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Pancreatic Intraepithelial Neoplasia Revisited and Updated

B. Sipos[a] S. Frank[a] T. Gress[b] S. Hahn[c] G. Klöppel[a]

[a]Department of Pathology, University of Kiel, Kiel, [b]Department of Internal Medicine, University of Marburg, Marburg, and [c]Department of Internal Medicine, University of Bochum, Bochum, Germany

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Abstract
Most pancreatic neoplasms are classified as ductal adenocarcinoma because they show a ductal phenotype, making a ductal origin very likely. The duct lesions that may give rise to pancreatic ductal adenocarcinoma have been called pancreatic intraepithelial neoplasia (PanIN). A classification system for these lesions distinguishes between three grades of PanIN. Molecular studies revealed that PanIN-2 and PanIN-3 lesions represent a distinct step towards invasive carcinoma. While high-grade PanINs are extremely rare in the normal pancreas, low-grade PanINs are common in individuals older than 40 years and may be associated with lobular fibrosis and intraductal papillary mucinous neoplasms of the gastric type. This disease spectrum has also been described in members of kindreds with familial pancreatic cancer. The natural history and cause of PanINs are unknown. As PanIN-1 lesions entail little risk, while PanIN-3 lesions are high-risk lesions, it would be of interest to target PanIN-2 lesions, which can be regarded as the starting point of progressive neoplastic changes that lead to invasive pancreatic ductal adenocarcinoma. Global gene expression analysis identified several differentially expressed genes which show enhanced expression in PanINs and may be used as potential biomarkers to facilitate diagnosis and therapy.

Introduction

Progress in cancer treatment has largely been achieved by three measures: prevention, removal after early detection, and effective chemotherapy in advanced stages. In pancreatic ductal adenocarcinoma (PDAC), often referred to as pancreatic cancer, all three measures have so far failed to noticeably improve the overall survival of the patients. Therefore, a great deal of effort has been put into increasing the effectiveness of the treatment options for PDAC.

The identification and detection of PDAC at its beginning, preferably in its preinvasive stage, is one option for improving patient survival by treating the disease at a potentially curable stage. Such a strategy has been very successful in improving the prognosis of other cancers such as carcinomas of the cervix, colon, prostate and breast. In PDAC, the clinical progress toward improving the 5-year survival rate has been slow, though our knowledge of the histopathology and our understanding of the molecular pathology and biology of precursor lesions has increased extensively during recent years.

The current concept of the development of PDAC is largely based on data that have accumulated from studies dealing with the histopathological and molecular characterization, recognition and tracing of pancreatic lesions and diseases that appear to progress to invasive PDAC. The pancreatic lesions that are thought to precede PDAC are known as pancreatic intraepithelial neoplasia (PanIN); the precursor diseases as intraductal papillary

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PD Dr. Bence Sipos
Department of Pathology, University of Kiel
Michaelisstrasse 11
DE–24105 Kiel (Germany)
Tel. +49 431 597 3402, Fax +49 431 597 3462, E-Mail s.bence@path.uni-kiel.de

mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs). Because IPMN and MCN are rare diseases and the IPMNs are additionally heterogeneous and therefore have a complex relationship to PDAC, this review focuses only on the PanINs and the recent efforts to recognize them and to understand their role in pancreatic carcinogenesis.

PanIN in Pancreatic Cancer

Fifty-four years ago, Sommers et al. [1] drew attention to a possible relationship between pancreatic duct lesions and pancreatic adenocarcinoma, later called ductal adenocarcinoma [2, 3]. This observation was substantiated by a number of studies in the late 70s, when Cubilla and Fitzgerald published a large series [4], and the early 80s [5, 6]. Further evidence that some of the duct lesions may be precursors to invasive PDAC came from a few case reports. In 1998, 5 patients were reported who developed invasive ductal carcinoma 1.5–10 years after partial pancreatectomy, which had revealed atypical pancreatic duct lesions. One obstacle in all these studies was that various terms were used to describe the observed lesions, making it difficult to compare the results and their interpretations. Therefore, the term pancreatic intraepithelial neoplasia (PanIN) was introduced by David Klimstra and Daniel Longnecker to summarize all the ductal changes that are thought to be forerunners of PDAC, already anticipating their neoplastic nature [7]. As these ductal changes included meta-, hyper- and dysplastic lesions, a three-tiered PanIN classification system was defined (see below).

