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

Citation
Patra, Krushna C, Nabeel Bardeesy, and Yusuke Mizukami. 2017. “Diversity of Precursor Lesions For Pancreatic Cancer: The Genetics and Biology of Intraductal Papillary Mucinous Neoplasm.” Clinical and Translational Gastroenterology 8 (4): e86. doi:10.1038/ctg.2017.3. http://dx.doi.org/10.1038/ctg.2017.3.

Published Version
doi:10.1038/ctg.2017.3

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:33029737

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

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

Krushna C. Patra, PhD1, Nabeel Bardeesy, PhD1 and Yusuke Mizukami, MD, PhD1,2

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.

Clinical and Translational Gastroenterology (2017) 8, e86; doi:10.1038/ctg.2017.3; published online 6 April 2017

Subject Category: Clinical Review

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.17

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,

1Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, Massachusetts, USA and 2Center for Clinical and Biomedical Research, Sapporo Higashi Tokushukai Hospital, Sapporo, Hokkaido, Japan

Correspondence: Yusuke Mizukami, MD, PhD, Division of Cancer Research, Center for Clinical and Biomedical Research, Sapporo Higashi Tokushukai Hospital, 3-1, North-33, East-14, Higashi-Ku, Sapporo, Hokkaido 065-0033, Japan. E-mail: ymizu_ccbr@tohtoku.jp or ymizukami@mh.harvard.edu

Received 23 July 2016; accepted 3 January 2017
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.\textsuperscript{10} Moreover, recurrence arises in 1.3\textasciitilde8\% and 46\textasciitilde67\% of resected patients with noninvasive IPMNs and invasive carcinomas associated with IPMN, respectively.\textsuperscript{21\textendash23} At present, there are no objective criteria—other than broad morphologic features—for guiding whether patients diagnosed with IPMN should undergo surgical interventions.\textsuperscript{10,14} Furthermore, there is still a debate regarding the appropriate guidelines for the management of asymptomatic pancreatic cysts.\textsuperscript{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.\textsuperscript{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."\textsuperscript{26\textendash28} 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

---

**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\textsuperscript{a} | Immunostaining markers | Progression to invasion | Type of associated adenocarcinoma |
|--------------------|---------|-------------------|-----------------|-----------------|------------------------|-----------------------------|
|                     |         |                   | KRAS | GNAS | RNF43 |                          |                             |
| Gastric type        | 60\textasciitilde70\% | MD < < BD | 53\textasciitilde87\% | 39\textasciitilde65\% | ? | Muc1\textasciitilde-, Muc2\textasciitilde-, Muc5AC\textasciitilde+, Muc6\textasciitilde- | Low | Tubular $\approx$ classic PDA? |
| Intestinal type     | 30\textasciitilde40\% | MD > > BD | 40\textasciitilde46\% | 48\textasciitilde83\% | ? | Muc1\textasciitilde-, Muc2\textasciitilde+, Muc5AC\textasciitilde+, Muc6\textasciitilde- | High | Colloid |
| Overall             | (100\%) | MD < BD | 41\textasciitilde79\% | 41\textasciitilde75\% | 14\textasciitilde75\% |                             |                             |

BD, branch-duct type; MD, main-duct type.

\textsuperscript{a}\% of case with mutation.
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 146.28,38 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 tumors49 (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
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. 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 ubiquitylation and lysosomal degradation of FZD receptors. 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. 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. 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.

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). A subset of these tumors shows inactivating mutation or deletion of STK11 (LKB1), and patients with heterozygous germine LKB1 mutations (i.e., Peutz–Jeghers syndrome patients) show elevated incidence of IPMN. 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, SMARC2, and SMARCA4. 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. Both LKB1 and BRG1 have been proposed to suppress Wnt signaling, 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.
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. 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.

IPMN PROGRESSION
Approximately 20–30% of surgically resected IPMN patients are found to have invasive cancer. 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). 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). 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. Although some studies have indicated that the outcomes for tubular carcinoma have a more favorable diagnosis than classic PDA, this reflect earlier diagnosis. Notably, invasive tubular carcinomas result in significantly worse patient outcomes than colloid carcinoma.

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. 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. 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.

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. The view that these tumors, sometimes referred to as “concurrent de novo PDAs”, arise as clonally distinct lesions is based on the observation 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). 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. 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. Supporting data have been described in the literature particularly among patients with a familial susceptibility who have undergone pancreatic resection. 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...
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.

