J Gastroenterol (2015) 50:520–532
DOI 10.1007/s00535-015-1037-4

The Japanese Society of Gastroenterology

REVIEW

The genetic classification of pancreatic neoplasia

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Received: 18 December 2014 / Accepted: 30 December 2014 / Published online: 21 January 2015

Abstract Cancer is caused by the accumulation of inherited and/or acquired alterations in specific genes. The recent decline in the cost of DNA sequencing has allowed tumor sequencing to be conducted on a large scale, which, in turn, has led to an unprecedented understanding of the genetic events that drive neoplasia. This understanding, when integrated with meticulous histologic analyses and with clinical findings, has direct clinical implications. The recent sequencing of all of the major types of cystic and noncystic neoplasms of the pancreas has revealed opportunities for molecular diagnoses and for personalized treatment. This review summarizes the results from these recent studies focusing on the clinical relevance of genomic data.

Part of this review was presented at the 4th International Forum of the 100th General Meeting of the Japanese Society of Gastroenterology.

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Keywords Pancreatic tumors · Pancreatic cancer · DNA sequencing · Next generation sequencing

Abbreviations

ACC	Acinar cell carcinoma
IPMN	Intraductal papillary mucinous neoplasm
LOH	Loss of heterozygosity
MCN	Mucinous cystic neoplasm
mTOR	Mechanistic target of rapamycin
PanIN	Pancreatic intraepithelial neoplasia
PanNET	Pancreatic neuroendocrine tumor
PDA	Pancreatic ductal adenocarcinoma
PI3K	Phosphatidylinositol 3′-kinase
SCA	Serous cystadenoma
SPN	Solid-pseudopapillary neoplasm
STK11/ LKB1	serine-threonine kinase 11/ liver kinase B1
TGFBR2	transforming growth factor, beta receptor II
TP53	tumor protein 53
VEGF	vascular endothelial growth factor
VHL	von Hippel Lindau
WHO	World Health Organization
ZIM2	zinc finger, imprinted 2

Introduction

It is becoming increasingly clear that cancer is a disease caused by mutations and other alterations to specific genes. This growing perception, combined with the introduction of next-generation sequencing, has led to an unprecedented understanding of the pathogenesis of cancer, and to the opportunity for personalized diagnoses and treatment [1].

In what can be described only as a revolution, the exomes and/or genomes of all of the commonest types of human cancer have been sequenced. In 2007, Wood et al. [2] were the first to sequence the exome of a solid tumor when they sequenced the exomes of a series of well-characterized human breast and colorectal cancers. On the basis in large part of the success of these early sequencing efforts, the International Cancer Genome Consortium and The Cancer Genome Atlas separately launched large multi-institutional efforts to sequence the genomes of 50 major cancer types [3]. These and other studies have revealed that most cancer types are characterized by a small number of genes that are targeted in most tumors of those types, and by a larger number of genes that are altered in only a small percentage of tumors of those types. Vogelstein et al. [1] designated these as "mountains" and "hills" in the cancer genomic landscapes, and unified the mountains and the hills by identifying the common cellular pathways that they target. Vogelstein et al. also made the important distinction between "driver" mutations (those that are functionally significant and promote neoplastic growth) and "passenger" mutations (somatic mutations that are present in a neoplasm but which do not have functional significance). The cancer sequencing revolution has struck tumors of the pancreas, and the results have been dramatic.

All of the major types of tumors of the pancreas, including all of the cystic and all of the solid tumors, have been sequenced [4–11]. The results of these sequencing studies have enormously improved our understanding of pancreatic neoplasia, they have created a foundation for novel research [8, 12–14], and they have significant clinical applications [15–17] (Table 1).

In this review we summarize the salient genetic alterations of the most important pancreatic neoplasms and we emphasize possible clinical applications. A detailed understanding of the gross and microscopic pathologic features of a tumor is important because the pathologic features will guide how we study a disease and because a good understanding of pathologic features can form a foundation for clinical applications. Because of the focus of this review is on genetic changes in pancreatic neoplasms, we will only briefly summarize the individual gross and histopathologic features of each entity.

Pancreatic ductal adenocarcinoma

Pancreatic cancer is the fourth leading cause of cancer death, and it is projected to become the second leading cause of cancer death in the USA by 2030 [18]. In spite of significant cost-intensive research efforts, the 5-year survival rate for patients with pancreatic ductal adenocarcinoma (PDA) remains approximately 5 %, a statistic that has virtually not changed in the past 50 years. PDA is often diagnosed in an advanced stage with primary tumors invading surrounding structures and/or with distant metastases, leaving only palliative treatment options [19].