In PDACs and their variants, PanINs of all grades are observed. They occurred even in the so-called undifferentiated (anaplastic) carcinoma, whose presumably ductal origin [8] is further supported by this finding [9]. While large numbers of low-grade PanINs can be found diffusely distributed in the nonneoplastic pancreatic tissue, the few high-grade PanINs are usually observed in the immediate vicinity of the infiltrating tumor [10]. This often makes it very difficult to decide whether a particular high-grade PanIN lesion is an intraductal growth of the carcinoma along a preexisting benign duct [5, 11] or a separate neoplastic duct change. In a study by the authors of 37 total pancreatectomy specimens containing infiltrating PDACs in the pancreatic head, high-grade PanIN, i.e. intraductal carcinoma, was found in the main pancreatic duct beyond the usual Whipple resection line, i.e. in the body and tail of the pancreas in three cases. In each case, it proved to be an intraductal extension of the carcinoma in the head of the pancreas [12]. A recent study in which this issue was also addressed concluded that most high-grade PanIN lesions occur within an area of 10 mm adjacent to the infiltrating carcinomas and were thus interpreted as intraductal extensions of the tumor rather than independent neoplastic proliferations [13]. On the other hand, extensive LOH analysis of PanIN lesions revealed cases with clonal heterogeneity between high-grade PanIN lesions and their corresponding PDAC [14], which contradicts the interpretation that most high-grade PanINs simply represent 'cancerization of the ducts'.

The early descriptions of PanINs already mention that they occur not only in association with PDAC but also with other uncommon types of pancreatic cancer [1]. Thus, they were found in the nonneoplastic portion of pancreata harboring acinar cell carcinomas, MCNs, serous cystadenomas, solid pseudopapillary neoplasms and pancreatic endocrine tumors [15]. In almost all cases, the PanINs found in these conditions are of low grade and their prevalence tends to correlate with patient age.

PanIN in the Normal or Chronically Inflamed Pancreas

Low-grade PanINs have been identified in the normal pancreas in 16–80% of adult pancreata not harboring an invasive PDAC or any other cancer [16–18]. High-grade PanINs, i.e. PanIN-3 lesions, were reported in <5% of such pancreata in three studies [10], and were not detected in the two most recent studies [16, 17]. The frequency of low-grade PanINs correlates with age, being low in patients younger than 40 years and increasing sharply beyond 40 years [16, 17]. PanINs have also been reported more commonly in the head of the pancreas than elsewhere [4, 10, 17, 19]. This was not confirmed, however, in our studies on total pancreas specimens [5, 16].

Among patients with chronic pancreatitis low-grade PanIN lesions were mostly reported. They affected only a small proportion (11%) of patients. High-grade PanIN lesions were only found in two studies [17, 20], but were not detected in others [21–23]. Patient age and duration of chronic pancreatitis did not correlate with the grade of PanIN lesions and their K-ras status. Duration was, however, significantly associated with K-ras positivity, as a meta-analysis of published data showed that no case of chronic pancreatitis with a disease duration of less than 3 years had K-ras mutations [24]. Another genetic change found in PanINs of chronic pancreatitis was p16 inactivation [25].