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) 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 PDAs/PanIN 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.
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] fluoro-deoxyglucose–positron emission tomography in the assessment of IPMN malignancy has suggested that this method has a high diagnostic accuracy as a prognostic factor. However, using fluoro-deoxyglucose–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, 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. Other serum metabolite levels are also emerging diagnostic tools for early detection of the PDA and predictors of the prognosis, and may have particular value in the context of inherited disease. 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. 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. 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. 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. 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. 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, 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. 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 track the genomic evolution of cancer systematically. 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, 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. 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. Given the high sensitivity of droplet digital PCR, 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. 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 mechanisms and specifying different tumor subtypes. Studies in GEMMs indicate that PanINs arise from pancreatic acinar cells that incur Kras mutations 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, it is not known whether human PDA also goes through an ADM stage. Notably, GEMMs targeting ductal cells with Kras and p53 mutations can also
Clinical and Translational Gastroenterology

Also promotes the formation of IPMN-like tumors in mice, and thus, knockout of Gnas develop IPMNs that resemble human IPMNs, consistent with the recapitulation of the biology of cystic tumors in GEMMs. Several GEMMs of cystic neoplasms of the pancreas have been established (Table 2). Mice with combined activating mutations in Kras and Gnas develop IPMNs that resemble the human tumors. 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, however, these tumors arise without involving clear PanIN precursors.

Several GEMMs of cystic neoplasms of the pancreas have been established (Table 2). Mice with combined activating mutations in Kras and Gnas develop IPMNs that resemble the human tumors. 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, however, these tumors arise without involving clear PanIN precursors.

**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<sup>G12D</sup>,Smad4<sup>lox/lox</sup>             | Bardeesy et al.|
| Tgfα    | Growth factor                                  | Pdx1-Cre;LSL-Kras<sup>G12D</sup>,Ela-Tgfα                                     | Siveke et al.   |
| Titf1γ  | TGF-β signaling (tumor suppressor)            | Pdx1-Cre;LSL-Kras<sup>G12D</sup>,Titf1γ<sup>lox/lox</sup>                      | Vincent et al.  |
| Smara4/Brg1 | Chromatin remodeling complex (tumor suppressor) | Ptf1a-Cre;LSL-Kras<sup>G12D</sup>,Brg1<sup>lox/lox</sup>                         | von Figura et al.|
| Gnas    | Guanine nucleotide-binding protein              | Ptf1a-Cre;LSL-Kras<sup>G12D</sup>,Tgf(CAG-LSL-GNAS<sup>R201H</sup>)           | Taki et al.     |
| Acrv1β  | TGF-β signaling (tumor suppressor)            | Pdx1-Cre;LSL-Kras<sup>G12D</sup>,Acrv1β<sup>lox/lox</sup>                      | Qiu et al.      |

**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. 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**

Guarantor of the article: Yusuke Mizukami, MD, PhD.

Specific author contributions: KCP, NB and YM wrote and edited the manuscript. All the authors have critically reviewed the manuscript. The final version of the manuscript was approved by all the authors.

Financial support: This work was supported by JSPS KAKENHI Grant Number 25461029 and by a Pancreas Research Foundation in Japan to YM and support from the Granara-Skerry Trust, the Linda J. Verville Foundation, the Begg Family, and grants from the NIH (P01 CA117969-07, R01 CA133557-05) to NB. KCP is supported by a post-doctoral fellowship from the Department of Defense, USA (W81XWH-16-1-0285). The authors are Andrew L. Warshaw...
Institute for Pancreatic Cancer Research scholars. N.B. is the holder of the Gallagher Chair in Gastrointestinal Cancer Research at the Massachusetts General Hospital, USA. Potential competing interests: None.

Acknowledgments. We are thankful to members of the Bardeesy Lab, Drs Carlos Fernández-del Castillo (Massachusetts General Hospital), Hiroyuki Maguchi (Teine Keijinkai Hospital), and Hidenori Karasaki (Sapporo Higashi Tokushukai Hospital) for critical reading of the manuscript and helpful discussions. We appreciate Dr Yuko Omori (Teine Keijinkai Hospital) for providing pathology images.

