Abstract
Invasive ductal adenocarcinoma of the pancreas remains an almost universally lethal disease. Despite strenuous research efforts, the prognosis of the disease has not improved in the past decades. However, knowledge of pancreatic tumorigenesis and the identification and characterization of the precursor lesions that give rise to invasive pancreatic cancer have dramatically improved. This, coupled with the finding that it takes almost two decades for a pancreatic cell with an initial mutation to develop into a metastatic pancreatic cancer provides hope for the early detection of curable pancreatic neoplasms. We present a review of established precursor lesions of pancreatic cancer, including pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasms (including intraductal oncocytic papillary neoplasm and intraductal tubulopapillary neoplasm), and mucinous cystic neoplasm.

Keywords
cyst fluid; intraductal oncocytic papillary neoplasm; intraductal papillary mucinous neoplasm; intraductal tubulopapillary neoplasm; mucinous cystic neoplasm; pancreatic ductal adenocarcinoma; pancreatic intraepithelial neoplasia

Introduction
Invasive pancreatic ductal adenocarcinoma (PDAC, i.e. pancreatic cancer), is the fourth leading cause of cancer-related deaths in the USA. In 2008, an estimated 161,800 world-wide deaths were attributed to pancreatic cancer in the developed world, and the mortality rate approaches its incidence (10–12:100,000). The 5-year survival rate of pancreatic cancer is a bleak <6% which is due, at least in part, to the fact that the vast majority of patients (~80%) are diagnosed with locally advanced or metastatic disease. Chemo- and/or radiation therapies are only marginally effective, increasing survival by only a few months for only a subset of patients. Therefore, it is crucial that pancreatic neoplasia be detected earlier, before an invasive cancer develops, while the disease is still curable.

It is now recognized that invasive pancreatic cancer arises from histologically well-defined noninvasive precursor lesions. This, combined with the recent finding that it takes many years for a pancreatic cell with an initial mutation to progress to a metastatic cancer, highlights a significant window of opportunity for early detection.

The first step in developing an early detection test is to identify curable precursor lesions and to understand their biology. Herein, we present a review of the literature on four recognized precursor lesions of pancreatic cancer, beginning with the microscopic pancreatic intraepithelial neoplasia (PanIN) followed by the macroscopic intraductal papillary mucinous neoplasm (IPMN) including intraductal oncocytic papillary neoplasm (IOPN), then briefly covering intraductal tubulopapillary neoplasm (ITPN), and fourth a discussion of the mucinous cystic neoplasm (MCN). Finally, recent advances in cyst fluid analysis and its potential use in diagnosis (for biomarker detection) will be discussed, highlighting findings that could be of potential benefit for the detection and treatment of precursors to PDAC.

Pancreatic intraepithelial neoplasia
Clinical appearance and morphology of PanINs
Microscopic intraductal lesions believed to be precursors to invasive pancreatic cancer have been recognized for more than a century. It was only in the past 2–3 decades that molecular studies helped establish that these small lesions, now called PanINs, are definitely precursors to PDAC.

Like PDAC itself, PanINs are encountered mostly in the head of the pancreas and less in the body and/or tail. In one large autopsy study, the overall prevalence of PanINs was approximately 19% (Table 1). In addition, the prevalence of PanINs has been found to increase with age; 6.7% of patients aged ≤50 years, 28% of patients aged 50–65 and 37% of patients aged ≥65 harbour PanIN lesions. Cubilla and Fitzgerald reported that PanIN-2 (which they had designated as intermediate-grade ductal papillary hyperplasia) was three times more prevalent in pancreata with an associated invasive cancer than in those without, and the highest grade lesions (i.e. PanIN-3) were only found in pancreata with an associated PDAC. Similarly, Andea et al. reported that PanINs were more common in pancreata harbouring PDAC (82%), than in pancreata with pancreatitis (60%) and normal pancreata.
(16%). Thus, PanINs, particularly high-grade PanINs, increase with age and are associated with PDAC.\cite{13}

Pancreatic intraepithelial neoplasia occurs in smaller pancreatic ducts and are <5 mm in diameter which makes PanINs generally not grossly detectable. In fact, size is one of the features used to distinguish PanINs from IPMNs which, by definition, are usually >1 cm. PanINs are characterized by the replacement of the normal cuboidal ductal epithelium by columnar mucinous cells, either flat or papillary, with various degrees of dysplasia. PanINs are divided in three grades based on the degree of cytonuclear and architectural atypia (Figure 1).\cite{14,15} Typically, low-grade or PanIN-1A lesions have a flat epithelium consisting of columnar mucinous cells with basally located round to oval uniform nuclei (containing supranuclear mucin) which are perpendicularly oriented to the basement membrane. In contrast to PanIN-1A, PanIN-1B lesions have a (micro)papillary architecture. PanIN-2 lesions feature greater architectural complexity, including early loss of nuclear polarity, pseudostratification and hyperchromasia (i.e. darker nuclei), all consistent with intermediate-grade dysplasia. In PanIN-3 lesions (high-grade dysplasia or carcinoma-

