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INVITED REVIEW

Precursor lesions in pancreatic cancer: morphological and molecular pathology

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Summary

Pancreatic cancer has a dismal prognosis and is the fourth most common cause of cancer related death in Western societies. In large part this is due to its typically late presentation, usually as locally advanced or metastatic disease. Identification of the non-invasive precursor lesions to pancreatic cancer raises the possibility of surgical treatment or chemoprevention at an early stage in the evolution of this disease, when more amenable to therapeutic interventions. Precursor lesions to pancreatic ductal adenocarcinoma, in particular pancreatic intraepithelial neoplasia (PanIN), have been recognised under a variety of synonyms for over 50 years. Over the past decade our understanding of the morphology, biological significance and molecular aberrations of these lesions has grown rapidly and there is now a widely accepted progression model integrating the accumulated morphological and molecular observations. Further progress is likely to be accelerated by improved mouse models of pancreatic cancer and by insight into the cancer genome gained by the International Cancer Genome Consortium (ICGC), in which an Australian consortium is leading the pancreatic cancer initiative. This review also outlines the morphological and molecular features of the other two precursors of pancreatic ductal adenocarcinoma, i.e., intraductal papillary mucinous neoplasms and mucinous cystic neoplasms.

Key words: Cancer, pancreas, pathology, precursor.

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INTRODUCTION

Pancreatic carcinoma is the fourth most common cause of cancer related death in Western societies with an estimated 42 470 new cases and 35 240 deaths in the USA in 2009.[1] In Australia, deaths caused by pancreatic cancer closely parallel its incidence; in 2005 there were 2181 new cases and almost as many deaths, 2026.[2] Carcinoma of the exocrine pancreas is typically rapidly lethal with a median survival of less than 6 months and a 5 year survival of approximately 5%.[1,3] This dismal prognosis is, in large part, due to its characteristically late clinical presentation, usually as locally advanced or metastatic disease, since most patients initially have relatively mild and non-specific symptoms.[4,5] Moreover, pancreatic cancer usually has an abundant stromal reaction and there is emerging evidence that the stellate cells responsible for formation of the pancreatic stroma may facilitate disease progression, including early dissemination of disease (recently reviewed by Vonlaufen *et al.*[6]). Surgical resection is the only effective treatment with a 5 year survival of 15–25% following complete resection. However, only the 10–20% of patients without clinical or imaging evidence of advanced disease are operative candidates.[7–9]

One of the keys to improving survival rates in this disease lies in the detection of tumours, or more importantly, their non-invasive precursor lesions, at an earlier stage when more amenable to therapeutic interventions. Hence, there has been considerable interest in the morphology and molecular pathology of pancreatic tumour precursor lesions and advances in our understanding of the natural history of pancreatic carcinoma. Ductal adenocarcinoma, including its variants, accounts for approximately 85% of pancreatic cancer and this review will focus on its precursors which include microscopic lesions, i.e., pancreatic intraepithelial neoplasia (PanIN), and macroscopic mass forming (cystic) lesions, intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN).

PANCREATIC INTRAEPITHELIAL NEOPLASIA (PANIN)

General features and terminology

Microscopic lesions associated with pancreatic cancer have been recognised in the smaller calibre pancreatic ducts (less than 5 mm diameter) for well over 50 years under a wide variety of names, including flat hyperplasia, ductal papillary hyperplasia, ductal papillary hyperplasia with atypia, mucinous cell hyperplasia, mucinous hypertrophy, goblet cell metaplasia, atypical papillary hyperplasia, severe ductal dysplasia and carcinoma *in situ*, amongst others.[10–15] The current classification of these lesions, based on the terminology 'pancreatic intraepithelial neoplasia' (PanIN) proposed by Klimstra and Longnecker in 1994, was adopted by a National Cancer Institute sponsored Pancreas Cancer Think Tank in 1999.[16,17] The PanIN terminology was chosen by the participants at that meeting to emphasise that the spectrum of these lesions reflects

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a tumour progression model with increasing neoplastic potential, in a similar manner to the well established progression models for other organs such as cervical, prostate and breast carcinoma.[16] In those organs, standardisation of terminology has facilitated studies that have led to an increased understanding of disease progression and underpinned early clinical interventions.

Microscopic features and grading

There are three grades of PanIN representing a proposed progression from PanIN-1 with minimal nuclear atypia through to PanIN-3 representing severe dysplasia/carcinoma *in situ.* PanIN-1 is further divided into two groups PanIN-1A and PanIN1-B.[16] PanIN-1 are defined as flat epithelial lesions composed of tall columnar cells with regular, basally located nuclei and abundant supranuclear mucin (Fig. 1B). Nucleoli are inconspicuous and mitotic figures are not seen. PanIN-1B has similar cytological features but displays a papillary or micropapillary rather than flat architecture (Fig. 1C).[16,18,19] PanIN-2 lesions are characterised by a papillary, micropapillary, or occasionally flat architecture, with mild to moderate nuclear atypia. There may be some loss of polarity and pseudostratification with nuclear crowding, enlargement and hyperchromasia (Fig. 1D). Mitoses are rarely seen and, when present, are basal and not atypical. Cribriform structures and necrosis are not a feature. PanIN-3 lesions have a papillary, micropapillary, or occasionally flat architecture. Cribriform structures and luminal necrosis are often seen, as is 'budding off' of small clusters of epithelial cells into the gland lumen. Cytologically, there is enlargement and hyperchromasia of nuclei with loss of polarity and increased nuclear:cytoplasmic ratios (Fig. 1E). Nucleoli may be prominent. Mitoses, including atypical forms, are sometimes present and may be luminal rather than basally located. By definition, PanIN-3 is an *in situ* malignancy with an intact basement membrane.[16,18,19] To promote the PanIN classification system and aid the standardisation of diagnostic criteria internationally, the NCI sponsored expert panel advocating its use has established a useful Web based teaching resource illustrating examples of each grade (http://pathology.jhu.edu/pancreas_panin).[16] In addition, the morphological spectrum of PanIN lesions has recently been expanded with the addition of intestinal, foamy gland and oncocytic variants of PanIN; however, all these variants appear to be rare and are unlikely to be encountered by most pathologists.[20,21]

Evidence for role as cancer precursors

There are four main lines of evidence for regarding these lesions as neoplastic and for incorporating them into a progression model. Firstly, autopsy studies of patients with

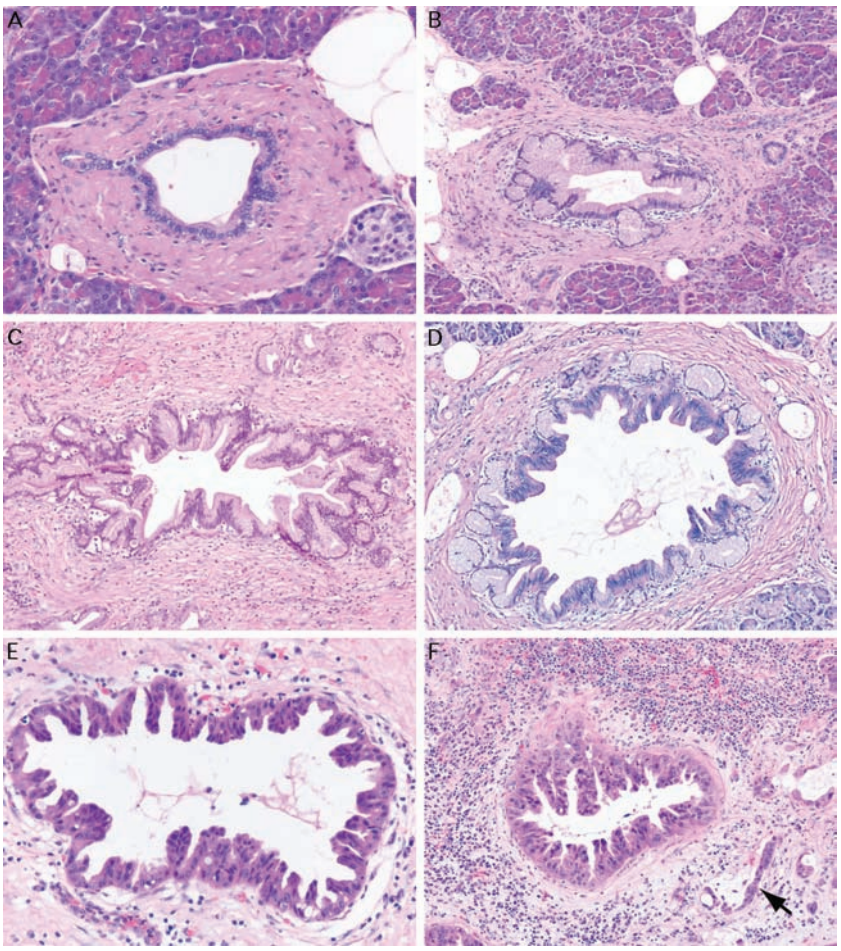

Fig. 1 Examples of different grades of pancreatic intraepithelial neoplasia (PanIN). (A) Normal interlobular duct lined by regular cuboidal epithelium. (B) PanIN-1A lined by tall columnar mucinous epithelium with minimal nuclear atypia. (C) PanIN-1B with short papillary infoldings but minimal nuclear atypia. (D) PanIN-2 with a similar papillary architecture to PanIN-1B but moderate nuclear atypia. (E) PanIN-3 with severe nuclear atypia equivalent to carcinoma *in situ*. (F) Intraductal spread of carcinoma ('cancerisation of the ducts'); note proximity to invasive ductal adenocarcinoma (black arrow). (H&E stain.)

pancreatic ductal adenocarcinoma have identified the frequent occurrence of hyperplastic ductal lesions (now designated PanIN) in the non-involved pancreatic tissue; an early study found ductal hyperplasia in 41% of the cases with pancreatic cancer versus 9% of the control pancreases without malignancy.[15] A later study in 1976 by Cubilla and Fitzgerald of 227 pancreatic resection and autopsy specimens versus 100 age and sex matched controls, reported ductal papillary hyperplasia (PanIN-1B and 2) in 37% of the pancreatic cancer cases and only 12% of the control pancreases. Moreover, marked atypical hyperplasia and carcinoma *in situ* (PanIN-3) were found respectively in 20% and 12% of pancreases with carcinoma but in none of the control cases.[13] More recent studies have confirmed their observation and failed to detect PanIN-3 in pancreases not harbouring an invasive carcinoma.[22,23] Additionally, other investigators have noted that like pancreatic cancer, the prevalence of duct lesions increases with age and that they are more common in the head than tail of the pancreas.[24] It has been also recognised that PanIN lesions occur in patients with familial pancreatic cancer and hereditary pancreatitis.[25,26] However, it should be noted that PanIN, particularly when of lower grade or in older patients, may be a coincidental finding in the pancreas adjacent to uncommon, non-ductal pancreatic tumours, including acinar cell carcinoma, mucinous cystic neoplasm, pancreatic endocrine tumours, serous cystadenomas and solid pseudopapillary neoplasms.[27] Hence, PanIN should not be used histologically to assist in the diagnosis and classification of pancreatic tumours.

