Diversity of Precursor Lesions For Pancreatic Cancer: The Genetics and Biology of Intraductal Papillary Mucinous Neoplasm

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CLINICAL AND SYSTEMATIC REVIEWS

Diversity of Precursor Lesions For Pancreatic Cancer: The Genetics and Biology of Intraductal Papillary Mucinous Neoplasm

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Pancreatic ductal adenocarcinoma (PDA), one of the most lethal cancers worldwide, is associated with two main types of morphologically distinct precursors—pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN). Although the progression of PanIN into invasive cancer has been well characterized, there remains an urgent need to understand the biology of IPMNs, which are larger radiographically detectable cystic tumors. IPMNs comprise a number of subtypes with heterogeneous histopathologic and clinical features. Although frequently remaining benign, a significant proportion exhibits malignant progression. Unfortunately, there are presently no accurate prognosticators for assessing cancer risk in individuals with IPMN. Moreover, the fundamental mechanisms differentiating PanIN and IPMN remain largely obscure, as do those that distinguish IPMN subtypes. Recent studies, however, have identified distinct genetic profiles between PanIN and IPMN, providing a framework to better understand the diversity of the precursors for PDA. Here, we review the clinical, biological, and genetic properties of IPMN and discuss various models for progression of these tumors to invasive PDA.

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OVERVIEW

Pancreatic ductal adenocarcinoma (PDA) is among the most aggressive cancer types, with surgery offering the only possibility of cure for early stage tumors, and with only a modest response to current chemotherapeutics. At present, there are no reliable methods for early PDA detection, nor is there a comprehensive classification system that links PDA subtypes to specific pharmacological vulnerabilities.[1–3] PDA is associated with several distinct precursor lesions that likely impact disease biology, efficacy of therapy, and prognosis. These include pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN).[4] The stepwise progression of microscopic PanIN lesions to invasive PDA has been well characterized (for reviews, see refs 5–7). MCN are relatively rare, slow growing cystic tumors, arising primarily in women, and their potential for progression to PDA remains uncertain (reviewed in refs 8,9). IPMNs are more common cystic tumors, that are being increasingly appreciated as important PDA precursors, and have overlapping, but distinct genetic alterations as compared with PanINs (**Figure 1**).[10,11] Here we focus on IPMNs and the relationship of this tumor type to PanIN and to invasive cancer.[10,11]

Although the overall incidence of IPMN is not known, the frequency of the disease diagnosis is increasing.[12] As the disease has become more well characterized, the diagnostic criteria for IPMN have been refined,[13] highlighting the diversity of PDA precursors.[14] Many patients with IPMN are asymptomatic, and these tumors typically do not progress rapidly;[15] however, the presence of IPMN may result in other complications such as abdominal pain, pancreatitis, and jaundice.[14,16] Moreover, IPMNs comprise a heterogeneous group of tumors with a wide range of grades and histotypes. These tumors have variable prognosis including subsets with unequivocal malignant potential. Therefore, physicians need to categorize the tumors based on symptoms, imaging modalities, and cytological findings to predict the risk of progression to invasive disease (see review on guidelines defined by International Association of Pancreatology (IAP) and European Study Group on Cystic Tumors of the Pancreas).[14,16] Given the increasing number of benign cases identified by advanced imaging modalities, the incidence of the tumors associated with invasive carcinoma is low (~10%). Among surgically resected cases (histologically proven IPMN), the frequency of invasive tumors is found to be approximately 30%. Notably, IPMN patients with "high-risk stigmata" defined by IAP guidelines have a 5-year risk of PDA development of approximately 50%.[17] Another property of this tumor type is its multifocal nature and the appearance of recurrences distant from the resection margin.[18–20] It is not clear at the time whether these features reflect the presence of a field defect,

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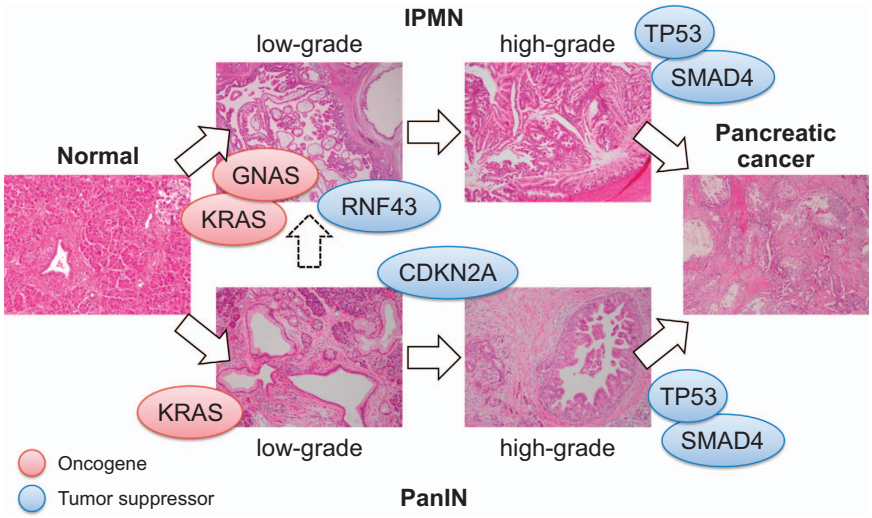

Figure 1 Precursors of pancreatic cancer. Pancreatic ductal adenocarcinoma (PDA) can arise from the progression of IPMN (top) or PanIN (bottom). It is not known whether early IPMNs (incipient IPMN) originate from low-grade PanIN, or develop independently from normal pancreatic ducts or other pancreatic cell lineages. Red and blue circles indicate oncogenes and tumor-suppressor genes, respectively. The precise timing of *RNF43* mutations and the order of *GNAS* and *KRAS* mutations have not been fully established. IPMN, intraductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia.