<table>
<thead>
<tr>
<th>General characteristics of precursor lesions of pancreatic cancer</th>
<th>PanIN</th>
<th>IPMN</th>
<th>MCN</th>
<th>ITPN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex ratio (female: male)</strong></td>
<td>1:1</td>
<td>2:3</td>
<td>20:1</td>
<td>1:1</td>
</tr>
<tr>
<td><strong>Predominant age of diagnosis</strong></td>
<td>Increasing with age</td>
<td>60–70</td>
<td>40–50</td>
<td>56 (mean)</td>
</tr>
<tr>
<td><strong>Intrapancreatic location</strong></td>
<td>Head &gt; body/tail</td>
<td>Head &gt; body/tail</td>
<td>Body/tail</td>
<td>Head &gt; body &gt; tail</td>
</tr>
<tr>
<td><strong>Relation with pancreatic duct(s)</strong></td>
<td>Occur in small ducts</td>
<td>Occur in main and/or branch ducts</td>
<td>None</td>
<td>Occur in dilated ducts</td>
</tr>
<tr>
<td><strong>Diagnostic features by imaging techniques</strong></td>
<td>Chronic pancreatitis-like changes in very few patients</td>
<td>Dilated pancreatic duct, filling defects, cyst(s)</td>
<td>Cystic mass with thick walls that compresses/displaces duct</td>
<td>Resemble pancreatobiliary-type IPMNs</td>
</tr>
<tr>
<td><strong>Macroscopic features</strong></td>
<td>Mostly not grossly visible (&lt;5 mm)</td>
<td>Dilated pancreatic ducts with abundant mucin</td>
<td>Well-defined cysts with thick walls containing mucin or hemorrhagic material</td>
<td>Solid nodular masses within ducts, no mucin</td>
</tr>
<tr>
<td><strong>Microscopic features</strong></td>
<td>Columnar/papillary mucinous epithelium, adjacent parenchymal atrophy may be present</td>
<td>Flat, micro/grossly papillary epithelium, parenchymal atrophy may be present</td>
<td>Mucin-producing columnar cells with associated ovarian-like stroma</td>
<td>Cribriform or solid with necrosis</td>
</tr>
</tbody>
</table>

PanIN, pancreatic intraepithelial neoplasia; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; ITPN, intraductal tubulopapillary neoplasm.

Table 1

In contrast to the negative effect of invasive cancer at a margin, PanIN, even high-grade PanIN-3 at a margin appears to have no clinical significance for patients with an invasive cancer.\cite{16}

PanINs are often surrounded by lobular parenchymal atrophy (i.e. lobulocentric atrophy) (Figure 1f).\cite{17,18,19} Observations in humans suggest that PanIN lesions obstruct exocrine outflow in the ducts and the subsequent release of acinar enzymes to the parenchyma leads to autodigestion, thus creating localized pancreatitis-like atrophy. By contrast, observations in genetically engineered animal models suggest that genetic changes in acinar cells drive acinar-ductal metaplasia imparting the appearance of lobulocentric atrophy. Patients with a strong family history of pancreatic cancer sometimes have multiple PanIN lesions which produce multifocal lobulo-centric atrophy and radiological changes similar to those seen in chronic pancreatitis. This suggests that a specific at-risk population can be screened for the presence or absence of PanINs by looking for these changes.\cite{19}

A number of intraductal lesions should be considered in the differential diagnosis of PanINs. First, repeated ductal epithelium injury can lead to the replacement of normal cuboidal cells by mature stratified or pseudostratified squamous epithelium, commonly referred to as squamous metaplasia. Squamous metaplasia is distinguished from PanINs by the direction of differentiation of the cells, and the absence of significant atypia in squamous metaplasia. Second, duct inflammation and repair can produce reactive atypia with enlarged nuclei and nucleoli. The inflammation helps distinguish reactive atypia from PanINs. Third, ‘cancerization of ducts’ can occur as invasive cancer grows from the stroma back into and along previously non-neoplastic ducts. Two features distinguish PanINs from this “cancerization.” Cancerization is almost always associated with an invasive carcinoma in the stroma next to the lesion in...
question, and cancerization is characterized by an abrupt transition between normal and abnormal epithelium. Finally, and most challenging, PanINs are sometimes almost impossible to distinguish from smaller branch-duct IPMNs (see below), especially the gastric-type. As there is histological overlap, IPMNs are often multifocal, and histologically tend to have longer papillae and more abundant mucin production than do PanINs. PanINs are defined as lesions <5 mm (microscopic) and IPMNs are generally macroscopically appreciated (>10 mm). We and others have suggested the term “incipient IPMN” for the large grey zone between larger PanINs and smaller IPMNs (i.e. <10 mm). The presence of a radiologically detectable lesion favours the diagnosis of IPMN.

**Molecular alterations in PanINs**

PanINs have been extensively studied at the molecular level. The “PanINgram” is a widely accepted progression model in which an accumulation of molecular alterations is seen with increasing grades of dysplasia. Although the genetic alterations in PanINs do not necessarily occur in a specific order, they eventually lead to uncontrolled cell growth, and the development of an invasive carcinoma. These molecular changes include DNA mutations, epigenetic modification of the DNA, and changes in RNA and protein expression.