Secondly, evidence for the temporal progression of these lesions is provided by animal models. Syrian golden hamsters treated with *N*-nitroso-bis-(2-oxopropyl)amine (BOP) show a progression from mildly hyperplastic lesions at 8 weeks, to papillary hyperplasia and carcinoma *in situ* at 12 weeks and invasive ductal adenocarcinoma at 24 weeks post-injection.[28] Genetically engineered mouse models for pancreatic cancer have also been developed (these are discussed in more detail below) which demonstrate a variety of non-invasive precursor lesions morphologically analogous to human PanIN. Indeed, a recent NCI sponsored consensus meeting has established a uniform nomenclature for the pathology of genetically engineered mouse models of pancreatic exocrine neoplasia (mouse pancreatic intra-epithelial neoplasia or mPanIN).[29]

Furthermore, in humans there are case reports of patients whose Whipple's resection specimens showed chronic pancreatitis and 'atypical papillary hyperplasia' or 'atypical ductal hyperplasia and carcinoma *in situ*' (PanIN-3), presenting 17 months to 9 years after the resection with pancreatic ductal adenocarcinoma.[10,11] Another patient, who had a pancreatic adenocarcinoma completely resected but in whom there was a focus of PanIN-3 noted at the pancreatic neck margin, presented 9 years later with ductal adenocarcinoma in the remaining pancreas.[10] These reports also suggest that the progression of high grade PanIN to invasive carcinoma may take many years. Similarly, in a recent study of the genomes of seven pancreatic cancer metastases, a quantitative analysis of clonal relationships indicated at least a decade between the initiating mutation and the development of the non-metastatic founder cell.[30]

Finally, molecular pathology studies (discussed in more detail below) have shown that the precursor lesions (PanIN) harbour some, but not all, of the molecular aberrations found in pancreatic ductal carcinoma. Moreover, the prevalence of these genetic alterations increases with the grade of the PanIN.[31,32]

Clinical implications

The practical implications of each grade of PanIN are the subject of ongoing study to clarify issues such as how often or rapidly progression to invasive carcinoma occurs. The case reports discussed above indicate that PanIN-3 is a risk factor for the development of invasive carcinoma and that it is highly desirable that pancreatic resection margins are clear of PanIN-3 as well as invasive carcinoma. In contrast, the high frequency of occurrence of PanIN-1 in non-neoplastic pancreases strongly suggests that the risk of progression to invasive carcinoma is very low for PanIN-1 alone. In line with this, the current College of American Pathologists (CAP) Protocol for reporting carcinoma of the exocrine pancreas recommends that the highest grade of PanIN present in a specimen is recorded in diagnostic pathology reports, and in particular the presence of PanIN-3 at the resection margin.[33] PanIN-2 represents a more problematic lesion, complicated by the relatively poor interobserver variation in its recognition. In the 2001 paper proposing the current standardised definitions for pancreatic ductal precursor lesions, the agreement between pathologists on the expert panel in the diagnosis of PanIN-2 was poor (kappa value = 0.14).[16] However, approximately half of the ductal lesions selected for evaluation by the panel in that study were specifically chosen because they represented difficult 'borderline' lesions for which a certain amount of interobserver variation may be expected.[16] A follow-up study published in 2005 found better agreement on grading of precursor lesions with 75.9% overall agreement on the second slide review (kappa = 0.523, moderate agreement).[34] A recent review has suggested that PanIN-2 lesions in particular should be targeted for further investigation.[35]

Differential diagnosis

Mimickers of PanIN lesions exist and may also complicate histological assessment and present problems in day-to-day reporting. So-called 'cancerisation of the ducts' occurs when adenocarcinoma invades into adjacent ducts and spreads along them mimicking PanIN-3 (Fig. 1F).[16,36,37] Clues to the recognition of this phenomenon include proximity of the involved duct to an invasive carcinoma, which is often more apparent in deeper sections, and a relatively abrupt transition between the malignant epithelium and normal duct epithelium. In contrast, PanIN-3 is commonly surrounded by zones of lesser grade dysplasia which more gradually merge with the adjacent morphologically normal ductal epithelium.[18,20] Reactive epithelial changes may also mimic PanINs but usually lack the architectural features and often have significant inflammatory infiltrates. Nuclei in reactive cells may be enlarged with distinct nucleoli but tend to have smooth contours and less variability. Intraductal papillary mucinous neoplasms have similar appearing epithelium to PanIN lesions and may cause diagnostic difficulties when they involve the smaller branch ducts (this will be discussed in the section on IPMNs below).

Molecular aberrations in PanIN

The molecular analysis of evolving pancreatic ductal adenocarcinoma has provided a compendium of genetic lesions, often implicating known cancer-related genes and classical cancer signalling cascades. Many of these molecular events have been correlated with defined histopathological stages of the putative progression model for pancreatic ductal adenocarcinoma (PDAC) (Fig. 2), with molecular aberrations occurring

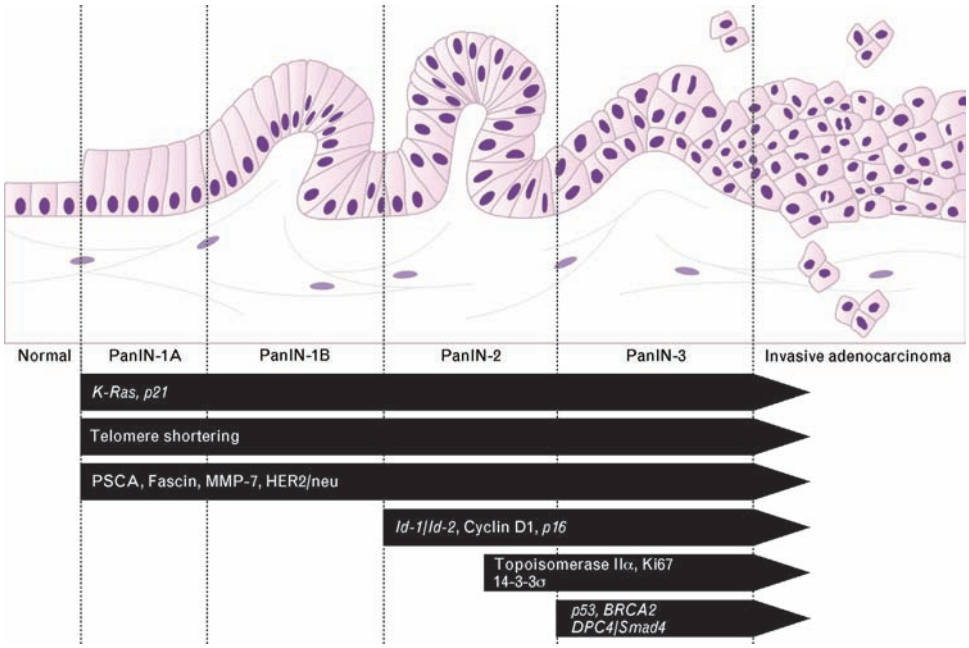

Fig. 2 Schematic representation of the molecular aberrations in PanIN. Progression of normal ductal epithelium through PanIN to invasive ductal adenocarcinoma from left to right. Arrows denote the temporal occurrence of genetic aberrations in PanIN. Aberrations observed from early PanIN-1A lesions include activating *K-Ras* mutations, overexpression of $p21^{WAF1/CIP1}$, *HER-2/neu*, PSCA, Fascin and MMP-7 as well as telomere shortening; followed by overexpression of *Id-1/Id-2* and cyclin D1, loss of $p16^{INK4A}$ expression (PanIN-2); with overexpression of TopoIIα, 14-3-3σ and Ki-67 along with *p53* protein accumulation or mutation, loss of expression of *DPC4/Smad4* and inactivation of *BRCA2* occurring late in the progression of PanIN (PanIN-3). See text for more detail.

throughout pancreatic carcinogenesis as a consequence of multiple changes occurring at the genomic, transcriptomic, epigenetic and proteomic level. Specifically, alterations in cancer-related genes, which can be classified into either oncogenes (tumour promoting) or tumour suppressor genes, result in changes at the genomic level that have the potential to drive carcinogenesis by providing cells with specific advantages allowing them to survive and proliferate in their microenvironment. Genes important in the development of pancreatic cancer will be described in the order generally observed in the progression of PanINs through to PDAC.

Genetic aberrations that occur very early in the development of PanIN include those of *K-Ras, $p21^{WAF1/CIP1}$, HER-2/neu*, PSCA, Fascin, MMP-7, HOXB2 as well as telomere shortening (PanIN-1A/B); followed by *Id-1/Id-2, $p16^{INK4A}$*, cyclin D1 (PanIN-2); with *Tp53, DPC4/Smad4, BRCA2* occurring late in the progression of PanIN (PanIN-3) along with TopoIIα, 14-3-3σ and Ki-67. Each will be discussed in more detail below.