Table 1 Major subtype of IPMN and their characteristics

Histologic subtype	Frequency	Morphologic subtype	Genetics[a]			Immunostaining markers	Progression to invasion	Type of associated adenocarcinoma
			KRAS	*GNAS*	*RNF43*			
Gastric type	60–70%	MD << BD	53–87%	39–65%	?	Muc1–, Muc2–, Muc5AC+, Muc6+	Low	Tubular ≅classic PDA?
Intestinal type	30–40%	MD >> BD	40–46%	48–83%	?	Muc1–, Muc2+, Muc5AC+, Muc6–	High	Colloid
Overall	(100%)	MD < BD	41–79%	41–75%	14–75%			

BD, branch-duct type; MD, main-duct type.
[a]% of case with mutation.

migration of the neoplastic cells, or the initiation of multiple independent lesions.

Currently, the surgical removal of IPMN remains the best option for cure, but this also can be associated with significant morbidity and mortality.[10] Moreover, recurrence arises in 1.3%–8% and 46%–67% of resected patients with noninvasive IPMNs and invasive carcinomas associated with IPMN, respectively.[21–23] At present, there are no objective criteria—other than broad morphologic features—for guiding whether patients diagnosed with IPMN should undergo surgical interventions.[10,14] Furthermore, there is still a debate regarding the appropriate guidelines for the management of asymptomatic pancreatic cysts.[24]

Therefore, the current challenges in the field include the need to better understand the tumor biology of IPMN subtypes and to assemble a detailed registry of IPMN patients that provides a complete population view of disease progression.[25] Studying the influence of gene mutations and the associated pathways on IPMN development and malignant potential will enable better clinical decisions (e.g., surgery vs. monitoring). Finally, defining the molecular signatures of PDAs that arise from IPMNs may unveil more effective and specific therapeutic approaches for this subset of PDA patients.

DIVERSITY OF PRECURSOR LESIONS FOR PANCREATIC CANCER

PanINs are microscopic lesions, whereas IPMNs are larger and can be detected radiographically. Histologically, IPMNs are characterized by intraluminal mucin and papillary growth. These phenotypes are also seen in PanINs, but are less pronounced. The definitions of these lesions are partially based on size, with PanINs considered as microscopic lesions typically <5 mm in size and IPMNs as grossly visible masses usually >10 mm in size. At the microscopic level, there is a "gray area" presented by intermediate sized lesions (between 5 and 10 mm) for which pathologists have recently proposed the term "incipient IPMN".[26–28] Overall, the considerable overlap between PanINs and early IPMNs is one of the key issues of debate at present in the clinical community. For example, it remains unclear whether lesions fated to become IPMN arise *de novo* or whether IPMN arise from early PanIN lesions, or whether both scenarios exist.

IPMNs involve the main pancreatic duct (main-duct type), its side branch (branch-duct type), or both (mixed type). There are significant differences in biological behavior depending on the anatomic involvement: the main-duct type has highest risk of

malignant progression, followed by the mixed-type IPMN and branch-duct lesions. However, there are no clear molecular differences that distinguish the anatomical types.[29]

Fully formed IPMNs can be classified into multiple subtypes (i.e., gastric, intestinal, pancreatobiliary, and oncocytic) according to well-characterized histopathologic features.[30,31] The two most common histologic subtypes, gastric and intestinal, have distinct locations and mucin profiles (**Table 1**). Gastric-type IPMNs are usually associated with branch duct lesions, whereas the intestinal type typically involves the main pancreatic duct. Regions with intestinal or pancreatobiliary histology can often be observed mixed with gastric-type IPMN, and therefore may represent transitional disease.[32] It should also be noted that in the current criteria gastric-type IPMN are histologically defined as lesions with "basally located nuclei".[33] Therefore, by definition, this subtype tends to have lower grade dysplasia. On the other hand, "complex papillae" and "enlarged hyperchromatic nuclei" are included in the criteria for pancreatobiliary IPMN, restricting this subtype to severely dysplastic lesions. Hence, although the subtype classification is a significant predictor of survival,[34] this may simply reflect distinct histological grades. Finally, the oncocytic subtype is a rare variant, characterized by complex (arborizing) papillae lined by multilayers of cuboidal cells with large nuclei and abundant mitochondria.[32] These tumors are mostly associated with the main duct and are characterized by frequent high-grade dysplasia and/or invasion. However, the prognosis for these patients is better than that for invasive tumors associated with the other IPMN subtypes (see IPMN progression section below).[35] Overall, this complexity highlights the need for the more detailed study of the molecular underpinnings and natural history of these heterogeneous tumors.

GENETICS OF PDA AND IPMN

KRAS and other key mutations implicated in pancreatic cancer. The classic route to PDA, as established by studies of human specimens and by the development of genetically engineered mouse models (GEMMs), involves the early acquisition of *KRAS* mutations resulting in the development of PanIN that have low malignant potential (**Figure 1**).[36,37] *KRAS* is mutated in the great majority of PDA, and this genetic event has been identified at earliest stages of PanINs.[28] Approximately 80–95% of PDA patients have major hot spot mutations at *KRAS* codon 12 (G12D, G12V, and G12R) or other less frequent variants at codons 13, 61, and 146.[2,38] There is emerging evidence suggesting that there may be differences in the biologic/clinical impact of these various mutant alleles, with potential differential effects on resistance to apoptosis,[39] metastatic efficiency,[40] and patient survival.[38] Further functional studies using cellular and *in vivo* models will be required to answer how different *KRAS* variants potentially regulate unique oncogenic functions.

