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Pancreatic Ductal Adenocarcinoma: Cellular Origin, Signaling Pathways and Stroma Contribution

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Abstract
Pancreatic cancer has a very poor prognosis, in part due to its diagnosis at late stages of the disease and to limited response to chemotherapy and radiotherapy. The vast majority of pancreatic cancers are classified as pancreatic ductal adenocarcinomas (PDACs). Despite advances in knowledge on the cellular origin of PDAC or the involvement of signal transduction pathways therein, many questions remain unanswered. In this review, we summarize recent findings and current hypotheses regarding these two questions. Since pancreatitis is a risk factor for human PDAC, and the latter proceeds with an intense fibrotic reaction, we also analyze the role of the stroma in PDAC progression. An improved understanding of these key aspects for PDAC ontogeny will open new avenues for tumor prevention, early detection, and improved therapy.

Introduction

The vast majority of pancreatic cancers are classified as pancreatic ductal adenocarcinomas (PDACs) [1]. Although these tumors represent less than 2% of cancer cases, they are the fifth leading cause of cancer-related death. One reason for this dismal prognosis is that most patients are diagnosed at a late stage of the disease. Furthermore, PDAC disseminates very rapidly and the 2-year survival of patients with a 2-cm tumor is only 20%. Therefore, the identification of the PDAC precursor lesions is an urgent objective that could help to diagnose pancreatic cancer early [for further reviews, see 2, 3].

At the morphological level, PDACs are composed of epithelial cells with varying degrees of ductal differentiation, surrounded by a dense, reactive fibrotic stroma. Here, we will address two crucial questions related to these characteristics of PDAC: (1) the cell type and origin of the neoplastic cells and the pathways involved in malignant transformation, and (2) the contribution of the tumor microenvironment (stroma) to malignant transformation.

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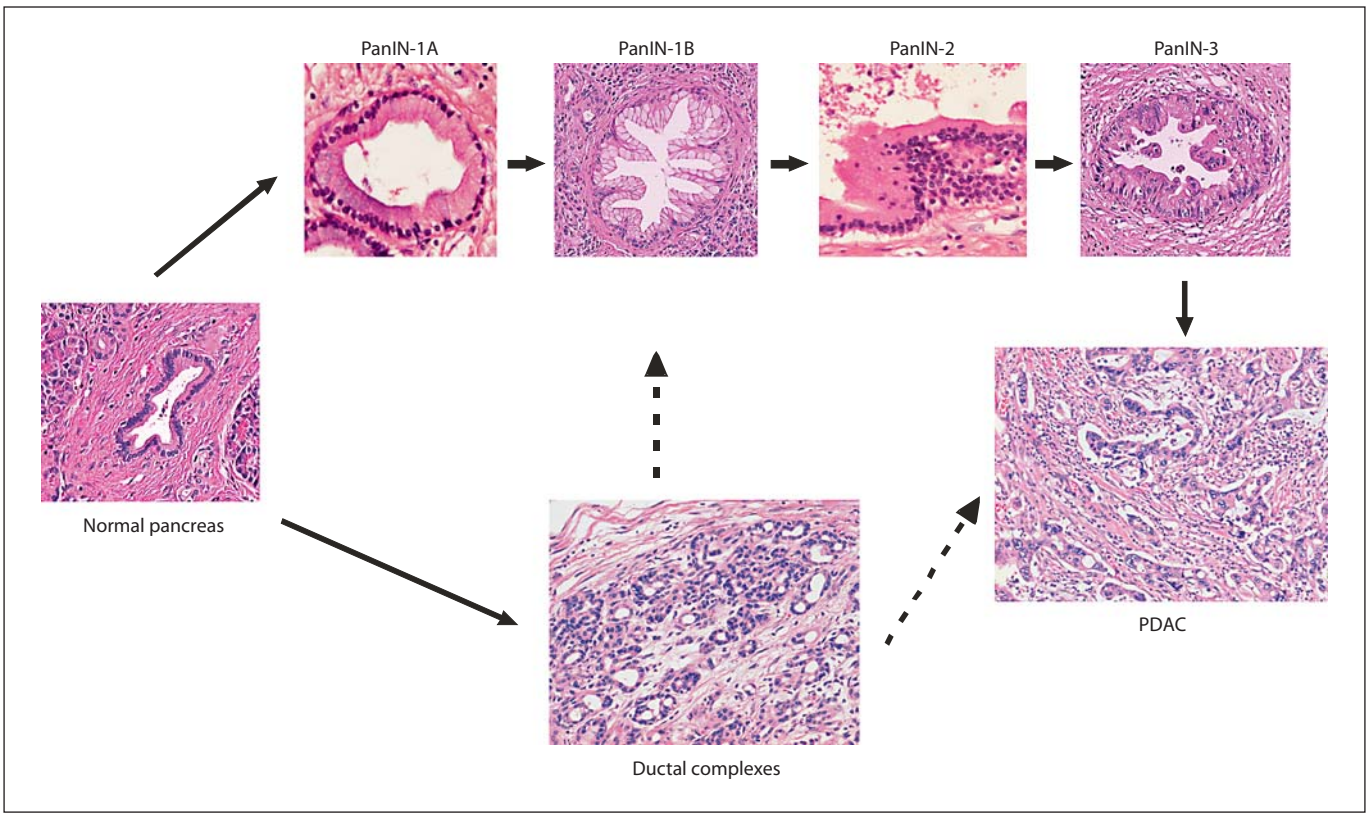