One of the most important oncogenes in pancreatic carcinogenesis is *K-Ras*. *K-Ras* is a member of the RAS family of GTP-binding proteins that mediates cell cycle progression, cellular proliferation, differentiation and survival.[38,39] Activating *K-Ras* point mutations at codon 12 (substitution of glycine with aspartate, valine or arginine) have been detected in approximately 30% of early PanIN-1A lesions, over 40% of PanIN1-B lesions, rising to over 90% in advanced PanIN-2 to PanIN-3 lesions, and invasive pancreatic ductal adenocarcinoma.[17,40–43] Activated *K-Ras* engages multiple effector pathways, such as the *Raf*-mitogen-activated kinase (MAPK), phosphoinositide 3-kinase (PI3K) and *Ral*-GDS pathways. These downstream targets may provide effective points of therapeutic intervention given the difficulties encountered with inhibition of *K-Ras*.[44]

Deregulation of cell-cycle machinery, such as overexpression of $p21^{WAF1/CIP1}$, is another early event in the development of PanIN (16% in PanIN-1A) (Fig. 3A), with an increase in frequency concomitant with an increase in PanIN progression. Over 80% of PanIN-3 lesions are reported to overexpress $p21^{WAF1/CIP1}$.[32] Overexpression of $p21^{WAF1/CIP1}$ has been show to occur independently of *p53, DPC4/SMAD4* and cyclin D1 status, which may be the consequence of activated *K-ras* mutations.[32]

Aberrations in the *HER2/neu* gene have also been reported in an immunohistochemical study,[45] which demonstrated that HER2/neu protein overexpression was as high as 82% in PanIN-1A lesions, increasing to over 95% in PanIN-3. More recently, Moriya *et al.*[46] confirmed these data by demonstrating an increase in HER2/neu protein overexpression from PanIN-1 (50%) through to 80% of PanIN-3 lesions. However, care should be taken in interpreting these data as the frequency of *HER2/neu* gene amplification using fluorescent *in situ* hybridisation (FISH) has revealed discordance between *HER2/neu* gene amplification and protein expression in PDAC.[47] Saxby *et al.* demonstrated that ~3% of invasive PDAC exhibited *HER2/neu* gene amplification, in comparison to 17% of PDAC overerexpressing HER2/neu protein. This is likely to be a manifestation of polysomy of chromosome 17 in the tumour cells, as 30% of samples in this study demonstrated a raised gene copy number with normal HER2:Chr17 ratio in this study.[47]

Prostate stem cell antigen (PSCA) is reportedly expressed in 30% of PanIN-1 lesions, increasing to 40–60% of PanIN-2 and PanIN-3 lesions, respectively, while not expressed in normal pancreatic ducts.[48] This was observed using immunohistochemical assessment of tissue microarrays following transcript profiling. Further, the actin-binding skeletal protein fascin was reported to be present in early PanIN-1A lesions (25%), increasing in frequency in PanIN-2 (57%) and PanIN-3 (57%) lesions. Matrix metalloproteinase-7 (MMP-7) is a member of the MMP family of zinc-dependent extracellular proteases and is an important regulator of cell surface proteolysis.[49] MMP-7 is not expressed in normal pancreatic tissue, while expression of MMP-7 has been shown in the earliest

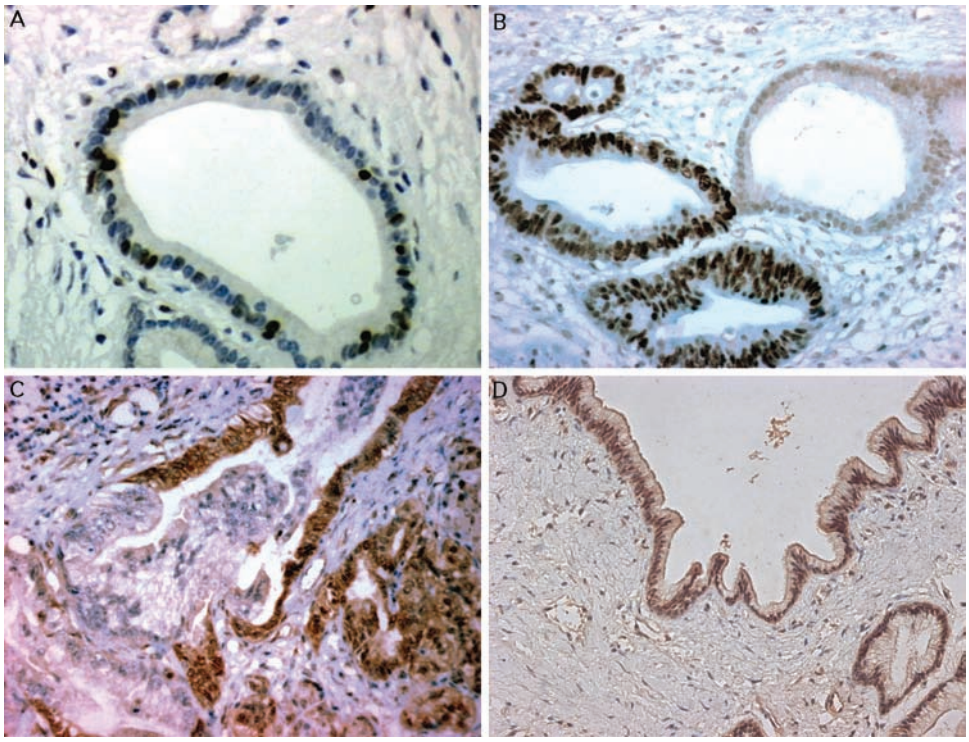

Fig. 3 Immunohistochemical demonstration of molecular aberrations in PanIN. (A) p21 expression in PanIN-1A. (B) p53 immunostaining in PanIN-3 (left) with contrasting lack of staining PanIN-1A (right). (C) Smad4 expression in PanIN-2 (upper right) with loss of staining in adjacent PanIN-3 (lower left). (D) Ectopic HOXB2 expression in PanIN-1B.

PanIN-1 lesions (73%) with a constant frequency observed through to PanIN-3 lesions, suggesting that in pancreatic carcinogenesis, MMP-7 has a function that precedes the onset of malignant invasion.[50]

In a recent study, nuclear expression of the Homeobox transcription factor, HOXB2, was identified in 48 of 128 PDAC (38%). In cases when HOXB2 expression was present within the tumour, >80% of the nuclei stained positively. HOXB2 expression was detected in the histologically normal pancreatic ducts of two of 26 (8%) patients, in one of 24 (4%) PanIN-1A lesions, three of 20 (15%) PanIN-1B (Fig. 3D), three of 10 (30%) PanIN-2, and one of four (25%) PanIN-3 lesions, suggesting that HOXB2 expression occurs in a spectrum of PanIN and may play a role in the evolution of PanIN.[51]

Telomeric repeat sequences (TTAGGG), present at the end of linear chromosomes to prevent fusion, have been reported to become aberrantly shorter during development of early PanIN-1A (90%) lesions[52] through to over 95% of all PanIN. Shortening of the telomeres has been suggested to lead to increased fusion events between chromosomes, and thus chromosomal instability, to further promote oncogenesis and neoplastic progression to pancreatic ductal adenocarcinoma.[53]

$p16^{INK4A}$, two 9p21 locus/INK4A/ARF tumour suppressors

INK4A plays a central role as a pancreatic ductal adenocarcinoma tumour suppressor.[42,54,55] Loss of *INK4A* function, brought about by mutation, deletion or promoter hypermethylation, occurs in 80–95% of sporadic ductal adenocarcinomas, and *INK4A* loss is generally seen in moderately advanced lesions (PanIN-2) that show features of dysplasia.[42,56] *INK4A* germline mutations are associated with the familial atypical mole-malignant melanoma (FAMMM) syndrome, which is characterised by a high incidence of melanoma and a 13-fold increased risk of pancreatic cancer.[57] The *9p21* locus encodes two overlapping tumour suppressors *INK4A* and *ARF*, and their respective protein products $p16^{INK4A}$ and $p19^{ARF}$, and as a result of homozygous deletion of *9p21* in approximately 40% of tumours, many pancreatic cancers sustain loss of *INK4A* and *ARF* tumour suppression pathways.[58]

Overexpression of cyclin D1 occurs as an intermediate event in PanIN progression, notably observed at low frequency from PanIN-2 (19–27%), increasing to 40–57% of PanIN-3 lesions reflecting an intermediate step in the carcinogenesis process.[48,59] Id proteins antagonise basic helix-loop-helix proteins, inhibit differentiation and enhance cellular proliferation. When compared with early PanIN-1A/B lesions, Id-1 and Id-2 are overexpressed in later stage PanIN 2/3 lesions as well as invasive PDAC.[60]

Tp53, p53 tumour suppressor

p53 is located on chromosome 17p and is mutated in approximately 75% of pancreatic ductal carcinomas.[61,62] The *p53* gene encodes for a tumour suppressor protein that functions as a short lived transcription factor, crucial in cell cycle regulation and apoptosis, and is activated and stabilised in response to a wide variety of genotoxic cellular stresses.[62,63] Consistent with a role in constraining malignant progression, *p53* mutation appears in the later stage PanIN-3 that have acquired significant features of dysplasia (Fig. 3B). Therefore, loss of *p53* function could serve to enable the growth and survival of cells harbouring procarcinogenic chromosomal aberrations.[44,48,64]

DPC4/Smad4, SMAD4/DPC4 tumour suppressor and TGF-β

Loss of the transcriptional regulator *SMAD4* (or *DPC4*) is another frequent event associated with the progression of pancreatic ductal adenocarcinoma. The *SMAD4* gene is a

tumour suppressor gene mapped to chromosome *18q21*, which serves as a central component in the TGF-β signalling cascade and is targeted for deletion or intragenic point mutations in approximately 50–55% of adenocarcinoma cases.[44,65] *SMAD4* has been designated a progression allele due to being intact in PanIN-1 and PanIN-2 lesions, with loss observed in later stage PanIN-3 lesions (Fig. 3C).[66] Further, tumours with an intact *SMAD4* may have a higher propensity for showing poorly differentiated features at a histopathological level.[48,67–69] The mechanism by which *SMAD4* loss contributes to tumorigenesis is likely to involve its central role in TGF-β-mediated growth inhibition.

The biological role of the TGF-β pathway in human malignancy is complex as it exerts both growth inhibitory and growth promoting effects depending on the cell type and context.[70] The importance of TGF-β signalling in pancreatic cancer is highlighted by the fact that 90% of pancreatic tumours show loss of heterozygosity (LOH) at the *SMAD4* locus with 50% of ductal adenocarcinomas having either homozygous deletion or mutational inactivation of the second allele, which may have a primary role in modulating the interaction of the tumour with the microenvironment rather than growth control of the tumour cells themselves.[44] TGF-β family ligands are overexpressed in ductal adenocarcinoma and may also help to promote the characteristic desmoplastic response.[71]

BRCA2

Inherited *BRCA2* mutations are usually associated with familial breast and ovarian cancers but they also carry a significant risk for the development of pancreatic cancer, and have been reported in up to 17% of pancreatic cancers occurring in a familial setting harbouring mutations in this gene.[72] The BRCA2 protein may function as a genomic maintenance gene by preventing DNA strand breaks that occur during normal cell cycle division. Loss of the wild-type allele occurs as a late event in those inheriting germline heterozygous mutations of *BRCA2* and appears to be restricted to PanIN-3 lesions and ductal adenocarcinoma, confirming that biallelic inactivation of *BRCA2* is a late event in pancreatic carcinogenesis.[73]

Topoisomerase IIα (Topo IIα) and Ki-67 are proliferation antigens used as markers of unrestrained cellular proliferation, an intrinsic feature of neoplasia. Both Topo IIα and Ki-67 have been demonstrated to express concordantly in 7% of PanIN-2 lesions and 71% of PanIN-3 lesions, with no significant expression of these proliferation antigens in early PanIN-1A or -1B lesions.[48] Further, Klein *et al.*[74] also demonstrated an increasing Ki-67 labelling with increasing grades of dysplasia in PanINs.