1. Singh A, Greninger P, Rhodes D et al. A gene expression signature associated with ‘K-Ras addiction’ reveals regulators of EMT and tumor cell survival. Cancer Cell 2009; 15: 489–500.
2. Collisson EA, Sadanandam A, Olson P et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011; 17: 500–503.
3. Waddell N, Pajic M, Patch AM et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2016; 538: 495–501.
4. Matthai H, Schülick RD, Hruban RH et al. Cystic precursors to invasive pancreatic cancer. Nat Rev Gastroenterol Hepatol 2011; 8: 141–150.
5. Bardeesy N, Aguirre AJ, Chu GC et al. Both p16(ink4a) and the p19(ARF)/p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc Natl Acad Sci USA 2006; 103: 5947–5952.
6. Kopp JL, von Figura G, Mayes E et al. The genetic and epigenetic landscape of human pancreatic carcinomas. Nature 2016; 540: 495–500.
7. Morris JPt, Wang SC, Hebrok M. KRAS, Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. Gut 2013; 62: 141–150.
8. Sakorafas GH, Szymiotis V, Reid-Lombardo KM et al. Primary pancreatic cystic neoplasms revisited: part II. Mucinous cystic neoplasms. Surg Oncol 2011; 20: e93–101.
9. Yamao K, Yamagawara A, Takahashi K et al. Clinicopathological features and prognosis of mucinous cystic neoplasm with ovarian-type stroma, a multi-institutional study of the Japan pancreas society. Pancreas 2011; 40: 67–71.
10. Fernández-del Castillo C, Adsay NV. Intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology 2010; 139: 708–713; 713.e1–2.
11. Maguchi H, Tanno S, Mizuno N et al. Natural history of branch duct intraductal papillary mucinous neoplasms of the pancreas: a multicenter study in Japan. Pancreas 2011; 40: 394–370.
12. Klibansky DA, Reid-Lombardo KM, Gordon SR et al. The clinical relevance of the increasing incidence of intraductal papillary mucinous neoplasms. Clin Gastroenterol Hepatol 2012; 10: 555–558.
13. Obara T, Saitoh Y, Maguchi H et al. Papillary adenoma of the pancreas with excessive mucin secretion. Pancreas 1992; 7: 114–117.
14. Tanaka M, Fernandez-del Castillo C, Adsay V et al. International consensus guidelines 2012 for the management of IMPMN and MCN of the pancreas. Pancreatology 2012; 12: 183–197.
15. Tanno S, Nakano Y, Nishikawa T et al. Natural history of branch duct intraductal papillary-mucinous neoplasms of the pancreas without mural nodules: long-term follow-up results. Gut 2008; 57: 339–343.
16. Del Chiaro M, Verbeke C, Salvia R et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015; 6: 6744.
17. Maguchi H, Takagi Y, Dogita K et al. Clinical characteristics and outcome of patients with intraductal papillary mucinous neoplasm of the pancreas. Jpn J Clin Oncol 2015; 45: 1038–1109 e1.
18. Notta FR, Kloppe G, Volkan Adsay N et al. Classification of types of intraductal papillary-mucinous neoplasms of the pancreas and other tumoral intraductal neoplasms of pancreaticobiliary tract: recommendations of Verona Consensus Meeting. Ann Surg 2016; 263: 162–177.
19. Adsay NV, Merati K, Basturk O et al. Pathologically and biologically distinct types of epithelial tumors in intraductal papillary mucinous neoplasms: delineation of an ‘intestinal’ pathway of carcinogenesis in the pancreas. J Am Surg Pathol 2004; 28: 839–848.
20. Nino-Murcia M, Fernandez-del Castillo C, Baba Y et al. Prognosis of invasive papillary mucinous neoplasm depends on histological and precursor epithelial subtypes. Gut 2011; 60: 1712–1720.
21. Marchegiani G, Minckowski M, Ferrone CR et al. Oncoctytic-type intraductal papillary mucinous neoplasms: a unique malignant pancreatic tumor with good long-term prognosis. J Am Coll Surg 2019; 229: 839–844.
22. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer 2002; 2: 897–909.
23. Hingorani SR, Petrouin EF, Malta A et al. Preinvasive and invasive papillary ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003; 4: 437–450.
24. Witkiewicz AK, McMillan EA, Balaj U et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015; 6: 6744.
25. Guerrero S, Casanova I, Farre L et al. Kras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. Cancer Res 2000; 60: 6750–6756.
26. Alamo P, Gallardo A, Di Nicolantonio F et al. Higher metastatic efficiency of KRAS G12V than KRAS G13D in a colorectal cancer model. FASEB J 2015; 29: 464–476.
27. Bardeesy N, Cheng KH, Berger JH et al. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of cancer. Genes Dev 2006; 20: 3130–3146.
28. Murphy SJ, Hart SN, Lima JF et al. Genetic alterations associated with progression from pancreatic intraductal neoplasia to invasive pancreatic tumor. Gastroenterology 2013; 145: 1098–1109 e1.
29. Notta F, Chan-Seng-Yue M, Lemire M et al. A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns. Nature 2016; 538: 378–382.
30. Bailey P, Chang DK, Nones K et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016; 531: 47–52.
31. Wu J, Mattei H, Malta A et al. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. Sci Transl Med 2011; 3: 92ra86.
32. Furukawa T, Kuboki G, Yatomi Y et al. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep 2011; 1: 161.
33. O’Hayre M, Vaquero-Prado J, Kufareva I et al. The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. Nat Rev Cancer 2013; 13: 412–424.
34. Dal Molin M, Mattei H, Wu J et al. Clinicopathological correlates of activating GNAS mutations in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg Oncol 2013; 20: 3802–3808.
35. Weinstein LS, Shinker A, Gejman PV et al. Activation of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med 1981; 305: 1695–1695.
36. Gaiou J, Salamone S, Florio M et al. Hepatobiliary and pancreatic neoplasms in patients with McCune-Albright Syndrome. J Clin Endocrinol Metab 2014; 99: E97–E101.
37. Mantovani G, Lania AG, Spada A. GNAS imprinting and pituitary tumors. Mol Cell Endocrinol 2010; 326: 15–18.
38. Yamada M, Sekine S, Ogawa R et al. Frequent activating GNAS mutations in villous adenoma of the colon. J Pathol 2012; 228: 113–118.
39. Naut JC, Fabre M, Couchy G et al. GNAS-activating mutations define a rare subgroup of inflammatory liver tumors characterized by STAT3 activation. J Hepatol 2012; 56: 184–191.

