Molecular Pathology of Pancreatic Cancer Precursor Lesions

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Abstract Pancreatic cancer is the fourth leading cause of cancer-related death in the USA. Each year about 44,000 patients are newly diagnosed with pancreatic cancer in the USA. Most of these patients present with advanced disease and have a very poor prognosis.

Given this dismal prognosis, the challenge is to identify pancreatic cancer in an early stage or, better, patients at risk for pancreatic cancer before an incurable invasive carcinoma has developed. Several distinctive precursor lesions of pancreatic cancer are now known, which theoretically allows for detection of patients at risk of developing pancreatic cancer. These precursor lesions are the microscopic pancreatic intraepithelial neoplasia (PanIN) and the macroscopic cystic precursor lesions intraductal papillary mucinous neoplasia (IPMN), intraductal tubulopapillary neoplasm (ITPN), and mucinous cystic neoplasia/mucinous cystadenoma (MCN).

Insight in the molecular biology of pancreatic adenocarcinoma and these precursor lesions has substantially increased during the past decades. Accurate understanding of the successive molecular genetic alterations in these lesions may eventually lead to biomarkers that can predict biological behavior and guide treatment of patients at risk of invasive pancreatic cancer. This chapter reviews the clinical, diagnostic, and molecular genetic aspects of these pancreatic cancer precursor lesions.

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the USA. In 2012, an estimated 44,000 patients are diagnosed with pancreatic cancer and about 37,000 patients will die of this disease (Siegel et al. 2012). Worldwide,

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approximately 277,000 new patients are diagnosed with pancreatic cancer each year (Maisonneuve and Lowenfels 2010). Depending on stage, the median survival varies from 2.5 to 6.8 months in patients without surgical therapy to 4.5–24.1 months in patients receiving surgery. The overall 5-year survival rate is 3–5 %, whereas the 5-year survival rate is 15–30 % for patients with early-stage disease treated by curative resection. However, more than 70 % of patients present with stage III or IV disease and have a poor prognosis (Bilimoria et al. 2007; Hidalgo 2010; Hruban et al. 2010; Vincent et al. 2011; Siegel et al. 2012). The asymptomatic nature of early pancreatic cancer, the lack of sensitive and specific tools to diagnose early disease, and the lack of response to most forms of treatment all contribute to the high mortality rate of pancreatic cancer. Despite intensive research prognosis of invasive pancreatic cancer has barely improved in the past decades. Postoperative adjuvant chemo- and/or radiation therapies are only marginally effective and there is a high level of chemo- and radioresistance (Hidalgo 2010; Vincent et al. 2011). The most promising way to reduce pancreatic cancer mortality is therefore to identify and treat patients at risk for pancreatic cancer before an incurable invasive carcinoma develops (Maitra et al. 2005; Hruban et al. 2007b).

Although still evolving, knowledge of pancreatic tumorigenesis has significantly improved during the past decades and it is now clear that invasive pancreatic cancer develops from several distinctive precursor lesions. The most common precursor lesion is the microscopic pancreatic intraepithelial neoplasia (PanIN). Less common are the macroscopic cystic precursor lesions intraductal papillary mucinous neoplasm (IPMN), intraductal tubulopapillary neoplasm (ITPN), and mucinous cystic neoplasm/mucinous cystadenoma (MCN) (Maitra et al. 2003; Hruban et al. 2007b, 2010). Detection and treatment of these precursor lesions and thereby preventing development of full-blown invasive pancreatic adenocarcinoma is an important strategy to reduce pancreatic cancer mortality. However, definitive preoperative diagnosis and prediction of biological behavior of these lesions is often difficult but essential for further treatment decisions. Accurate knowledge of molecular genetic alterations in these lesions may lead to biomarkers that can differentiate between and predict biological behavior of these lesions, and thus guide further treatment of patients with these lesions. In this chapter, clinical, histopathological, and molecular aspects of the different pancreatic cancer precursor lesions are discussed.

Pancreatic Intraepithelial Neoplasia

Definition, Clinical Appearance, and Histopathology

PanIN is the most common precursor lesion of conventional pancreatic ductal adenocarcinoma. PanIN is a microscopic precursor lesion arising in small caliber pancreatic ducts and has been recognized for more than a century (Hruban et al. 2004, 2010; Maitra et al. 2005). PanINs occur most frequently in the head of the pancreas

and to a lesser extent in the body or tail. The overall prevalence of PanIN is estimated to be about 20 % and the incidence increases with age, present in 6.7 % of people ≤50 years of age, 28 % in people between 50 and 65 years of age, and 37 % of people ≥65 years of age (Kozuka et al. 1979; de Wilde et al. 2012). In addition, PanIN lesions occur more often in pancreata harboring adenocarcinoma (82 %) than pancreata with pancreatitis (60 %) or normal pancreata (16 %) (Andea et al. 2003; Hruban et al. 2008). Moreover, multiple PanINs of all grades are frequently observed in individuals with inherited susceptibility to pancreatic cancer (Shi et al. 2009).

PanINs occur in smaller pancreatic ducts and are less than 5 mm in diameter which is in fact one of the features used to distinguish PanIN from IPMNs which are usually >1 cm diameter. PanINs are microscopic lesions and are not macroscopically detected (Hruban et al. 2004).

Histologically, PanINs are lined by columnar mucinous epithelium instead of the normal cuboidal pancreatic duct epithelium (Hruban et al. 2004). Most PanINs express MUC1, MUC5AC, and MUC6 suggesting gastric foveolar differentiation (Kim et al. 2002). MUC2 expression is not present in PanIN, a distinctive feature to differentiate it from IPMN (Hruban et al. 2004; Maitra et al. 2005).

PanINs are divided in three grades based on the degree of cytonuclear and architectural atypia (Fig. 1a–e) (Hruban et al. 2004, 2010). Low-grade or PanIN-1A lesions typically have flat epithelium consisting of columnar mucinous cells oriented perpendicularly to the basement membrane with basally oriented uniform round to oval nuclei and supranuclear mucin. PanIN-1B lesions have a (micro)papillary architecture, whereas PanIN-2 lesions show even more architectural complexity with pseudostratification, nuclear hyperchromasia, and beginning loss of nuclear polarity consistent with intermediate-grade dysplasia. PanIN-3, or high-grade dysplasia/carcinoma-in situ, is characterized by significant cytological atypia and includes complete loss of nuclear polarity, nuclear hyperchromasia, conspicuous nucleoli, and the presence of (atypical) mitotic figures. In addition, PanIN-3 is characterized by architectural changes including (micro)papillary epithelium and cribriform growth, and there is sometimes luminal necrosis (Hruban et al. 2004; de Wilde et al. 2012). Interestingly, PanINs are often surrounded by lobular parenchymal atrophy which can be detected by imaging techniques (e.g., endoscopic ultrasound) and may be used as a biomarker in a subset of patients with a high-risk pancreatic cancer (Meckler et al. 2001; Detlefsen et al. 2005; Brune et al. 2006).

Molecular Characteristics of PanIN

Molecular genetic alterations in PanIN confirm the stepwise progression from normal epithelium to low-grade, subsequent high-grade dysplasia and invasive carcinoma. A simplified model of this histologic–genetic progression is called the "PanINgram" and shows that accumulation of molecular alterations correlates with increasing grades of dysplasia (Hruban et al. 2000) (Fig. 2). Early genetic alterations that can initiate PanIN development are mainly found in the *KRAS* oncogene and