The *p53*-regulated gene *14-3-3σ* belongs to the 14-3-3 family of proteins that play a role in a number of cellular process such as cell signalling, cytoskeletal reorganisation, cell cycle regulation and apoptosis.[61,75] Overexpression of *14-3-3σ* has been demonstrated in ~21% of PanIN-2 lesions, increasing to 85% in PanIN-3 lesions,[48] perhaps occurring as an inverse correlation to the abrogation of *p53*.

INTRADUCTAL PAPILLARY MUCINOUS NEOPLASMS (IPMN)

General features and definition

Intraductal papillary mucinous neoplasms have been increasingly recognised over the past 3 decades since Ohhashi and colleagues highlighted the key features differentiating IPMN from ductal adenocarcinoma and mucinous cystic neoplasms in the early and mid 1980s.[75,76] Most of the early case series were from Japan but awareness of IPMNs increased rapidly in Western countries and this entity now accounts for 3–5% of all pancreatic neoplasms and 20% of cystic neoplasms.[77] The early reports used a confusing variety of names, including mucinous ductal ectasia, ductectatic mucinous cystic neoplasm, villous adenoma of the main duct, intraductal papilloma, papillary adenoma with excessive mucin secretion, intraductal mucin hypersecreting neoplasm and mucin-producing tumour, amongst others.[75,76,78–82] These were superseded by intraductal papillary-mucinous neoplasm, a term coined by Sessa *et al*. in 1994, and adopted in the 1997 Series 3 AFIP Fascicle on Tumors of the Pancreas and 2000 WHO Classification.[14,83,84] The Series 4 AFIP Atlas defines IPMN as 'a grossly visible, mucin-producing epithelial neoplasm that predominantly grows within the main pancreatic duct or one of its branches; it often, although not always, has a papillary architecture. IPMNs frequently produce copious amounts of mucin, and this mucin usually significantly dilates the pancreatic ducts'.[77]

Patients with an IPMN often present with abdominal pain, back pain, weight loss and anorexia, related to recurrent episodes of pancreatitis caused by intermittent obstruction of the pancreatic ducts by thick mucin or tumour.[80,85] A growing number of tumours are discovered incidentally during investigations for other conditions. The average age at presentation is approximately 65 years with a wide age range.[86–89] IPMNs are slightly more common in men than women.[86–89] Endoscopy is often diagnostic showing enlargement of the main duodenal papilla and a widely open orifice through which mucin may extrude.[90,91] Endoscopic retrograde cholangiopancreatography (ERCP) demonstrates dilatation of the main pancreatic duct and/or cystic dilation of a branch duct without a stricture. Filling defects due to intraluminal mucin plugs, sometimes in association with solid tumour, are a characteristic feature when present.[75,80,85,92] Computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP) typically show a distended main pancreatic duct or cystic dilatation of major branch ducts.[75,93] Most IPMNs are localised in the head of the pancreas (70%) rather than the body or tail. Between 20 and 30% of cases are multifocal, including the 5–10% IPMN which diffusely involve the entire gland raising the possibility of a field change within the pancreatic ducts.[78,94,95]

Macroscopic features

The macroscopic appearances of IPMN are variable depending on factors such as their location within the duct system, the degree of duct dilatation and the amount of mucin secreted. The gross appearances closely match the radiological findings such that tumours are classified as main duct type, branch duct type or mixed type involving main pancreatic duct and its major branches in a segmental or diffuse pattern.[83,87] Tumours are usually at least 10 mm in diameter ranging up to 150 mm and the better differentiated lesions usually contain abundant viscous mucin.[95,96] The epithelium lining the dilated ducts may be relatively flat and smooth or granular (ductectatic pattern); alternatively papillary excrescences or solid masses may fill the lumen (villous growth pattern), particularly in the more dysplastic tumours.[83,88,96] An invasive component may be suspected by the presences of sclerotic or mucoid (colloid-like) areas.[96] However, it should also be noted that the pancreatic tissue surrounding IPMNs is generally pale and firm, reflecting the fibrosis and atrophy associated with chronic

obstructive pancreatitis due to plugging of the ducts by thick mucin.

Microscopic features and grading

Well differentiated tumours are typically characterised by tall, well-formed papillae with fibrovascular cores lined by a layer of columnar, mucin-secreting epithelium with varying degrees of atypia (Fig. 4). A minority of tumours, usually the gastric subtype (see below) have predominantly flat epithelium. Focally the epithelial lining of the lesion may be denuded, sometimes with extravasation of acellular mucin into the stroma that must be distinguished from invasion by mucinous non-cystic carcinoma.[97] The low power architecture of higher grade tumours is more complex with branching papillae,

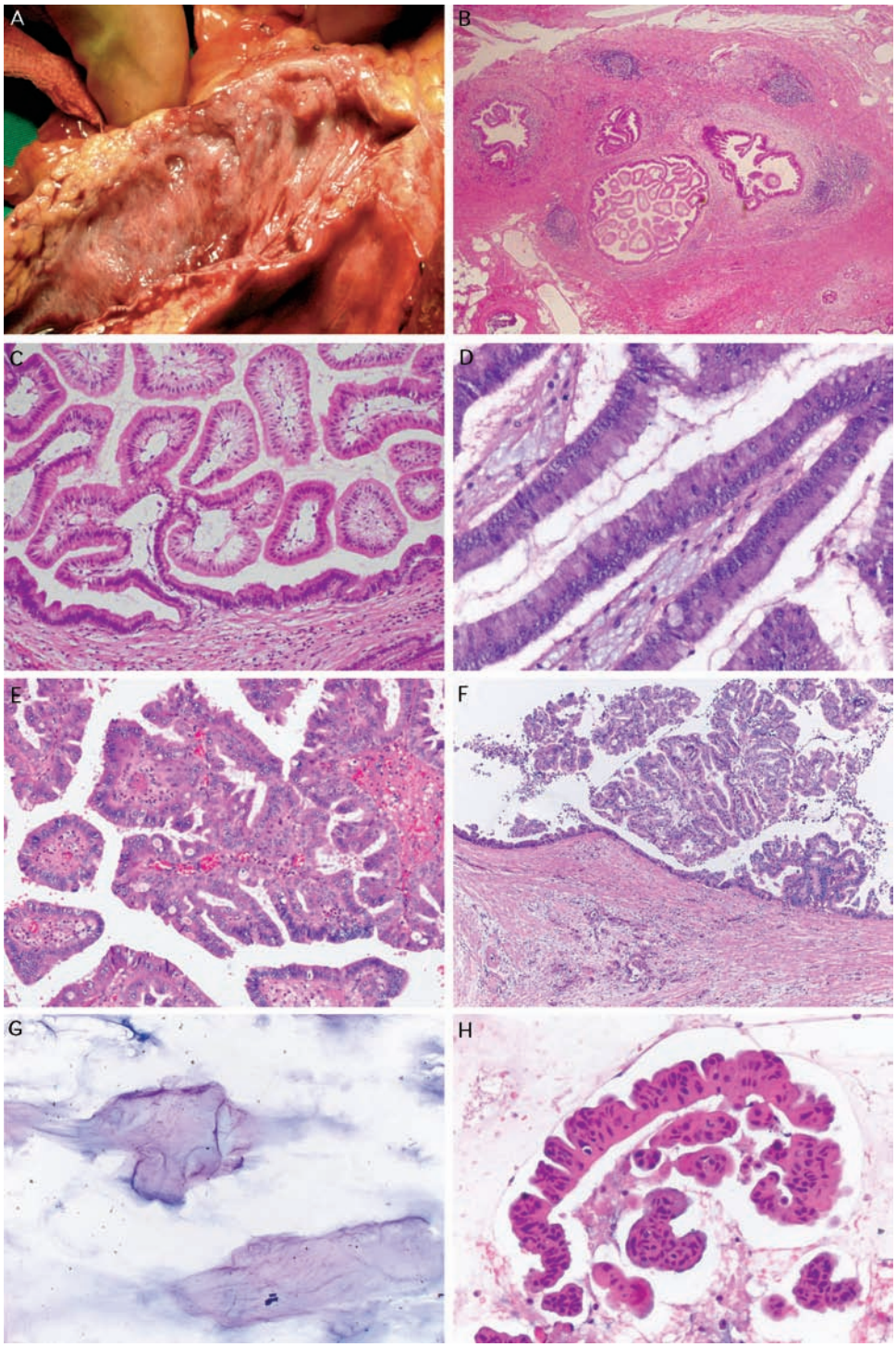

Fig. 4 Intraductal papillary mucinous neoplasms (IPMN). (A) Macroscopic photograph of papillae of IPMN in dilated main pancreatic duct. (B) Branch duct involvement by IPMN. Dilated ducts have rounded, relatively regular contours. (C) Higher power view of B; IPMN, low-grade dysplasia, gastric subtype. (D) Long slender villi of intestinal type IPMN with moderate dysplasia. (E) Complex arborising architecture of pancreatobiliary type IPMN with high-grade dysplasia. (F) Pancreatobiliary type IPMN with high-grade dysplasia (top) and invasive ductal adenocarcinoma, tubular type (lower). (G) Thick mucus from EUS-FNA of IPMN. (H) Cell block section prepared from EUS-FNA of IPMN. Strips of columnar epithelium with moderate dysplasia. (B–F,H, H&E stain; G, Papanicolaou stain). (Fig. 4A reproduced from Chang *et al*. J Gastroenterol Hepatol 2008; 23: 1036–45 with permission.[174]).

pseudopapillary structures, cribriform glands and budding off of clusters of neoplastic cells into the lumen.[77,83,88,96]