Mutant KRAS-expressing PanIN undergo progression to higher grade PanIN lesions and to invasive PDA upon subsequent acquisition of inactivating mutations in tumor-suppressor genes such as *CDKN2A* and/or *TP53* (and frequently *SMAD4*).[41–45] Multiple other recurrent mutations have been identified in PDA, most notably in chromatin regulators (e.g., ARID1A and KDM6A),[3,38] although the timing of such mutations and their roles in disease pathogenesis remain under study.

IPMN-associated genetic alterations. Recent in depth sequencing studies have revealed a recurrent set of mutations that define IPMN and distinguish these tumors genetically from PanIN lesions. The data also reveal that the gastric, pancreatobiliary, and intestinal subtypes of IPMN have overlapping mutational spectra whereas oncocytic IPMN are distinct and will be discussed separately at the end of the following section.

GNAS as a key IPMN oncogene. The discovery of oncogenic *GNAS* mutations in IPMNs provides an opening for the elucidation of molecular mechanisms driving IPMN and IPMN-related PDAs.[46,47] *GNAS* encodes the G-protein alpha stimulatory subunit of heterotrimeric G proteins.[48] Somatic *GNAS* mutations are found in each major IPMN subtype, with highest frequency in intestinal-type tumors[49] (**Table 1**). Notably, increased risk of IPMN has been described in McCune-Albright syndrome, which is caused by post-zygotic mosaic autosomal dominant activating mutations of *GNAS*.[50,51] Oncogenic *GNAS* has also been identified in other tumor types including those from the pituitary, liver, and colon.[52–54]

Catalogs of somatic mutations in the earliest stage of PanINs demonstrated *GNAS* mutation albeit with low frequency, either occurring alone or in combination with oncogenic *KRAS*,[28] suggesting a biological overlap between PanIN and IPMN. Although these pathways have yet to be examined in depth in IPMN, some insights can be gleaned from studies in other contexts. Although wild-type GNAS cycles between its inactive GDP bound form and its active GTP bound form in response upstream activation of associated G-protein-coupled receptors, oncogenic GNAS (typically R201C and R201H mutations in IPMN) is constitutively activated.[55] In turn, active GNAS stimulates adenylyl cyclase, leading to elevated synthesis of the second messenger, cyclic AMP (cAMP). cAMP acts through multiple effectors including activating protein kinase A (PKA), and EPAC1 and 2, which are nucleotide exchange factors for the Rap subfamily of RAS-like small GTPases, as well as regulating the opening of cyclic nucleotide-gated ion channels (**Figure 2a**).

The pathways relevant to pancreatic tumorigenesis have not been established. However, both PKA and EPAC1/2 have been implicated in many cancer relevant processes.[56,57] For example, PKA can activate the MAP-kinase pathway in some cell types and has central roles in controlling cell metabolism in many tissues.[58,59] In addition, EPAC1 and 2 have important functions in the control of cell adhesion and migration in multiple contexts.[57,60,61] cAMP has been shown to induce mucin secretion that is dependent on PKA in some cell types,[62–64] which potentially accounts for the characteristic mucinous phenotype of IPMN. Interestingly, GNAS also has a tissue-specific tumor-suppressor function relating to a role of GNAS–PKA signaling in inactivation of the GLI and YAP oncogenic transcriptional regulators.[65,66] Therefore, it will be of significant importance to not only determine the critical downstream program for GNAS-mediated tumorigenesis but

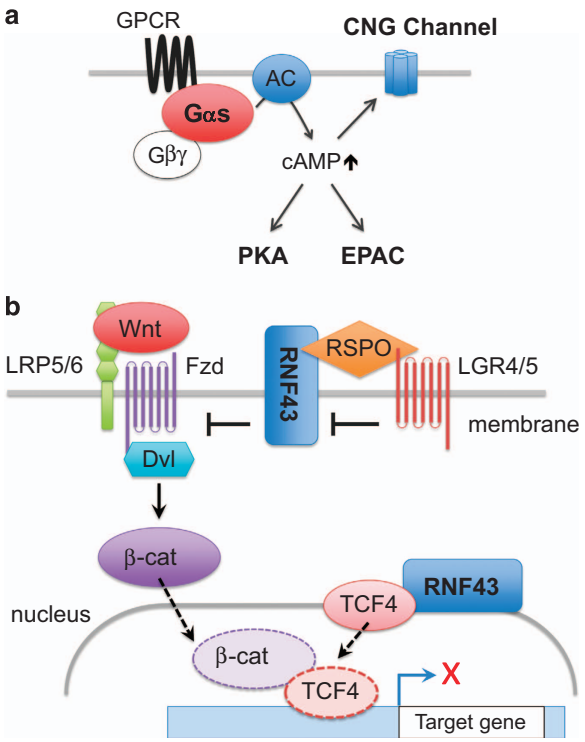

Figure 2 Signaling pathways controlling IPMN and associated PDA. (**a**) Mutant *GNAS* (encoding the Gαs protein) constitutively activates Adenylyl cyclase (AC), leading to generation of cyclic AMP (cAMP) and consequent induction of diverse intracellular signaling pathways. Effectors of cAMP include protein kinase A (PKA), exchange protein directly activated by cAMP (EPAC1 and EPAC2) and cyclic nucleotide-gated (CNG) channels. The relevant pathways mediating the oncogenic functions of mutant *GNAS* in pancreatic tumorigenesis have not been elucidated. (**b**) RNF43 is a transmembrane E3 ubiquitin ligase that inhibits Wnt/β-catenin signaling by reducing the level of Frizzled receptors (Fzd). RNF43 has also been suggested to localize to the nuclear envelope localization where it inactivates Tcf4, a transcription factor that cooperates with β-catenin to induce Wnt target genes. Therefore, loss-of-function mutations in *RNF43* can enhance Wnt signaling. Gαs, G-protein alpha stimulatory subunit; IPMN, intraductal papillary mucinous neoplasm; PDA, pancreatic ductal adenocarcinoma.