Fig. 1. Cellular pathways involved in PDAC development and progression. The upper panel pathway involves the development of PanINs originating from ductal cells; the lower panel pathway involves the appearance of ductal complexes occurring as an intermediate stage that may – with or without PanINs – lead to PDAC.

Cellular Origin of PDAC

The ductal morphology of PDAC led to postulate that ductal cells were at the origin of transformation. Supporting this hypothesis, PDAC occurs with high frequency in association with dysplastic and hyperplastic ductal lesions [4, 5]. In this respect, three different ductal preneoplastic lesions have been described: pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasms and intraductal papillary mucinous neoplasms [6, 7]. Common and distinct molecular events have been found among these lesions, suggesting that each precursor lesion may reflect variations leading to malignant transformation [8, 9] (fig. 1). The observation that these lesions display some of the genetic alterations found in PDAC strengthened the hypothesis that PDACs arise from preexisting duct cells. However, this hypothesis has been surprisingly difficult to prove, as direct targeting of oncogenic K-Ras, the most frequent genetic alteration in PDAC, to mature ductal cells using the cytokeratin 19 promoter fails to induce PanINs or PDAC in mice [10].

Other studies using experimental animal models suggest that PDAC may derive from endocrine or acinar cells undergoing metaplasia (or transdifferentiation) processes.

The endocrine hypothesis was based on experiments performed using transformed islet cell cultures that, when transplanted into mouse and hamster, progressed to PDAC. Furthermore, endocrine lineage markers are frequently expressed in pancreatic cancer cells [11]. These results have led to speculate that endocrine cells are potential PDAC precursors [for review, see 12]. However, recent data using in vivo lineage tracing in mice suggest that β-cell transdifferentiation plays no role in regeneration, metaplasia or carcinogenesis [13].

The frequent observation of acinar-to-ductal metaplasia in humans [14] and in mouse models using acinar-specific promoters suggests an acinar origin of PDAC. In

this respect, in Ela-*TGF-α* transgenic mice, acinar cells lose zymogen granules and become transitional cells, which subsequently acquire the features of ductal cells. These acinar-derived ductal cells show progressive proliferation and dysplasia – supporting their premalignant nature – and can lead, after long latency, to tumors [15]. Similarly, in the Ela-myc model, mice develop pancreatic cancer with 100% penetrance at an early age, half of which are pure acinar carcinomas and the remaining half are ductal adenocarcinomas or mixed ductal and acinar carcinomas [16].

Mouse models using activated K-Ras have provided further insights into the cell of origin of PDAC. These models have used knock-in strategies for conditional activation of a $Kras^{G12D}$ allele by Cre-loxP recombination. Pancreas-specific activation of K-Ras leads to the development of PanIN lesions [17, 18]. Moreover, when K-Ras activation is combined with mutations in p53 [19] or Ink4a/Arf [17], a rapid progression to PDAC is observed. Unfortunately, these studies can not address the cell-origin of PDAC, as they used Pdx1-*Cre* or p48-Ptf1α-*Cre* driver strains, which mediate *Cre*-loxP recombination in all pancreatic cell types.