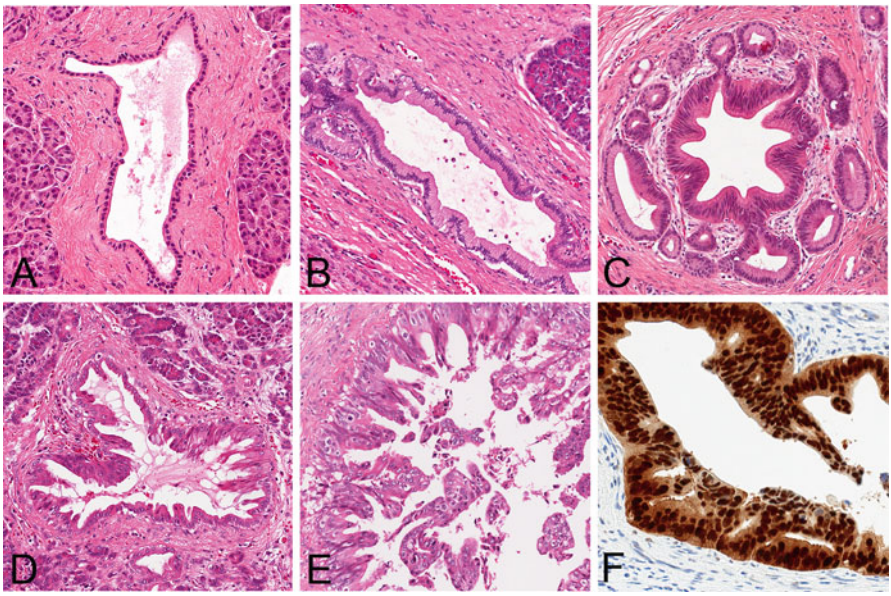

Fig. 1 Histology of PanIN lesions. (**a**) Normal pancreatic duct lined by cuboidal epithelium. (**b**) PanIN-1A. Pancreatic duct lined by flat epithelium consisting of columnar mucinous cells with basally oriented uniform round to oval nuclei and supranuclear mucin. (**c**) PanIN-1B. Pancreatic duct lined by epithelium consisting of columnar mucinous cells and micropapillary architecture. (**d**) PanIN-2. Pancreatic duct lined by columnar cells with nuclear hyperchromasia, pseudostratification, and papillary architecture. (**e**) PanIN-3. Pancreatic duct lined by columnar cells with severe cytonuclear pleiomorphism, loss of nuclear polarity, and complex architecture with (micro)papillary epithelium and cribriform growth pattern. (**f**) p53 immunohistochemistry in a PanIN-3 lesion showing accumulation of the p53 protein consistent with *TP53* mutation

less frequently in *p16/CDKN2A*, *GNAS*, or *BRAF* (Kanda et al. 2012). In addition, telomere shortening is found in >90 % of PanIN lesions of all grades but this may rather be a consequence of activation of oncogene stress-induced senescence programs than an initiator of PanIN (van Heek et al. 2002; Kanda et al. 2012).

Previous studies have shown an increase of *KRAS* mutations correlating with neoplastic progression (i.e., 36 % in PanIN-1A, 44 % in PanIN-1B, 87 % in PanIN-2/3, and >90 % in PDAC), suggesting that *KRAS* mutation is more involved after PanIN initiation than responsible for initiation of tumorigenesis (Moskaluk et al. 1997; Hruban et al. 2000; Lohr et al. 2005). However, a recent study using more sensitive mutation detection methods identified *KRAS* mutations in >90 % of both low- and high-grade PanIN lesions. Interestingly, the average concentration of mutant *KRAS* alleles increased in subsequent PanIN grades, which is consistent with a gradual expansion of the *KRAS*-mutant clone during progression of PanIN. This finding can also explain the lower prevalence of *KRAS* mutations in low-grade lesions found in prior studies that used less sensitive sequence methods (Kanda et al. 2012). *BRAF* mutations were only found in a small subset of *KRAS*-wild-type

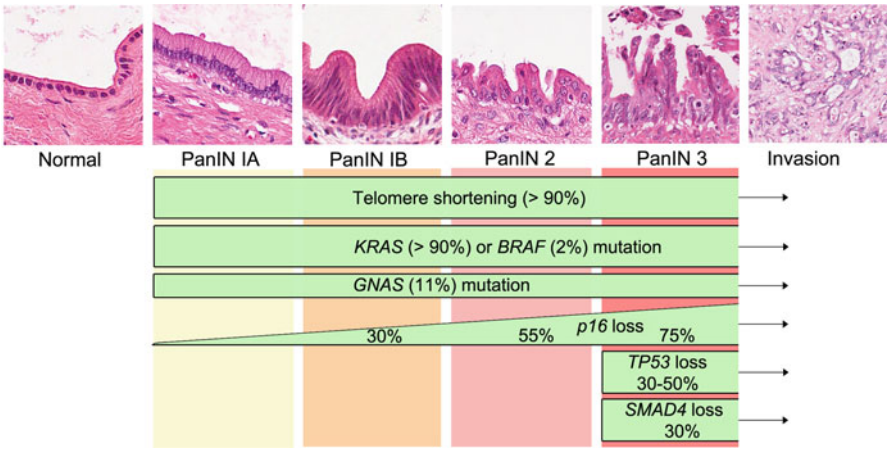

Fig. 2 Progression model of pancreatic cancer. Each step in the progression from normal epithelium to low-grade PanIN, subsequent high-grade PanIN and eventually invasive adenocarcinoma is accompanied by additional genetic alterations. More than 99 % of the earliest stage PanIN-1 lesions contain mutations in *KRAS*, *p16/CDKN2A*, *GNAS*, or *BRAF*

PanINs and pancreatic cancers (Jones et al. 2008; Kanda et al. 2012). A subset of PanINs (~11 %) harbored a *GNAS* mutation, an oncogene that was recently discovered to be mutated in ~60 % of IPMNs (Wu et al. 2011b; Kanda et al. 2012). Interestingly, in some PanINs a *GNAS* mutation was the only identified mutation and in other PanINs the *GNAS* mutation seemed to have occurred earlier than the *KRAS* mutation. In total, >99 % of the earliest stage PanIN-1 lesions contain mutations in *KRAS*, *p16/CDKN2A*, *GNAS*, or *BRAF*, indicating that somatic mutations are required for the early development of all PanIN lesions which can be used as an argument against the hypothesis that PanINs begin as metaplasia. However, it also appears that *KRAS* mutation alone provides only a modest selective advantage over neighboring cells and that additional genetic or epigenetic events are needed for neoplastic progression (Kanda et al. 2012).

p16/CDKN2A mutation is a relatively early event in PanIN and may be the additional genetic event needed for PanINs with *KRAS* mutation to progress (Hruban et al. 2000; Kanda et al. 2012). *p16/CDKN2A* mutations were found in 11 % of low-grade (i.e., PanIN-1/2) lesions and were more often found in PanIN lesions without a *KRAS* mutation (Kanda et al. 2012). Previously, loss of p16/CDKN2 protein expression was already shown to increase with PanIN grade (i.e., p16/CDKN2 protein expression was lost in 30 % of PanIN-1A/B, 55 % of PanIN-2, and 71 % of PanIN-3 lesions) (Wilentz et al. 1998). Loss of function occurs through homozygous deletions, mutation and loss of heterozygosity (LOH) or promotor hypermethylation, each of these mechanisms accounting for approximately one-third of p16 silencing (Schutte et al. 1997). In addition, overexpression of cyclin D1 is noted in 29 % of PanIN-2 and 57 % of PanIN-3 lesions (Maitra et al. 2003).

Inactivation of p53 through intragenic mutation and LOH of the *TP53* gene is a late event in pancreatic tumorigenesis and appears to be limited to PanIN-3 and invasive pancreatic cancer where it is found in 30–50 % of cases (Fig. 1f) (Hruban et al. 2000; Luttges et al. 2001). Inactivation of the tumor suppressor gene *SMAD4* (*DPC4*) is found in approximately 30 % of PanIN-3 and 50 % of PDAC cases and is therefore another late event in pancreatic tumorigenesis (Hruban et al. 2000; Wilentz et al. 2000). Loss of the wild-type *BRCA2* allele has been found in PanIN-3 in a patient with a germline *BRCA2* mutation (Goggins et al. 2000). In addition to mutational inactivation of tumor suppressor genes, epigenetic inactivation by hypermethylation of tumor suppressor genes is a frequent event early in PanIN development and increases with increasing grade of dysplasia (Sato et al. 2008). Also aberrant overexpression of oncogenes such as components of EGFR, Notch and Hedgehog signaling occurs in PanIN and is associated with invasive adenocarcinomas (Day et al. 1996; Miyamoto et al. 2003; Thayer et al. 2003). Lastly, PanIN lesions show aberrant expression of many microRNAs, which is likely to be important in pancreatic carcinogenesis. Interestingly, expression of some microRNAs, such as miR-196b, appears specific for high-grade lesions (PanIN-3 and PDAC) and may therefore be useful as diagnostic markers (Yu et al. 2012).