The 2000 WHO classification divided IPMNs into benign (IPMN adenoma), borderline (IPMN borderline) and malignant non-invasive (intraductal papillary mucinous carcinoma, IPMC) or invasive (IPMC + invasion) categories according to the greatest degree of dysplasia present.[83] The 2007 edition of the AFIP Atlas of Tumor Pathology has revised this nomenclature as the use of term 'borderline' raised the potential for clinical confusion.[77] The recommended WHO 2010 terminology for non-invasive IPMN is now: IPMN with low-grade dysplasia, IPMN with intermediate-grade dysplasia and IPMN with high-grade dysplasia.[77,98] IPMN with low-grade dysplasia are lined by epithelium composed of regular tall columnar cells demonstrating only slight or minimal atypia (Fig. 4B). Nucleoli are inconspicuous and only very occasional mitotic figures are present. IPMN with intermediate-grade dysplasia show moderate cytological atypia with some nuclear crowding, nuclear enlargement and hyperchromatism plus an increased nuclear: cytoplasmic ratio and pseudostratification (Fig. 4C). Mitoses may be identified. In IPMN with high-grade dysplasia there is marked cytological atypia with more loss of polarity, higher nucleocytoplasmic ratios, reduction in cytoplasmic mucin, cellular pleomorphism and more frequent mitotic figures (Fig. 4D).[14,83] Associated invasive carcinoma is present in 20–40% of IPMN and may be in the form of mucinous non-cystic carcinoma/colloid carcinoma (40% cases) or conventional tubular ductal adenocarcinoma (60%).[96,99] The grade of dysplasia commonly varies from area to area within an IPMN, hence it is important to sample these tumours thoroughly. For this reason, in a recent consensus publication it was recommended that all IPMN should be wholly embedded and examined microscopically to avoid missing areas of high-grade dysplasia or invasion.[19] The neoplasm should be graded according to the highest degree of dysplasia present and, if invasion is present, the type (tubular ductal adenocarcinoma, colloid carcinoma or oncocytic carcinoma) and extent should be recorded along with standard parameters such as grade, lymphovascular invasion, excision margins and lymph node status.

Histological subtypes

Differences in the direction of differentiation of the neoplastic epithelium have also been observed and in 2004 Adsay and colleagues proposed that IPMN can be subclassified into intestinal, pancreatobiliary, null and mixed types on morphological and immunohistochemical grounds.[96,100,101] In Adsay's study these histological subtypes varied in terms of grade of dysplasia generally present, the prevalence and type of associated invasive carcinoma and in the expression of mucin glycoproteins MUC2 and MUC1 and of the transcription factor CDX2, a key determinant of intestinal differentiation.[100] Similarly defined subtypes were subsequently incorporated into a consensus subclassification of IPMN with Adsay's null type being renamed as the gastric type.[102] The intestinal type is the most common (approximately 35% cases) and is characterised by long villous papillae lined by columnar cells with basally located or pseudostratified, hyperchromatic elongated nuclei resembling the epithelium of colorectal villous adenomas. Typically the cells have abundant apical mucin and often scattered goblet cells are present. Most intestinal type IPMNs have intermediate- or high-grade dysplasia. Immunostaining for MUC2 and CDX2 is positive and MUC1 negative.[100,102] The pancreatobiliary type is less common (approximately 20% cases) and shows thin, complex arborising papillae lined by cuboidal cells with round or ovoid nuclei, vesicular chromatin and prominent nucleoli. These cells contain less mucin and pancreatobiliary IPMNs typically have high grade dysplasia. MUC1 staining is usually positive while MUC2 and CDX2 are generally negative.[100,102] Gastric type IPMNs are flat or have thick, finger-like papillae lined by epithelium resembling gastric foveolar epithelium with abundant apical mucin and basally located nuclei. Only low grade dysplasia is usually present. Staining for MUC1, MUC2 and CDX2 are negative.[100,102]

The biological significance of these morphological types of IPMN are still unclear, but interestingly, mucinous non-cystic carcinoma is more commonly found in association with intestinal type IPMN while pancreatobiliary type IPMN is associated with conventional tubular ductal adenocarcinoma.[100] The pancreatobiliary type IPMN tend to have a more aggressive clinical course while the gastric type is rarely associated with invasion.[100] Areas of lower grade gastric type epithelium are often associated with larger, higher grade areas of other types and it has been suggested that the null/gastric type epithelium may represent a common precursor to the other types.[100] It is well recognised that IPMNs are commonly composed of more than one cell type and it has been proposed that each IPMN should be subclassified by the dominant component.[102] Interestingly, the intestinal and pancreatobiliary types rarely occur together in the same IPMN.

Intraductal oncocytic papillary neoplasm (oncocytic type IPMN)

Intraductal oncocytic papillary neoplasm (IOPN) is a closely related tumour that has been recently incorporated into the proposed consensus subclassification of IPMN as the oncocytic type.[102] This tumour has a similar gross appearance to conventional IPMN appearing either as a unilocular or multilocular cystic mass filled with reddish-brown friable tumour.[103] Histologically, oncocytic IPMNs characteristically fill cystically dilated ducts and have a complex architecture comprising arborising papillae and cribriform glands. The papillae are lined by stratified oncocytic epithelial cells with eosinophilic finely granular cytoplasm. Small intraepithelial mucin-containing 'lumina' are often prominent and goblet cells may be seen.[83,103] Most oncocytic type IPMNs are included in the high-grade dysplasia (carcinoma *in situ*) category based on the architectural complexity more than cytological features.[103] When invasive carcinoma is present the malignant cells may retain an oncocytic phenotype. Immunohistochemical staining for MUC1 is generally focally positive while MUC2 and CDX2 are negative.[102]

Differential diagnosis

The differential diagnoses of IPMN include other cystic lesions of the pancreas as well as other precursor lesions and mucinous non-cystic carcinoma. Intraductal papillary mucinous neoplasm was often misdiagnosed as mucinous cystic neoplasm (MCN) prior to the widespread recognition of IPMN in the late 1980s and early 1990s.[81] IPMN often presents as a mucin-filled cystic mass like MCN and microscopically both lesions are lined by variably dysplastic, mucin secreting columnar epithelium that is strikingly similar. However, in MCNs the papillae are generally shorter and less well developed and characteristic cellular ovarian-type stroma is present, at least focally. Moreover, MCNs usually occur in women at a younger

median age, are generally located in the tail or body of the pancreas, and rarely communicate with the pancreatic ductal system, unlike IPMNs.[78,82,91,104] In addition, MCNs tend to have a dense fibrous capsule, often with calcium deposition.[105,106] This is an important distinction to make as MCNs are much less likely to be multifocal than IPMN.

Differentiating between IPMNs and some PanINs can also be problematic given that both lesions consist of intraductal neoplastic proliferations of histologically similar mucin-producing columnar cells with a variable degree of papilla formation.[19] Although PanINs typically arise in the small ducts, they may occasionally involve larger ducts. Conversely, while IPMNs generally arise in the larger ducts, they can involve smaller ducts. Indeed, both lesions may coexist in the one pancreas, possibly accounting for the development of pancreatic ductal carcinoma several years after 'curative' resection for non-invasive IPMN and also the presence of ductal adenocarcinoma distant from an IPMN.[95,96,107,108] To clarify this issue, a consensus conference in 2003 revised the definitions for these lesions with PanIN being characterised as a microscopic lesion usually involving ducts less than 5 mm in diameter, while IPMN is typically a grossly visible lesion greater than 10 mm in diameter arising from the main pancreatic duct or branch ducts.[19] These new guidelines have led to improvement in consistency in classifying these lesions although lesions in the grey zone between 5 and 10 mm in diameter remain problematic.[34] The presence of tall papillae with stromal cores, macroscopically visible mucin or expression of MUC2 favours the diagnosis of IPMN over a PanIN, although it should be noted that MUC2 staining is not particularly sensitive.[19,100]

Retention cysts occurring following obstruction of a major pancreatic duct may also mimic IPMN. In contrast to most IPMN they have flat non-mucinous epithelium, minimal cytological atypia and are usually unilocular.[19] Retention cysts contain thin mucin, if any, unlike the thick viscous mucin characteristic of IPMNs.

Frozen section diagnosis

Frozen section examination has been advocated principally for assessment of the pancreatic transection margin intraoperatively during resection of intraductal papillary mucinous neoplasms. Recent studies have reported that routine frozen section examination during pancreatico-duodenectomy or distal pancreatectomy resulted in the detection of IPMN at the resection margin, followed by additional resection, in 23–30% of patients.[86,109,110] Discrepancy between frozen section and subsequent definitive paraffin section examination was reported as low and there was no recurrence of non-invasive IPMN.[109–111] However, in practice the interpretation of the pancreatic resection margin may be problematic, especially for the non-invasive component, as IPMNs may extend into the smaller ducts and ductules mimicking PanIN.[19,108] IPMNs tend to be larger that PanIN with taller papillae and more mucin.[19,108] Additionally, if the IPMN is a main duct type but the lesion at the margin is located in a small duct, a PanIN is more likely. Denudation of the duct epithelium at the margin may also complicate assessment of frozen sections since the epithelial lining in IPMN is often focally eroded and deeper sections may help clarify the diagnosis in this situation. Moreover, given that a small proportion of IPMN are multifocal within the pancreatic duct system, some patients will inevitably still have undetected tumour in the pancreatic remnant even when the surgical resection margin is clear.[78,94,95]

Prognosis and biological behaviour

The prognosis of patients with resected non-invasive IPMN is generally good with 5 year survival rates of 77–96%, although a number of patients with a non-invasive IPMN die from pancreatic adenocarcinoma months or years after resection of the IPMN.[99,108,112,113] There are a number of possible explanations depending on the circumstances of each case: (1) this could represent a sampling phenomenon with focal stromal invasion in the original tumour not being represented in the blocks taken at macroscopic dissection; (2) it may relate to incomplete resection of a solitary tumour; (3) to multifocal IPMN; or (4) an adenocarcinoma may have arisen from co-existing PanIN, the latter not an uncommon finding.[108]

The outlook for patients whose IPMN has an invasive component is less favourable, with 5 year survival rates of 34–70%.[97,99,114–119] However, in the six studies that have compared the behaviour of invasive IPMN to a matched cohort of common/standard pancreatic ductal adenocarcinomas after resection, the invasive IPMN group has had a significantly better prognosis, i.e., 34–62% versus 9–21% 5 year survival.[114–119] Interestingly, the difference in outcome disappears in the presence of any one of the following adverse features: advanced tumour stage (T2–4); regional lymph node metastasis; high grade tumour; vascular invasion; perineural invasion; or margin involvement.[114,116,119] This probably reflects, at least in part, the propensity of invasive IPMN to be diagnosed relatively early, i.e., at a less advanced stage, due to the associated larger, often cystic, intraductal component, which is more likely to become symptomatic or to be found on imaging.[116,117] The difference in the rate of survival for stage I tumours and the lower rates of nodal involvement, vascular invasion and perineural invasion for invasive IPMNs versus conventional pancreatic ductal adenocarcinomas may be due to a less aggressive biological nature.[115–117] There is some evidence that the mucinous non-cystic (colloid) carcinoma found in 40% of invasive IPMN is less deadly than the conventional/tubular pancreatic ductal adenocarcinoma; in one study the 24 patients with colloid type invasive IPMN had a 5 year survival of 57% compared to the overall survival rate of 42%.[113,116]