Activating point mutations of the KRAS2 oncogene, usually of codons 12 or 13, play a central role in the development of PanINs. KRAS2 gene mutations have been detected in 36% of PanIN-1A lesions, 44% of PanIN-1B lesions, and 87% of PanIN-2/3 lesions in one study; the latter closely approximating the >90% prevalence of KRAS2 gene mutations observed in PDAC (Table 2). The RAS family of proto-oncogenes encode small GTP-binding proteins that mediate cell-cycle progression and cell proliferation, differentiation and survival through pathways, including the RAF-mitogen-activated protein kinase

**Figure 1** Progression of pancreatic intraepithelial neoplasia (PanIN) lesions. (a) Normal pancreatic duct lined by cuboidal epithelium without atypia. (b) PanIN-1A lesion featuring flat columnar, mucinous epithelium with basally-oriented nuclei with only minimal cytological atypia. (c) PanIN-2 lesion with papillary architecture, pseudostratified tall columnar epithelium and moderate architectural and cytological atypia. (d) PanIN-3 lesion with significant architectural and cytological atypia, i.e. note the cribriform areas and budding off of clusters of cells as well as the enlarged, hyperchromatic nuclei with loss of polarity. (e) Moderately differentiated invasive pancreatic ductal adenocarcinoma (note the abundant stroma). (f) Lobulocentric atrophy surrounding a pancreatic duct with low-grade PanIN.
Interestingly, shortened telomeres can also be seen in PanIN-3s and 1A/B, 55% of PanIN-2 and 71% of PanIN-3 lesions. Inactivation of the cell-cycle gene recognized and earliest of these alterations include inactivation been demonstrated in PanIN lesions. Briefly, the most widely
shortening, which has been observed in
90% of PanIN
30% of PanIN-
90% Methylated 95%
Variation greatly 50–75%
~ 50%

Table 2

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene/protein</th>
<th>Cellular function</th>
<th>PanIN-1A/B</th>
<th>PanIN-2</th>
<th>PanIN-3</th>
<th>IPMN</th>
<th>PDAC</th>
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<tr>
<td>Tumour suppressor genes</td>
<td>p16/CDKN2A</td>
<td>G1/S-transition</td>
<td>~ 30%</td>
<td>55%</td>
<td>71%</td>
<td>&gt; 50% Methylated</td>
<td>95%</td>
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<tr>
<td></td>
<td>TP53</td>
<td>Cell-cycle arrest</td>
<td>Observed</td>
<td>~ 30%</td>
<td>80%</td>
<td>Varied greatly</td>
<td>50–75%</td>
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<tr>
<td></td>
<td>SMAD4</td>
<td>TGF-β signalling</td>
<td></td>
<td></td>
<td></td>
<td>~ 50%</td>
<td>~ 50%</td>
</tr>
<tr>
<td></td>
<td>p21/CDKN1</td>
<td>G1/S-transition</td>
<td>16%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>LKB1/STK11</td>
<td>Cell polarity</td>
<td></td>
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<td>Oncogenes</td>
<td></td>
<td>MAPK/ERK signalling</td>
<td>36–44%</td>
<td>87%</td>
<td>25%</td>
<td>&gt; 90%</td>
<td>66%</td>
</tr>
<tr>
<td></td>
<td>KRAS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5%</td>
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<tr>
<td></td>
<td>GNAS</td>
<td>Signal transduction</td>
<td>G,α</td>
<td>31–81%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>PIK3CA</td>
<td>Cell proliferation/survival</td>
<td>7–10%</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>BRAF</td>
<td>MAPK/ERK signalling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genome maintenance genes</td>
<td></td>
<td>DNA damage repair</td>
<td>Observed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>BRCA2</td>
<td></td>
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</table>

PanIN, pancreatic intraepithelial neoplasia; IPMN, intraductal papillary mucinous neoplasia; PDAC, pancreatic ductal adenocarcinoma; TGF-β, transforming growth factor β; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; G, G protein α-subunit.

While some genes are somatically inactivated in PanINs, in other cases a germline mutation is coupled with a somatic mutation in the remaining allele. For example, in patients harbouring a germline BRCA2 gene mutation, LOH at the BRCA2 locus (13q12.3) has been demonstrated in PanIN-3 lesions. BRCA2 codes for a protein involved in repairing double strand DNA breaks, and germline mutations in BRCA2 are associated with an increased risk of breast, ovarian and pancreatic cancer.

The patterns of protein expression in PanINs have also been defined by immunolabeling. Typically, the apomucins MUC1, MUC4 and MUC5AC are expressed in PanINs and immunolabeling for specific mucins can help distinguish a large PanIN from a small intestinal-type IPMN (Table 3). Other proteins of interest overexpressed in PanIN lesions include mesothelin, a GPI-anchored protein, and prostate stem cell antigen (PSCA). Of interest, the aberrant overexpression of the pericentromeric satellite repeat HSATII in PanIN and PDAC has recently been reported, and it has been suggested that HSATII is a possible specific biomarker for the progression of dysplasia.

