventh edition ⁷⁸
TX	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	
Tis	Carcinoma <i>in situ</i> (this includes high-grade pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasm with high-grade dysplasia, intraductal tubulopapillary neoplasm with high-grade dysplasia, and mucinous cystic neoplasm with high-grade dysplasia)	Carcinoma <i>in situ</i>
T1	Tumor ≤2 cm in greatest dimension	Tumor limited to the pancreas, ≤2 cm in greatest dimension
T1a	Tumor ≤0.5 cm in greatest dimension	
T1b	Tumor >0.5 and <1 cm in greatest dimension	
T1c	Tumor 1–2 cm in greatest dimension	
T2	Tumor >2 and ≤4 cm in greatest dimension	Tumor limited to the pancreas, >2 cm in greatest dimension
T3	Tumor >4 cm in greatest dimension	Tumor extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery
T4	Tumor involves the celiac axis, superior mesenteric artery, and/or common hepatic artery, regardless of size	Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor)

AJCC, American Joint Committee on Cancer.

immunostaining or maintained SMAD4 expression does not exclude PDAC.⁵⁹

The recent development of large-scale genome sequencing studies provides insights in genetic alteration of PDAC. On average, PDAC has 50 to 80 exomic nonsilent mutations. Larger structural variations, including intra-chromosomal rearrangement, deletions, and amplifications, are also common in PDAC.⁶⁰ Mutation of the *KRAS* oncogene occurs early in neoplastic progression and is observed in almost all precursor lesions of PDAC, even in low-grade PanIN and in >90% of PDACs. Subsequent mutations that drive neoplastic progression are tumor suppressor genes, such as *CDKN2A*, *TP53*, and *SMAD4*. Further accumulation of genetic and epigenetic alterations drives neoplastic progression of precursor lesions into invasive carcinoma.⁶¹ Most PDACs harbor abnormalities in *CDKN2A* because of mutation or hypermethylation of the promoter region. A *TP53* mutation occurs in 50% of PDACs, and loss of *SMAD4* expression is observed in 55% of PDACs.^{60,62} Loss of *SMAD4* is associated with poor prognosis, whereas mutations in chromatin-regulating genes (*MLL*, *MLL2*, *MLL3*, and *ARID1A*) are associated with improved survival.^{62–64} Clinical trials targeting specific pathways and mutations in PDAC are being conducted. The development of personalized treatment may drastically change the outcome of this disease. In addition to genetic alterations, tumor microenvironment also plays an important role in PDAC.^{65,66} Tumor stromal expression profile was found to have prognostic significance.⁶⁷ Distinct immune cell populations are present in the PDAC microenvironment, which affect tumor progression and therapeutic outcomes. Regulatory T cells likely play a role in the early stage of disease, and high intratumoral regulatory T cell/CD4⁺ T cell

ratio is a prognostic factor of poor survival. Investigation is underway in targeting regulatory T cells in malignancy.^{68,69}

The prognosis of PDAC is poor, with an overall 5-year survival rate at approximately 8%. Surgical resection is the only curative modality,⁷⁰ but only 20% of PDACs are resectable at the time of diagnosis. The 5-year survival rate for patients after surgery ranges from 3% to 31%, depending on clinical stage, and the median survival time after resection is 12 to 18 months.⁷¹ Initial borderline and non-resectable PDACs may be down-staged to resectable tumors in approximately 30% to 40% of patients after neoadjuvant chemotherapy.⁷² Only 3% of patients survive after 8 years. In the past decade, several landmark trials in the treatment of PDAC have been published and have brought some optimism. Studies showed that as monotherapy, FOLFIRINOX (leucovorin calcium, fluorouracil, irinotecan hydrochloride, and oxaliplatin) provides a survival benefit of >4 months compared with gemcitabine, but with higher toxicity. Nab-paclitaxel has demonstrated antitumor activity, and the combination of gemcitabine and nab-paclitaxel has become another first-line option for patients. Nano-liposomal irinotecan in combination with fluorouracil/leucovorin has recently been approved for second-line treatment.⁷³ There is also evidence that a small proportion of PDACs that are deficient for mismatch repair system benefit from immunotherapy, such as pembrolizumab.⁷⁴

The main differential diagnosis for conventional ductal adenocarcinoma is the distinction of a well-differentiated carcinoma from reactive ductules in atrophic chronic pancreatitis. Invasive glands are usually distributed haphazardly, whereas benign ductules in areas of chronic pancreatitis retain lobular configuration. The cytoplasm of tumor cells is more eosinophilic than in benign conditions.