Molecular aberrations in IPMN

IPMNs share many of the molecular aberrations observed in PDAC and PanIN (reviewed by Thosani *et al.*[120]), although to a lesser extent than PDAC, and mostly in more advanced, invasive IPMNs.[121] Such abnormalities include activation of oncogenic pathways, including the *K-ras* and protein kinase B/Akt pathways; inactivation of the tumour suppressor genes *p53*, *CDKN2A/p16/MTS1*; as well as deregulation of *STK11/LKB1*, dual specificity phosphatase 6 (DUSP6) and hypermethylation of several tumour suppressor genes.[120]

K-ras mutations are frequently observed in IPMNs with both low grade and high grade dysplasia and the frequency of mutation increases in proportion to the degree of cellular atypia. In particular, a stepwise increase in *K-ras* mutation frequency from normal epithelium to hyperplasia, through to adenoma (IPMN with low grade dysplasia) and finally to carcinoma (IPMN with high grade dysplasia) has been reported.[122] Protein kinase B, also referred to as Akt,[123] promotes cell proliferation, rescues cells from apoptosis and is frequently overexpressed in IPMNs, particularly in those with high grade epithelial atypia.[124]

As observed in PanIN, mutation of *p53* is frequently encountered in high grade IPMN. Disturbances in the DNA damage

checkpoint mechanisms following *p53* mutation, lead to genomic instability and consequently have been implicated in the malignant progression of IPMNs.[84,88,125] The *MTS1* gene encodes for p16 protein that, via downstream mechanisms, leads to cell-cycle arrest.[126,127] In IPMNs, *CDKN2A* pathway function can be lost through gene mutation, deletion or hypermethylation of its promoter region leading to abrogation of gene function and unrestricted cell-cycle progression, especially in high-grade IPMN atypia.[128,129]

In a subset of IPMNs, biallelic inactivation of the Peutz–Jeghers syndrome gene *STK11/LKB1* has been demonstrated.[130,131] In addition, abrogation of *DUSP6* has been identified in a small subset of IPMNs. Hemizygous deletion of *DUSP6* also occurs in some PDAC, indicating a potential role of *DUSP6* mutation in malignant transformation of IPMNs.[129,132]

Interestingly, expression of *SMAD4/DPC4*, which is lost in PanIN-3 and approximately 50% of pancreatic ductal adenocarcinomas, is retained in most IPMN with the *SMAD4/DPC4* gene almost always remaining intact in intestinal-type IPMN.[133,134] No mutation of *SMAD4/DPC4* is seen in non-invasive IPMNs irrespective of the degree of cellular atypia, and despite frequent hemizygous deletion of the allele,[135] suggesting that inactivation/loss of *SMAD4/DPC4* is a late genetic aberration occurring in tubular type ductal carcinogenesis arising from pancreatobiliary-type IPMN.[120]

Alterations in DNA methylation have also been demonstrated, principally associated with silencing of tumour suppressor genes such as *p16* and *ppENK*,[136] and are significantly increased in frequency in high grade IPMNs when compared with low grade IMPNs and thus may contribute to the malignant transformation of IPMNs.[136]

MUCINOUS CYSTIC NEOPLASMS (MCN)

General features and definition

Mucinous cystic neoplasms were recognised and described several decades before IPMNs but they were not clearly differentiated from serous microcystic adenomas until the 1970s or from IPMNs until the 1980s.[75,76,104,137] They account for approximately 2–5% of pancreatic tumours and are found almost exclusively in women with a mean age of 40–50 years and wide age range (18–95 years).[105,106,138–140] MCNs are defined as 'neoplasms composed of mucin producing epithelial cells associated with an ovarian-type of stroma' in the latest AFIP Atlas of Tumor Pathology and, less succinctly, as 'cystic epithelial neoplasms occurring almost exclusively in women, showing no communication with the pancreatic ductal system and composed of columnar, mucin-producing epithelium, supported by ovarian-type stroma' in the 2000 WHO Classification.[140,141] Most MCNs present with non-specific symptoms such as abdominal pain or discomfort, weight loss, nausea and vomiting, and diarrhoea. Between 5 and 30% have a palpable mass.[105,106] The duration of symptoms ranges from a few days to greater than 30 years.[105] A proportion of MCNs, 10–29%, are discovered incidentally during routine physical examination or imaging for other indications.[105,106,142] CT, ultrasound and MRCP scanning typically show a well demarcated, multilocular cystic mass with septation. Calcifications are often seen around the periphery in the capsule.[105,141] ERCP is often useful in demonstrating displacement of the pancreatic duct and the usual lack of communication between the cyst and the pancreatic ductal system.

Macroscopic features

The large majority of mucinous cystic neoplasms are found in the body and tail of the pancreas; in one large series 80% were located in the tail, an additional 13.8% in the tail and body and only 4% in the head of the pancreas, while in other studies a slightly larger proportion of tumours (10%) were present in the pancreatic head.[105,143] Most MCNs are large at the time of diagnosis; the mean size is between 5 and 12.5 cm with reported tumours ranging from 1.5–30 cm in diameter.[105,106,137–139,143,144] In contrast to IPMNs, MCNs are rarely multifocal.[138] The tumours generally have a smooth surface, often covered with engorged dilated blood vessels and thick fibrous pseudocapsule.[105,106] Nearly all MCNs are multilocular, although occasional unilocular tumours have been documented (Fig. 5A).[105,106] The cystic spaces usually contain thick mucin, although they may be filled by haemorrhagic fluid or necrotic debris.[105,106,141] Individual locules vary between a few millimetres and greater than 10 cm in diameter and are separated by fibrous septa of varying thickness. They do not communicate with the pancreatic duct system, except in rare cases where there is erosion of an adjacent duct wall by the expanding tumour.[141,145] The cyst lining can be smooth and glistening, trabecular or show scattered frond-like papillary projections.[105,106] Solid mural nodules may represent areas of stromal invasion and should be thoroughly blocked for histological examination.[106] The surrounding pancreatic tissue is often fibrous and atrophic, secondary to displacement and obstruction of adjacent pancreatic ducts.[105]

Microscopic features and grading

The cystic spaces are lined by a mucinous columnar epithelium with a variable degree of atypia, very similar in appearance to that seen in IPMNs, however, no intraductal spread is present. The epithelium is usually flat but low papillary projections, cribriform and glandular arrangements may be present, especially in higher grade tumours (Fig. 5B–D).[105,106] Scattered goblet cells and endocrine cells are often observed as well as occasional cases containing foci of pseudopyloric or squamous metaplasia.[105,106,143] Areas of denudation of the epithelial lining are commonly seen.[105,141] The epithelium shows immunostaining for CEA while MUC1 is positive only in scattered goblet cells and in areas with an invasive component.[143] Characteristically, there is a layer of cellular ovarian-type stroma comprising crowded spindle cells with overlapping fusiform nuclei and intermingled capillaries immediately beneath the cyst epithelium (Fig. 5E). Some tumours contain clusters of large eosinophilic cells resembling luteinised cells within the cellular ovarian-type stroma.[141,144] Immunohistochemically, the stromal cells are positive for oestrogen in approximately 25% cases and progesterone in 50–75% of cases (Fig. 5F). This typical ovarian-type stroma is replaced by densely collagenous fibrous stroma in some areas, often where there has been erosion of the epithelial lining. Calcification of the dense collagenous fibrous tissue in the pseudocapsule and septa is common and occasionally metaplastic bone may be seen.[105,106]

The 2000 WHO Classification divides non-invasive MCNs into three categories based on the degree of architectural and cytological atypia: mucinous cystadenoma; mucinous cystic neoplasm, borderline type; and mucinous cystadenocarcinoma (carcinoma *in situ*).[140] As for IPMNs, the 2007 AFIP classification has dropped the confusing term 'borderline' and