Intraductal papillary mucinous neoplasm

Clinical appearance and morphology of IPMNs

IPMNs are mucin-producing epithelial lesions in the ductal system of the pancreas that are, in contrast to PanINs, macroscopic lesions. IPMNs arise more often in the head than in the body or tail of the gland, and IPMNs are slightly more common in males (~60% of cases) (Table 1). The usual age at which patients with an IPMN are diagnosed is 60–70 years, and patients with noninvasive IPMNs tend to be 3–5 years younger than patients with an IPMN with an associated invasive carcinoma. Patients with an IPMN can present with nonspecific symptoms such as abdominal discomfort/pain, back pain,
nausea, vomiting, anorexia, weight loss and recurrent episodes of pancreatitis. In some instances patients report having had symptoms for over 10 years. IPMNs have been reported in individuals with a family history of pancreatic cancer and in patients with Peutz-Jeghers syndrome.19,20,39

By far the most important prognosticator in an IPMN is the presence or absence of an associated invasive carcinoma. The 5-year survival rate for patients with an IPMN with an invasive carcinoma is 34\(^\text{e}\)62% compared to 77\(^\text{e}\)100% for patients with an IPMN without an associated invasive carcinoma.40,42 The “Sendai criteria,” international consensus guidelines developed at a meeting in Sendai, Japan, provide consensus criteria on when to clinically monitor and when to resect IPMNs.43,44 Briefly, the criteria advocate surgical resection of all main duct IPMNs and the resection of branch-duct IPMNs that are symptomatic, \(>3\text{ cm}\), harbour a mural nodule, or associated with significant dilatation of the pancreatic duct. If cytology is positive for carcinoma, those lesions should also be resected.

Currently, some of the best noninvasive modalities to evaluate an IPMN include high-resolution computerized tomography (CT), magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP). These imaging techniques will generally reveal a pancreatic cyst with ectasia of the main duct, multiple cysts with dilatation of branch ducts, or a mixture of these. Endoscopic ultrasonography (EUS) is slightly more invasive than these other technologies, but provides greater resolution and can be used for sampling cyst fluid contents by fine needle aspiration (FNA). The finding of mucin oozing from a prominent ampulla of Vater (i.e. “fish mouth papilla”) on EUS is virtually diagnostic of an IPMN.

IPMNs can be divided in three types: main duct, branch duct, and combined- or mixed-type based on their macroscopic appearance.45 Main-duct IPMNs usually occur in the head of the gland with often the presence of copious thick mucin which gives rise to a (diffusely) dilated main pancreatic duct. Branch-duct IPMNs involve side branches of the main pancreatic duct, as the name suggests, and occur mainly in the head and uncinate process. Branch-duct IPMNs are often multicystic grape-like structures with thin cyst walls (Figure 2). Mixed-type IPMNs involve both the main and branch ducts. Both main and branch-duct IPMNs can be associated with atrophy of the adjacent pancreatic parenchyma.

IPMNs grossly contain either papillary projections (villous growth) or flat epithelium (ductectatic pattern) as opposed to the thin, translucent normal ductal epithelium. Differentiation between the types of IPMNs is clinically relevant as main duct and combined-type IPMNs tend to harbour higher grades of dysplasia and are more likely to be associated with infiltrating adenocarcinoma than are branch-duct IPMNs. Specifically, a recent analysis found infiltrating carcinoma associated with 48% of main-duct IPMNs, 42% of combined-type IPMNs and only 11% of branch-duct IPMNs.46 Pathologists should be aware that invasive carcinoma can arise focally within an IPMN, and therefore IPMNs need to be thoroughly sampled histologically.

### Apomucin expression patterns in pancreatic precursor lesions

<table>
<thead>
<tr>
<th></th>
<th>MUC1</th>
<th>MUC2</th>
<th>MUC4</th>
<th>MUC5AC</th>
<th>MUC6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal duct</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PanINs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PanIN-1A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PanIN-1B</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PanIN-2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PanIN-3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IPMNs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Weak</td>
</tr>
<tr>
<td>Pancreatobiliary</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Some</td>
</tr>
<tr>
<td>Gastric</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Oncocytic</td>
<td>+</td>
<td>Goblet cells only</td>
<td>-</td>
<td>Goblet cells only</td>
<td>++</td>
</tr>
<tr>
<td>ITPN</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Invasive</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*PanIN, pancreatic intraepithelial neoplasia; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; ITPN, intraductal tubulopapillary neoplasm.*

Table 3

Figure 2 Branch-duct IPMN located in the body of the pancreas where a single cyst is visible with flat lining. The main pancreatic duct (arrows) is not involved and is dilated at the level of the IPMN. Note that the cyst wall is relatively simple without mural nodules.
Several gross features can be suggestive of invasive adenocarcinoma in IPMNs. These include irregular heterogeneous thickening of cyst walls, fibrotic foci, and the presence of solid/gelatinous stromal nodules.

Invasive carcinoma arising in IPMNs have significantly better survival than primary PDAC not arising in association with an IPMN, but much of this difference is attributable to the lower stage at which IPMN-associated carcinomas are diagnosed. Colloid adenocarcinomas, almost always arise from intestinal-type IPMNs, have a significantly more favourable outcome than do tubular adenocarcinomas.