also to understand this dichotomy between oncogenic and tumor suppressive functions. Another key clinically relevant question is to address to what extent mutant *GNAS* supports the maintenance of established IPMN or resulting PDA. This will indicate whether GNAS or its downstream effectors may be potential therapeutic targets. In this regard, as a mutated GTPase, oncogenic GNAS poses similar challenges to drug development as does activated KRAS, and thus, deciphering its key downstream pathways would be of central importance.

Loss of function of RNF43 in cystic pancreatic cancers. Loss of function *RNF43* mutations have recently been found in IPMN and mucinous cystic neoplasm.[46,47] Although such mutations were first reported in high-grade IPMN,[67] it is now clear that they are also present in low-grade tumors.[68,69] Moreover, subsequent studies have indicated that inactivation of *RNF43* is not unique to the cystic pancreatic neoplasms and associated PDA, but can also be found in classic PDA.[3,38,70] Other cancer types exhibiting frequent *RNF43* mutations include colorectal and endometrial cancers.[71]

The *RNF43* gene encodes a transmembrane E3 ubiquitin ligase that can antagonize Wnt signaling via internalization and turnover of low-density lipoprotein receptor-related protein 5 (LRP5) and LRP6, which are Wnt ligand co-receptors.[71–73] The binding of Wnt ligands to Frizzled receptors (FZD) and the LRP5/LRP6 co-receptors activates canonical WNT signaling via the stabilization of β-catenin (**Figure 2b**). RNF43 and its related protein ZNRF3 are β-catenin target genes, and hence serve as negative feedback regulators to limit Wnt signaling by inducing the ubiquitinylation and lysosomal degradation of FZD receptors.[72,73] The negative regulatory function of RNF43 is blocked when R-spondin, a Wnt signaling enhancer, binds to LGR4 or LGR5; the R-spondin-LGR4/LGR5 complex then sequesters RNF43, thereby preventing it from interfering with Wnt activity. Another model has suggested that RNF43 inhibits Wnt signaling downstream of the Wnt receptors, acting to sequester the T-cell factor 4 (TCF4) transcription factor, a binding partner of β-catenin in the regulation of target genes.[74] Taken together, both models predict that loss-of-function mutations in RNF43 confer Wnt activation. Notably, studies of PDA cell lines harboring RNF43 loss-of-function mutations exhibit heightened sensitivity to pharmacologic inhibition of the Wnt-specific acyltransferase, porcupine, which is required for Wnt ligand secretion.[70,75] These data suggest a clinical path forward to treat RNF43 mutant tumors with porcupine inhibitors, such as the Novartis drug, LGK974, that is presently in clinical trials. It will also be interesting to determine whether RNF43 regulates additional pathways. In this regard, *in vitro* studies have suggested that RNF43 inactivation dampens the ATR/ATM-mediated DNA damage response pathway, which may involve Wnt-dependent or -independent mechanisms.[76]

Other tumor-suppressor genes potentially involved in IPMN tumorigenesis. Mutations in *TP53*, *CDKN2A*, and *SMAD4* also found in IPMN, particularly in high-grade lesions, and are candidate regulator of the malignant progression of these tumors to PDA (**Figure 1** and **Table 1**).[68,77–79] A number of other molecular alterations have been associated with IPMN. A subset of these tumors shows inactivating mutation or deletion of *STK11* (LKB1), and patients with heterozygous germline LKB1 mutations (i.e., Peutz–Jeghers syndrome patients) show elevated incidence of IPMN.[80] The LKB1 tumor suppressor encodes a serine–threonine kinase that is central to the control of cellular energy metabolism. Another pathway that is recurrently altered in pancreatic cancers involves the SWI/SNF chromatin remodeling complex, including mutations in *ARID1A*, *ARID1B*, *PBRM1*, *SMARCA2*, and *SMARCA4*.[38,81] Although mutations in components of SWI/SNF complex have not been specifically observed in IPMNs, expression of SMARCA4/(*BRG1*) is downregulated in some of these tumors.[82] Both LKB1 and BRG1 have been proposed to suppress Wnt signaling,[83,84] suggesting that intersection between RNF43 and these pathways may be associated with IPMN pathogenesis. The patterns in which tumor-suppressor genes are impaired may be tightly linked to unique phenotypes of the tumor, pharmacologic vulnerabilities, and outcome.[38,70]

Genetic features of oncocytic IPMN. The genetics of oncocytic IPMN have been subject to less study than the other IPMN subtypes. Nevertheless, it appears clear that oncocytic IPMNs are distinct. Targeted sequencing of a panel of 300 cancer genes in nine histologically typical oncocytic IPMNs failed to identify mutations in *KRAS* and *GNAS*, and an *RNF43* mutations was seen in only a single case.[85] A number of variants in other genes were found, although the clear identification of the key mutations underlying this IPMN subtype will require sequencing a larger number of tumors and possibly a broader set of genes. Immunohistochemical analysis of a series of markers reinforces the molecular differences between oncocytic tumors and the other IPMN subtypes.[86]