Intraductal Papillary Mucinous Neoplasm

Definition, Clinical Appearance, and Histopathology

IPMN is a macroscopically visible cystic mucin producing tumor arising in a main pancreatic duct or one of its branches. IPMNs are quite common lesions and account for approximately 3 % of exocrine pancreatic neoplasms and for 20 % of cystic pancreatic neoplasms (Kosmahl et al. 2004; Adsay et al. 2010; Shi and Hruban 2012). Most IPMNs are found in patients between 60 and 70 years of age and the mean age of diagnosis varies from 63 to 66 years (Fukushima et al. 1997; Chari et al. 2002). Patients with an IPMN with an associated invasive carcinoma tend to be 3–5 years older than patients with an IPMN without invasive carcinoma. IPMNs are slightly more common in males (~60 % of cases) than females (Shi and Hruban 2012).

IPMNs have been reported in individuals with a family history of pancreatic cancer and in patients with Peutz-Jeghers syndrome (Sato et al. 2001; Canto et al. 2012).

IPMNs are divided in main duct, branch duct, and combined or mixed type, which is mainly based on its appearance on imaging and to a lesser extent on gross pathologic examination (Crippa et al. 2010; Shi and Hruban 2012). Main-duct IPMNs usually occur in the pancreatic head and often produce copious thick mucin which gives rise to a (diffusely) dilated main pancreatic duct and associated symptoms. These symptoms include abdominal or back pain, nausea, vomiting, weight loss, or recurrent episodes of pancreatitis. Approximately 60 % of main-duct IPMNs harbor high-grade dysplasia and associated invasive carcinoma is found in about

45 % of main-duct IPMNs (Salvia et al. 2004; Kawamoto et al. 2006; Crippa et al. 2010; Shi and Hruban 2012). Branch-duct IPMNs occur mainly in the head and uncinate process and are often multicystic grapelike structures with thin cyst walls involving side branches of the main pancreatic duct. Branch-duct IPMNs are usually asymptomatic and are therefore often incidental findings on imaging studies for other medical reasons. One study found an unsuspected pancreatic cyst (most of which were probably IPMN) in 2.6 % of asymptomatic patients and this number increased with age (Laffan et al. 2008). Most branch-duct IPMNs are low-grade lesions with an indolent behavior, although high-grade dysplasia and invasive carcinoma are found in about 25 and 20 % of branch-duct IPMNs meeting the "Sendai criteria", respectively (Terris et al. 2000; Kawamoto et al. 2006; Rodriguez et al. 2007; Crippa et al. 2010; Shi and Hruban 2012). Mixed-type IPMNs involve both the main and branch ducts. Both main and branch-duct IPMNs can be associated with atrophy of the adjacent pancreatic parenchyma.

Prognosis of IPMN is mainly determined by the presence or absence of associated invasive carcinoma. The 5-year survival rate for patients with an IPMN without an associated invasive carcinoma is 90–100 %, whereas this is about 30–60 % for patients with an IPMN with associated invasive carcinoma (Chari et al. 2002; Maire et al. 2002; Raimondo et al. 2002; D'Angelica et al. 2004; Salvia et al. 2004; Nara et al. 2008; Crippa et al. 2010). Invasive carcinoma in IPMN has a better prognosis than primary PDAC which maybe mainly due to the lower stage at which IPMN-associated adenocarcinoma is usually diagnosed (Poultsides et al. 2010).

The "Sendai criteria" are international consensus guidelines for the management of IPMNs (Tanaka et al. 2006). These criteria advise surgical resection of all main-duct IPMNs and resection of branch-duct IPMNs that are symptomatic, >3 cm, harbor a mural nodule, or are associated with significant dilatation of the pancreatic duct. In addition, lesions should be resected if cytology shows severe cytonuclear atypia (Tanaka et al. 2006; Shi and Hruban 2012).

Grossly, IPMNs can be lined by flat epithelium (ductectatic pattern) or by epithelium with papillary projections (villous growth). By definition, IPMNs are >0.5 cm and most IPMNs are >1 cm, with the size varying from 1 cm to the entire pancreas (Hruban et al. 2007a). Careful gross examination to differentiate between main-duct and branch-duct IPMNs is important in view of the higher risk of high-grade dysplasia and invasive carcinoma in the main-duct type (Crippa et al. 2010). Because invasive carcinoma can be very focal within an IPMN, these lesions should be thoroughly sampled for histological examination. Gross features suggestive of invasive adenocarcinoma are irregular heterogeneous thickening of cyst walls, fibrotic foci, and the presence of solid nodules (Shi and Hruban 2012; de Wilde et al. 2012).

Microscopically, IPMNs are classified according to the degree of dysplasia and the direction of differentiation of the neoplastic epithelium, which can be intestinal-, pancreatobiliary-, gastric-, or oncocytic type. Because multiple histological types of epithelium can often be found in an IPMN, the dominant component defines the subtype (Adsay et al. 2010; Shi and Hruban 2012). It is important to recognize the histological subtype of an IPMN because this appears to be an independent predictor of patient prognosis (Furukawa et al. 2011). Moreover some IPMN subtypes

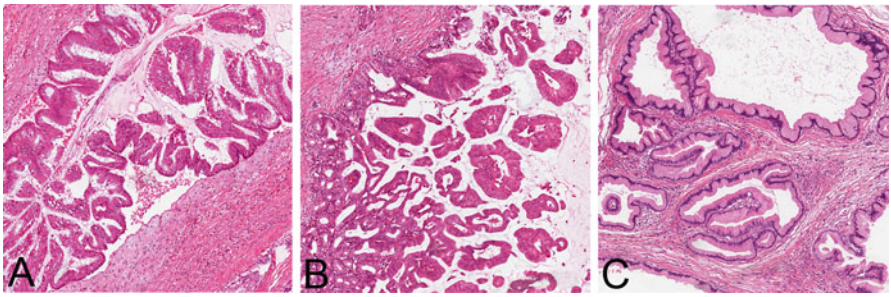

Fig. 3 Histologic subtypes of IPMN. (**a**) Intestinal-type IPMN with intermediate-grade dysplasia lined by columnar mucin-producing cells with cigar-shaped pseudostratified nuclei and scattered goblet-like cells. (**b**) Pancreatobiliary IPMN with high-grade dysplasia lined by cuboidal cells with round hyperchromatic nuclei with prominent nucleoli, cytoplasm containing less mucin than in the intestinal-type IPMN and more complex papillary architecture. (**c**) Gastric-type IPMN with low-grade dysplasia lined by a single layer of cells with basally oriented small nuclei and abundant apical cytoplasmic mucin resembling gastric foveolar epithelium

are associated with distinct types of invasive carcinoma with varying prognosis. For instance, colloid carcinoma (associated with intestinal-type IPMN) and oncocytic carcinoma (associated oncocytic-type IPMN) have better a prognosis than the tubular type carcinoma (associated with gastric-, pancreatobiliary-, or intestinal-type IPMN) which has a course similar as PDAC (Mino-Kenudson et al. 2011).

Main-duct IPMNs are usually lined by intestinal- and pancreatobiliary-type epithelium, whereas branch-duct IPMNs are typically lined by gastric-type epithelium (Adsay et al. 2010). The intestinal-type IPMN (Fig. 3a) shows long papillae lined by columnar mucin-producing cells with cigar-shaped pseudostratified nuclei and basophilic cytoplasm, resembling a villous adenoma of the colon. Often goblet-like cells are encountered. Intermediate to high-grade dysplasia is usually seen in this type (Adsay et al. 2010; Shi and Hruban 2012). The neoplastic cells of intestinal-type IPMN do not express MUC1, weakly express MUC6 and strongly express MUC5A, MUC2, and CDX2 (Adsay et al. 2004; Basturk et al. 2010).

Pancreatobiliary IPMNs (Fig. 3b) are lined by cuboidal cells with round hyperchromatic nuclei with prominent nucleoli and cytoplasm containing less mucin than in the intestinal-type IPMN. These IPMNs are further characterized by more complex thin papillae with branching and cribriform growth and therefore tend to be high-grade lesions (Adsay et al. 2010; Shi and Hruban 2012). Pancreatobiliary IPMNs have an immunohistochemical expression pattern similar to that of PanIN and usually express MUC1 and MUC5A, sometimes MUC6 but not MUC2 (Adsay et al. 2004; Ban et al. 2006; Basturk et al. 2010).

Gastric foveolar-type IPMNs (Fig. 3c) are lined by cells with abundant apical cytoplasmic mucin and basally oriented small nuclei, resembling gastric foveolar epithelium (Furukawa et al. 2005). These IPMNs are usually lined by a single flat layer of epithelium lining dilated ducts. Papillary projections are uncommon in these lesions and there is mostly low-grade dysplasia. The neoplastic cells often extend along the pancreatic ducts into adjacent pancreatic tissue resulting in acinar-ductal metaplasia, acinar atrophy, and fibrosis. Gastric foveolar-type IPMNs strongly

express gastric-type mucins MUC5A and MUC6 but not MUC1 and MUC2 (Furukawa et al. 2005; Ban et al. 2006; Basturk et al. 2010).