Table 3 Comparison of Cytologic Features of PDAC and Chronic Pancreatitis

Cytologic features for PDAC	Cytologic features for chronic pancreatitis or autoimmune pancreatitis
<ul style="list-style-type: none"> • Moderate to high cellularity • Overlapping nuclei/three-dimensional crowded groups; disorganization (drunken honeycomb) • Isolated single cells • Nuclear enlargement, anisonucleosis (>4:1 variation in diameter) • Macronucleoli; irregular chromatin distribution (clumping and parachromatin clearing); nuclear membrane irregularity; increased N/C ratios • Cytoplasmic mucin vacuoles • Background necrosis; mitoses • Tumor cells embedded in desmoplastic stromal fragments 	<ul style="list-style-type: none"> • Low cellularity • Flat, cohesive sheets with evenly spaced or slightly crowded nuclei • Absent (or only rare) isolated atypical cells • Enlarged nuclei, but little variation in size (<4:1 diameter ratio) • Discernible nucleoli but not macronucleoli; round to oval nucleus and smooth nuclear membranes; low N/C ratio • No cytoplasmic mucin vacuoles • Background inflammation, fat necrosis, calcific debris; rare mitosis • Cellular stromal fragments (especially autoimmune pancreatitis)

N/C, nuclear/cytoplasmic; PDAC, pancreatic ductal adenocarcinoma.

Perineural or vascular invasion, invasion into the duodenal muscular wall or stroma adjacent to a muscular vessel,^{75,76} and isolated glands in the peripancreatic soft tissue⁷⁷ are diagnostic of carcinoma. Additional differential diagnosis includes other histologic subtypes of pancreatic neoplasms and metastasis.

Update of Tumor Staging and Surgical Pathology Report of PDAC

Post-resection prognosis of patients with pancreatic carcinoma is primarily determined by anatomical extent of the disease, as defined by TNM staging. According to the newly published eighth edition of the AJCC Cancer Staging Manual (AJCC, eighth edition),³ the T categories (T1 to T3) are defined by the tumor size instead of the extent of tumor invasion, as in the AJCC, seventh edition⁷⁸ (Table 2). The new T categories allow a more reproducible system of T staging and provide better prognostic stratification.³ For invasive carcinoma associated with precursor lesions, size of invasive component should be used to determine T stage. In cases of multifocal invasion, maximum linear dimension of the largest invasive focus should be used for staging.

In the setting of neoadjuvant therapy, gross assessment of tumor size may not always correlate with microscopic measurement of residual tumor. Scattered residual tumor glands may disperse within a large fibrotic tumor bed, which complicates the assessment of tumor size. Currently, there is no consensus guideline or protocol to guide gross and microscopic evaluation of pancreatic cancer after neoadjuvant therapy. Hartman and Krasinskas⁷⁹ suggested that if the tumor bed is ≤ 3 cm, the entire tumor bed should be submitted for microscopic examination, and if the tumor bed is ≥ 3 cm, sections should be submitted serially at 0.5-cm intervals along the largest dimension of the tumor bed. If no residual tumor is identified in the initial sections, then the entire tumor bed should be submitted for microscopic examination.^{79,80} Pai and Pai⁸⁰ noticed that tumor in the duodenal wall is often not affected by neoadjuvant therapy;

therefore, sampling of duodenal wall adjacent to the tumor bed should be performed.

The N stage was changed from two categories in the AJCC, seventh edition (N0 with no nodal metastasis and N1 with positive nodes) into three categories in the AJCC, eighth edition, to provide better prognostic stratification: N0 with no positive nodes, N1 with one to three positive nodes, and N2 with four or more positive nodes. Microscopic examination of at least 12 regional lymph nodes is recommended.^{81,82}

Standardized assessment of resection margins in pancreaticoduodenectomy specimens is important. Resection margin with tumor cells at or within 1 mm is considered a positive margin.⁸³ Adsay et al⁸⁴ recommended submitting the uncinate margin entirely for histologic examination, given that grossly invisible tumor is often seen in this area and the poor prognosis of a positive uncinate margin. Vascular groove is the site of the confluence of portal and superior mesenteric veins and is often considered as a margin. Although not considered true margins, status of anterior and nonuncinate posterior surface involvement needs to be included in the surgical pathologic report.