Tuveson et al. [20] have recently shown that the *K-Ras* knock-in in the locus of *Mist-1*, a transcription factor required for acinar organization, induces acinar metaplasia and dysplasia, leading to invasive and metastatic pancreatic cancer with mixed acinar, cystic, and ductal features. More recently, Guerra et al. [21] have developed a conditional system for Cre expression under the Elastase promoter. They found that expression of activated $K\text{-}Ras^{G12V}$ in nonductal embryonic cells results in PanINs and invasive PDAC. These results suggest that PDAC can originate by transdifferentiation of acinar/centroacinar cells or their precursors into ductal-like cells. Interestingly, centroacinar cells have been also proposed as the origin of ductal lesions in a pancreas-specific knock-out of *Pten*, a negative modulator of the PI3K/Akt pathway. These mice develop tubular complexes that replace acinar parenchyma and are considered to be the result of the centroacinar compartment expansion [22]. Centroacinar cells lie at the interface between acinar cells and adjacent ductal epithelium and could represent a stem- or progenitor-like population in the adult pancreas, particularly in response to pancreatic injury conditions.

Independently of their origin, putative cancer stem cells, defined by their ability to self-renew, to differentiate into the bulk tumor population, and their potential for tumor formation have been recently identified from human PDAC using cell surface markers [23, 24].

Stem cell biology has contributed important information regarding epithelial stem cells as potential precursors for human cancer.

During normal pancreatic development, undifferentiated precursor cells are responsible for generating mature acinar, ductal and islet cell types. Although the location and identity of the stem cells in adult pancreas is currently an area of intensive investigation, a precursor cell type could participate in the generation of PDAC [17, 18]. Pointing to this possibility, recent studies have demonstrated that metaplastic and neoplastic ductal epithelium shares features with embryonic pancreas, suggesting that further insights into factors regulating pancreatic development may be useful in identifying initiating events in pancreatic cancer. In the following section, we will briefly introduce key transcription factors and transduction signals involved in pancreas organogenesis and their relationship with PDAC.

Signaling Pathways Involved in Pancreatic Development and Cancer

Both endocrine and exocrine progenitors are derived from a common precursor population located in the foregut endoderm. As organogenesis progresses, this population is exposed to signaling cues of neighboring tissues, leading to the expression of a hierarchy of transcription factors required for normal cell specification and differentiation. Among them, Pdx1 and p48/Ptf1α play a dual role during development: at early stages, they are expressed in pancreatic progenitor cells which will give rise to all cells of the pancreas (duct, acinar, endocrine) in a cell-autonomous manner, as shown by lineage tracing analyses in mice; subsequently, they regulate cell type-specific differentiation. Thus, as cytodifferentiation occurs, Ptf1α is restricted to acinar cells and regulates acinar gene expression, whereas Pdx1 is downregulated in these cells, remains absent in adult ducts and is highly expressed in β-cells. The correct integration of extrinsic and intrinsic signals is therefore required for the proper organ development [reviewed in 25, 26].

During development, the Notch signaling pathway promotes pancreatic progenitor self-renewal or exocrine lineage commitment and inhibits cell differentiation as well. Thus, overactivation of this pathway in pancreatic progenitors impairs the differentiation into both endocrine and exocrine lineages [27, 28], whereas its inactivation leads to premature differentiation of endocrine pancreas [29, 30]. Notch blocks pancreatic acinar cell differ-

entiation by inactivating the PTF1 complex through several distinct mechanisms [31, 32].

In normal adult pancreas, Notch and its ligands are expressed at low or undetectable levels [30]. However, this pathway is activated during acinar to ductal transdifferentiation in vitro and in vivo [33, 34]. In addition, enforced expression of cytoplasmic Notch in normal mouse pancreas induced acinar-ductal metaplasia [34]. In preneoplastic lesions, as well as invasive PDAC, Notch receptors, ligands and downstream targets such as Hes1 are upregulated both in mice and humans, suggesting that Notch activation is an early event resulting in the accumulation of undifferentiated precursor cells. Moreover, by using in vitro and in vivo systems it was demonstrated that Notch activation resulted from EGF receptor activation, another signaling route involved in acinar to ductal metaplasia that is upregulated in human and mouse pancreatic neoplasia [34, 35]. Recently, matrix metalloproteinase 7, which is expressed in most metaplastic epithelia in vivo, was found to be required for Notch activation [36], leading to dedifferentiation of acinar cells. This mechanism would provide an alternate way of Notch activation during exocrine pancreatic disease, in contrast to the ligand-dependent cleavage of Notch during pancreatic development. Intriguingly, Notch signaling remains active in normal adult centroacinar cells, making this population a potential source for pancreatic cancer genesis [22].