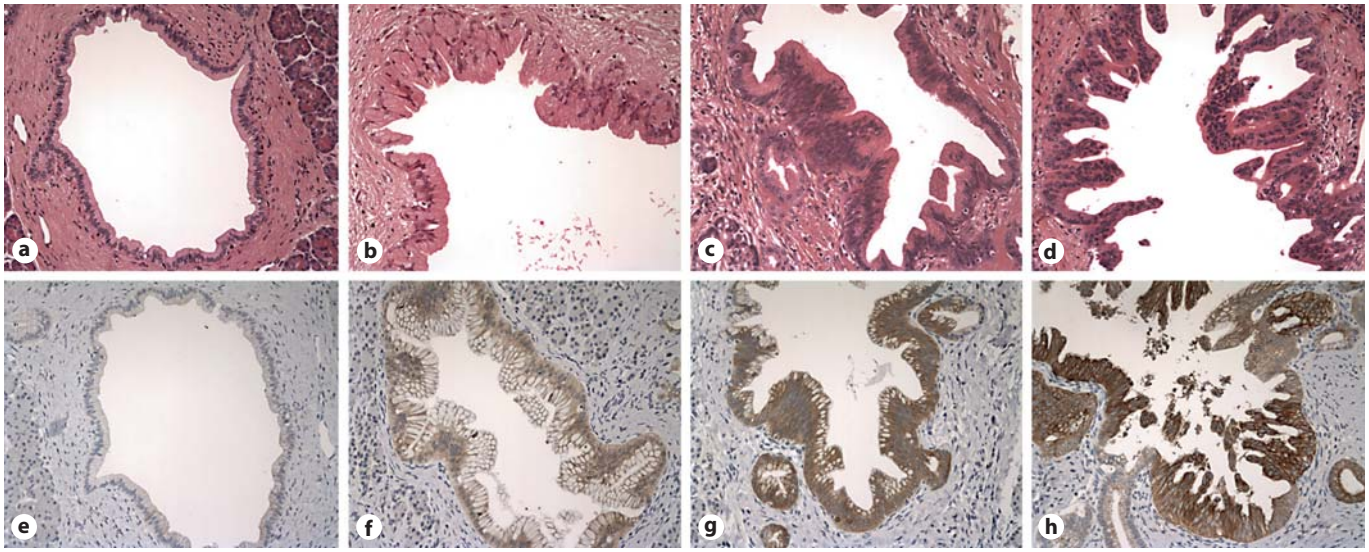

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Fig. 1. Histology of normal pancreatic duct (**a**) and PanIN lesions: PanIN-1 (**b**), PanIN-2 (**c**) and PanIN-3 (**d**). Immunohistochemical detection of 14-3-3σ in normal duct (**e**) and PanINs of different grades: PanIN-1 (**f**), PanIN-2 (**g**) and PanIN-3 (**h**). ×200 magnification.

PanIN and the Development of Ductal Adenocarcinoma

The cellular phenotype of most neoplasms that arise in the pancreas imitates, to differing degrees, the cell types present in the normal pancreas, ductal cells, acinar cells and endocrine cells. This imitation of the normal cellular phenotype is also reflected in the expression of cell lineage markers in the tumor cells, which are therefore used to distinguish and categorize these tumors. According to this histogenetical classification concept, ductal adenocarcinomas originate from ductal cells, leaving open the question whether this cell comes from the compartment of the large or small ducts or the centroacinar units. Of course, in the light of the stem cell concept it is debatable whether the cellular phenotype of a neoplasm reflects the true cell of origin, or whether cells with stem cell properties become cancer stem cells that, depending on the misactivation or suppression of certain molecular pathways by mutated oncogenes and/or tumor suppressor genes, give rise to neoplasms with various differentiation phenotypes. Despite these uncertainties regarding the cellular origin of tumors, the current concept assumes that the precursors of ductal adenocarcinomas are found in the ductal compartment of the pancreas (including the centroacinar units) and that the cells of the other compartments might only be involved in a very early precursor stage by transdifferentiation into a ductal cell type. The latter development seems to play a role in the K-ras mouse model [26].

The classification of the lesions that are thought to be precursors of PDAC distinguishes three PanIN grades on the basis of histological and cytological criteria. Briefly, PanIN-1 lesions have a flat (PanIN-1A) or papillary (PanIN-1B) mucinous epithelium without cellular atypia, whereas PanIN-2 lesions show increasing signs of cellular atypia and a prevalence of papillary architecture [27, 28]. Finally, PanIN-3 lesions correspond to carcinoma in situ, i.e. intraductal carcinoma (fig. 1a–d). The reproducibility of the PanIN classification revealed good observer agreement for PanIN-1 and PanIN-3, but rather poor for PanIN-2 [29].