Resected PDAs are usually solid, firm, diffusely infiltrative and white-yellow [4]. Microscopically, PDAs, by definition, are composed of infiltrating neoplastic glands. Very characteristically, these neoplastic glands elicit an intense desmoplastic reaction, such that much of the tumor is actually composed of nonneoplastic fibroblasts and inflammatory cells [20]. It is important to appreciate the desmoplastic stroma when conducting genetic analyses because if one does not dissect the stroma away from the neoplastic cells, one will end up with samples with a low neoplastic cellularity, making analyses difficult to interpret. As is true for other epithelial neoplasms, invasive PDA appears to arise from histologically well-characterized noninvasive precursor lesions. These include microscopic precursor lesions called pancreatic intraepithelial neoplasia (PanIN) lesions, and larger cystic precursor lesions called intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) [21, 22]. These precursor lesions are important because they represent an opportunity to detect and treat pancreatic neoplasia before it progresses to an incurable invasive carcinoma [23].

The genetic changes in invasive PDAs are complex, with genomes harboring complex karyotypes and multiple copy number alterations often spanning very large genomic regions [6, 24, 25]. At the individual gene level, DNA changes include activating mutations in oncogenes, inactivating mutations in tumor suppressor and caretaker genes, epigenetic modifications, and altered telomere length. The first large sequencing study of invasive PDA, by Jones et al. [6] in 2008, confirmed that an oncogene, *KRAS*, and three tumor suppressor genes, *CDKN2A* (also known as *P16*), *TP53*, and *SMAD4* (also known as *DPC4*), are targeted in most of these cancers [26]. The other genes targeted less often included *MLL3*, *TGFBR2*, *ARID1A*, and *ATM*. Other sequencing studies have followed up on the original study by Jones et al., and these have validated the genes and pathways identified by Jones et al. as targeted in PDA, and have suggested other genes that are targeted at lower prevalence. For example, the International Cancer Genome Consortium project suggested that genes in axon guidance pathways (e.g., SLIT/ROBO) may also be targeted in PDAs [25, 27].

One of the major driver mutations in PDA first described by Almoguera et al. [28] in 1988 is *KRAS* on chromosome arm 12p, which is activated by point mutations in more than 90 % of PDAs. *KRAS* mutations usually target a "hot spot" at codon 12, but they may also affect codons 13 and 61 [4–6, 29, 30]. Akin to other proteins of the Ras family, *KRAS* encodes a GTPase that works as an activation/

Table 1 Genes altered in pancreatic neoplasia and possible clinical implications

Tumor type	Targeted gene	Clinical implication if inactivated
PDA	*KRAS*	
	CDKN2A/P16	
	TP53	Mutations detected in pancreatic juice may indicate high-grade dysplasia
	SMAD4/DPC4	Mutations may be associated with metastatic spread with possible biomarker function to guide therapy in borderline resectable PDA
	MLL3	
	TGFBR2	
	ARID1A	
	ATM	
Familial PDA	*BRCA1*	Tumors harboring mutations may be more amenable to DNA cross-linking drugs and to PARP inhibitors
	BRCA2	
	PALB2	
	ATM	
	CDKN2A/P16	
	PRSS1	
	SPINK1	
	MLH1	Tumors harboring mutations may be less sensitive to 5-fluorouracil
	MSH2	
IPMN	*KRAS*	Mutations detected in cyst fluid may help guide the therapy in pancreatic cysts: tumor-specific mutational profiles may indicate cyst type
	GNAS	
	RNF43	
MCN	*KRAS*	
	RNF43	
SCA	*VHL*	
SPN	*CTNNB1*	
PanNET	*MEN1*	
	DAXX	
	ATRX	
	TSC2	Tumors harboring mutations may be amenable to mTOR pathway inhibitors
	PTEN	
	PIK3CA	
	PHLDA3	LOH may be associated with negative outcome and disease progression
ACC	*SMAD4/DPC4*	
	TP53	
	GNAS	
	BRAC2	Tumors harboring mutations may be more amenable to DNA cross-linking drugs and to PARP inhibitors
	PALB2	
	ATM	
	BAP1	
	JAK1	Tumors harboring a mutation may be amenable to Jak1 inhibitors
	BRAF	Tumors harboring a mutation may be amenable to BRAF inhibitors
	BRAF and *CRAF* fusions	Tumors harboring a mutation may be amenable to MEK inhibitors
PB	*APC*	
	CTNNB1	

ACC acinar cell carcinoma, *BRAF* v-Raf murine sarcoma viral oncogene homolog B, *IPMN* intraductal papillary mucinous neoplasm, *Jak1* janus kinase 1, *LOH* loss of heterozygosity, *MCN* mucinous cystic neoplasm, *MEK* mitogen-activated protein/extracellular-signal-regulated kinase kinase, *mTOR* mechanistic target of rapamycin, *PanNET* pancreatic neuroendocrine tumor, *PARP* poly(ADP-ribose) polymerase, *PB* pancreatoblastoma, *PDA* pancreatic ductal adenocarcinoma, *SCA* serous cystadenoma, *SPN* solid-pseudopapillary neoplasm

inactivation switch that controls a number of downstream pathways that affect proliferation, differentiation, and cell survival [31–34]. A third of PDAs without a *KRAS* mutation harbor *BRAF* mutations, highlighting the importance of Ras signaling for PDA formation [35]. Many of the remaining *KRAS* wild-type PDAs appear to be microsatellite unstable, and will be discussed later [36].