Cytopathology of PDAC

EUS-FNA biopsy is widely used in pathologic diagnosis of pancreatic tumors with high sensitivity and specificity.⁸⁵ In most institutions, a rapid on-site evaluation is performed to increase the diagnostic yield of solid mass and to reduce nondiagnostic rate.

EUS-FNA biopsy of normal pancreas parenchyma consists predominantly of cohesive, grapelike groups of acinar cells, and a few flat sheets of bland, evenly distributed ductal epithelial cells. Aspirates of pancreatic adenocarcinoma are often highly cellular. The background can be clean, necrotic, inflammatory, or mucinous. Neoplastic ductal epithelial cells are arranged in groups, forming sheets, clusters, and three-dimensional aggregates. Several

distinct nuclear features are diagnostic of pancreatic adenocarcinomas,⁸⁶ such as nuclear pleomorphism, hyperchromasia, macronucleoli, high nuclear/cytoplasmic ratio, and nuclear overlapping and irregular nuclear membranes (Figure 2). High-grade ductal adenocarcinoma often shows more overt features of malignancy, making the diagnosis straightforward. Single isolated tumor cells and mitosis are commonly seen in the background of a high-grade tumor. On the other hand, well-differentiated tumor cells have less nuclear pleomorphism, low nuclear/cytoplasmic ratio, and abundant intracellular mucin. The cells arranged in sheets become disorganized, not evenly spaced, resembling a drunken honeycomb. Marked anisonucleosis (>4:1 variation in diameter) can be a helpful feature (Table 3). Demonstrating an invasion into desmoplastic stroma in cell blocks from aspirates or core biopsy specimens can also aid the final diagnosis.

When cytomorphic features are qualitatively or quantitatively insufficient for making a definitive malignant diagnosis, atypical or suspicious for malignancy can be rendered. The cytologic or architectural features in the atypical category are beyond those associated with reactive changes, but insufficient in the degree or quantity to be categorized as either neoplastic or suspicious for malignancy.⁴⁰ The suspicious for malignancy category is defined as a sample with significant cytologic or architectural atypia suggestive of malignancy, but qualitatively or quantitatively insufficient for a definitive diagnosis.⁴⁰

In chronic or autoimmune pancreatitis, the FNA specimen is hypocellular, containing a few clusters of ductal epithelial cells with reactive atypia, cellular stromal fragments with crush artifact, and lymphoplasmacytic inflammation (Table 3). Islet cells sometimes can also be seen in the aspirate of chronic pancreatitis. Immunostain for IgG4 can sometimes be helpful for the diagnosis of autoimmune pancreatitis, but serum IgG4 is clinically more diagnostic. If ductal epithelial atypia is concerning for malignancy, immunohistochemical stains for SMAD4 and p53 can be helpful. Loss of SMAD4 expression and strong positivity for p53 support a malignant diagnosis.

Conclusion

Pancreatic cancer is one of the deadliest diseases, with poor outcomes and short-lived treatment response; therefore, there is an urgent need for early detection and more effective treatment strategies. In the past decade, comprehensive next-generation sequencing analyses have been performed on samples from large cohorts of patients. These studies have defined the genomic landscape of PDAC, identified novel gene mutations involving pancreatic tumorigenesis, and clarified the genetic alterations that underlie multistep tumorigenesis.⁸⁷

Furthermore, genetic alterations can be used to develop diagnostic markers for early detection, biomarkers for

predicting disease progression or therapeutic response, and novel targets for therapies.

The evolutionary patterns of PDAC suggest a 10-year window from an initiating mutation to an invasive disease, which provides a broad time period for early detection. The application of molecular alteration has been used in liquid biopsy of pancreatic lesions, including FNA samples of pancreatic cysts, duodenal fluid collected endoscopically after secretin stimulation, and circulating tumor DNA in peripheral blood.⁸⁷ Molecular analysis of cyst fluid cannot only distinguish neoplastic precursors from benign cystic lesions, but can also identify precursor lesions with high-grade dysplasia and associated invasive carcinoma. Circulating tumor DNA can be detected in patients with metastatic pancreatic cancer and almost half of patients with localized disease.^{63,88} Despite low sensitivity in detection of noninvasive lesions, circulating tumor DNA can be used to detect invasive cancers at an earlier stage and to follow up patients with known cancer. Circulating tumor DNA has the potential to be used as a surrogate for detecting genetic alteration in the main tumor and providing information for prognosis and therapeutic resistance.⁸⁸ These new techniques highlight the promise of early diagnosis of advanced pancreatic cystic lesion and PDAC.

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