Oncocytic-type IPMNs, also known as intraductal oncocytic papillary neoplasms (IOPNs), are composed of cells with abundant granular eosinophilic cytoplasm due to accumulation of mitochondria. The architecture of IOPNs is very complex with arborizing papillae, cribriform growth, and solid nests, growing into the lumen of the dilated duct. Intraepithelial and intracellular mucin is frequently present and scattered goblet cells can be observed. The stratified oncocytic neoplastic cells have abundant eosinophilic granular cytoplasm and large round uniform nuclei. Because of the marked cytonuclear and architectural atypia most IOPNs are classified as having high-grade dysplasia (Adsay et al. 2010; Shi and Hruban 2012). Sometimes it can be difficult to appreciate the intraductal nature of this lesion. IOPNs express MUC1 and MUC6, whereas expression of CDX2, MUC2, and MUC5A is restricted to the goblet cells (Basturk et al. 2010; Liszka et al. 2010; Shi and Hruban 2012). Invasive carcinoma arising from IOPN is a relatively well-circumscribed tumor composed of cells with the characteristic oncocytic features growing in the periductal stroma as small solid nests and glands (Patel et al. 2002). Although only few cases have been described, genetic changes seem distinct from typical pancreatic adenocarcinoma which may explain the indolent clinical behavior of IOPN (Patel et al. 2002; Xiao et al. 2011).

In the fourth edition of WHO classification of tumors of the digestive system, ITPN is recognized as a subtype of the intraductal pancreatic neoplasms and is therefore discussed separately (Adsay et al. 2010).

Molecular Characteristics of IPMN

A recent study investigating eight IPMNs by whole-exome sequencing showed that IPMNs contain an average of 26 somatic mutations (Wu et al. 2011a). The most common genetic alteration in IPMN is mutation of codon 12 and to a lesser extent codon 13 of the *KRAS* gene which is found in >80 % of IPMNs (Wu et al. 2011b). Previous studies have shown that the prevalence of *KRAS* mutation increases with increasing grade of dysplasia (Sessa et al. 1994; Satoh et al. 1996; Schonleben et al. 2007). In addition, this study identified mutations in *GNAS*, a well-known oncogene functioning as a signal transducer between hormonal receptors and adenylyl cyclase, to be present in 66 % of IPMNs. Interestingly, it was suggested that *GNAS* mutations are specific for IPMN since mutations in this gene were not found in other types of cystic pancreatic neoplasms (i.e., serous cystadenoma, MCN, and solid pseudopapillary neoplasm) or in invasive adenocarcinomas not associated with IPMNs, whereas *GNAS* mutations were found in adenocarcinomas developing in association with IPMNs (Wu et al. 2011b).

Taken together, about 50 % of IPMNs harbor both a *GNAS* and a *KRAS* mutation, whereas either a *KRAS* or a *GNAS* mutation can be found in 96 % of IPMNs. Because *KRAS* and *GNAS* gene mutations can be detected in cyst fluid, mutation analysis of these genes in cyst fluid aspirates may prove to be a valuable asset for

preoperative diagnostic workup of IPMNs (Wu et al. 2011b). Importantly, both *KRAS* and *GNAS* mutations are restricted to specific codons (*GNAS* codon 201 and *KRAS* codon 12 or 13) which makes analysis of these molecular alterations relatively straight forward and suitable for routine diagnostics (Wu et al. 2011b).

Different subtypes of IPMN appear to follow different pathways of neoplastic progression. For instance, gastric- and pancreatobiliary-type IPMNs show higher rates of *KRAS* mutation than intestinal-type IPMNs, whereas *GNAS* mutations are most prevalent in the intestinal-type IPMNs and absent in IOPN (Mohri et al. 2012; Wu et al. 2011b). In addition, *KRAS* mutation and p53 overexpression are less prevalent in IOPN than in pancreatobiliary-type IPMN (17 % vs. 58 % and 11 % vs. 58 %, respectively) (Xiao et al. 2011). Whole-exome sequencing also identified *RNF43*, encoding a protein with intrinsic E3 ubiquitin ligase activity, as a gene that is frequently mutated in IPMN (6 of 8 cases). Although *RNF43* mutations were not specific for IPMN, since mutation of this gene was also found in a subset of MCNs, this finding highlights the importance of inactivation of ubiquitin ligase in cystic pancreatic tumors (Wu et al. 2011a).

The mTOR pathway may be involved in IPMN tumorigenesis via loss of *LKB1/STK11* which is a serine threonine kinase upstream of mTOR. *LKB1/STK11* loss is found in IPMNs arising in patients with Peutz-Jeghers syndrome (caused by germline *LKB1/STK11* mutation) and also in about 25 % of sporadic IPMNs (Su et al. 1999; Sato et al. 2001). In addition, *PIK3CA*, which also encodes a protein upstream of AKT-mTOR, is mutated in a subset of IPMNs (~10 %), but *PIK3CA* mutation may be more specific for ITPNs than for IPMNs (Schonleben et al. 2008b; Yamaguchi et al. 2011).

Other genetic alterations in IMPN are found with variable frequencies. *TP53* mutation represents a late event in neoplastic development of IPMN and is found in 0–50 % of IPMNs (Sessa et al. 1994; Kawahira et al. 2000; Sasaki et al. 2003; Xiao et al. 2011). Loss of *p16/CDKN2A* has been reported in 0–80 % of IPMNs and increases with grade of dysplasia (Biankin et al. 2002; Sasaki et al. 2003). SMAD4 is only rarely inactivated in noninvasive IPMN and protein expression is preserved in most IPMNs regardless of grade of dysplasia (Iacobuzio-Donahue et al. 2000a; Biankin et al. 2002). *APC* and *HER2* mutations are very rare in IPMN (Schonleben et al. 2008a; Schonleben et al. 2008b; Wu et al. 2011a; Xiao et al. 2011). Allelic loss of at least one chromosome region is found in most IPMNs (7 of 8) (Fritz et al. 2009). By array-CGH it has been shown that copy number alterations are frequently found in IPMNs with moderate- and high-grade dysplasia but not in IPMNs with low-grade dysplasia. Commonly lost regions were located on chromosomes 5q, 6q, 10q, 11q, 13q, 18q, and 22q (Fritz et al. 2009).

Gene expression analysis of IPMN has identified a number of genes that are associated with progression to invasive carcinoma, including *claudin 4*, *CXCR4*, *S100A4*, and *mesothelin*, which may serve as biomarkers to identify high-risk IPMNs (Sato et al. 2004; Habbe et al. 2009; Tsutsumi et al. 2011; Jury et al. 2012). Expression of *MSX-2* has been linked to neoplastic progression of branch-duct IPMN (Satoh et al. 2010). Overexpression of Sonic Hedgehog is an early event in the development of IPMN (Ohuchida et al. 2006). In addition, aberrant DNA

methylation occurs frequently in IPMNs and contributes to inactivation of tumor suppressor genes and neoplastic progression (Sato et al. 2002; Hong et al. 2008, 2012). Interestingly, methylation of specific genes, including *BNIP3*, *PTCHD2*, *SOX17*, *NXPH1*, and *EBF3*, may predict the presence of high-grade dysplasia in an IPMN (Hong et al. 2012). Also, overexpression of microRNAs, in particular miR-21 and miR-155, has been described in IPMN (Habbe et al. 2009). Lastly, telomere shortening has been shown in IPMN and the average telomere length decreases with tumor progression (Hashimoto et al. 2008).

Intraductal Tubulopapillary Neoplasm

Definition, Clinical Appearance, and Histopathology

ITPN is a recently described rare variant of an intraductal neoplasm of the pancreas accounting for <1 % of all exocrine pancreatic neoplasms and for 3 % of pancreatic intraductal neoplasms (Tajiri et al. 2005; Yamaguchi et al. 2009; Adsay et al. 2010). Limited data is available about prognosis for patients with ITPN, but 5-year survival is likely more than 30 %. No significant correlation between invasive growth and survival has been found which may be due to the microscopic nature of the invasion or because small foci of invasion may have been missed due to inadequate sampling (Adsay et al. 2010).