The most frequently inactivated tumor suppressor gene in PDA is *CDKN2A* on chromosome arm 9p [37]. *CDKN2A* is inactivated in 95 % of PDAs: in 40 % by intragenic mutations coupled with loss of the second allele (loss of heterozygosity, LOH), in 40 % by homozygous deletion, and in 15 % by epigenetic silencing [30, 38, 39]. The protein p16 plays a crucial role in cell cycle control as it slows the transition from G_1 phase to S phase. Hence, loss of expression of p16 in pancreatic cancers leads to a decreased inhibition of the cell cycle and therefore promotes unrestrained proliferation of the neoplastic cells.

The tumor suppressor gene *TP53* on chromosome arm 17p is inactivated in approximately 75 % of PDAs [40]. The protein p53 has a number of important functions that help to prevent neoplastic transformation. The gene is inactivated in PDA primarily through intragenic mutations coupled with LOH of the wild-type allele [30, 41]. A third tumor suppressor gene, *SMAD4* (on chromosome arm 18q), is inactivated in 55 % of PDAs. *SMAD4* is inactivated by either homozygous deletion or the combination of an intragenic mutation coupled with LOH [42]. The gene product of *SMAD4* is a critical component in the transforming growth factor β pathway, regulating among other things the proliferation and production of the extracellular matrix [6, 43].

Many of the genetic changes found in invasive PDA are also present in PanIN lesions. For example, almost all PanIN lesions, even the lowest-grade PanIN-1 lesions, harbor somatic *KRAS* mutations [44]. In addition, the other genes targeted in invasive PDA, *CDKN2A*, *TP53*, and *SMAD4*, have all been found to be mutated in PanIN lesions, particularly high-grade PanIN lesions [45, 46]. These findings help establish that PanIN lesions can indeed be precursors to invasive PDA. Supporting this conclusion, is the observation in genetically engineered mouse models that activation of Kras in the pancreas produces mouse PanIN lesions [33].

Although most pancreatic cancers appear to be sporadic, a significant fraction have a strong familial component, and some of these familial cancers have unique genetic drivers. Approximately 5–10 % of PDAs arise on the background of a genetic predisposition [47]. The lifetime risk of PDA grows with the number of relatives an individual has with PDA. The risk is doubled in individuals with a single affected first-degree relative [48], culminating in a 32-fold greater risk for individuals with three or more affected first-degree relatives [49]. Although it is clear that there is a strong familial component to pancreatic cancer, a causative germline mutation in a known pancreatic cancer susceptibility gene can be identified in only approximately 20 % of familial cases [50, 51]. The genes that are known to predispose to pancreatic cancer (Table 1) include genes causing hereditary breast–ovarian cancer (caused by germline mutations in *BRCA1*, *BRAC2*, or *PALB2*; with a relative risk of 3.5–10 for *BRCA2*), familial atypical multiple mole melanoma syndrome (caused by germline mutations in *p16/CDKN2A*; with a relative risk of 13–22), Peutz–Jeghers syndrome (caused by germline mutations in *STK11*; with a relative risk of 132), hereditary pancreatitis (caused by germline mutations in *PRSS1* or *SPINK1*; with a relative risk of 50–80), Lynch syndrome (caused by germline mutations in *MLH1*, *MSH2*, etc.; with a relative risk of 8.6) [47, 51, 52], and ataxia–telangiectasia (caused by germline mutations in *ATM*) [7, 53, 54].

The elucidation of the genetic alterations that drive PDA and its variants has had direct clinical implications.

First, genetic alterations can form the basis for screening and early detection. Studies of genetic evolution have been used to estimate that it takes at least 10 years from a first mutation in a pancreatic ductal epithelial cell until the development of a primary invasive cancer [55]. This suggests a reasonably large window for the early detection of curable pancreatic neoplasia [56]. Pancreatic cancer is, however, a relatively rare disease, and screening efforts will have to be focused on populations with an elevated risk of developing the disease [23]. Again, our genetic understanding can help here as well, as individuals with a strong family history of PDA and individuals who carry a known genetic predisposition, because of their quantifiably increased risk, would be the first to benefit from early detection efforts [57, 58]. An understanding of the genetics of PDA and its precursors can also form the basis for the actual tests used to screen for early disease. For example, Kanda et al. [13, 14] demonstrated that molecular screening is possible in secretin-stimulated pancreatic juice samples harvested through noninvasive endoscopy. They found *TP53* mutations in pancreatic juice samples collected with the help of endoscopic ultrasonography from patients with histologically proven high-grade dysplasia or PDA [13] as well as *GNAS* mutations in patients with radiologically suspected IPMNs [14]. These promising methods have to be validated in a larger prospective trial before their introduction in clinical use.