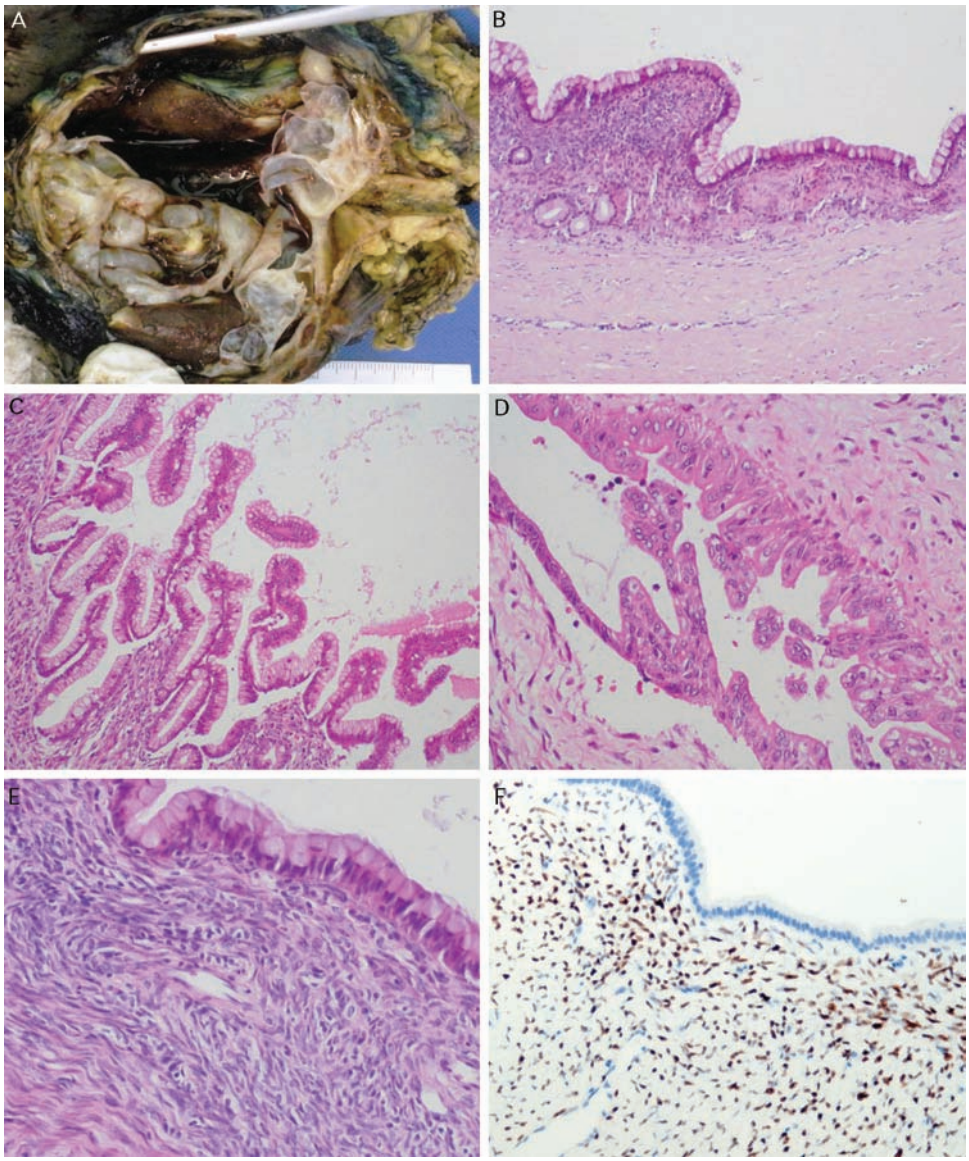

Fig. 5 Mucinous cystic neoplasms (MCN). (A) Macroscopic photograph of cystic locules in MCN. (B) MCN with low-grade dysplasia and subjacent ovarian type stroma. (C) MCN with papilla. These are generally shorter and less well developed than those in IPMN. (D) MCN with high-grade dysplasia. Note that subepithelial stroma is more densely collagenous than in B, probably reflecting previous ulceration. (E) Higher power view of cellular ovarian-type stroma. (F) Positive immunostaining for progesterone receptor in ovarian-type stroma. (B–E, H&E stain).

categorises the three types as mucinous cystic neoplasm with low grade dysplasia, mucinous cystic neoplasm with moderate dysplasia (intermediate-grade in the 2010 WHO classification) and mucinous cystic neoplasm with high grade dysplasia.[141] MCN with low-grade dysplasia has a single layer of cuboidal to columnar epithelium with only minimal to mild cytological atypia. The cells have regular, small, basally located nuclei and abundant apical mucin.[14,140,141] Mitoses are not seen and the cyst lining is usually flat rather than papillary. MCN with intermediate-grade dysplasia shows mild to moderate architectural and cytological atypia. The epithelium may be 2–7 layers thick with more cellular crowding.[105] The nuclear:cytoplasmic ratio is increased with some anisokaryosis, nucleoli, occasional mitoses and slight loss of polarity. MCNs with high-grade dysplasia display cribriforming and papillae without fibrovascular cores. The epithelial cells are crowded with loss of polarity, increased nuclear:cytoplasmic ratios, significant pleomorphism, prominent nucleoli and more frequent mitotic figures.[14,140,141] Typically, the epithelium of MCNs shows a mixture of different grades of dysplasia, often with an abrupt transition between bland and malignant epithelium, underscoring the need to submit the entire tumour for microscopic examination. The tumour should be graded on the worst (most severe) grade present rather than the average or most prevalent grade.

An invasive component is identified in approximately one-third of these tumours (mucinous cystadenocarcinoma, invasive in the 2000 WHO classification), although a recent Japanese multi-institutional study of 156 cases found invasion in only 3.9%.[105,106,138–140,144] The invasive carcinoma is usually a conventional ductal (tubular) adenocarcinoma, although mucinous non-cystic (colloid) carcinoma, undifferentiated (anaplastic) carcinoma, undifferentiated carcinoma with osteoclast-like giant cells, adenosquamous carcinoma and sarcomas may occasionally arise.[105,138,143,146] The invasion may only occur very focally within a tumour; again underlining the need to submit the whole tumour for microscopic examination to exclude invasion.[138,141] It is also important not to misinterpret

crypt-like invaginations or budding at the base of the cyst epithelium for true stromal invasion, which can be distinguished by the presence of severe nuclear atypia, single malignant cells or irregular or cribriform glands in a dense fibrous stroma.[14]

Differential diagnosis

The main differential diagnoses include other cystic lesions of the pancreas, in particular IPMN as discussed above. It is important to distinguish serous microcystic and oligocystic adenomas, which are benign in the vast majority of cases, from MCNs, many of which have significant potential for the development of malignancy.[140] Serous cystic neoplasms occur in men as well as women, typically in an older age group and are found as often in the head of the pancreas as in the body or tail. In contrast to MCN, serous cystic neoplasms are filled with thin watery fluid, often have a central stellate scar and are lined by cuboidal epithelial cells with clear, glycogen rich cytoplasm. Serous cystic neoplasms do not have an ovarian-type stroma.[141]

Pseudocysts may have thick fibrous walls like MCNs but are more common in men than women, are usually associated with previous episodes of pancreatitis and are unilocular. Pseudocysts contain necrotic debris and haemorrhagic fluid and lack an epithelial lining and ovarian-type stroma. Other cystic neoplasms of the pancreas, such as solid pseudopapillary neoplasms, cystic endocrine tumours and acinar cystadenocarcinoma lack mucin and usually do not present a diagnostic problem histologically.

Frozen section diagnosis

The location of the cyst in the body or tail of the pancreas, the patient's gender and the lack of communication between the cyst and the pancreatic ductal system, and the mucinous cyst content may suggest the possibility of a mucinous cystic neoplasm even before microscopic examination. Histologically, the presence of mucinous columnar epithelium distinguishes MCN from serous cystic neoplasms, while ovarian-type stroma excludes IPMN. Often the epithelium is focally denuded and more than one area of the tumour may need to be examined. Assessment of the grade of dysplasia is problematic in the limited sample assessed by frozen section and prone to sampling error. Likewise, even if a more macroscopically solid area is selected for frozen section, stromal invasion may be very focal and is impossible to definitively exclude.[138]

Cytological diagnosis

With the increasing use of endoscopic-guided fine needle aspiration (EUS-FNA) in the initial assessment of pancreatic lesions, there has been considerable interest in cytological diagnosis of the wide range of cystic and mucinous lesions of the pancreas.[147–153] However, pancreatic mucinous neoplasms, especially intraductal papillary mucinous neoplasm and mucinous cystic neoplasm, have significantly overlapping cytological features and often cannot be reliably differentiated from each other by aspiration cytology alone. Hence, the cytological appearances of these two entities are dealt with together in this section. The separation of IPMN and MCN with low grade dysplasia from those with moderate dysplasia is also problematic in aspiration specimens and the term benign mucinous neoplasm may be used cytologically as an umbrella term to encompass these diagnoses.

On FNA cytology, benign pancreatic mucinous neoplasms are composed of columnar cells with basally located nuclei and abundant cytoplasmic mucin (Fig. 4H). Variable amounts of extracellular mucin may be present in the background of the smears and separation from intestinal or gastric mucin may be difficult in EUS-FNA specimens. Similarly, misinterpretation of gastric or duodenal epithelium may result in an incorrect diagnosis of pancreatic mucinous neoplasm. The mucin from the gastrointestinal tract is usually thin and watery with few entrapped cells, whereas mucinous neoplasms, particularly IPMN, have thick, viscous mucin with entrapped cells (Fig. 4G). Another significant limitation of FNA diagnosis of these lesions is that often high-grade dysplasia or malignant transformation may be focal, resulting in potential sampling error and under-diagnosis of malignancy.[149,150,153–155]

As mentioned above, there is considerable overlap in cytological features between IPMN and other pancreatic lesions, especially MCN and mucinous ductal adenocarcinoma.[148,149,152] Reported values for the sensitivity of EUS-guided FNAC for the diagnosis of pancreatic mucinous neoplasms vary from 38–100%. Likewise there is considerable variation in reported specificity, in one series it was 98%, although in another there were five false positive diagnoses out of 18 cases.[148,149,153] These variations probably reflect a number of factors including the skill of the endoscopic aspirator and adequacy of the sample, the experience of the cytopathologist, whether the cytopathologist attends the FNA procedure, the use of adjunct techniques such as liquid based collection methods, and the rigor with which the clinicopathological correlation is performed.[148,152,153] The most consistent cytological finding in IPMN is abundant thick colloid-like mucin, while the presence of papilliform fragments and intracellular mucin appear to be the most specific features.[148,149]

Studies of the preoperative grading of IPMN utilising cytology have demonstrated features that correlate with low grade and moderate dysplasia (tight epithelial clusters) or carcinoma *in situ* (abundant background inflammation and chromatin clearing). The presence of necrosis has been found to be strongly suggestive of invasion.[151] The sensitivity of EUS-guided FNAC for the prediction of malignancy in IPMN is approximately 60%, while the specificity is 100%.[147,149] The sensitivity of FNAC is limited by the focal nature of malignancy in many IPMN and the attendant risk of sampling error leading to underestimation of tumour grade.[149,150] Brushing and biopsy specimens obtained during endoscopic retrograde pancreatography have also been utilised for the diagnosis and grading of IPMN with similar results to EUS-guided FNAC.[150]

The main differential diagnosis of benign mucinous neoplasms on FNA is the mucinous non-cystic carcinoma, an uncommon variant of conventional ductal adenocarcinoma which demonstrates more than 50% of mucinous epithelium. Cytologically these carcinomas are typified by atypical cells, some of which may have a signet ring morphology, in a background of extravasated mucin. Thorough sampling may reveal areas of more typical ductal adenocarcinoma.[155]

In summary, there is overlap between the cytological features of MCN, IPMN and mucinous non-cystic carcinoma. Recine *et al*. identified distinctive cytological features of IPMN and MCN. In their study, tall papillae lined by mucin-containing columnar cells with minimal atypia favoured IPMN, whereas MCN showed honeycomb sheets and clusters of mucin-containing columnar cells with rare small papillary clusters.[154] One of the most important aspects of FNA cytology

is to not only determine that a pancreatic lesion is mucinous in nature, but also to detect changes that may indicate malignant transformation. Malignant change within mucinous lesions may be demonstrated cytologically by mild to moderate background mucin, three dimensional clusters with nuclear crowding and overlapping, poorly cohesive clusters with singly dispersed cells, enlarged nuclei with high nuclear:cytoplasmic ratio, nuclear membrane irregularity, prominent nucleoli, and background necrosis. This spectrum of changes may be seen in malignant MCN, malignant IPMN and mucinous ductal carcinoma.[155] Ultimately, the accuracy of cytological diagnosis and its value as a tool in the preoperative assessment of IPMN and MCN of the pancreas is dependent on close correlation with the clinical and imaging information.