Since IPMNs can be multifocal, surgically resected patients should be followed after their surgery for metachronous disease.

Microscopically, IPMNs can be classified based on the degree of cyto-architectural atypia into low-, intermediate- and high-grade lesions. IPMNs can also be classified based on the direction of differentiation of the neoplastic epithelium into intestinal-, pancreatobiliary-, gastric-, and oncocytic-types, although this subtyping is less important clinically than the presence or absence of an associated invasive carcinoma (Figure 3). The recently recognized intraductal tubulopapillary neoplasm (ITPN) will be covered separately due to its “novelty.” Intestinal- and pancreatobiliary-type IPMNs are typically observed in main-duct IPMNs, while branch-duct IPMNs typically exhibit gastric-type epithelium. The intestinal-type IPMN consists of long papillae built up by columnar mucin-producing cells with cigar-shaped pseudostratified nuclei and basophilic cytoplasm. Intermediate- to high-grade dysplasia is usually seen in this type which closely resembles colonic villous adenomas. Pancreatobiliary-type IPMNs are characterized by more complex thin papillae with branching, built up by cuboidal cells containing less mucin and round hyperchromatic nuclei which thus explains its usual appearance as high-grade dysplasia. It is less common for gastric-type IPMNs to have papillary features as they instead are composed of single layers of flat cells (i.e. almost always a low-grade dysplasia) with basally-oriented nuclei. As the name suggests, gastric-type IPMNs resemble gastric-foveolar epithelium; the cytoplasm contents are abundant and pale coloured and occasional goblet cells may be appreciated. Oncocytic-type IPMNs, also known as intraductal oncocytic papillary neoplasms (IOPNs), are composed of cells with abundant eosinophilic cytoplasm. The architecture of IOPNs is complex, including arborizing papillae, solid nests, and cribiform growth pattern with intraepithelial lumina formation. The stratified oncocytic neoplastic cells contain abundant eosinophilic granular cytoplasm with large round (uniform) nuclei and typically feature high-grade dysplasia. As scattered goblet cells may also be observed, it might be hard to appreciate the intraductal nature of this lesion. Because regularly multiple histological types can be appreciated within an IPMN, the dominant component should be used to determine its subtype.

The different types of IPMNs have distinct patterns of apomucin expression that distinguishes them (Table 3). In short, the intestinal-type typically expresses MUC2 (and transcription factor CDX2), the pancreatobiliary-type expresses MUC1, the gastric-type only expresses MUC5AC, and lastly IOPNs express MUC1 and MUC6. In addition, the noninvasive pancreatobiliary-type also expresses MUC6 which is virtually absent in the intestinal- and gastric-type IPMNs. The latter suggests differentiation into a pyloropancreatic tumorigenic lineage rather than the intestinal MUC2/CDX2 pathway found in intestinal- and gastric-type IPMNs.

Figure 3: Histology of intraductal papillary mucinous neoplasm (IPMN) lesions. (a) Low-grade gastric-type IPMN. (b) Intestinal-type IPMN with high-grade dysplasia. (c) High-grade pancreatobiliary IPMN with cuboidal neoplastic epithelium forming complex papillae. (d) An IOPN composed of cells with abundant cytoplasm.
Molecular alterations in IPMNs

A growing body of evidence suggests that an accumulation of genetic alterations in specific genes drives the progression of IPMNs to invasive carcinoma. Recently, mutations in the oncogene GNAS were discovered in 66% of IPMNs.54 The major gene product of GNAS is the G protein-α-subunit (Gαs) which is a necessary signal transducer between hormonal (amongst other) receptors and adenylyl cyclase to produce cAMP production; activating mutations in GNAS are believed to cause continuous cAMP production, in turn leading to proliferation.53 Mutations in the GNAS gene appear to be specific for IPMNs as they are not present in non-IPMN-associated PDACs nor in MCNs and other cystic neoplasms of the pancreas.52,54 This finding is exciting because GNAS gene mutations can be detected in cyst fluid, suggesting that IPMNs could be definitively diagnosed by analyzing cyst fluid aspirates for GNAS gene mutations (see below). The most common mutations encountered in IPMNs are activating KRAS2 gene mutations (up to 80% of cases), with increasing prevalence from low- to higher-grade IPMNs (Table 2).52,55–64 Of note, KRAS2 gene mutations are not a feature of IOPNs.62 p53 overexpression, indicative of an underlying mutation in the TP53 gene has also been observed in high-grade IPMNs.59,60,62,65,66 A smaller percentage of IPMNs harbour LKB1 gene (also known as STK11) mutations. Loss of heterozygosity at the LKB1 locus (19p13.3) as well as loss of LKB1 expression has been reported in 25% of IPMNs.39,67 Lkb1 is a serine threonine kinase upstream of mTOR; germline mutation of LKB1 is associated with Peutz-Jeghers syndrome, and biallelic inactivation of LKB1 has been reported in IPMNs that arise in patients with Peutz-Jeghers syndrome. Up to 10% of IPMNs harbour mutations in the PIK3CA gene, and the protein product of PIK3CA, phosphatidylinositol 3-kinase, is also involved upstream of AKT-mTOR signalling.60,68 Recently, a BRAF mutation was reported in an IOPN.62