Second, some genetic alternations found in PDA have prognostic implications and may therefore serve as biomarkers. Blackford et al. [59] analyzed sequence data on 39 genes frequently mutated in PDA in a well-characterized series of 90 tumors and correlated the results with patient outcome. They found that loss of *SMAD4* in a

pancreatic cancer was associated with a shorter overall survival (11.5 months compared with 14.2 months in patients whose tumors had intact *SMAD4*). Taking these findings one step further, Iacobuzio-Donahue et al. [60] showed that PDAs with inactivated *SMAD4* are more likely to metastasize widely than are PDAs with intact *SMAD4*. Taken together, these findings suggest that *SMAD4* status could guide therapeutic approaches in patients with borderline resectable PDA. Patients with tumors that have *SMAD4* inactivation may benefit from systemic therapy, as their tumors are more likely to be metastatic (Fig. 1b).

Third, genetic changes may form the basis for individualized therapies. For example, in vitro studies have suggested that genetic testing may even be useful to preselect patients for treatment with chemotherapy agents [61]. Tumors harboring Fanconi anemia pathway gene mutations (such as *BRCA1*, *BRCA2*, and *PALB2* mutations) and tumors harboring *ATM* mutations are examples of potential individualized treatment targets. It has been shown that this subgroup of PDAs is more amenable to DNA cross-linking drugs and to poly(ADP-ribose) polymerase inhibitors [54, 62–66]. In addition, PDAs with microsatellite instability, on the basis of experience with colorectal cancers with microsatellite instability, are less likely to be sensitive to 5-fluorouracil than are microsatellite-stable pancreatic cancers [67]. Primary tumor xenografts may help to test the effect of multiple therapeutic agents on a patient's tumor in order to individualize the drug regimen for that patient, thereby avoiding unnecessary side effects from ineffective drugs administered [68].

Strategies to effectively block oncogenic *KRAS* signaling are particularly attractive approaches to therapy since virtually all PDAs harbor *KRAS* mutations. Unfortunately, these efforts have been largely ineffective. Nevertheless, expert groups such as the RAS Initiative led by Frank McCormick at Frederick National Laboratory for Cancer Research have dedicated their work to finding ways to block this apparently "undruggable" pathway.