IPMN PROGRESSION

Approximately 20–30% of surgically resected IPMN patients are found to have invasive cancer.[34,87] These invasive cancers are likely to arise through multiple distinct routes (**Figure 3**). First, IPMN can directly progress to two types of malignant tumor, colloid carcinoma, which is an atypical variant of PDA (~3% of total PDAs), and tubular carcinoma (~7% of total PDA diagnoses overall).[34,88,89,90] The direct evolution from IPMN to invasive carcinoma can be demonstrated histologically by documentation of transitions between high-grade and invasive PDA, however, there are cases where the possibility of collision between noninvasive and invasive lesion. Genetic approach is useful to clarify the origin of each tumor compartment if they are intimate by showing shared mutational signatures (e.g., common *KRAS* and *GNAS* mutations).[88,91] Tubular carcinoma is histologically indistinguishable from "classic" PDA (i.e., PDA not associated with cystic tumors), and these tumors are thought to be mainly derived from gastric- and pancreatobiliary-type IPMNs.[34,87,88] Although some studies have indicated that the outcomes for tubular carcinoma have a more favorable diagnosis than classic PDA, this reflect earlier diagnosis.[89] Notably, invasive tubular carcinomas result in significantly worse patient outcomes than colloid carcinoma.[34]

Colloid carcinoma, the second type of invasive cancer associated with IPMN, is highly distinctive, characterized by extensive stromal pools of acellular mucin with floating tumor cells and more favorable survival.[90] Pathologic analysis indicates that colloid carcinoma originates from intestinal subtype IPMN, which is supported by genetic data showing common driver mutations in adjacent noninvasive and invasive lesions.[46,88] Despite the documentation of accumulating genetic alterations in the low-grade to advanced disease sequence in some cases (**Figures 1** and **3**, and see below), the histologic progression of colloid carcinoma remains to be fully defined. It should be noted that, in this more indolent type of IPMN-associated adenocarcinoma, recurrence of the IPMN can be seen, even years after the initial diagnosis.[34,92]

Another subset of invasive cancers resembling "classic PDA" occurring in IPMN patients may be derived from independent neoplastic lesions rather than from direct progression of index IPMN lesions.[89] The view that these tumors, sometimes referred to as "concurrent *de novo* PDAs", arise as clonally distinct lesions is based on the observation

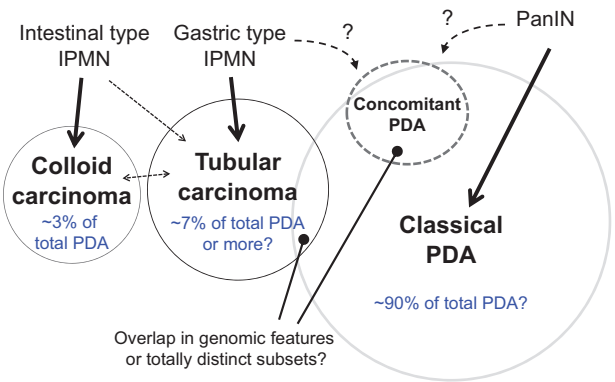

Figure 3 PDA subtypes and their precursors. Pathologic analysis indicates that intestinal-type IPMN can progress directly to an unusual subtype of invasive pancreatic cancer known as colloid carcinoma. In addition, a subset of, typically gastric- (and pancreatobiliary) type, progress to invasive tubular carcinomas, which are histologically indistinguishable from "classic PDA". The frequency shown is based on the studies on histologically confirmed IPMN-derived carcinomas and classic PDAs reported in the same time period.[34,88,89,90] Moreover, recent studies demonstrated that about 8–11% of PDA harbor GNAS mutations or amplifications,[38] (also see TCGA data set, http://www.cbioportal.org) and IPMN-derived PDA accounts for ~10% of all PDA resected; therefore, ~90% PDA are considered to originate from PanIN, however, there is significant variability between studies. Thus, this estimate largely depends on institutional definition of PanIN and IPMN. Note that the precise origins of concomitant PDA remain to be fully defined (see text for details). IPMN, intraductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma.

that some patients have PDA that is geographically separate from the IPMN. Such distal cancers have been reported in ~10% of IPMN patients during follow-up (the great majority having gastric-type IPMN[93]). Multicentric tumor development is one of the hallmarks of IPMNs, leading to the suggestion that IPMN create a field defect, which has a causal role in provoking concurrent PDA. Multicentric IPMN lesions range in size, most commonly >10 mm size and radiographically detectable, but sometimes microscopic and thus PanIN-like. Notably, distinct *KRAS* mutations have been detected in multiple such PanIN-like lesions in pancreata harboring IPMN, consistent with field cancerization.[18,94] The most likely explanation for the "concomitant PDA" is that patients with IPMN often have concurrent PanIN or small gastric-type IPMN lesions that develop into PDA.[26,93] Supporting data have been described in the literature particularly among patients with a familial susceptibility who have undergone pancreatic resection.[95,96] To fully understand biologic features of such PDA evolving from either microscopic PanIN lesions or visible gastric-type IPMN lesions, it will be critical to conduct systematic analysis of the signature IPMN gene mutations (*GNAS* and *RNF43*) in the histologically distinct precursor lesions. Overall, the models for the pathogenesis of "concurrent PDA" are largely conjectural, and are a further stimulus to study IPMN progression experimentally.