ITPN is a generally large (average size 6 cm; range 0.8–15.0 cm) macroscopically visible solid nodular tumor filling the dilated pancreatic duct. In contrast to IPMN, these tumors lack overt mucin production and have a predominantly tubular growth pattern although papillae can be found in some lesions (Suda et al. 1996; Yamaguchi et al. 2009, 2011). The tumor consists a proliferation of back-to-back acinar glands lined by cuboidal cells with modest amount of eosinophilic to amphophilic cytoplasm and round to oval moderately to marked atypical nuclei (Fig. 4). Typically ITPNs express cytokeratins 7 and 19 and MUC1. About 60 % of cases also express MUC6, whereas MUC2 and MUC5AC are not expressed, which can be helpful in distinguishing these lesion from IPMNs (Tajiri et al. 2005; Yamaguchi et al. 2009). There is homogenous high-grade dysplasia and complex architecture throughout the lesion and, in contrast to IPMNs, foci of necrosis are frequently encountered. In about 40 % of cases an associated invasive carcinoma is found (Suda et al. 1996; Yamaguchi et al. 2009).

Molecular Characteristics of ITPN

Few studies have investigated the molecular characteristics of ITPN. Abnormal expression of p53 and SMAD4 has been described in 1 case. No aberrant expression of β-catenin or mutations in *KRAS* of *BRAF* have been found (Yamaguchi et al.

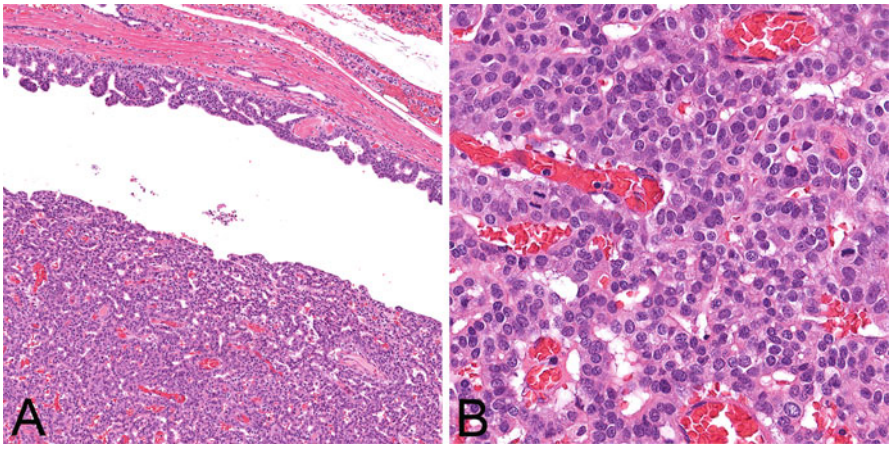

Fig. 4 Histologic appearance of ITPN. (**a**) Intraductal tubulopapillary neoplasm showing an intraductal proliferation of back-to-back acinar glands lined by cuboidal cells with marked cytonuclear pleiomorphism. (**b**) Detail of (**a**) showing proliferation of cuboidal cells with hyperchromatic anisomorphic nuclei and several mitoses

2009, 2011), whereas a *KRAS* mutation is found >80 % of IPMNs (Sarr et al. 2001; Crippa et al. 2008, 2010; Wu et al. 2011a). Interestingly, a recent study investigating molecular alterations in 11 ITPNs and 50 IPMNs found mutations in *PIK3CA* in a subset of ITPNs (3 of 11) but in none of the IPMNs. In addition, *PIK3CA* mutations were associated with strong expression of phosphorylated AKT. As previously reported, no *BRAF* of *KRAS* gene mutations were found in any of the ITPNs. These results suggest a role of the phosphatidylinositol 3-kinase pathway in ITPNs and the activated phosphatidylinositol 3-kinase pathway may therefore be a potential target for molecular diagnosis and therapy of ITPNs.

Mucinous Cystic Neoplasm

Definition, Clinical Appearance, and Histopathology

MCN of the pancreas is a macroscopically visible cystic neoplasm accounting for approximately 8 % of all resected cystic lesions of the pancreas (Kosmahl et al. 2004; Fukushima and Fukayama 2007; Zamboni et al. 2010). These lesions are most often found in the body and tail of the pancreas and, in contrast to IPMNs, usually do not communicate with the pancreatic duct system. Almost all MCNs occur in female patients with a female to male ratio of 20:1. However, male gender cannot be used to rule out the diagnosis since sporadic MCNs have been reported in males (Wouters et al. 1998). The mean age at diagnosis is between 40 and 50 years with a range of 14–95 years (Thompson et al. 1999; Wilentz et al. 1999; Zamboni et al.

1999; Fukushima and Fukayama 2007). On average, patients with an associated invasive carcinoma are 5–10 years older than patients with noninvasive MCN (Zamboni et al. 2010).

Clinical manifestations of MCN depend on the size of the lesion. Lesions smaller than <3 cm are often found incidentally in patients imaged for another indication. Larger lesions often give rise to nonspecific complaints such as abdominal discomfort and the sensation of a mass in the epigastric region. About one-third of resected MCNs have an associated invasive carcinoma, which usually resembles a common pancreatic ductal adenocarcinoma. However, the number of MCNs with associated adenocarcinoma may decrease since more MCNs are being detected incidentally in patients imaged for another reason (Wilentz et al. 1999; Zamboni et al. 1999, 2010; Tanaka et al. 2006; Fukushima and Fukayama 2007; Crippa et al. 2008; Yamao et al. 2011). Patients with a surgically resected noninvasive MCN have an excellent prognosis, but the 5-year survival rate for patients with an MCN with an associated invasive carcinoma is about 50–60 %. Since the invasive component can be very focal MCNs should undergo extensive histological examination before invasion is excluded (Wilentz et al. 1999; Zamboni et al. 1999; Fukushima and Fukayama 2007). In contrast to IPMNs, MCNs are almost always unifocal and after surgery for an MCN there is minimal risk of metachronous disease (de Wilde et al. 2012).

Macroscopically, MCNs are single spherical lesions with a mean diameter of 6–10 cm (range 2–35 cm) and a fibrous pseudocapsule. The tumor can be unilocular or multilocular with cysts varying from millimeters to several centimeters containing thick mucinous and/or hemorrhagic or necrotic material. Low-grade lesions usually have a smooth and glistering internal surface, whereas high-grade lesions often show papillary projections. MCNs with an associated invasive carcinoma are often large and multilocular and contain papillary projections or mural nodules (Zamboni et al. 1999; Fukushima and Fukayama 2007).

Histologically, MCNs are defined by the presence of distinctive ovarian-type stroma consisting of densely packed spindle cells with round to elongated nuclei and a small amount of cytoplasm expressing inhibin, estrogen and progesterone receptors, as well as vimentin, smooth-muscle actin, and desmin (Fig. 5) (Fukushima and Mukai 1997; Ridder et al. 1998; Thompson et al. 1999; Zamboni et al. 1999; Tanaka et al. 2006). In some lesions it may be difficult to identify the ovarian-type stroma since the stroma may become fibrotic and hypocellular and some areas can resemble corpora albicantia (Fukushima and Fukayama 2007; Zamboni et al. 2010).

The epithelium overlying the ovarian-type stroma and lining the cyst consists of mucin-producing tall columnar epithelial cells that can have pseudopyloric, gastric-foveolar, small- or large-intestinal differentiation. Rarely squamous differentiation is noted (Zamboni et al. 2010). The columnar epithelial cells express cytokeratins 7, 8, 18, and 19, the gastric-type mucin MUC5A, and pancreatic-type mucin DUPAN-2 and CA19-9. Scattered goblet-like cells express the intestinal mucin MUC2. MUC1 expression is observed in most MCNs with invasive ductal adenocarcinoma (Luttges et al. 2002). Within a single MCN the degree of epithelial atypia can vary greatly and change abruptly from minimal to severe dysplasia or even focal invasive growth. MCNs should therefore be extensively sampled for histologic examination before