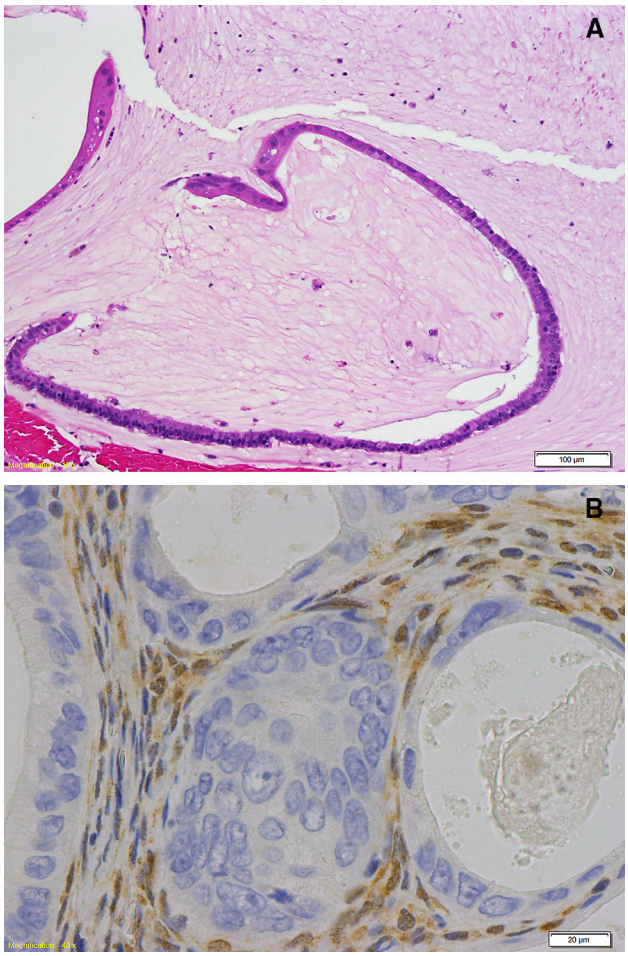

Fig. 1 Examples of how genetic biomarkers may contribute to histologic diagnosis and/or clinical outcome prediction. **a** A colloid adenocarcinoma (hematoxylin and eosin stain; original magnification ×10). These tumors are strongly associated with *GNAS* codon 201 mutations and an improved outcome compared with tubular ductal adenocarcinomas, which are almost always *GNAS* wild-type tumors. **b** A pancreatic ductal adenocarcinoma with loss of *SMAD4* (Immunostain for Smad4; original magnification ×40) as often mediated through a somatic mutation. This genetic alteration is usually associated with a particularly poor outcome with rapid widespread metastatic disease

Cystic lesions of the pancreas

Intraductal papillary mucinous neoplasms

IPMNs are mucin-producing epithelial lesions that arise in the larger pancreatic ducts [69]. IPMNs are, by definition, larger than 1 cm and can be solitary or multifocal [70–72]. IPMNs can involve the main pancreatic duct or they may arise in a branch off from the main duct. Some are combined or mixed, involving both the main duct and branch ducts [73]. IPMNs localized in the main pancreatic duct are more frequently associated with an invasive adenocarcinoma than are branch-duct-type IPMNs [74, 75]. IPMNs can be categorized histologically on the basis of the direction of differentiation of the neoplastic cells into intestinal, gastric-foveolar, pancreatobiliary, and oncocytic subtypes. The epithelial subtype has been shown to correlate with the likelihood that the IPMN harbors high-grade dysplasia or an associated invasive carcinoma [76, 77].

The main clinical problem in treating patients with an IPMN is determining if and when the lesion should be resected. IPMNs that are likely to have high-grade dysplasia or a resectable associated invasive carcinoma should be resected, but many IPMNs will have only low-grade dysplasia and their clinical course can be safely followed without surgery [75, 78, 79].

Previous studies have shown that IPMNs harbor specific genetic alterations, including copy number changes [80] and intragenic mutations [81]. Recent comprehensive sequencing studies have shown that IPMNs harbor an average of 26 nonsynonymous coding mutations, with *KRAS* as the most frequently mutated gene (approximately 80 %) [8, 9, 82]. Although *KRAS* is also a hallmark of PDA, two other genetic alterations are highly prevalent in IPMNs (Table 1). In contrast to "common" PDAs (arising in the absence of an associated IPMN), *GNAS* codon 201 mutations are present in two thirds of IPMNs and inactivating *RNF43* mutations can also be seen [82, 83]. Somatic mutations in *GNAS* lead to an activation of an alpha subunit of a G protein causing increased adenyl cyclase activity and high cyclic AMP production. *GNAS* mutations appear to be most prevalent in intestinal-type IPMNs and their associated invasive colloid adenocarcinomas [84]. Patients with colloid cancers usually have a more favorable outcome compared with those with a tubular PDA (Fig. 1a). Inactivating mutations in the tumor suppressor gene *RNF43* are also found in a subset of IPMNs. The associated protein, a ubiquitin E3 ligase, has been implicated as an inhibitor of Wnt signaling [8]. The four most frequently involved genes in PDA play a role in IPMNs too. As mentioned, somatic *KRAS* mutations occur in a high proportion of IPMNs, with higher incidence in gastric and pancreatobiliary subtypes and the associated tubular adenocarcinomas [85–88]. Somatic *TP53* mutations are almost exclusively found in areas of high-grade dysplasia or invasion, which indicates that this mutation is a relatively late event in carcinogenesis [88–91]. Most noninvasive IPMNs show intact expression of the tumor suppressor gene *SMAD4*, whereas SMAD4 loss can be seen in invasive carcinomas arising in association with IPMNs, again suggesting that *SMAD4* is targeted late in neoplastic progression [92, 93]. Similarly, loss of p16 is very common in invasive carcinoma arising in association with an IPMN, whereas p16 is preserved in most lower-grade IPMNs [93]. Other mutations that can be found in IPMNs include mutations of *PIK3CA* (approximately 10 %) [88], *STK11* (also known as *LKB1*) (5 % of sporadic IPMNs) [94] and *BRAF* (2.7 % of IPMNs) [95, 96].

Mucinous cystic neoplasms

MCNs are cyst-forming mucin-secreting epithelial neoplasms with a distinctive ovarian-type stroma [4]. They are almost always found in the tail of the pancreas, and are much commoner in women than in men. In contrast to IPMNs, the cysts of MCNs do not communicate with the pancreatic duct system. MCNs, like IPMNs and PanIN lesions, can be a precursor to invasive adenocarcinoma [21, 72, 97]. Microscopically, MCNs are characterized by a neoplastic mucin-rich columnar epithelium with varying degrees of dysplasia sitting on an ovarian-type stroma composed of spindle-shaped cells [72, 98]. Owing to their predominance in women, their distal location in the pancreas, and their ovarian-type stroma, it has been speculated that MCNs do not originate from pancreatic tissue, but instead arise from cells left behind by the ovaries as they descend past the pancreas embryologically.