The hedgehog family of secreted signaling proteins regulates the growth of many organs during embryogenesis [37]. During pancreatic development, this pathway is negatively regulated. Sonic hedgehog (Shh) is thus undetectable in the prepatterned pancreatic territory and in the adult pancreas. Shh knockout mice display an increased pancreatic mass, whereas Pdx1-driven misexpression of Shh leads to pancreatic agenesis [38, 39]. Therefore, correct regulation of the pathway is required for pancreatic specification.

Importantly, Shh activation has been implicated in both the initiation of pancreatic ductal neoplasia and in the maintenance of advanced tumors [40, 41], Shh being activated in PanINs and expressed at highest levels in later-stage lesions and carcinomas [42]. Moreover, misexpression of Shh in transgenic Pdx1-*Shh* mice results in the formation of PanIN-like lesions containing mutations in K-Ras and overexpressing Her-2/neu, which are genetic alterations found early in the progression of human PDAC [42]. Treatment with cyclopamine, an antagonist of the pathway, induced apoptosis and blocked proliferation in a subset of pancreatic cancer cells both in vitro and in vivo [42]. Indeed, PanIN development is associated, in part, with activation of an epithelial differentiation program characteristic of adjacent foregut epithelium [43], a gene expression signature which can be induced by ectopic Shh activation in nontransformed ductal cells [43]. Enhanced pancreatic ductal cell proliferation, protection from apoptosis and cooperation with activated K-Ras [44] have been shown as other molecular mechanisms by which Shh contributes to pancreatic tumorigenesis.

Wnt signaling is also tightly regulated during pancreatic embryogenesis and its inappropriate activation during early pancreatic development results in agenesis of this organ. Enforced Wnt activation in early Pdx1-expressing cells induces a severe reduction in pancreatic mass [45], similar to what occurs when pancreata develop without β-catenin [46–48] or by overexpression of a dominant-negative form of Frz8 [49]. Impaired Wnt signaling leads to a complete lack of acinar cells. Conversely, activation of the pathway predominantly in acinar cells induces an increased pancreatic mass, due to an enhanced exocrine cell proliferation without pancreatic tumor formation [45, 50].

Accordingly, mutations in APC and β-catenin in various types of nonductal pancreatic cancers have been reported, but are rare in PDAC [51–54]. Nonetheless, aberrant cytoplasmic and nuclear expression of β-catenin were reported in preneoplastic lesions and a number of PDAC [55, 56]. Recently, Pasca di Magliano et al. [57] demonstrated similar results in a large cohort of patients and in mouse models of PDAC. Inhibition of the pathway in several PDAC cell lines reduced their ability to proliferate and was associated with increased apoptosis, as a result of the transcriptional activity of β-catenin rather than to E-cadherin/β-catenin binding. As this requires a physical interaction between nuclear β-catenin and TCF/lef transcription factors, the authors investigated TCF4 expression, observing that it was high in cultured pancreatic cancer cells whereas it was only detected in a small proportion of normal duct cells. Interestingly, Shh signaling enhanced TCF4 expression, and in vivo overexpression of Gli2 (a Shh target) in Pdx1-expressing cells resulted in the formation of undifferentiated tumors displaying cytoplasmic and nuclear β-catenin expression. Similar results were obtained when both K-Ras and Shh signaling were deregulated, thus positioning Wnt activation downstream of Shh signaling during pancreatic tumorigenicity.