More broadly, it is critical to identify the core machinery by which an apparently indolent IPMN is transformed into an aggressive carcinoma. In this respect, the temporal order of the different genetic mutations in IPMN is an important area in need of investigation, which can be addressed using both refined genetic analysis and genetically engineered mouse models. Such studies will provide new insight into IPMN

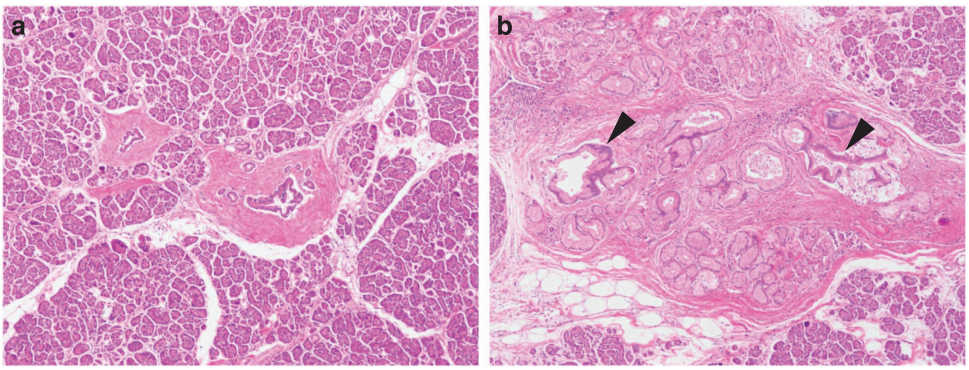

Figure 4 Human PanINs observed in resected specimens of PDA. Classic PDAs are often accompanied by multiple PanIN-like lesions, with varying grade and morphology. The presence of these microscopic lesions have been the subject of investigation as potentially predicting post-surgery recurrence. Such lesions may include early IPMNs, as some have been reported to harbor *GNAS* mutations (see refs 31,48). There are two patterns of morphology. (**a**) Solitary PanINs observed in normal pancreatic tissue (acinar compartment without pancreatitis) apart from PDA. (**b**) PanINs (arrowheads) associated with acinar-to-ductal metaplasia (ADM). This pattern is more frequently seen in IPMN patients. IPMN, intraductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma.

pathogenesis, potentially offering a framework for patient management and informing novel therapeutic strategies for subsets of PDA patients. Finally, it is important to determine whether invasive cancers associated with IPMN are indeed distinct from "classic" PDA in terms of underlying molecular circuitry and therapeutic responsiveness.[93]

The issues of clonality and field defect are presently under discussion as potentially having more general importance in PDA. Classic PDAs are often accompanied by multiple PanIN-like lesions with diverse grades of atypia in the "normal area" of the resected specimens.[97,98] These lesions are usually solitary, localized in the normal acinar compartment without pancreatitis. However, PanINs associated with regions of acinar-to-ductal metaplasia (ADM; see below for discussion of these lesions) can also be seen (**Figure 4**). The value of these lesions as a prognostic factor in postoperative recurrence of patients with PDAs has been controversial.[26,98–100] It has been shown that the presence of PanINs in the pancreatic transection margin does not influence outcome in patients with R0 resected PDA.[101] In addition, the presence of incidentally discovered PanINs found in patients who underwent pancreatectomy for non-PDA did not result in an appreciable cancer risk in the pancreatic remnant after short-term follow-up (median 3.7 years).[102] Nevertheless, such satellite lesions have been proposed by some investigators to be either early sub-clones of the associated PDA or independent clones reflecting a field defect.[26,97,103] To better understand these lesions, it will be necessary to conduct careful analysis by immunostaining for TP53 and SMAD4 and sequencing various PDA/IPMN oncogenes and tumors suppressors.[28,103] In addition, considering the fact that even individuals with non-IPMN pancreatic cysts have higher risk of developing PDA,[104] the definitions of precursor lesions for human PDA will need to be carefully re-considered at the molecular level.

IMAGING OF HIGH-GRADE IPMN AND ASSOCIATED INVASIVE FOCI

Features detected by imaging modalities that are predictive of invasive carcinoma with an associated IPMN include involvement and marked dilatation of the main pancreatic duct, diffuse or multifocal involvement, the presence of a large mural nodule (i.e., solid mass within the cystic tumor), and obstruction of the common bile duct.[14] A number of studies also highlight the presence of large mural nodules as a reliable sign indicative of malignant IPMN.[105,106] High-resolution imaging tools such as endoscopic ultrasound can visualize fine morphology of the IPMN cyst and related solid components, and the modality is highly recommended to risk-stratify the lesion.[107–109] Magnetic resonance can provide three-dimensional reconstruction images of the entire pancreatic ductal system, magnetic resonance cholangiopancreatography, rendering it a common diagnostic tool.[107,110] However, it should be noted that thus far, no imaging techniques have reliably demonstrated a significant capability to distinguish invasive from noninvasive disease.

What then is the first radiologic sign suggestive of high-grade IPMN and early invasion? Such signs would be critical for clinical management. It remains to be determined whether an invasive lesion can originate from mural nodules *per se*. Previous studies have not specifically addressed the localization of the most severely dysplastic lesions in resected IPMN specimens, and it is noteworthy that flat/low-papillary IPMN lesions outside of mural nodules can be potential precursors of invasive carcinoma.[111] The presence of a mural nodule does correlate with the histological grade of the entire lesion, supporting the notion that massively growing papillary tumors are highly suggestive of malignancy in IPMN.[105,106] However, we have demonstrated that invasive carcinomas continuously associated with mural nodules were found only in the pancreatobiliary subtype.[111] By contrast, high-grade dysplasia and invasive carcinoma with components of colloid and tubular carcinoma were observed in the areas apart from mural nodules in the intestinal and gastric type. Therefore, the emergence of invasion from IPMN is not always limited to the mural nodules, and so flat/low-papillary lesions need to be carefully surveyed.[111,112] To better understand clonal evolution during IPMN progression, systematic evaluation of the molecular signatures in each tumor compartment including both mural nodules and surrounding flat/low-papillary areas

will be necessary. These studies may support the development of novel molecular imaging that allow us to monitor the emergence of invasion at earlier time points.