Diversity of Precursor Lesions for Pancreatic Cancer
Patra et al.

Clinical and Translational Gastroenterology
Diversity of Precursor Lesions for Pancreatic Cancer
Patra et al.

55. O'Hayre M, Degese MS, Gulksnd JS. Novel insights into G protein and G protein-coupled receptor signaling in cancer. Curr Opin Cell Biol 2014; 27: 126–135.
56. Ji J, Mei FC, Johnson BH et al. Protein kinase A, not Epac, suppresses hedgehog activity and regulates glucocorticoid sensitivity in acute lymphoblastic leukemia cells. J Biol Chem 2007; 282: 33730–33737.
57. Burchardt T, Conant A, Haynes L et al. cAMP inhibits migration, ruffling and paxillin accumulation in focal adhesions of pancreatic ductal adenocarcinoma cells: effects of PKA and EPAC. Biochim Biophys Acta 2013; 1833: 2664–2672.
58. Almahairi M, Mei FC, Cheng X. Cyclic AMP sensor EPAC proteins and energy homeostasis. Trends Endocrinol Metab 2014; 25: 60–71.
59. Kim EJ, John YS. Cyclic AMP signaling reduces sirtuin 6 expression in non-small cell lung cancer cells by promoting ubiquitin-proteasomal degradation via inhibition of the Ras-MEK-ERK (Raf/mitogen-activated extracellular signal-regulated kinase/extracellular signal-regulated kinase) pathway. J Biol Chem 2015; 290: 9604–9613.
60. Yu JL, Deng R, Chung SK et al. Epac activation regulates human mesenchymal stem cells adhesion. Stem Cells 2016; 34: 948–959.
61. Howe AK, Juliano RL. Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. Nat Cell Biol 2000; 2: 593–600.
62. Chinn EC, Harris TE, Atyasayeura DR. Changes in CAMP-dependent protein kinase (PKA) and progestrone secretin in luteinizing human granulosa cells. J Endocrinol 2004; 183: 39–50.
63. Baskrov N, Prota M. Tumour suppressor protein PKA-a-mediated secretion of mucin from human colon epithelial cells. J Cell Physiol 2000; 185: 408–415.
64. Nakamura M, Endo K, Nakata K. Mucin-like glycoprotein secretion is mediated by cyclic-AMP and protein kinase C signal transduction pathways in rat corneal epithelium. Exp Eye Res 1998; 66: 513–519.
65. He X, Zhang L, Chen Y. E259. – Nature SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor remodeling subunit BRG1/SMARCA4 is frequently observed in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol 2011; 17: 793–803.
66. Sakamoto H, Kuboki Y, Hatori T. Methylation of BRCA1/BRCA2 gene in fresh frozen pancreatic tissue. Jpn J Clin Oncol 2015; 45: 957–960.
67. Okabayashi T, Shima Y, Kosaki T. Inactivation of the BRCA1 gene in pancreatic ductal adenocarcinoma. Proc Natl Acad Sci USA 2015; 112: 9246–9251.
68. Amato E, Molin MD, Matticini A et al. Tumour suppressor gene NF1 is a tumour suppressor in pancreatic cancer. Proc Natl Acad Sci USA 2016; 113(13): 3589–3594.
69. Premi MS, Horiuchi M. Inactivation of the BRCA1/BRCA2 gene in fresh frozen pancreatic tissue. Jpn J Clin Oncol 2015; 45: 957–960.
70. Wang G, Fu Y, Yang X et al. Brg-1 targeting of novel miR505a-5p/NF3/Wnt signaling axis regulates colorectal cancer metastasis. Oncogene 2016; 35: 651–661.