Sequencing of the exomes of a series of histologically well-characterized MCNs detected 16 mutations on average in each tumor [8]. Mutations in *KRAS* are common (approximately 80 %) [99–101], and 40 % of MCNs harbor an inactivating mutation in *RNF43*. *CDKN2A* [100], *TP53* [101, 102], and *SMAD4* [92, 103] can also be targeted in MCNs, especially those with high-grade dysplasia or an associated invasive carcinoma. In contrast to IPMNs, *GNAS* does not appear to be targeted in MCNs [8, 104].

Serous cystadenomas

Serous cystadenomas (SCAs) are cystic neoplasms that are almost always benign and only extremely rarely behave in a malignant manner [4]. Grossly, most SCAs appear as multilocular or unilocular microcystic lesions. They sometimes have a characteristic central star-shaped scar with calcifications [33]. Microscopically, the well-demarcated cysts have a single layer of cuboidal glycogen-rich epithelium.

Whole-exome sequencing revealed an average of ten nonsynonymous mutations per SCA [8]. All SCAs sequenced harbored at least one region with LOH, predominantly located at chromosome arm 3p. The tumor suppressor gene *VHL* is located on 3p, and sequencing also revealed intragenic mutations in *VHL*, helping to establish *VHL* as a driver of this tumor type [105–107]. The gene product of *VHL* has an E3 ubiquitin ligase activity responsible for "marking" proteins for subsequent degradation. Patients with von Hippel–Lindau syndrome are predisposed to develop SCAs, as well as a variety of other clear cell neoplasms. The syndrome is caused by a germline (autosomal dominant) mutation of *VHL* with inactivating alteration or loss of the other functioning allele, further highlighting the critical role of this gene in SCA formation [108]. *VHL* alterations (intragenic mutations or LOH at 3p) can be used to help identify a cystic tumor as an SCA, and since virtually all SCAs are benign, these patients, if asymptomatic, can be safely followed up and unnecessary surgery can be avoided.

Solid-pseudopapillary neoplasms

Solid-pseudopapillary neoplasms (SPNs) are rare exocrine pancreatic neoplasms predominantly seen in younger female

patients [4]. Grossly, they have solid and cystic areas, and the cystic areas are often filled with hemorrhagic debris. SPNs are malignant; 15 % of patients have distant metastases at the time of diagnosis [109–111]. Histologically, the neoplastic cells are characteristically poorly cohesive. They have uniform nuclei, sometimes with a nuclear groove. Other histologic features that can help establish the diagnosis include hyaline globules, foam cells, and cholesterol clefts [112]. Sequencing of SPNs has revealed that they harbor only a few DNA alterations [8, 113]: LOH is rare in SPNs, and they harbor only three somatic mutations on average. A missense mutation in *CTNNB1*, encoding β-catenin, is found in virtually all SPNs [8, 114]. β-Catenin is a component of the cadherin protein complex with critical function in cell–cell adhesion and gene transcription within Wnt signaling. *CTNNB1* mutations inhibit ubiquitination and degradation of β-catenin, leading to an accumulation of β-catenin in the nucleus. In the nucleus, β-catenin induces the expression of its target proteins [115].

Clinical implications

Cystic lesions of the pancreas are increasingly diagnosed owing to an ever-increasing use of modern cross-sectional imaging. At autopsy as many as 25 % of individuals without any pancreas-linked symptoms or diseases have cystic changes in their pancreas [116]. Most of these sometimes minute cysts can now be visualized by high-resolution computed tomography, magnetic resonance imaging, or endoscopic ultrasonography [117–119]. This "epidemic" of pancreatic cysts is, on the one hand, an unprecedented chance to treat pancreatic neoplasia before it progresses to an invasive carcinoma. On the other hand, there is a risk of harmful overtreatment if, for example, a patient undergoes unnecessary surgery for an asymptomatic entirely benign pancreatic cyst. Therefore, it is essential that clinicians have sensitive and specific tools to distinguish benign neoplastic cysts (e.g., SCAs) from cysts with the potential to progress to metastatic cancer (e.g., IPMNs, MCNs, or SPNs). Advances in our understanding of the genetics of cystic neoplasms of the pancreas suggest a new approach to characterizing pancreatic cysts. Fluid can be aspirated from pancreatic cysts at the time of endoscopic ultrasonography. This fluid will contain molecular material shed from the neoplastic cells and therefore will contain the specific DNA alterations that can be used to characterize the cyst type (i.e., IPMN, *KRAS*, *GNAS*, and *RNF43*; MCN, *KRAS* and *RNF43*; SCA, *VHL* and LOH of chromosome 3; SPN, *CTNNB1*). Additionally, cyst fluid may be evaluated for microRNAs or for mucins. When integrated with clinical findings, such molecular approaches have the potential of fundamentally changing the way cystic neoplasms of the pancreas are managed [120–124].