Loss of expression of p16 and SMAD4 has been reported in IPMNs, but SMAD4 inactivation is not as common in IPMNs as it is in high-grade PanNPs.66,69,70 Microsatellite analysis in IPMNs revealed allelic losses at chromosomes 9p in 62%, and 17p and 18q in both 38%.71 Since these regions harbour the genes p16, TP53 and SMAD4 this suggests that biallelic inactivation of these genes is targeted during IPMN progression. Microsatellite analyses and analyses of the patterns of GNAS and KRAS2 gene mutations have supported the histologic finding of multifocality of IPMNs.71

Aberrant methylation also occurs widely in IPMNs, more in invasive carcinomas associated with IPMNs than in noninvasive lesions.72–74 Specifically, methylation of p16, p73 and APC (respectively in >50, >50 and 10% of noninvasive IPMNs), methylation of mismatch repair genes MLH1 and MGMT (20% of noninvasive IPMNs) and methylation of CLDN5 (33% of low-, 42% of intermediate- and 62% of high-grade IPMNs) have been observed.72,73 Oligonucleotide microarray expression analyses have revealed significantly higher claudin-4, S100A, CXCR4 and mesothelin expression in IPMNs associated with an invasive carcinoma than in noninvasive IPMNs.75 Moreover, simultaneous overexpression of at least two of these proteins in one lesion was observed in 73% of invasive carcinomas arising from IPMNs.

Aberrant expression of microRNAs, a class of noncoding RNAs with a regulatory translational function of mRNA transcripts, has been reported in IPMNs. The microRNAs miR-21 and miR-155 have been shown to be expressed in IPMNs at levels >10-fold compared to those seen in normal ductal cells.76 It has been speculated that microRNAs might serve as a biomarker for early pancreatic neoplasia since aberrant expression of these microRNAs can also be detected in pancreatic juice (see below).

Shortened telomeres have also been reported in IPMNs.30,77 Specifically, 50% of low-grade IPMNs have shortened telomeres, with average length decreasing with IPMN progression. This finding suggests that telomere shortening is also an early event in the IPMN tumorigenesis. Alterations found in cyst fluid associated with IPMNs will be discussed at the end of this review.

ITPN: clinical appearance, morphology and molecular alterations

ITPNs are rare (~3% of pancreatic intraductal neoplasms) grossly visible intraductal lesions characterized by solid growth pattern, minimal mucin production, tubulopapillary architecture, and frequent necrosis. ITPNs are generally large (on average 6 cm) solid nodular masses (Table 1). Microscopically the neoplastic cells have a cribriform growth pattern with only occasionally papillae (Figure 4).78 ITPNs are usually not associated with cyst formation and they do not, by definition, produce abundant mucin. Most ITPNs have high-grade dysplasia and in less than half of the cases an associated invasive component is found.50,79 The rare incidence of the lesion leaves extensive (prognostic) data unavailable, but 5-year survival is >30%.

ITPNs typically express cytokeratin 7 and 19, underlining their ducital differentiation, and almost all express MUC1. Apomucin 6 is expressed in roughly 60% of cases but MUC2 and MUC5AC are not expressed, features that can help distinguish these lesion from IPMNs (Table 3). Mutations in the KRAS2 and BRAF genes have not been observed in ITPNs, and aberrant expression of β-catenin and loss of Smad4 and E-cadherin expression is rare (<10%). However, abnormal expression of p53 and p16 has been observed more frequently (20% and 54%, respectively).80

As stated before, ITPNs should be differentiated from IPMNs, especially the pancreatobiliary-type. Apart from the already

![Figure 4 Histologic feature of a high-grade ITPN with cribriform architecture and minimal mucin production.](image-url)
Mucinous cystic neoplasm

Clinical appearance and morphology of MCNs

MCN can also be a precursor to invasive pancreatic cancer. \(^46\) MCNs arise more often in the body or tail of the pancreas than in the head of the gland, and over 90% of the patients are females, usually between the ages of 40–50 years (Table 1). \(^81\) The median age of diagnosis of MCN with an associated invasive carcinoma is around 55 years. Patients with MCNs usually present with nonspecific symptoms such as abdominal discomfort and the sensation of a mass in the epigastric region. At the time of diagnosis, about one-third of MCNs have an associated invasive carcinoma, but this number may drop as more and more MCNs are being detected in asymptomatic patients imaged for another indication. Patients with a surgically resected noninvasive MCN are essentially cured, while the 5-year survival rate for patients with an MCN with an associated invasive carcinoma is 50–60%. \(^82,83\) In contrast to IPMNs, MCNs are almost always unifocal, and the risk of metachronous disease after surgery for an MCN is therefore minimal.