BIOMARKERS FOR SURVEILLANCE OF IPMN AND STRATEGIES FOR RISK ASSESSMENT

Despite significant advances in the imaging of IPMN during past decades, there are still limitations in discriminating invasive lesions from benign/indolent tumors. Better prediction of histological grades using noninvasive tools is imminently needed for IPMN patients to make appropriate management decisions. One promising approach is monitoring metabolism of the tumor. A prospective study evaluating [F-18] fluorodeoxyglucose–positron emission tomography in the assessment of IPMN malignancy has suggested that this method has a very high diagnostic accuracy as a prognostic factor.[113,114] However, using fluorodeoxyglucose–positron emission tomography in standard diagnosis is not feasible due to its prohibitive cost. Considering that serum metabolomic analysis offers potential discrimination of PDA from chronic inflammation,[115] such an approach can also be utilized as a cut-off tool to distinguish malignant vs. benign IPMNs. Fatty acid synthase, a metabolic enzyme that catalyzes the synthesis of long-chain fatty acids, is expressed at high levels not only in PDA but also in IPMN, and fatty acid synthase expression in IPMNs correlated with histologic grade and with the presence of an associated invasive cancer.[116] Other serum metabolite levels are also emerging diagnostic tools for early detection of the PDA and predictors of the prognosis,[117,118] and may have particular value in the context of inherited disease.[119] However, our understanding of tumor cell metabolism during the progression of IPMN has not been well elucidated, in part due to a paucity of human IPMN-derived cell lines.[120,121] Establishing such materials for research may be vital to characterize IPMN cell metabolism and potentially develop novel diagnostic tests that may capitalize on that knowledge.

Among the currently available techniques, direct tumor sampling by means of endoscopic ultrasound-guided fine needle aspiration is recommended due to its high specificity to predict malignancy.[122,123] In addition to cytology and CEA levels in cyst fluid, this procedure has some additional benefits that cannot be obtained by other modalities. This approach can provide levels of inflammatory cytokines such as IL-1β and IL-8 that have significant diagnostic value to identify cysts at a high risk of malignancy.[124] High-risk/malignant IPMNs and lesions with low-intermediate grade have been reported to be distinguished via ELISA of the cyst fluid using the Das-1 monoclonal antibody, which detects an intestinal epithelial antigen and can recognize premalignant conditions of the upper GI tract.[125] These new biomarkers may improve our ability to predict patient outcomes. The genetic alterations that are responsible for the initiation and progression of IPMN and PanIN could serve as immediate biomarkers for diagnosis. Indeed, genetic analysis of the cyst fluid utilizing recent sequencing technologies can also offer accurate classification of cystic neoplasms of the pancreas and identify cysts that require surgery.[126] However, in clinical practice, it is often difficult to obtain sufficient amount of tissue and cyst fluid from the index IPMN lesion by endoscopic ultrasound–fine needle aspiration and the success rate is largely operator dependent. There are still arguments over the risk for the procedure-associated tumor dissemination,[127,128] and serial sampling is not feasible as a routine assessment for the tumor grading. In addition, IPMN often presents as a multicentric lesion and even a single cyst can be composed of multiple clones with distinct sets of driver mutations.[18,79] Thus, the diversity of IPMN clonality may be an obstacle to discrimination between benign and malignant IPMN.

Circulating cell-free DNA (cfDNA) shed from tumors into the blood has been studied for monitoring tumor genetics and offers opportunities to trace the genomic evolution of cancer systematically.[129] Although the level of cfDNA is generally higher in cancer patients than healthy individuals, specifically detecting the rare fraction of circulating tumor-derived DNA in patient plasma remains a technical challenge. Initial efforts have been made to quantify the circulating tumor-derived DNA in cancer patients using conventional PCR,[130,131] but the low sensitivity of this approach has limited its feasibility as a routine clinical test. New technologies for quantifying cfDNA are now sensitive enough for reliable application in the clinic.[132,133] Targeted sequencing of major driver mutations such as *KRAS* and *GNAS* in cfDNA is currently under investigation in Japanese patients who have pancreatic tumors and cysts (UMIN000012810). Given the high sensitivity of droplet digital PCR,[133] somatic mutations in plasma cfDNA from patients with localized pancreatic neoplasms, including low-grade IPMNs, can be successfully quantified. Sometimes referred to as liquid biopsy, this approach could provide a powerful molecular test to predict the likelihood of malignancy. By setting an appropriate protocol for clinical tests, serial blood sampling allows physicians to conduct real-time monitoring of tumor genomic alterations (manuscript in preparation). Cancer-derived exosomes in the blood are another intensively studied area, and cell surface markers have been reported to be specifically enriched in cancer cell-derived exosomes.[134] These technologies may further expand the capabilities of liquid biopsies although large validation studies are required before introduction into the clinic.