Pancreatic neuroendocrine tumors

Pancreatic neuroendocrine tumors (PanNETs) are the second most frequently diagnosed primary tumor of the pancreas [125]. Compared with PDA, however, the prognosis is usually much better, with approximately 40 % of affected patients surviving 10 years after diagnosis [126–128]. PanNETs are heterogeneous and clinically can be divided into functioning (syndromic) or nonfunctioning (nonsyndromic) tumors. Among all functioning PanNETs, insulinomas are the commonest subgroup (30–45 %) [127], followed by gastrinomas (16–30 %), VIPomas, glucagonomas (less than 10 % each), and somatostatinomas (less than 5 %) [129]. Most PanNETs occur sporadically, although up to 10 % arise in individuals with a cancer-predisposition syndrome, such as von Hippel–Lindau syndrome (caused by inherited mutations in *VHL*), tuberous sclerosis (caused by inherited mutations in *TSC1* or *TSC2*), or multiple endocrine neoplasia 1 (caused by inherited mutations in *MEN1*) [130].

PanNETs are grossly distinct from PDAs. They usually form well-demarcated soft, solid, or rarely cystic neoplasms [4]. Histologically, PanNETs are characterized by epithelial cells with round to oval nuclei containing a "salt and pepper" type chromatin. PanNETs are graded by their proliferative activity as assessed by Ki-67 immunohistochemical labeling. Grade 1 PanNETs have a Ki-67 labeling index of 0–2 %, grade 2 PanNETs have a Ki-67 labeling index of 3–20 %, and grade 3 PanNETs (designated neuroendocrine carcinomas) have a Ki-67 labeling index of more than 20 % [4].

Jiao et al. [10] sought to elucidate the genetics of PanNETs. They sequenced the exomes of ten nonfamilial PanNETs and validated their findings in a series of 58 independent surgically resected PanNETs. They detected a mean of 16 mutations per tumor, and the genes affected included *MEN1*, *DAXX*, *ATRX*, and mechanistic target of rapamycin (mTOR) pathway genes (*TSC2*, *PTEN*, and *PIK3CA*). Intragenic mutations in *MEN1* were most frequent and were detected in 44 % of the PanNETs, and 70 % of the PanNETs had LOH in this region. *DAXX* (25 %) and *ATRX* (18 %) were also targeted in PanNETs [10]. *DAXX* and *ATRX* code for proteins that function in a chromatin remodeling complex. These proteins have a pivotal impact in telomere preservation by incorporating histone variant H3.3 at the telomeric ends of chromosomes [131, 132]. PanNETs with *DAXX*/*ATRX* mutations almost always have the "alternative lengthening of telomeres" (ALT+) phenotype [133, 134]. Other genes targeted in PanNETs include genes coding for members of the phosphatidylinositol 3′-kinase (PI3K)–AKT–mTOR pathway. In approximately 16 % of PanNETs, an alterations in *PTEN*, *TSC2*, or *PIK3CA* was found [10]. The PI3K–AKT–

mTOR pathway is an important regulator of energy metabolism, cell growth, and cell proliferation. The tumor suppressor gene *PTEN* encodes a phosphatase that inhibits an unrestrained activation of the mTOR pathway, therefore protecting a cell from uncontrolled proliferation [135]. *PIK3CA*, on the other hand, is thought be involved in the tumorigenesis of many forms of cancers by coordinating a wide range of cellular activities [136]. In a recent study, Ohki et al. [137] reported that PanNETs often show LOH in the *PHLDA3* gene, a genetic change which seems to be associated with a negative outcome and disease progression. Previously, it was shown that the gene product of *PHLDA3* acts as a p53-regulated tumor suppressor in PanNETs through an inactivation of the PI3K–AKT–mTOR pathway [138].

The genes targeted in PanNETs suggest a personalized approach to treatment [139, 140]. Since 16 % of PanNETs harbor mutations in genes involved in PI3K–Akt–mTOR signaling, drugs that target the mTOR pathway should be effective in PanNETs with one of these mutations. A variety of mTOR inhibitors have been developed, and everolimus has been successfully used to treat patients with advanced PanNETs; extending the relapse-free survival from 4.6 months with placebo to 11 months in the treated cohort [141–143]. Another promising therapeutic option may be the inhibition of the angiogenesis of the tumor. Here sunitinib, a receptor tyrosine kinase inhibitor, was approved by the Food and Drug Administration for treatment of advanced PanNETs after successful phase III trials [144].