In a next step, it was examined whether the morphologically suggested progression from PanIN-1 to PanIN-2 and -3 lesions [30] is associated with genetic changes that fit into the genetic profile of invasive PDAC. The first investigations focused on K-ras mutations, because of their high incidence (up to 90%) in invasive PDACs [31–33]. These studies using a combination of microdissection and genetic and immunohistochemical techniques revealed a highly varying frequency of K-ras mutations in duct lesions, ranging from 0–95% [20, 34], depending on the lesions selected for analysis and the method of detection applied. Using the new PanIN classification, the results of some of the earlier studies can be reclassified. In a study with a high prevalence of papillary lesions with

severe dysplasia (probably PanIN-3), K-ras mutations were present in 75% of the lesions [35], whereas in unselected material (including a large number of PanIN-1A and 1B lesions) the frequency was only 39% [36]. When lesions such as mucinous hypertrophy of ductal cells, which are now included in the category PanIN-1A, were analyzed separately, the K-ras mutation rate was 20% [37]. In addition, it was demonstrated that K-ras mutations may even occur in normal duct cells [16, 37]. These data showed that K-ras mutations are a frequent event in pancreatic duct cells, but they were not useful for discriminating PanINs according to their cytologic grade of malignancy. It was therefore necessary to look for other genetic changes that characterize the development of PDACs. Several molecular studies focused on an LOH analysis of p16, p53 and DPC4 [14, 38, 39], because they are the next most frequent genetic alterations in invasive PDACs [35, 40, 41]. These studies revealed a rising incidence of LOH of the above-mentioned genes with increasing PanIN grade. In PanIN-3 lesions, almost as many LOHs had accumulated as in the corresponding invasive carcinomas. By contrast, losses at one or two chromosomal loci were only found in 67% of PanIN-2 lesions with moderate dysplasia [14], while in PanIN-1 either no [14, 38] or only very few losses at one chromosomal locus [39] were detected. Later shortened telomeres were also demonstrated in PanIN lesions of all grades [42] and were thought to predispose PanINs to accumulate progressive chromosomal abnormalities. Whether BRCA2, a tumor suppressor gene known to be involved in breast tumorigenesis, plays an important role in the late development of PDAC is doubtful because its inactivation was observed in less than 10% of PanIN-3 lesions [43].

These findings provided a basis for a progression model for the development of PDAC, which postulates that a successive accumulation of alterations in the cancer-associated genes with increasing PanIN grades leads to invasive PDAC [30]. The question as to the earliest genetic event in this model has not yet been clearly answered. Among the genes that set the stage for the development of preinvasive carcinoma in the pancreas are K-ras, erb B2 (HER-2/neu) and the p16 tumor suppressor gene on chromosome 9p. However, the significance of these genes in this scenario is still unclear. K-ras is already found in normal-appearing epithelium [16, 37] and innocent-looking PanIN-1 lesions in nonneoplastic pancreata [16, 18, 22, 37, 44]. erb B2 (HER-2/neu) is suspected because it may be found to be overexpressed in PanIN-1 lesions [45], and a p16 mutation was described in a single PanIN-1 lesion next to an invasive carcinoma [35]. Furthermore, immunohistochemical data, though difficult to interpret, suggest a reduction in p16 protein expression starting already at the stage of PanIN-1 lesions [46]. Thus, the neoplastic potential of the PanIN-1 lesions remains unclear so far. A decisive step, however, seems to be the inactivation of the tumor suppressor genes p53 and DPC4. This probably already occurs in the PanIN-2 stage [35, 38]. Since abnormal p53 and SMAD4/DPC4 protein expression was mainly observed in PanIN-3, while LOH at the chromosomal loci 17p (p53) and 18q (DPC4) was already found in PanIN-2, allelic deletion may precede the mutational event in the biallelic inactivation of these two suppressor genes [14]. In a study employing microdissection of PanIN lesions, whole genome amplification, microsatellite analysis and PanIN tissue microarrays, it was shown that the greatest changes in gene expression occur between PanIN stages 1B and 2, suggesting that PanIN-2 may represent the first truly preneoplastic stage in PDAC progression [47]. Since these expression data nicely correspond with the initiation of chromosomal alterations at the PanIN-2 stage [14], genetic instability and increasing changes in gene expression patterns appear to be linked to PDAC progression. The PanIN-1 stage, on the other hand, is characterized by lesions without atypia that are genetically stable. Their K-ras mutation rate ranges between 0–61%, depending strongly on the detection method applied (mutation rates above 30% were generally only found if some type of mutation enrichment methodology was used) [24]. This stage shows little if any risk of advancing towards PDAC.