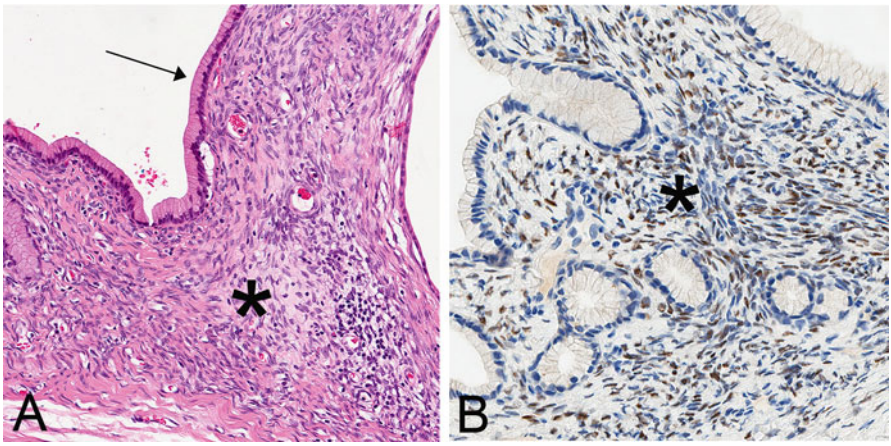

Fig. 5 Histologic appearance of MCN. (**a**) Mucinous cystic neoplasm showing ovarian type stroma (*asterisk*) and lining by mucin-producing tall columnar epithelial with low-grade dysplasia (*arrow*). (**b**) Estrogen receptor expression in stromal cells (*brown staining*) (*asterisk*)

excluding invasive growth. MCNs are categorized based on the highest degree of architectural and cytonuclear atypia present, as MCN with either low-grade, intermediate-grade, or high-grade dysplasia (Zamboni et al. 2010).

Two theories about pathogenesis of MCN prevail in the literature. One hypothesis argues that MCNs are a result of ectopic gonadal mesenchyme that may be incorporated in the pancreas during the fourth and fifth weeks of embryogenesis as a result of the close proximity of the left primordial gonad to the dorsal pancreatic anlage which gives rise to the body and tail of the pancreas (Zamboni et al. 1999; Erdogan et al. 2006). However, this theory does not explain the rare occurrence of MCNs in male patients. An alternative theory suggests that neoplastic epithelial cells of MCNs induce ovarian stromal differentiation in cells that are normally present in the pancreas (Zamboni et al. 2010).

Molecular Characteristics of MCN

A recent study investigated genetic alterations in MCN by whole-exome sequencing and found that MCNs contain an average of 16 somatic mutations and relatively few allelic losses (Wu et al. 2011a). *KRAS* is the most frequently mutated gene in MCN and correlates with the degree of neoplastic progression. *KRAS* mutations have been found in 26 % (7/27) of MCNs with low-grade dysplasia, 38 % (5/13) of MCNs with intermediate-grade dysplasia, and 89 % (8/9) of MCNs with high-grade dysplasia or carcinoma (Jimenez et al. 1999). *p53* mutation appears to be a relatively late event occurring only in areas with severe dysplasia or carcinoma (Jimenez et al. 1999).

A newly discovered and relatively frequently mutated gene in MCN is *RNF43* which was mutated in three of eight MCNs and encodes a protein with intrinsic E3 ubiquitin ligase activity (Wu et al. 2011a). Allelic loss at 3p25, the chromosomal location of *VHL* gene, has been reported in 17 % (2/12) of MCNs (Kim et al. 2003). In addition, loss of SMAD4 and p16/CDKN2A expressions is found in lesions with associated invasive carcinoma (Iacobuzio-Donahue et al. 2000b). Hypermethylation of *p14* and *p16* has been reported in about 15 % of benign or borderline MCNs (Kim et al. 2003).

Global gene expression profiling identified a number of genes that are upregulated in the epithelium of MCNs, including *S100P*, *PSCA*, *c-myc*, *STK6/STK15*, *cathepsin E*, *TCF4*, and *pepsinogen C*. In addition, activation of the Notch pathway was shown in the epithelial component by the demonstration of overexpression of Jagged1 and the downstream Notch pathway member Hes1. Overexpression of steroidogenic acute regulatory protein (*STAR*) and estrogen receptor 1 (*ESR1*) occurs in the stroma (Fukushima et al. 2004).

Conclusions

Molecular genetic alterations in pancreatic cancer have largely been unraveled in the past decade and knowledge about pancreatic cancer precursor lesions has substantially grown. Recently, some important steps have been made in the molecular characterization of pancreatic cancer precursor lesions which may ultimately prove to be useful in diagnostic workup of patients with these lesions and may lead to new targets for therapy.

All pancreatic cancer precursor lesions share a high frequency of somatic mutation of the *KRAS* oncogene. In PanIN, it was recently shown that somatic mutations in *KRAS* or *GNAS* are already present in virtually all of the earliest PanIN lesions. In addition, *GNAS* mutations are found in the majority of IPMNs but not in other cystic pancreatic tumors such as MCN or serous cystic adenoma. Furthermore, cystic pancreatic tumors appear to share defects in genes that play a role in the ubiquitin ligase complex. *RNF43* mutations were identified in IPMNs and MCNs but not in serous cystic adenomas or solid pseudopapillary neoplasms. Serous cystic adenomas are characterized by mutations in *VHL* and solid pseudopapillary neoplasms by mutations in *CTNNB1*.

Testing for these and other molecular genetic alterations in pancreatic cyst fluid can potentially be used to distinguish different cyst types on a molecular level and may lead to more accurate diagnosis (Wu et al. 2011a). However, further studies are needed to validate these findings and to test the potential of these genetic alterations for diagnostic use. In addition, it is important to develop biomarkers that can distinguish between high-grade or low-grade lesions and predict biological behavior.

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References

Adsay NV, Merati K, Basturk O et al (2004) Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: delineation of an "intestinal" pathway of carcinogenesis in the pancreas. Am J Surg Pathol 28(7):839–848

Adsay NV, Fukushima N, Furukawa H et al (2010) Intraductal neoplasms of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, Theise N (eds) WHO Classification of Tumours of the Digestive System World Health Organisation Classification of Tumors. IARC, Lyon, pp 304–313

Andea A, Sarkar F, Adsay VN (2003) Clinicopathological correlates of pancreatic intraepithelial neoplasia: a comparative analysis of 82 cases with and 152 cases without pancreatic ductal adenocarcinoma. Mod Pathol 16(10):996–1006

Ban S, Naitoh Y, Mino-Kenudson M et al (2006) Intraductal papillary mucinous neoplasm (IPMN) of the pancreas: its histopathologic difference between 2 major types. Am J Surg Pathol 30(12):1561–1569

Basturk O, Khayyata S, Klimstra DS et al (2010) Preferential expression of MUC6 in oncocytic and pancreatobiliary types of intraductal papillary neoplasms highlights a pyloropancreatic pathway, distinct from the intestinal pathway, in pancreatic carcinogenesis. Am J Surg Pathol 34(3):364–370

Biankin AV, Biankin SA, Kench JG et al (2002) Aberrant p16(INK4A) and DPC4/Smad4 expression in intraductal papillary mucinous tumours of the pancreas is associated with invasive ductal adenocarcinoma. Gut 50(6):861–868

Bilimoria KY, Bentrem DJ, Ko CY et al (2007) Validation of the 6th edition AJCC Pancreatic Cancer Staging System: report from the National Cancer Database. Cancer 110(4):738–744

Brune K, Abe T, Canto M et al (2006) Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. Am J Surg Pathol 30(9):1067–1076

Canto MI, Hruban RH, Fishman EK et al (2012) Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology 142(4):796–804

Chari ST, Yadav D, Smyrk TC et al (2002) Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. Gastroenterology 123(5):1500–1507

Crippa S, Salvia R, Warshaw AL et al (2008) Mucinous cystic neoplasm of the pancreas is not an aggressive entity: lessons from 163 resected patients. Ann Surg 247(4):571–579

Crippa S, Fernandez-Del Castillo C, Salvia R et al (2010) Mucin-producing neoplasms of the pancreas: an analysis of distinguishing clinical and epidemiologic characteristics. Clin Gastroenterol Hepatol 8(2):213–219

D'Angelica M, Brennan MF, Suriawinata AA et al (2004) Intraductal papillary mucinous neoplasms of the pancreas: an analysis of clinicopathologic features and outcome. Ann Surg 239(3):400–408

Day JD, Digiuseppe JA, Yeo C et al (1996) Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. Hum Pathol 27(2):119–124

de Wilde RF, Hruban RH, Maitra A et al (2012) Reporting precursors to invasive pancreatic cancer: pancreatic intraepithelial neoplasia, intraductal neoplasms and mucinous cystic neoplasm. Diagn Histopathol 18(1):17–30

Detlefsen S, Sipos B, Feyerabend B et al (2005) Pancreatic fibrosis associated with age and ductal papillary hyperplasia. Virchows Arch 447(5):800–805