MCNs generally form well-circumscribed cystic masses with thick septa (visible on EUS) (Figure 5). Unilocular tumours have a smooth glistening internal surface whereas papillary projections may be present in higher-grade lesions. \(^84\) MCNs have not been studied as well as PanINs and IPMNs at the molecular level, mostly because of their lower incidence. The expression of MUC5AC in low-grade dysplastic MCNs was briefly mentioned before, but MUC2 can also be expressed (restricted to goblet cells within the epithelium) in noninvasive lesions, whereas MUC1 expression is associated with invasive MCNs (Table 3). \(^88\) Similar to PanINs and IPMNs, MUC6 expression can be observed in the early stage of noninvasive MCNs. \(^51,88,89\) An almost identical pattern of Claudin expression was found in MCNs and IPMNs, in which Claudin-2 expression decreases with increasing grade of dysplasia and Claudin-4 expression which increases with increasing grade of dysplasia. \(^90\) Other genes that have been reported to be overexpressed in MCNs include cathepsin E (CTSE), proto-oncogene MET (encoding hepatocyte growth factor binding receptor), proto-oncogene MYC, PSCA, and S100 calcium binding protein P (S100P) in the epithelium, and steroidogenic acute regulatory protein (STAR) and oestrogen receptor 1 (ESR1) in the ovarian-type stroma. \(^91\)

Genetic alterations that have been identified in MCNs include activating mutations in the KRAS2 gene, and inactivating SMAD4 and TP53 mutations in more advanced MCNs. \(^92,93\)
Pancreatic cyst fluid: implications for translational research

Distinguishing pancreatic cystic lesions

Cysts are common in the pancreas; they have been reported in >20% of autopsied patients and ~3% in patients undergoing abdominal imaging (CT or MRI). The increasing use and accuracy of abdominal imaging techniques will further increase the incidence of asymptomatic pancreatic cysts (pancreatic incidentalomas) in the near future.96 The fact that IPMNs and MCNs are recognized precursors to pancreatic cancer, in contrast to other pancreatic cystic lesions, underlines the need to accurately predict the nature of a cystic lesion so that it can be treated appropriately (safely monitored or surgically resected).

Pancreatic cyst fluid as a diagnostic modality

Cyst fluid obtained through FNA during EUS has been analyzed in several studies. CEA has been the most commonly used biomarker for differentiating between mucinous (i.e. IPMNs and MCNs) and nonmucinous cysts (i.e. cysts without malignant potential such as serous cystadenomas (SCAs)). Using 800 ng/ml as cut-off level in differentiating between mucinous and non-mucinous lesions, a meta-analysis of 12 studies calculated a sensitivity of 48% and specificity of 98%.97 In one of these studies, CEA was significantly better in differentiating mucinous from nonmucinous lesions than EUS when 192 ng/ml (sensitivity 73%, specificity 84%) was used as cut-off level.98 In a more recent and larger series from the same institution, an improved accuracy (sensitivity 81%, specificity 98%) was reported in differentiating mucinous from nonmucinous cysts at an optimal determined CEA cut-off level of 109.9 ng/ml. Though, the accuracy in the latter study did not differ significantly between a CEA cut-off level of 109.9 ng/ml versus the previously determined 192 ng/ml. In contrast with this finding is a more recent report by Haab et al. in which at this cut-off level (192 ng/ml) CEA alone had a sensitivity of 37% and specificity of 80% in differentiating between mucinous and nonmucinous cysts.100

The fact that CEA levels of >450 ng/ml were reported in lymphoepithelial cysts, which are benign lesions, reiterates the inability to distinguish definitively between malignant and benign cystic lesions using CEA.101,102 On the other end of the spectrum, low levels (<5 ng/ml) of CEA strongly support a diagnosis of SCA or pseudocyst as opposed to mucinous neoplasms (IPMNs and MCNs).103

CEA expression in combination with 13 other proteins was found to distinguish IPMNs from SCAs with 92% diagnostic accuracy which suggests that CEA in combination with other factors might be a more sensitive and specific diagnostic modality. Several studies have examined the combination of molecular and CEA analysis in distinguishing mucinous from nonmucinous cysts and found improved diagnostic sensitivity compared to molecular analysis by itself. Other tumour markers besides CEA, such as CA15.3, CA19-9, CA72-4 and CA125 have been evaluated but were found to be of lesser diagnostic value than CEA.104-107

Expression of apomucins may also be of use. MUC5AC expression was used to discriminate mucinous cysts from non-mucinous cysts (78% sensitivity, 80% specificity) with increasing sensitivity and specificity (87% and 86%, respectively) when CA19-9 was added.100 In high-grade IPMNs, in a different study, MUC2 and MUC4 levels were found...
significantly elevated and serum MUC5AC was significantly elevated compared to low-grade lesions. The accuracy of MUC1 to differentiate high-grade/invasive IPMNs from low-/intermediate-grade IPMNs was 81% in pancreatic juice. These results show that the aberrantly expressed apomucins in precursor lesions are reflected in cyst fluid.

Another group of biomarkers in cyst fluid that warrant further elucidation are aberrantly expressed microRNAs. One small study found upregulated expression of miR-155 in 60% of IPMN-associated cyst fluid specimens compared to none in control cases. A recent study by Ryu et al. found that this microRNA transcript showed a trend towards differential expression in mucinous and nonmucinous cyst fluids. On the other hand, the authors found significantly higher expression of microRNAs miR-17-3p, miR-221, and miR-21 in the cyst fluid of mucinous precursors versus nonmucinous precursors. Moreover, the authors determined the diagnostic performance of the latter microRNA (miR-21) for mucinous underlying cysts and found a sensitivity of 80% with 76% specificity and a sensitivity of 96% with specificity of 50%. These findings affirm microRNAs to be helpful in differentiating between mucinous and nonmucinous cysts based on cyst fluid analysis.

