Differential role of Hedgehog signaling in human pancreatic (patho-) physiology: An up to date review

Eckhard Klieser, Stefan Swierczynski, Christian Mayr, Tarkan Jäger, Johanna Schmidt, Daniel Neureiter, Tobias Kiesslich, Romana Illig

Abstract

Since the discovery of the Hedgehog (Hh) pathway in drosophila melanogaster, our knowledge of the role of Hh in embryonic development, inflammation, and cancerogenesis in humans has dramatically increased over the last decades. This is the case especially concerning the pancreas, however, real therapeutic breakthroughs are missing until now. In general, Hh signaling is essential for pancreatic organogenesis, development, and tissue maturation. In the case of acute pancreatitis, Hh has a protective role, whereas in chronic pancreatitis, Hh interacts with pancreatic stellate cells, leading to destructive parenchym fibrosis and atrophy, as well as to irregular tissue remodeling with potency of initiating cancerogenesis. In vitro and in situ analysis of Hh in pancreatic cancer revealed that the Hh pathway participates in the development of pancreatic precursor lesions and ductal adenocarcinoma including critical interactions with the tumor microenvironment. The application of specific inhibitors of components of the Hh pathway is currently subject of ongoing clinical trials (phases 1 and 2). Furthermore, a combination of Hh pathway inhibitors and established chemotherapeutic drugs could also represent a promising therapeutic approach. In this review, we give a structured survey of the role of the Hh pathway in pancreatic development, pancreatitis, pancreatic carcinogenesis and pancreatic cancer as well as an overview of current clinical trials concerning Hh pathway inhibitors and pancreas cancer.

Key words: Pancreatic cancer; Hedgehog; Pancreatitis; Pancreas; Development
Core tip: The Hedgehog (Hh) pathway is a ligand-dependent and evolutionary conserved cellular signaling mechanism with various physiologic (development) and pathogenetic functions (especially carcinogenesis). Concerted Hh signaling is essential for human pancreatic development and homeostasis of the gastrointestinal tract. Aberrant expression within the Hh signaling pathway results in malformations like annular pancreas. The Janus aspect of Hh in pancreatitis is reflected by the protective role of Hh in acute pancreatitis vs the disease-progressive function of Hh in chronic pancreatitis (CP), whereby CP is linked to pancreatic cancerogenesis via pancreatic intraepithelial neoplasia (PanIn). Starting with PanIn and ending up at metastatic disease, Hh pathway is expressed in ductal pancreatic cancer thereby influencing and being paracrine influenced by the tumor microenvironment.

INTRODUCTION

Hedgehog (Hh) genes were originally identified in drosophila melanogaster as regulators of body patterning during embryonic development[1]. Today it is known that the Hh pathway plays a central role in diverse biological processes in mammals, such as embryonic development, cell proliferation, differentiation, tissue repair and maintenance of stem cell status in the adult[2].

In general, activation of the Hh pathway relies on the binding of a secreted ligand to its receptor. Three ligand homologues are known in mammals: Desert hedgehog (Dhh), Indian hedgehog (Ihh) and Sonic hedgehog (Shh). The ligands are produced as precursors and are secreted after extensive modifications to bind to their membrane bound receptor, called Patched. In mammals, two homologues exist, Patched1 (Ptc1) and Patched2 (Ptc2). After signal transduction via the co-receptor Smoothened (Smo), the executing transcription factors of the Hh pathway are the Gli proteins, of which three homologues are known in mammals: Gli1, Gli2 and Gli3[3]. Using a simplified model, the canonical Hh signaling can be described as follows[2,4]: In the absence of a Hh ligand, Ptc inactivates Smo - probably by preventing its localization into the primary cilium, a cell organelle that is thought to be essential for proper Hh signaling[5,6]. As a consequence, the Gli proteins are processed in such a way that they act as transcriptional repressors of the Hh target genes. However, upon binding of the Hh ligand to the receptor Ptc, inactivation of Smo is ended, allowing Smo to translocate to the primary cilium and initiate a cascade of events that ultimately lead to the conversion of Gli factors into their active form. The latter then shuttle into the nucleus and enable transcription of Hh target genes, including components of the pathway itself, such as Ptc1 and Gli1, indicating a built-in feedback loop within the Hh signaling cascade[5]. In addition to the “classical and canonical” Hh signaling described above, also non-canonical (Gli-independent), non-classical (ligand-independent) and aberrant Hh signaling (driven by activating mutations) have been identified at different stages of carcinogenesis (Figure 1)[7].