Erdogan D, Lamers WH, Offerhaus GJ et al (2006) Cystadenomas with ovarian stroma in liver and pancreas: an evolving concept. Dig Surg 23(3):186–191

Fritz S, Fernandez-del Castillo C, Mino-Kenudson M et al (2009) Global genomic analysis of intraductal papillary mucinous neoplasms of the pancreas reveals significant molecular differences compared to ductal adenocarcinoma. Ann Surg 249(3):440–447

Fukushima N, Fukayama M (2007) Mucinous cystic neoplasms of the pancreas: pathology and molecular genetics. J Hepatobiliary Pancreat Surg 14(3):238–242

Fukushima N, Mukai K (1997) "Ovarian-type" stroma of pancreatic mucinous cystic tumor expresses smooth muscle phenotype. Pathol Int 47(11):806–808

Fukushima N, Mukai K, Kanai Y et al (1997) Intraductal papillary tumors and mucinous cystic tumors of the pancreas: clinicopathologic study of 38 cases. Hum Pathol 28(9):1010–1017

Fukushima N, Sato N, Prasad N et al (2004) Characterization of gene expression in mucinous cystic neoplasms of the pancreas using oligonucleotide microarrays. Oncogene 23(56): 9042–9051

Furukawa T, Kloppel G, Volkan Adsay N et al (2005) Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. Virchows Arch 447(5):794–799

Furukawa T, Hatori T, Fujita I et al (2011) Prognostic relevance of morphological types of intraductal papillary mucinous neoplasms of the pancreas. Gut 60(4):509–516

Goggins M, Hruban RH, Kern SE (2000) BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: evidence and implications. Am J Pathol 156(5):1767–1771

Habbe N, Koorstra JB, Mendell JT et al (2009) MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. Cancer Biol Ther 8(4):340–346

Hashimoto Y, Murakami Y, Uemura K et al (2008) Telomere shortening and telomerase expression during multistage carcinogenesis of intraductal papillary mucinous neoplasms of the pancreas. J Gastrointest Surg 12(1):17–28, discussion 28-9

Hidalgo M (2010) Pancreatic cancer. N Engl J Med 362(17):1605–1617

Hong SM, Kelly D, Griffith M et al (2008) Multiple genes are hypermethylated in intraductal papillary mucinous neoplasms of the pancreas. Mod Pathol 21(12):1499–1507

Hong SM, Omura N, Vincent A et al (2012) Genome-wide CpG island profiling of intraductal papillary mucinous neoplasms of the pancreas. Clin Cancer Res 18(3):700–712

Hruban RH, Goggins M, Parsons J et al (2000) Progression model for pancreatic cancer. Clin Cancer Res 6(8):2969–2972

Hruban RH, Takaori K, Klimstra DS et al (2004) An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol 28(8):977–987

Hruban RH, Pitman MB, Klimstra DS (2007a) Intraductal neoplasms. In: Hruban RH, Pitman MB, Klimstra DS (eds) Tumors of the pancreas: AFIP atlas of tumor pathology. AFIP atlas of tumor pathology, vol 6. American Registry of Pathology in collaboration with Armed Forces Institute of Pathology, Washington, DC, pp 75–110

Hruban RH, Takaori K, Canto M et al (2007b) Clinical importance of precursor lesions in the pancreas. J Hepatobiliary Pancreat Surg 14(3):255–263

Hruban RH, Maitra A, Goggins M (2008) Update on pancreatic intraepithelial neoplasia. Int J Clin Exp Pathol 1(4):306–316

Hruban RH, Boffetta P, Hiraoka N et al (2010) Ductal adenocarcinoma of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) WHO Classification of tumors of the digestive system World Health Organisation Classification of Tumors. IARC, Lyon, pp 281–291

Iacobuzio-Donahue CA, Klimstra DS, Adsay NV et al (2000a) Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas: comparison with conventional ductal adenocarcinomas. Am J Pathol 157(3):755–761

Iacobuzio-Donahue CA, Wilentz RE, Argani P et al (2000b) Dpc4 protein in mucinous cystic neoplasms of the pancreas: frequent loss of expression in invasive carcinomas suggests a role in genetic progression. Am J Surg Pathol 24(11):1544–1548

Jimenez RE, Warshaw AL, Z'Graggen K et al (1999) Sequential accumulation of K-ras mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg 230(4):501–509, discussion 509-11

Jones S, Zhang X, Parsons DW et al (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321(5897):1801–1806

Jury RP, Thibodeau BJ, Fortier LE et al (2012) Gene expression changes associated with the progression of intraductal papillary mucinous neoplasms. Pancreas 41(4):611–618
Kanda M, Matthaei H, Wu J et al (2012) Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology 142(4):730–733
Kawahira H, Kobayashi S, Kaneko K et al (2000) p53 protein expression in intraductal papillary mucinous tumors (IPMT) of the pancreas as an indicator of tumor malignancy. Hepatogastroenterology 47(34):973–977
Kawamoto S, Lawler LP, Horton KM et al (2006) MDCT of intraductal papillary mucinous neoplasm of the pancreas: evaluation of features predictive of invasive carcinoma. AJR Am J Roentgenol 186(3):687–695
Kim GE, Bae HI, Park HU et al (2002) Aberrant expression of MUC5AC and MUC6 gastric mucins and sialyl Tn antigen in intraepithelial neoplasms of the pancreas. Gastroenterology 123(4):1052–1060
Kim SG, Wu TT, Lee JH et al (2003) Comparison of epigenetic and genetic alterations in mucinous cystic neoplasm and serous microcystic adenoma of pancreas. Mod Pathol 16(11):1086–1094
Kosmahl M, Pauser U, Peters K et al (2004) Cystic neoplasms of the pancreas and tumor-like lesions with cystic features: a review of 418 cases and a classification proposal. Virchows Arch 445(2):168–178
Kozuka S, Sassa R, Taki T et al (1979) Relation of pancreatic duct hyperplasia to carcinoma. Cancer 43(4):1418–1428
Laffan TA, Horton KM, Klein AP et al (2008) Prevalence of unsuspected pancreatic cysts on MDCT. AJR Am J Roentgenol 191(3):802–807
Liszka L, Pajak J, Zielinska-Pajak E et al (2010) Intraductal oncocytic papillary neoplasms of the pancreas and bile ducts: a description of five new cases and review based on a systematic survey of the literature. J Hepatobiliary Pancreat Sci 17(3):246–261
Lohr M, Kloppel G, Maisonneuve P et al (2005) Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. Neoplasia 7(1):17–23
Luttges J, Galehdari H, Brocker V et al (2001) Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. Am J Pathol 158(5):1677–1683
Luttges J, Feyerabend B, Buchelt T et al (2002) The mucin profile of noninvasive and invasive mucinous cystic neoplasms of the pancreas. Am J Surg Pathol 26(4):466–471
Maire F, Hammel P, Terris B et al (2002) Prognosis of malignant intraductal papillary mucinous tumours of the pancreas after surgical resection. Comparison with pancreatic ductal adenocarcinoma. Gut 51(5):717–722
Maisonneuve P, Lowenfels AB (2010) Epidemiology of pancreatic cancer: an update. Dig Dis 28(4–5):645–656
Maitra A, Adsay NV, Argani P et al (2003) Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. Mod Pathol 16(9):902–912
Maitra A, Fukushima N, Takaori K et al (2005) Precursors to invasive pancreatic cancer. Adv Anat Pathol 12(2):81–91
Meckler KA, Brentnall TA, Haggitt RC et al (2001) Familial fibrocystic pancreatic atrophy with endocrine cell hyperplasia and pancreatic carcinoma. Am J Surg Pathol 25(8):1047–1053
Mino-Kenudson M, Fernandez-Del Castillo C, Baba Y et al (2011) Prognosis of invasive intraductal papillary mucinous neoplasm depends on histological and precursor epithelial subtypes. Gut 60(12):1712–1720
Miyamoto Y, Maitra A, Ghosh B et al (2003) Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell 3(6):565–576
Mohri D, Asaoka Y, Ijichi H et al (2012) Different subtypes of intraductal papillary mucinous neoplasm in the pancreas have distinct pathways to pancreatic cancer progression. J Gastroenterol 47(2):203–213

Moskaluk CA, Hruban RH, Kern SE (1997) p16 and K-ras gene mutations in the intraductal precursors of human pancreatic adenocarcinoma. Cancer Res 57(11):2140–2143