MOUSE MODELS OF PANCREATIC CANCER

GEMMs can help dissect the molecular mechanisms underlying progression of pancreatic cancer precursors and specifying different tumor subtypes. Studies in GEMMs indicate that PanINs arise from pancreatic acinar cells that incur Kras mutations[6] and undergo the process of ADM. ADM involves the conversion of acinar cells to those with a morphology and marker profile resembling that of the pancreatic ductal cells. ADM can be transiently induced upon pancreatic injury and may represent acinar de-differentiation, creating a state that is at increased vulnerability to oncogenic transformation. Kras mutations appear to fix ADM cells toward a course leading to PanIN lesions, therefore, ADMs has been recognized as the initial lesion in GEMMs for PDA. Although human acinar cells also possess plasticity to trans-differentiate to ductal cells,[135] it is not known whether human PDA also goes through an ADM stage.[136] Notably, GEMMs targeting ductal cells with Kras and *p53* mutations can also

Table 2 Genes that cooperate with mutant Kras to produce an IPMN-like phenotype in GEMMs

Gene	Function	Genotype of GEMM	Reference
Smad4	TGF-β signaling (tumor suppressor)	*Pdx1 (or Ptf1a)-Cre;LSL-Kras^{G12D};Smad4$^{lox/lox}$*	Bardeesy *et al.*[41]
Tgfa	Growth factor	*Pdx1-Cre;LSL-Kras^{G12D};Ela-Tgfa*	Siveke *et al.*[143]
Tif1g	TGF-β signaling (tumor suppressor)	*Pdx1-Cre;LSL-Kras^{G12D};Tif1g$^{lox/lox}$*	Vincent *et al.*[140]
Smarca4/Brg1	Chromatin remodeling complex (tumor suppressor)	*Ptf1a-Cre;LSL-Kras^{G12D};Brg1$^{lox/lox}$*	von Figura *et al.*[142]
Gnas	Guanine nucleotide-binding protein	*Ptf1a-Cre;LSL-Kras^{G12D};Tg(CAG-LSL-GNAS^{R201H})*	Taki *et al.*[139]
Avcr1b	TGF-β signaling (tumor suppressor)	*Pdx1-Cre;LSL-Kras^{G12D};Acvr1b$^{lox/lox}$*	Qiu *et al.*[141]

give rise to PDA, however, these tumors arise without involving clear PanIN precursors.[137]

Several GEMMs of cystic neoplasms of the pancreas have been established (**Table 2**).[5,138] Mice with combined activating mutations in *Kras* and GNAS develop IPMNs that resemble the human tumors.[139] As *Kras* mutations alone result in PanINs, this model suggests that mutant GNAS may reprogram Kras-induced early PanIN into IPMN-like tumors. This model does not appear to undergo progression to PDA, and thus, knockout of *Smad4* in the context of *Kras* activation also promotes the formation of IPMN-like tumors in mice,[41] although mutational inactivation of TGF-β signaling (e.g., mutation of *SMAD4* or *TGFBR*) is not thought to be a major event in the early stages of human IPMN. Knockout of TIF1 gamma (*Tif1γ*) and activin A receptor type IB (*Acvr1B*), which are other potential TGF-β pathway components, demonstrated similar IPMN phenotypes.[140,141] The loss of chromatin regulators Brg1 is also known to form cystic pancreatic neoplasms that resemble human IPMN via the cooperation with oncogenic *Kras*.[142] This suggests that Brg1 has an inhibitory role against IPMN tumorigenesis. Expression of the EGF receptor ligand, Tgf-α, is also able to synergize with oncogenic *Kras* to promote development of cystic papillary lesions resembling human IPMNs,[143] consistent with the relevant role of EGFR expression to assess the clinical course of IPMN.[144] Its remains to be seen whether loss of Rnf43 also recapitulates the biology of cystic tumors in GEMMs.

To date, all available GEMMs for IPMN-like tumors have been developed in the context of oncogenic *Kras*, in which ADM-PanINs axis may be directed to cystic and papillary growing phenotype instead of their typical progression with marked desmoplasia. Therefore, it is reasonable to speculate that small PanINs can develop into IPMN depending on the nature of co-existing mutations (such as *GNAS*), although this has not been formally demonstrated in these models, and moreover, there is no clinical evidence showing this transition. IPMNs may have been proposed to originate from the large pancreatic ducts—either the main duct or a large branch duct.[142] However, it is presently unknown whether IPMN may also arise from ADM and whether the differences in cell of origin might contribute to the distinct biology of IPMN and PanIN. To address this question experimentally, it will be interesting to examine cohorts of mice with combinations of mutations in *Kras*, *Gnas*, and *Rnf43* targeted specifically to the acinar cells or ductal cells. Such an effort may provide a new insight regarding the fundamental diversity of the precursor lesions for pancreatic cancer.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Over the past several decades, a growing number of studies have contributed to establishing guidelines for the management of IPMN patients.[14,16] Clinical efforts to gain further insights into the natural history and biology of the IPMN are necessary for a better consensus in the field. An improved risk stratification algorithm needs to be established not only for index IPMN lesions but also for unexpected emergence of concomitant PDA. Mouse models harboring combinations of *Kras*, *Gnas*, and *Rnf43* mutations will be important tools in the functional dissection of the different types of IPMN. Detailed genetic studies in surgical specimens of IPMN-associated "early" PDA based on minute pathological navigation are also greatly needed. In addition, establishment of patient-derived IPMN cell lines (and cell lines from IPMN-originating PDA) is also critical to allow experimental study of the biology of these tumors. Outstanding questions to be addressed include the nature of the core machinery leading specific subtypes of the precursors as well as their cell of origin. In addition, the potential role of a field defect and multicentric precursors and the associated molecular pathways that may influence invasive tumor development are other important issues to be clarified. To ultimately reduce the mortality from PDA, first a more reliable screening strategy based on genetics and molecular signature is necessary to identify individuals with imminent risk. Second, understanding the pathways supporting the growth of invasive cancers associated with IPMN will be critical for the development of more specific and effective therapies. The current dilemma regarding the management of IPMN patients will likely only be overcome through collaboration between clinicians and basic scientists.

CONFLICT OF INTEREST

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