The pancreas is a fundamental organ of the digestive system with specialized endocrine and exocrine functions. The acinar cells within the exocrine pancreatic compartment produce and secrete numerous digestive enzymes into the duodenum. In the endocrine compartment, specialized cells produce hormones and directly release them into the blood stream - most importantly to control and regulate the blood glucose concentration. It is known from previous studies that physiologic Hh pathway signaling is crucial for correct development of the pancreas[8,9]. With this review, we give an overview of the current understanding of the role of Hh signaling in pancreatic development, cell differentiation and functional specialization. In a second part, the pathomechanistic implications of deregulated Hh signaling are discussed for the clinically most important pancreatic pathologies.

Hh SIGNALING IN HUMAN PanCREATIC DEVELOPMENT

Development of the pancreas

Pancreatic development is based on: (1) The fusion of two evaginations of the foregut to one single organ; and (2) endodermal growth by dichotomy branching. According the classical Carnegie stages[10,11], in stage 13, the dorsal pancreatic bud arises at first as a thickening of the endodermal tube, which proliferates, into the dorsal mesogastrium. In close, in stage 14, the ventral pancreatic bud evacinates to the liver primordium. As a result of differential growth of the duodenum, which rotates 90 degrees clockwise and becomes “C”-shaped, the ventral pancreatic bud comes to lie below and behind the dorsal pancreatic bud in stage 15. Until stage 17, both pancreatic buds have fused: The ventral pancreatic bud forms the posterior part of the head and the posterior part of the uncinated process, whereas the rest of the pancreas is formed by the dorsal pancreatic bud (the anterior part of the head, the body and the tail). Failure of the ventral pancreatic bud to migrate will result in an annular pancreas with consequent duodenal stenosis[12]. The main pancreatic duct (of Wirsung) is formed by the fusion of the distal part of the dorsal pancreatic duct and the entire ventral pancreatic duct and enters the duodenum combined with the bile duct at
As described in detail in chapters Cellular differentiation and maturation of the tissue, Hh signaling occurs at low levels during early organogenesis to ensure the correct establishment of organ boundaries and tissue architecture, and is up-regulated in development, because ectopic expression of Shh leads to transformation of pancreatic mesoderm into intestinal mesenchyme in mice [15]. In single mutant mice (i.e., Shh-/- or Ihh-/-), gastrointestinal defects of the developing endoderm like annular pancreas or other malformations have been reported, suggesting similarities to human gut malformations [8,16].

It was shown that the graded response to Hh-signaling controls regular pancreatic development in mice, in which Hh signaling occurs at low levels during early organogenesis to ensure the correct establishment of organ boundaries and tissue architecture, and is up-regulated in late developmental stages to promote proliferation and maturation of the tissues [9,17-19]. Nielsen et al [20] confirmed the suggested concerted Hh signaling also in human pancreatic organogenesis: In early pancreatic development (7.5 wk), Gli3 was highly expressed in developing pancreatic ducts - while Smo and Gli2 were absent. In contrast, Smo and Gli2 were highly expressed between weeks 14 to 18, whereas the expression of Gli3 was reduced.

PDX1 (a pancreatic-promoting transcription factor; syn.: Insulin promotor factor 1) is also expressed in the preduodenal endoderm - including the sites of dorsal and ventral pancreatic bud formation. Total absence of the acinar structures is determined by pancreatic mesenchyme which gives rise to connective tissue between the ducts resulting in pancreatic proliferation and maintaining the relative proportions of acinar, α- and β-cells. Additionally, it provides cell lines for smooth muscle within the pancreatic tissue, and angiogenic mesenchyme produces blood and lymphatic vessels.

Molecular regulation of pancreatic development by Hh signaling

Pancreas development is regulated by the activation/inactivation of Hh signaling members, which are ex-/repressed either within pancreatic tissue (e.g., Ihh) or in adjacent tissue (e.g., Shh) [14]. Initial absence of Shh signaling is required for regular pancreatic development, because ectopic expression of Shh leads to transformation of pancreatic mesoderm into intestinal mesenchyme in mice [15]. In single mutant mice (i.e., Shh-/- or Ihh-/-), gastrointestinal defects of the developing endoderm like annular pancreas or other malformations have been reported, suggesting similarities to human gut malformations [8,16].

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pancreas is observed in homozygous PDX1 mutant mice that suggest that PDX1 is necessary for the formation of the pancreas and may be essential in the differentiation of pancreatic precursor cells[21,22]. Although all of the involved downstream effectors of human pancreas development have not been determined in detail yet, it appears that expression of the paired homeobox genes PAX4 and PAX6 specifies the endocrine cell lineage: Cells expressing both become β-, δ-, and γ-cells; whereas those expressing only PAX6 become α-cells.