The Cause of Low-Grade and High-Grade PanINs

What may induce PanIN-1 lesions in the pancreas? As they are frequently associated with K-ras mutations, the same factor that is discussed in the induction of mutated K-ras, i.e. smoking, may also play a role in the development of PanINs [48]. However, a recent study addressing this issue was unable to find any correlation between smoking, coffee consumption, or diabetes and the incidence of PanINs [17]. This supports the view that PanIN-1 lesions most likely arise independently of K-ras mutations rather than in concert with these genetic alterations. The question then remains why K-ras mutations prefer the ductal cells, and particularly the ductal cells in PanIN-1 lesions that show mucinous metaplasia, to the acinar cells and the endocrine cells of the pancreas.

The cause of high-grade PanINs is also not known. However, as high-grade PanINs have the same genetic profile as infiltrating PDACs, they most likely also share the risk factors identified for PDAC. Smoking, which is by far the strongest risk factor, probably plays the most important role, but the pathomechanisms are unclear so far. In alcoholic chronic pancreatitis, which is among the minor risk factors for PDAC [49], the synergistic effect of ethanol with smoking on the formation of acetaldehyde might play a role in the development of PDAC [50], because acetaldehyde generates reactive oxygen and nitrogen species [51]. This results in reactive aldehydes, which are known to induce DNA adducts and mutations [52]. Some of the reactive aldehydes detected in chronic pancreatitis [53] could then make the pancreatic duct epithelium receptive to malignant transformation and thus accelerate the development of PDAC.

Natural History of PanINs

The natural history of PanINs may be viewed as a progression from low- to high-grade PanIN lesions or as the development of de novo high-grade PanINs that occur in association with low-grade PanINs.

In the first case, the time axis of the progression of low-grade PanIN lesions to high-grade PanINs and then to invasive PDAC is probably long, because low-grade PanIN lesions occur already early in life [17], while PDAC has its highest incidence between 60 and 70 years of age [16]. This suggests that low-grade PanINs may exist for years before one of them is suddenly transformed into a high-grade PanIN and invasive PDAC by accidental accumulation of genetic events involving not only K-ras but also p16, p53 and SMAD4/DPC4. The long phase between the first occurrence of a low-grade PanIN lesion and its final outcome, which is harmless for most people but may be fatal for a few in whom additional genetic hits cause PanIN3 and subsequently invasive PDAC, is for the affected individuals like a 'dance on Vesuvius' that luckily most survive.

In the second case, when a high-grade PanIN develops as a de novo lesion, the time axis is short, because PanIN3 lesions are exceptional findings in the normal pancreas (see above), indicating that if they occur they rapidly turn into invasive PDAC. What role associated low-grade PanINs have in this scenario is totally unclear, but they might represent an indicator lesion signaling that the involved pancreas is receptive to PDAC development.