71. Basturk O, Tan M, Bhanot U et al. The oncogenic subtype is genetically distinct from other pancreatic intraductal papillary mucinous neoplasms subtypes. Mod Pathol 2016; 29: 1058–1069.
72. Basturk O, Chung SM, Hruban RH et al. Distinct pathways of pathogenesis of intraductal oncocytic papillae neoplasms and intraductal papillary mucinous neoplasms of the pancreas. Virchows Arch 2016; 469: 523–532.
73. Yamada S, Fuji T, Shimmoya Y et al. Clinical implication of morphological subtypes in management of intraductal papillary mucinous neoplasms. Gastroenterology 2014; 147: 226–237.
74. Dal Molin M, Hong SM, Hebbar S et al. Convergent structural alterations define pancreatic ductal adenocarcinoma concomitant with IPMN. Pancreas 2011; 40: 571–580.
75. Yopp AC, Kabata N, Jianakos M et al. Invasive carcinoma arising in intraductal papillary mucinous neoplasms of the pancreas: a matched control study with conventional pancreatic ductal adenocarcinoma. Ann Surg Oncol 2011; 18: 868–877.
76. Matsuzaka S, Karasaki H, Ono Y et al. Tracking the clinical evolution of endoscopic pancreatectomy, a rare variant of intraductal papillary mucinous neoplasm of the pancreas. Pancreas 2016; 45: 915–918.
77. Winter JM, Jiang W, Basturk O et al. Recurrence and survival after resection of small intraductal papillary mucinous neoplasm-associated carcinomas (< 20-mm invasive component); a multi-institutional analysis. Ann Surg 2016; 263: 793–801.
78. Ideno N, Ohtsuka T, Kono H et al. Intraductal papillary mucinous neoplasms of the pancreas with distinct pancreatic ductal adenocarcinomas are frequently of gastric subtype. Ann Surg Oncol 2013; 20: 141–151.
79. Matthaei H, Norris AL, Tsitsias AC et al. Clinicopathological characteristics and molecular analyses of multifocal intraductal papillary mucinous neoplasms of the pancreas. Ann Surg 2012; 255: 326–333.
80. Shi C, Klein AP, Goggins M et al. Increased prevalence of precursor lesions in familial pancreatic cancer patients. Clin Cancer Res 2009; 15: 7737–7743.
81. Bartsch DK, Dietzel K, Bargello M et al. Multiple small ‘‘imaging’’ branch-duct type intraductal papillary mucinous neoplasms (IPMNs) in familial pancreatic cancer: indicator for concomitant high grade pancreatic intraepithelial neoplasia? Fam Cancer 2013; 12: 89–96.
82. Imi K, Karasaki H, Ono Y et al. Metachronous pancreatic cancer originating from disseminated founder pancreatic intraepithelial neoplasia (PanINs). J Pathol Clin Res 2015; 1: 76–80.
83. Matsuzaka S, Karasaki H, Ono Y et al. Tracking the clinical evolution of endoscopic pancreatectomy, a rare variant of intraductal papillary mucinous neoplasm of the pancreas. Pancreas 2011; 40: 571–580.
84. Yu JL, Deng R, Chung SK et al. Epac activation regulates human mesenchymal stem cells adhesion. Stem Cells 2016; 34: 948–959.
85. Howe AK, Juliano RL. Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. Nat Cell Biol 2000; 2: 593–600.
Diversity of Precursor Lesions for Pancreatic Cancer
Patra et al.

Clinical and Translational Gastroenterology is an open-access journal published by Nature Publishing Group.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/