Nara S, Shimada K, Kosuge T et al (2008) Minimally invasive intraductal papillary-mucinous carcinoma of the pancreas: clinicopathologic study of 104 intraductal papillary-mucinous neoplasms. Am J Surg Pathol 32(2):243–255

Ohuchida K, Mizumoto K, Fujita H et al (2006) Sonic hedgehog is an early developmental marker of intraductal papillary mucinous neoplasms: clinical implications of mRNA levels in pancreatic juice. J Pathol 210(1):42–48

Patel SA, Adams R, Goldstein M et al (2002) Genetic analysis of invasive carcinoma arising in intraductal oncocytic papillary neoplasm of the pancreas. Am J Surg Pathol 26(8):1071–1077

Poultsides GA, Reddy S, Cameron JL et al (2010) Histopathologic basis for the favorable survival after resection of intraductal papillary mucinous neoplasm-associated invasive adenocarcinoma of the pancreas. Ann Surg 251(3):470–476

Raimondo M, Tachibana I, Urrutia R et al (2002) Invasive cancer and survival of intraductal papillary mucinous tumors of the pancreas. Am J Gastroenterol 97(10):2553–2558

Ridder GJ, Maschek H, Flemming P et al (1998) Ovarian-like stroma in an invasive mucinous cystadenocarcinoma of the pancreas positive for inhibin. A hint concerning its possible histogenesis. Virchows Arch 432(5):451–454

Rodriguez JR, Salvia R, Crippa S et al (2007) Branch-duct intraductal papillary mucinous neoplasms: observations in 145 patients who underwent resection. Gastroenterology 133(1): 72–79, quiz 309-10

Salvia R, Fernandez-del Castillo C, Bassi C et al (2004) Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg 239(5):678–685, discussion 685-7

Sarr MG, Kendrick ML, Nagorney DM et al (2001) Cystic neoplasms of the pancreas: benign to malignant epithelial neoplasms. Surg Clin North Am 81(3):497–509

Sasaki S, Yamamoto H, Kaneto H et al (2003) Differential roles of alterations of p53, p16, and SMAD4 expression in the progression of intraductal papillary-mucinous tumors of the pancreas. Oncol Rep 10(1):21–25

Sato N, Rosty C, Jansen M et al (2001) STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. Am J Pathol 159(6):2017–2022

Sato N, Ueki T, Fukushima N et al (2002) Aberrant methylation of CpG islands in intraductal papillary mucinous neoplasms of the pancreas. Gastroenterology 123(1):365–372

Sato N, Fukushima N, Maitra A et al (2004) Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. Am J Pathol 164(3):903–914

Sato N, Fukushima N, Hruban RH et al (2008) CpG island methylation profile of pancreatic intraepithelial neoplasia. Mod Pathol 21(3):238–244

Satoh K, Shimosegawa T, Moriizumi S et al (1996) K-ras mutation and p53 protein accumulation in intraductal mucin-hypersecreting neoplasms of the pancreas. Pancreas 12(4):362–368

Satoh K, Hamada S, Kanno A et al (2010) Expression of MSX2 predicts malignancy of branch duct intraductal papillary mucinous neoplasm of the pancreas. J Gastroenterol 45(7):763–770

Schonleben F, Qiu W, Bruckman KC et al (2007) BRAF and KRAS gene mutations in intraductal papillary mucinous neoplasm/carcinoma (IPMN/IPMC) of the pancreas. Cancer Lett 249(2):242–248

Schonleben F, Allendorf JD, Qiu W et al (2008a) Mutational analyses of multiple oncogenic pathways in intraductal papillary mucinous neoplasms of the pancreas. Pancreas 36(2):168–172

Schonleben F, Qiu W, Remotti HE et al (2008b) PIK3CA, KRAS, and BRAF mutations in intraductal papillary mucinous neoplasm/carcinoma (IPMN/C) of the pancreas. Langenbecks Arch Surg 393(3):289–296

Schutte M, Hruban RH, Geradts J et al (1997) Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res 57(15):3126–3130

Sessa F, Solcia E, Capella C et al (1994) Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-ras, p53 and c-erbB-2 abnormalities in 26 patients. Virchows Arch 425(4):357–367

Shi C, Hruban RH (2012) Intraductal papillary mucinous neoplasm. Hum Pathol 43(1):1–16
Shi C, Klein AP, Goggins M et al (2009) Increased prevalence of precursor lesions in familial pancreatic cancer patients. Clin Cancer Res 15(24):7737–7743
Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62(1):10–29
Su GH, Hruban RH, Bansal RK et al (1999) Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. Am J Pathol 154(6):1835–1840
Suda K, Hirai S, Matsumoto Y et al (1996) Variant of intraductal carcinoma (with scant mucin production) is of main pancreatic duct origin: a clinicopathological study of four patients. Am J Gastroenterol 91(4):798–800
Tajiri T, Tate G, Inagaki T et al (2005) Intraductal tubular neoplasms of the pancreas: histogenesis and differentiation. Pancreas 30(2):115–121
Tanaka M, Chari S, Adsay V et al (2006) International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 6(1–2):17–32
Terris B, Ponsot P, Paye F et al (2000) Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less aggressive pathologic features as compared with those involving the main pancreatic duct. Am J Surg Pathol 24(10):1372–1377
Thayer SP, di Magliano MP, Heiser PW et al (2003) Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 425(6960):851–856
Thompson LD, Becker RC, Przygodzki RM et al (1999) Mucinous cystic neoplasm (mucinous cystadenocarcinoma of low-grade malignant potential) of the pancreas: a clinicopathologic study of 130 cases. Am J Surg Pathol 23(1):1–16
Tsutsumi K, Sato N, Cui L et al (2011) Expression of claudin-4 (CLDN4) mRNA in intraductal papillary mucinous neoplasms of the pancreas. Mod Pathol 24(4):533–541
van Heek NT, Meeker AK, Kern SE et al (2002) Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. Am J Pathol 161(5):1541–1547
Vincent A, Herman J, Schulick R et al (2011) Pancreatic cancer. Lancet 378(9791):607–620
Wilentz RE, Geradts J, Maynard R et al (1998) Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. Cancer Res 58(20): 4740–4744
Wilentz RE, Albores-Saavedra J, Zahurak M et al (1999) Pathologic examination accurately predicts prognosis in mucinous cystic neoplasms of the pancreas. Am J Surg Pathol 23(11):1320–1327
Wilentz RE, Iacobuzio-Donahue CA, Argani P et al (2000) Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res 60(7):2002–2006
Wouters K, Ectors N, Van Steenbergen W et al (1998) A pancreatic mucinous cystadenoma in a man with mesenchymal stroma, expressing oestrogen and progesterone receptors. Virchows Arch 432(2):187–189
Wu J, Jiao Y, Dal Molin M et al (2011a) Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. Proc Natl Acad Sci USA 108(52):21188–21193
Wu J, Matthaei H, Maitra A et al (2011b) Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. Sci Transl Med 3(92):92ra66
Xiao HD, Yamaguchi H, Dias-Santagata D et al (2011) Molecular characteristics and biological behaviours of the oncocytic and pancreatobiliary subtypes of intraductal papillary mucinous neoplasms. J Pathol 224(4):508–516
Yamaguchi H, Shimizu M, Ban S et al (2009) Intraductal tubulopapillary neoplasms of the pancreas distinct from pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol 33(8):1164–1172
Yamaguchi H, Kuboki Y, Hatori T et al (2011) Somatic Mutations in PIK3CA and Activation of AKT in Intraductal Tubulopapillary Neoplasms of the Pancreas. Am J Surg Pathol 35(12): 1812–1817

Yamao K, Yanagisawa A, Takahashi K et al (2011) Clinicopathological features and prognosis of mucinous cystic neoplasm with ovarian-type stroma: a multi-institutional study of the Japan pancreas society. Pancreas 40(1):67–71

Yu J, Li A, Hong SM et al (2012) MicroRNA alterations of pancreatic intraepithelial neoplasias. Clin cancer res 18(4):981–992

Zamboni G, Scarpa A, Bogina G et al (1999) Mucinous cystic tumors of the pancreas: clinicopathological features, prognosis, and relationship to other mucinous cystic tumors. Am J Surg Pathol 23(4):410–422

Zamboni G, Fukushima N, Hruban RH et al (2010) Mucinous cystic neoplasms of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) WHO Classification of tumors of the digestive system World Health Organisation Classification of Tumors. IARC, Lyon, pp 300–303