Prognosis and biological behaviour

The malignant potential of mucinous cystic neoplasms was an area of controversy for many years. In 1978 Campagno and Oertel published a series of 41 MCNs in which three patients with non-invasive tumours died of disease. They concluded that all MCNs should be regarded as potentially malignant ('mucinous cystic neoplasm with latent or overt malignancy').[137] More recently, Thompson *et al*. reported a series of 130 MCNs and concluded that all MCNs are of low-grade malignant potential from the outset and proposed that the all tumours in this group should be designated 'mucinous cystadenocarcinoma of low grade malignant potential' and that the terms 'cystadenoma' and 'cystic neoplasm of indeterminate malignant potential (mucinous cystic neoplasm, borderline type)' should be discarded.[105] In their study, one patient with a tumour categorised as MCN without atypia died of disseminated disease, however, the tumours were not completely submitted for histological examination with only an average of 13 blocks taken from the tumours which averaged 10.5 cm in diameter.

In contrast, Wilentz *et al*. reported a series of 61 MCNs from Johns Hopkins Hospital which were completely resected and entirely submitted for histological examination and found that none of the 29 patients with non-invasive tumours died of disease (with a mean follow-up of greater than 5 years). On the other hand, eight of the 32 patients who had MCNs with an invasive component died directly of disease. Underscoring the need for extensive sampling of MCNs, they also noted that in one tumour there was only a single focus of invasive ductal adenocarcinoma in one slide, while another case, which showed only benign histology on incisional biopsy, contained foci of invasive malignancy on complete excision.[138] Similarly, Zamboni and colleagues reported a series of 56 cases of MCN which were extensively sampled, observing that all 40 cases of non-invasive MCN were alive without evidence of recurrence, after a median follow-up of 69.5 months for the group with moderate dysplasia. Interestingly, patients with invasion of the tumour wall or surrounding pancreatic tissue had a 9.7 times higher risk of death than those with invasion of intratumoural stromal septa or non-invasive mucinous cystic carcinomas, suggesting that the extent of invasion is a significant prognostic feature that should be recorded in the histopathology report.[106] Overall, the 5 year survival rate for MCN with an invasive component is approximately 50%.[106,138]

Molecular aberrations in MCN

The molecular landscape of MCN to some extent mimics that of PanIN, particularly in MCN with high grade dysplasia/*in situ* carcinoma or invasive carcinoma. It has been demonstrated that there is a sequential accumulation of genetic alterations in the carcinogenesis of MCN with histological progression from benign and borderline subtypes to *in situ* or invasive carcinoma, including activating *K-Ras* mutations as well as alterations in expression of *p53*, *p16* and *SMAD4/DPC4*.[156–160]

Several groups studying the molecular profile of pancreatic cystic lesions have reported activating *K-Ras* mutations in MCN at varying expression levels depending on the epithelial atypia within the MCN. Bartsch *et al.*[156] demonstrated *K-Ras* mutation in 100% of malignant cystic tumours and not in any of the resected benign lesions, whereas Jimenez *et al.*[159] identified *K-Ras* mutations in 20% of benign, 33% of borderline and 89% of malignant MCNs, respectively. More recently, Khalid *et al.*[161] have shown that malignant cyst fluid contains adequate DNA to allow mutational analysis and that a first hit *K-Ras* mutation followed by allelic loss is most predictive of the presence of malignancy in a pancreatic cyst. As with the progression of PanIN through to pancreatic ductal adenocarcinoma, *K-Ras* mutation appears to occur early and increases in frequency in parallel with the degree of dysplasia in MCN.

Similarly, overexpression of *p53* (whereby accumulation of p53 protein is indicative of an inactivating point mutation[162]) occurs late in the development of carcinoma in MCN, and in combination with *K-Ras* mutation.[159] It has also been reported that there is negligible or minimal overexpression of p53 in MCN with moderate dysplasia (benign/borderline MCN), while up to 50% of invasive, malignant MCN demonstrate p53 aberrations.[105,163] MCN also display abnormalities in $p16^{Ink4a}$ expression, as seen in PanIN and PDAC, with disease progression accelerated in the context of $p16^{Ink4a}$ deficiency and biallelic $p16^{Ink4a}/p19^{Arf}$ deletion,[164,165] particularly in invasive carcinomas.

Like PanIN, *SMAD4/DPC4* inactivation is a frequent event in invasive MCN.[158] Interestingly, the adjacent stroma appears to retain expression of SMAD4/DPC4, even in invasive MCN whereas epithelial SMAD4/DPC4 expression is lost. In contrast, SMAD4/DPC4 loss in non-invasive MCN, including MCN with low grade dysplasia, moderate dysplasia and high grade dysplasia, is an uncommon finding. This pattern of loss of SMAD4/DPC4 expression suggests that genetic inactivation of *SMAD4/DPC4* occurs late in the neoplastic progression of these tumours and is correlated with the development of stromal invasion.

FUTURE DIRECTIONS

Mouse models

Genetically engineered mouse (GEM) models of pancreatic cancer that recapitulate the human disease have been developed, with their pathology defined during a recent National Institutes of Health (NIH) convened consensus meeting.[29] Since their generation, the knowledge base of the molecular mechanisms underpinning pancreatic carcinogenesis has been substantially augmented. Mice expressing activated *K-ras* (endogenous locus) in developing pancreas as a result of *Pdx1* promoter driven Cre mediated recombination (Lox-STOP-Lox-$KRAS^{G12D}$)[166] develop the full spectrum of PanIN-like lesions (now designated mouse PanIN; mPanIN) observed in the human disease and invasive PDAC in a subset of mice by ~12 months. When crossed to incorporate a mutant $Trp53^{R172H}$ allele that is also dependent on Cre recombinase for expression (Lox-STOP-Lox-$Trp53^{R172H}$), mice develop

mPanIN lesions that progress to invasive, metastatic cancer at high frequency over 2–3 months.[167] The GEM models of pancreatic carcinogenesis generated by Hingorani and colleagues have allowed for a greater understanding of the molecular basis of pancreatic cancer progression, in particular highlighting the crucial participation of *K-ras* activation in driving this process. For example, activation of *K-ras* alone is sufficient to generate mPanIN lesions and eventually invasive carcinogenesis, with acceleration of carcinogenesis observed with the addition of $Trp53^{R172H}$ mutation, or biallelic deletion of *INK4a/Arf*. However, in the absence of *K-ras* activation in these models, neoplastic progression through PanIN to PDAC was not observed.[164,165] Importantly, these mouse models of PDAC driven by *K-ras* activation also display many of the molecular aberrations described above as well as overexpression of developmental signalling pathways such as Notch and Hedgehog.[166–168] In addition to GEM models that represent human PDAC (mPanIN), models have been described that mimic both human IPMN (mouse IPMN; mIPMN) and MCN (mouse MCN; mMCN). Whilst in their infancy, these models include the *Ela-K-ras*,[169] the *+Mist1-K-ras* mouse[170] and the $Kras^{LSL\text{-}G12D/+}$;$Dpc4^{flox/+}$;$p48^{Cre}$ mouse.[171] The *Ela-K-ras* mouse expresses Cre recombinase under the control of the rat *Elastase* promoter with expression of the endogenous $K\text{-}Ras^{G12V}$ oncogene in acinar/centroacinar cells. This model results in a full range of mPanINs and PDAC in a subset of mice histologically indistinguishable from those of the human disease, as well as mIPMN and mMCN lesions.[169] The *+Mist1-K-ras* mouse has a knock-in of mutant *K-ras* upstream of the *Mist1* coding region. *Mist1* is a transcription factor required for proper acinar organisation, and induces invasive and metastatic pancreatic tumours;[170] however, these tumours display mixed histological characteristics (such as mIPMN and mMCN in a subset of mice) that do not recapitulate the basic properties of human PDAC. Targeted expression of *K-ras* and *Dpc4* to the *p48*-specific compartment, which is more tightly confined to the pancreas when compared to *Pdx1* promoter driven Cre mediated recombination, results in macroscopic, mucinous cystic lesions in the body and tail of the pancreas. These animals also manifest a lower overall burden of macroscopic metastatic disease than $Kras^{LSL\text{-}G12D/+}$;$Trp53^{LSL\text{-}R172H/+}$;*Cre* mice.[167] In addition to low grade PanINs, cystic neoplasms are also apparent microscopically throughout the pancreas and demonstrate architectural evidence of progression from low grade, moderate, to high grade dysplasia.[171]

Mouse models of pancreatic cancer, such as those described above, have significantly advanced our ability to delineate the molecular aberrations driving pancreatic carcinogenesis, and will provide relevant preclinical model systems to address the issues that contribute to the lethality of the disease.[172]

The International Cancer Genome Consortium (ICGC)

Advances in sequencing technology have now made it feasible to perform massive scale, exhaustive, high throughput sequencing of nucleic acid. The International Cancer Genome Consortium (ICGC; http://www.icgc.org/) has been organised to launch and coordinate a large number of research projects with the common aim of elucidating the genomic changes present in the majority of cancers that contribute to the burden of disease world-wide.[173] Although not specifically directed at precursor lesions, the insights gained into invasive pancreatic cancer are likely to stimulate further studies of its premalignant lesions, e.g., in the same manner that detection of *K-Ras* and *p16* aberrations in PDAC led to investigations of their role in pancreatic carcinogenesis.

Australia is a member of the ICGC, and through the Australian Pancreatic Cancer Genome Initiative (APGI; http://www.garvan.org.au/apgi/overview.html) will contribute by sequencing the genome, epigenome and transcriptome of pancreatic cancer in a project primarily incorporating the Garvan Institute of Medical Research in Sydney and the Institute of Molecular Biosciences (UQ) in Brisbane, with significant contributions from the Walter and Eliza Hall Institute in Melbourne and the Australian Genome Research Facility.

CONCLUSION

Considerable progress has been made in our understanding of the morphological appearances, molecular pathology and biological behaviour of the three key precursor lesions of pancreatic ductal carcinoma, PanIN, IPMN and MCN. In the future, improvements in the identification of these lesions, insights into the pancreatic cancer genome and better mouse models of pancreatic cancer are likely to offer the opportunity to refine our current progression models and may provide targets for early intervention in this lethal disease.

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