Amylase is a biomarker of limited value in cyst fluid analysis, except that elevated amylase levels (>5-fold serum level) suggest either a pseudocyst or a connection between the cyst and the ductal system, thereby excluding MCNs and serous cystic neoplasms.

Cyst fluids have also been analyzed for KRAS2 gene mutations, and the results look promising. Khalid et al. analyzed cyst fluid for DNA quality/amount, for somatic gene mutations with microsatellite LOH was highly predictive for cysts with a high-grade/infiltrating IPMN (91% sensitivity, 93% specificity). In a larger follow-up study, this same group confirmed these findings and found that KRAS2 gene mutations were predictive for mucinous cysts (versus nonmucinous cysts). Moreover, elevated amounts of DNA in cyst fluid as well as the combination of KRAS2 gene mutations with microsatellite LOH were predictive for a high-grade and/or invasive cystic lesion. In a different study, LOH of genomic loci (amongst which TP53 and p16 coding) and KRAS2 gene mutations were together seen in two of four high-grade/invasive and one of 12 low-/intermediate-grade IPMNs.

Of interest, it has recently been shown that mutant Kras protein can be detected using proteomic techniques. Wang et al. successfully used the technique of “selected reaction monitoring” by mass spectrometry to detect and quantify Ras mutations at the protein level in cyst fluid of three IPMNs. DNA sequencing of the IPMN cyst fluid in this study confirmed mutant KRA2S2 genes, thereby stressing the potential of protein based technologies to detect DNA mutations.

The recent discovery by Wu et al. that GNAS gene mutations are specific for IPMNs, and that these mutations can be detected in cyst fluid, offers exciting promise for the development of a new marker. The authors of this study found that 96% of IPMNs harboured a KRA2S2 and/or a GNAS gene mutation. By contrast, none of the cyst fluids from 21 MCNs, 42 SCAs contained GNAS mutations, thus making GNAS mutations highly specific for IPMNs. Importantly, the GNAS mutations in the cyst fluids were identical to those identified in matched neoplastic epithelium from the same case, suggesting that the mutational profile of cyst fluids accurately reflects the lesional tissues. In addition, in IPMN-associated invasive PDACs identical GNAS mutations were found in the invasive cancers and the matched associated IPMN. These findings underline the usability and representation of cyst fluid in identifying potentially underlying disease.

In summary, the combined panel of mutations in KRA2S2 and GNAS appears to be very specific for IPMNs (with a 96% sensitivity and 100% specificity in distinguishing IPMNs from SCA), and the genetic analysis of cyst fluid accurately reflects the epithelium of the cyst wall. As mutations have only been observed in a single codon per gene involved, codon 12 of KRA2S2 and codon 201 of GNAS, these genes can be assessed with as little as 0.25 ml of aspirated cyst fluid using the ligation assay described by Wu et al.

Conclusions

As advanced invasive PDAC is almost a universally lethal cancer, the only hope for cure is to detect disease at an early stage. The identification and characterization of precursors to pancreatic cancer are crucial. In this review we discussed several precursor lesions, PanIN, IPMN, ITPN and MCN and their molecular characteristics; lesions about which knowledge is rapidly expanding. Translation of this knowledge into reliable early detection and treatment strategies remains a major clinical challenge. Research developments on the use and feasibility of cyst fluid analyses look promising and provide molecular targets to guide clinicians on the underlying precursor lesion and stage of disease. As the diagnostic criteria for pancreatic precursor lesions are still challenging, it is recommended to analyze and treat patients in a high-volume centre with an experienced multidisciplinary team.

REFERENCES


Research directions

- Further characterization of pancreatic precursor lesions will enable the identification of at-risk populations for pancreatic cancer and possibly reveal useful biomarkers.
- PanINs are microscopic lesions and therefore research into surrogate markers for their presence is needed.
- Molecular alterations in MCNs and IPMNs are increasingly being defined with the hope that molecular changes may also help classify these.
- The analysis of cyst fluid is promising and research into using cyst fluid to classify precursor lesions in the pancreas is urgently needed.
- In pancreatic cystic lesions GNAS mutations are IPMN-specific and combining GNAS and KRAS2 mutation analysis of cyst fluid distinguishes IPMNs from SCAs with 96% sensitivity and 100% specificity.

Practice points

- The identification of precursor lesions at an early, precursor, stage is crucial to outcome.
- The accumulation of genetic alterations drives the progression of precursor lesions into higher grades of dysplasia, and eventually to invasive carcinoma.
- Characteristic expression of apomucin proteins in precursor lesions can be used to differentiate between lesion types.
- The macroscopic nature of IPMNs, MCNs, IOPNs and ITPNs makes them detectable by imaging, thus enabling clinical follow-up of patients.
- Asymptomatic pancreatic cysts will increasingly be diagnosed due to the increasing use and accuracy of abdominal imaging techniques.