PanIN Disease in Familial PDAC

Meckler et al. [54] described fibrocystic changes and both low- and high-grade PanIN lesions in most pancreata of 11 family members among a kindred with a rare autosomal dominant pancreatic carcinoma syndrome. In a more recent study in another patient cohort with a strong family history of pancreatic cancer, lobular fibrosis with acinar atrophy of the pancreas was described in association with PanIN-1 and -2 lesions as well as small peripheral IPMNs, presumably of the gastric type [55, 56]. These findings, however, do not seem to be specific to patients with a familial history of pancreatic cancer. Similar changes have been reported in nonneoplastic pancreata, either as patchy lobular fibrosis associated with PanIN-1B lesions [57], as small peripheral cystic changes [58] and/or as tubular complexes [59]. Moreover, it has been recently recognized that low-grade PanIN lesions frequently occur next to gastric-type IPMNs and both low-grade PanIN lesions and gastric-type IPMNs stain for MUC5 in the absence of MUC1 and MUC2 positivity [60]. This raises the question whether the changes described in familial PDAC, normal pancreata and IPMNs of the gastric type are facets of one disease. If this were the case, the link between the three findings could be the PanIN-1B lesions. With their papillary proliferations they may, on the one hand, cause lobular fibrosis by obstructing the duct draining a lobule, and on the other hand they may undergo cystic transformation to IPMNs of the gastric type. The latter tumors would then be large cystic PanIN-1 lesions and thus a focal accentuation of a diffuse PanIN disease rather than a single lesion. This assumption would also explain why it is difficult to distinguish PanIN-1 lesions from some IPMNs [29] despite a consensus definition of both lesions [61]. Regarding the obviously common occurrence of low-grade PanIN lesions and associated gastric-type IPMNs in pancreata from patients with a family history of pancreatic cancer, it may be speculated that these pancreatic changes ('PanIN disease') could predispose to the development of a PDAC (see above).

PanINs and Their Markers

Detecting PDAC at the PanIN stage is a global aim. In recent years, numerous studies have analyzed the expression of proteins in PanIN lesions in the hope of finding biomarkers that would identify these duct changes and enable the early detection of PDAC. Such biomarkers

Table 1. Upregulated proteins in PanIN lesions compared to normal ducts[1]

	Function	Subcellular localization	Normal ducts	PanIN-1A	PanIN-1B	PanIN-2	PanIN-3	Source
MUC1	protection of epithelial surfaces, adhesive properties, cell-cell interactions	cell membrane, secreted	+	+	+	++	+++	[68]
MUC4	anti-adhesive properties, epithelial differentiation, stimulation of proliferation	cell membrane, secreted	–	+	+	++	+++	[70]
MUC5	protection of mucosa	secreted	–	+++	+++	+++	+++	[68]
MUC6	protection of epithelial surfaces, epithelial organogenesis	secreted	–	upregulated[2]		upregulated[2]		[71]
p16	inhibition of proliferation	nucleus	+	+	++	++	+++	[68]
Cyclin D1	cell cycle control	nucleus	–	–	–	+	++	[68]
Ki-67	proliferation marker	nucleus	–	–	–	+	+++	[68]
Topo II	control of DNA replication	nucleus	–	–	–	+	+++	[68]
p53	induction of apoptosis	cell membrane	–	–	–	–	++	[68]
Prostate SCA	unknown	cytoplasm, cell membrane	–	++	+	++	++	[68]
Fascin	organization of actin filaments	cytoplasm, secreted	–	+	+	++	++	[68]
14-3-3σ	adapter protein signaling pathways	cell membrane, secreted	–	+	+	+	+++	[68]
Mesothelin	cellular adhesion	cytoplasm	–	+	–	+	+	[68]
Class III tubulin	constituent of microtubules	cell membrane	–	+	+	+	++	[72]
Récepteur d'origine nantais	macrophage receptor	cell membrane	+	+	+	+++	+++	[73]
Integrin α6β4	receptor for laminin	cell membrane, secreted	+	+++	+++	+++	+++	[74]
Urokinase plasminogen activator	potent plasminogen activator	cell membrane	+	+	+	+++	++	[75]
Claudin 18	adhesive properties	cell membrane, secreted	–/+	+++	+++	+++	+++	[76]
Pepsinogen C	enzyme	cytoplasm secreted	–	upregulated[2]		upregulated[2]		[71]
TFF1	protection of mucosa	nucleus	–	upregulated[2]		upregulated[2]		[71]
KLF4	epithelial cell differentiation	cytoplasm secreted	–	upregulated[2]		upregulated[2]		[71]
Gastrin	hormone	nucleus	–	upregulated[2]		upregulated[2]		[71]
GATA4	transcriptional activator	nucleus	–	upregulated[2]		upregulated[2]		[71]
GATA5	transcriptional activator	nucleus	–	upregulated[2]		upregulated[2]		[71]
GATA6	transcriptional activator	nucleus	–	upregulated [2]		upregulated[2]		[71]
Sox-2	transcription factor	nucleus	–	upregulated[2]		upregulated[2]		[71]
HOXA5	transcription factor	nucleus	–	upregulated[2]		upregulated[2]		[71]
Forkhead6	transcription factor	nucleus	–	upregulated[2]		upregulated[2]		[71]
Villin	actin-binding protein	nucleus	–	upregulated[2]		upregulated[2]		[71]
HlxB9	transcription factor	cytoplasm	–	upregulated[2]		upregulated[2]		[71]