Acinar cell carcinoma

Acinar cell carcinomas (ACCs) are rare but important tumors. The outcome for patients with an ACC is almost as unfavorable as it is for patients with a PDA, and some (15 %) patients develop a dramatic syndrome of metastatic fat necrosis caused by lipase release from the tumor. Grossly, ACCs are often large (up to 10 cm) and relatively soft compared with PDAs [4]. Histologically, they have very little stroma, and the neoplastic cells form small acinar structures or sheets [15, 17]. The nuclei of the neoplastic cells often have single prominent nucleoli.

In 2014 Jiao et al. [11] sequenced the exomes of pancreatic neoplasms with acinar differentiation, and they found that ACCs have dramatic chromosomal instability and genetic heterogeneity. They detected an average of 64 somatic mutations per neoplasm (and this is not considering three outliers), which is higher than for PDAs. Genes implicated in other pancreatic neoplasms were altered in some of the ACCs, including *SMAD4* mutations in 25 %, *TP53* mutations in 15 %, and *GNAS* mutations in 10 %, as well as mutations in *RNF43* and *MEN1* (4 %) [11]. A small number of patients harbored somatic mutations in genes associated with familial pancreatic cancer syndromes. More recent genomic profiling of a large number of ACCs revealed recurrent and potentially actionable genomic alterations, including *BRAF* and *RAF1* fusions, and mutually exclusive DNA repair deficiencies [145].

Marked chromosomal instability may explain the general resistance of most ACCs to traditional systemic therapy regimens [11, 17]. In spite of a vast genetic heterogeneity in ACC, the whole-exome sequencing did identify genetic mutations in 40 % of the cancers that might be amenable for targeted therapies. These include, for example, *BRCA2*, *PALB2*, *ATM*, *BAP1*, *BRAF*, and *JAK1* mutations [11]. In particular, ACCs with an *ATM* mutation might be predicted to respond to poly(ADP-ribose) polymerase inhibitors and inhibitors of DNA-dependent protein kinase catalytic subunit [63, 64]. Novel strategies may also be use to try to treat RAF-gene-fusion-positive ACC with mitogen-activated protein/extracellular-signal-regulated kinase kinase inhibitors [145] as previously described in melanomas with *BRAF* fusions [146].

Pancreatoblastoma

Pancreatoblastomas often arise in the first decade of life, although cases in adults have been described [147]. Pancreatoblastomas have been reported in newborn children with the Beckwith–Wiedemann syndrome. Pancreatoblastomas are aggressive neoplasms with a median overall survival of only 15 months [148]. Histologically, pancreatoblastomas can show multiple directions of differentiation, including acinar, ductal, mesenchymal, neuroendocrine, and primitive embryonic differentiation [4]. By definition, an acinar component is present [147], as are characteristic squamous nests [149].

The hallmark DNA alteration in pancreatoblastomas is LOH at a highly imprinted region on chromosome arm 11p [149]. Most pancreatoblastomas harbor mutations in the Wnt signaling pathway, including inactivating mutations in *APC* and activating mutations in *CTNNB1* [149]. The genetic mutations frequently seen in PDA are rare (*KRAS*) or absent (*TP53*, *CDKN2A*, or *SMAD4*) [150]. Pancreatoblastomas may overlap in some morphological, immunohistochemical, and clinical features with ACCs. In contrast microsatellite instability is very uncommon in pancreatoblastomas [148, 149].

Owing to the rareness of pancreatoblastomas, no single study could reliably investigate a relation between genetic alternations and factors such as patient survival. No targeted therapies have been shown to be effective to date. Various adjuvant chemotherapy regimens have been

applied, and ambiguous results have been published as case reports [151]. As for most pancreatic malignancies, surgical resection is still the gold standard.

Conclusions

All of the major tumor types that arise in the pancreas have been sequenced in recent years. These sequencing studies have provided insight into key mechanisms of pathogenesis and have revealed a number of novel approaches to improving patient care. There are multiple challenges before personalized management based on the genetic signatures of a patient's tumor will become common practice, but we believe that hope is on the horizon. Well-organized work at multi-institutional and international levels will decrease the "bench-to-bedside" time to clinically use the valuable new data as soon as possible.

Acknowledgments This work was supported by a BONFOR research grant (O-1120051) provided to Hanno Matthaei by the University Medical Center, University of Bonn, Bonn, Germany, and by National Institutes of Health grant CA62924.

Conflict of interest Ralph H. Hruban receives royalty payments from Myriad Genetics for the invention linking PALB2 to familial pancreatic cancer. This is managed by Johns Hopkins University.

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