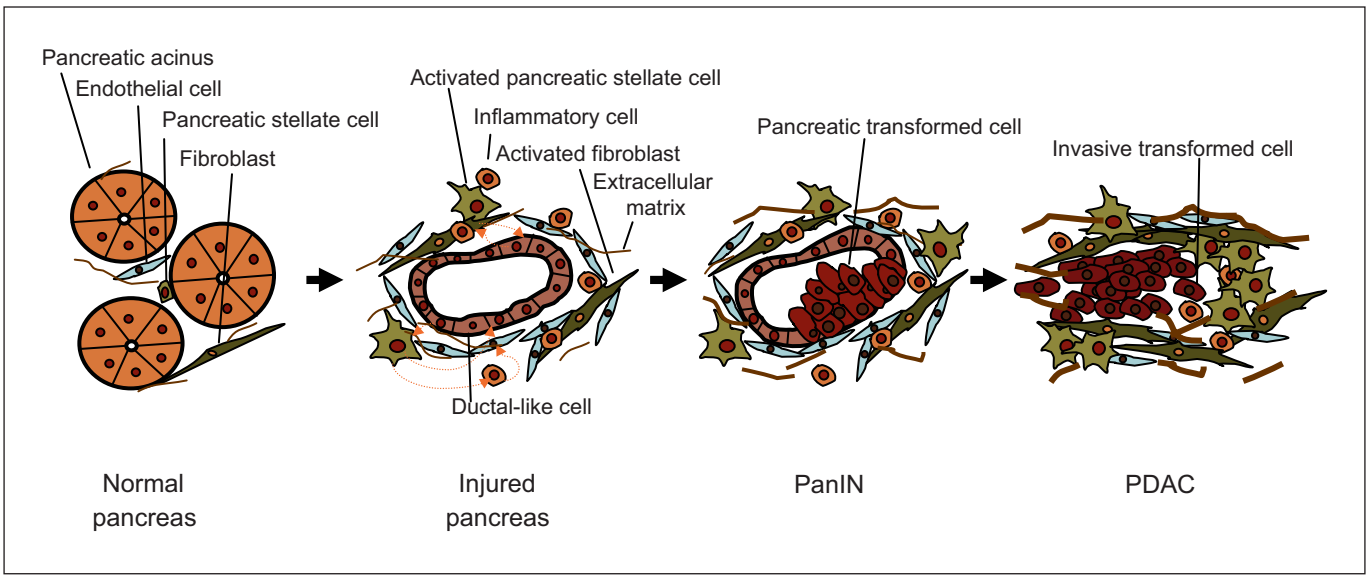

Fig. 2. Schematic representation of some tumor-stroma interactions during PDAC development.

Role of Desmoplasia in PDAC

As indicated above, one of the most important characteristics of PDAC is an intense fibrotic reaction, known as tumor desmoplasia. However, there is a dearth of information on the contribution of the stroma to the mechanisms responsible for PDAC progression.

The notion that the stroma can influence tumor development is now widely accepted because chronic inflammatory conditions – which induce stromal activation – have been frequently associated with cancer risk in numerous tissues. This premalignant condition, particularly in the hereditary form of chronic pancreatitis, also holds true for human PDAC [58], and is further supported by the inflammation-associated cancer model of Guerra et al. [21]. Importantly, these authors demonstrate that a mild experimental chronic pancreatitis dramatically sensitizes adult mice to K-Ras^{G12V}-driven PanIN/PDAC formation [21]. Therefore, an inflammatory environment may provide a landscape that fosters the transformation of epithelial pancreatic cells towards a neoplastic phenotype, and that eventually will result in PDAC.

This simplistic point of view is challenged by the observation that activated molecular pathways essential for PDAC development, i.e. K-Ras mutations or NF-κB activation, are already deregulated in chronic pancreatitis [59, 60]. Therefore inflammation and tumorigenesis are intimately linked from early stages of pancreatic injury, pointing out the need for a holistic understanding of the stroma-parenchyma relationship in PDAC.