Table 1 (continued)

	Function	Subcellular localization	Normal ducts	PanIN-1A	PanIN-1B	PanIN-2	PanIN-3	Source
Cathepsin E	proteasis	cell membrane	+	n.d.	+++	+++	+++	[47]
RAB1B	protein transport, probably involved in vesicular traffic	cell membrane	–	n.d.	+	+	++	[47]
CEACAM5	not known	nucleus, cytoplasm	–	n.d.	++	++	+++	[47]
S100P	calcium-binding protein	nucleus	–/+	+	+	+	++	[69]
S100P		cytoplasm	–	+++	+++	+++	+++	[77]
KOC: K homology domain containing protein overexpressed in cancer	regulation of mRNA translation	cell membrane	+	–	–	–	++	[78]
ADAM17	cleavage of TNF-α	cell membrane, cytoplasm	–/+	–	+	+	+++	[79]

+ = 1–33%, ++ = 34–66%, +++ = ≥ 67%. [1] Only results generated from a relevant number of PanIN lesions are listed. [2] No detailed information available.

could become potential targets for therapeutic interventions [62]. Array-based gene expression profiling studies have revealed the differential regulation of numerous genes in PDACs [63–67]. The expression of some of these genes at the protein level was subsequently validated by immunohistochemistry and detected not only in invasive PDAC but also in PanINs. Among the markers that emerged from these studies are S100P, PSCA, mesothelin, fascin, 14–3-3σ (fig. 1e–h), CEACAM5, cathepsin E and others [47, 68, 69]. Table 1 lists the PanIN markers that have been published so far and are available. It shows their known function, subcellular localization and the PanIN grades they recognize. From this table, it is obvious that many markers differentiate clearly between normal ducts and PanINs. In addition, a few markers label the 'risky' high-grade PanIN more strongly than the low-grade PanINs (fig. 1). However, table 1 also shows that there is no marker that identifies only 'risky' high-grade PanINs. The overexpression of most of these markers can not be assigned to common pathways, which are indicated in the tumorigenesis of PDAC (K-Ras, p53, DPC4, p16). It is also unclear whether these proteins contribute to development and progression of PDAC or represent only innocent bystanders, which nevertheless may be exploited for early detection of PanIN or PDACs. In addition, they may become targets for therapeutic interventions in order to prevent the development of 'risky' PanINs.

Conclusions

Exact morphological identification of preneoplastic changes combined with molecular analysis of microdissected tissue has broadened our understanding of cancer development and progression in the pancreas. The preneoplastic duct lesions that are thought to precede PDAC are classified as PanIN. As for the question of the risk that the various PanIN lesions entail, PanIN grade 1 lesions are probably of low risk, since, apart from an occasional K-ras mutation they do not seem to carry any other significant genetic change, such as LOH for p53 or DPC4, that would give them a significant neoplastic potential. Lesions with a clearly increased risk are PanIN-2 lesions, in which some genetic changes such as LOH for p53 and loss of p16 protein expression have already accumulated [47]. PanIN-3 lesions show the genetic profile of invasive PDACs and can be considered fully developed neoplastic changes. As PanIN-1 lesions entail little risk, while PanIN-3 lesions are high-risk lesions, it would be of interest to target PanIN-2 lesions, which can be regarded as the starting point of progressive neoplastic changes that lead to invasive PDAC.

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