Similarly to pancreatic inflammation, in early stages of the preneoplastic lesions there is a variable enhancement in the proliferation of mesenchymal cells, primarily resident fibroblasts, in the surrounding stroma. In addition, new blood vessels are generated and, in some instances, inflammatory cells infiltrate the stroma. As preneoplastic lesions progress to invasive PDAC, the stromal component is further activated. Pancreatic stellate cells proliferate, loose their cytoplasmic lipid droplets and acquire a spindled-shape, as in chronic pancreatitis. Other mesenchymal cells such as interlobular fibroblasts or the pericytes (derived at least in part from the bone marrow) also proliferate and contribute to tumor desmoplasia with an extensive deposition of extracellular matrix (ECM) components. Proliferation of stromal fibroblasts is further enhanced by the recruitment to the periphery of pancreatic tumors of a cohort of inflammatory immune cells expressing high levels of profibrotic and proangiogenic factors [61] (fig. 2). A key question is the ontogeny of some of the stromal cellular components, but the lack of specific markers for each population precludes the precise classification of these cells regarding their origin. This information is especially important in order to identify non-resident cells that are actively recruited to the stroma or those pancreatic cells that could have un-

dergone transdifferentiation or transition processes along PDAC development.

Although at histological level the composition of the stroma in chronic pancreatitis and in PDAC is similar, the sequential mutations and deletions that tumor cells undergo along the neoplastic process are likely to impinge on the cellular and molecular composition of the stroma. Conversely, tumor-conditioned stroma can modulate essential properties of the tumor cells such as their metastatic potential [62, 63].

A paradigm for tumor influencing the stroma is related to the status of the TGF-β signaling pathway. TGF-β is a cytokine secreted into the ECM by epithelial, endothelial, hematopoietic and mesenchymal cells [64, 65]. TGF-β cascade has a paradoxical role in pancreatic tumor development, first by acting as tumor suppressor through modulation of expression of cell cycle regulators and activation of apoptosis, and later on as tumor promoter [66]. Mutations or deletions of SMAD4, coding for a protein involved in TGF-β signaling, have been found in approximately 50% of pancreatic cancers. In vivo experimental models suggest that the Smad4 tumor suppressor activity could constrain the progression of K-Ras^{G12D}-initiated tumors in the early stages of the pancreatic neoplasia, mainly by affecting the growth of the epithelial transformed cells in the parenchyma [67].

In contrast, in high-grade PanIN and advanced cancers, the TGF-β signaling cascade is found to be active. Interestingly, human PDAC phenotypes vary from well differentiated to undifferentiated carcinoma. These phenotypes could be due to the ability of TGF-β to induce an epithelial to mesenchymal-like transition (EMT) of pancreatic tumoral cells, at least in vitro [68, 69]. Supporting this possibility, pancreatic tumors of Smad4-deficient mice retained epithelial differentiation, whereas tumors from Smad4 wild-type mice often display an EMT [67]. In this regard, direct evidence for the functional involvement of adult hepatocytes, closely related to pancreatic epithelial cells, in the accumulation of activated fibroblasts in the fibrotic liver via EMT has recently been reported [70]. Further investigation is necessary to elucidate the contribution of EMT to the pool of the fibroblasts in the pancreatic tumor stroma.

Besides this effect on tumor cells, there is increasing evidence for the paracrine role of TGF-β as tumor promoter that modulates the stroma, i.e. by altering the expression of ECM components, and stimulating angiogenesis and the immunosupression [61]. The fact that most cancer cells secrete higher amounts of TGF-β than their normal counterparts is highly suggestive, this overexpression being strongest in the most advanced stages of pancreatic cancer, whereas TGF-β RII expression is higher in the stroma of PDAC than in that of chronic pancreatitis. Collectively, these data imply that therapeutic approaches should take into account not only TGF-β status in the tumor, but also in the surrounding stroma, in order to evaluate beforehand, as much as possible, the efficacy of the treatment.

Other types of pancreatic autocrine and paracrine factors, in many instances secreted by both the epithelial tumor cell as well as by stromal cells, are cyclooxygenase 2, vascular endothelial factors, hepatocyte growth factor, or the family of matrix metalloproteinases [61]. Crosstalk between epithelial tumoral cells and different cells of the stromal compartment by means of these factors will result in the acquisition and enhancement of the pancreatic tumor abilities, such as angiogenesis and invasiveness, key properties for the fatal outcome of this disease.

Conclusion

The above-mentioned studies provide fascinating insights into the mechanisms that affect the plasticity of pancreatic cells in the context of neoplastic transformation. The identification of PDAC precursors, the signal transduction pathways involved in transformation, as well as the interactions between tumor cells and the surrounding microenvironment, are important objectives that can help to detect pancreatic cancer at earlier stages in order to render therapy more effective.

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