**Hh IN PANCREATITIS**

The cellular and molecular processes in acute pancreatitis (AP) and chronic pancreatitis (CP) were extensively elucidated in the last years providing valuable detailed insights which could be important in the next years for a further therapeutical approach in this partially lethal disease entity (reviewed in detail in[23-25]). In short, in the phase of AP, the major cellular key players are neutrophils, monocytes and macrophages which interact by building and secretion of cytokines and inflammatory mediators, mainly tumor necrosis factor α, interleukin (IL) 1β and 6, and monocyte chemotactic protein. In the phase of CP, pancreatic stellate cells (PSC), tissue infiltrating myeloid cells, and particularly macrophages are coming to the fore by induced and increased progressive fibrozing of the pancreas tissue, being mediated mainly by nuclear factor (NF)-κB[26,27]. Finally, the crosstalk of the mentioned cells is linked to T-subsets (CD-8+ /central memory cells as well as T-regulator cells) which are involved in the pathogenesis of CP[28,29]. Additionally, CP is commonly regarded as a relevant risk factor for ductal pancreatic cancer (DPC) by irregular ductal changes leading to acino-ductal metaplasia and pancreatic intraepithelial neoplasia (PanIN)[30,31].

Focusing on the linkage between the Hh pathway and AP as well CP, experimental investigations demonstrated that the members of the Hh pathway could be detected in different amounts in AP and CP, whereby the definitive functional role of Hh in AP and CP seems to be very different. Additionally, in the process of CP forward to DPC an irregular expression pattern of the Hh members are observed compared to the normal and structured embryonic development of the pancreas[9,32,33].

**AP**

Compared to CP, the role of Hh in AP has been dealt with only in few studies. Summarizing these data, activation of the Hh signaling is linked to injury and repair using the cerulein-mediated model, whereby the unequivocal conclusion of the available experimental data is that the Hh has protective function in AP. In 2008, Fendrich et al[33] presented a functional analysis of the Hh pathway in AP using pharmacologic and genetic techniques (like Ptc1-LacZ reporter mice and two different Cre-driven pancreas-specific depletion mice models of Smo) demonstrating that Hh is essentially involved in effective regeneration of the exocrine pancreas. By this approach, Shh, Ihh, and Gli1 are increasingly expressed in caerulein treated mice, whereby the pharmacologic and genetic inhibition lead to persistence of PDX1 expressing metaplastic intermediates and impaired tissue repair. Additionally, the group of Zhou et al[34] used a Cerulein-induced AP model in mice to show elegantly that: (1) Shh, not Ihh or Dhh, is involved in this model; (2) Shh-inhibition aggravates the AP; and (3) the anti-inflammatory autocrine effect of Shh is mediated by IL-10. A recent experimental study from 2014 showed that Gli1, the downstream member of the Hh cascade, could essentially influence the inflammatory reaction in the circumstances of remodeling processes of the pancreas. Based on genetic analysis of deletion of a single allele of Gli1, the authors postulated that the canonical Hh pathway, respectively the transcription factor Gli1, is essential for pancreatic recovery in inflammatory processes via Gli1 targeted cytokines, including IL-6, murine homolog of IL8, monocyte chemotactant protein-1, and Macrophage colony-stimulating factor M-csf, leading to pancreatic tumorgenesis via improper stromal remodeling and persistence of the inflammatory infiltrate[35].

**Chronic pancreatitis**

Empirc studies in humans with CP demonstrated a heterogeneous upregulated expression of Ihh, its receptors Ptc and Hedgehog-Interacting Protein, and Smo in different histological distribution and cellular localization of human tissue with CP using Northern blotting, immunohistochemistry and Western-blotting[36,37,38]. Interestingly, the members of the Hh pathway were localized mainly in the islet cells, whereas the Hh signaling members were present in degenerated acinar and tubular complexes of CP[36,37]. In addition, Kayed et al[37] could show that the inhibition of the Hh pathway via Cyclopamin led to growth inhibition of TAKA-1 pancreatic ductal cells through cell cycle arrest in vitro.

Based on cDNA microarrays, Bhanot et al[31] could support the findings, that the Hh pathway is altered in microdissected ectatic ducts of CP whereby dysregulation of Hh could enhance the probability of DPC via duct ectasia, acino-ductal metaplasia or intraepithelial neoplasia as reviewed by Bahnot et al[31] in 2008.

As mentioned above, PSCs are essentially involved in the pathogenesis of the CP, whereby the main question is, how the Hh pathway regulates the activation of these PSCs.

The experimental analysis of the group of Shinozaki et al[38] revealed that Ihh has no evident effect on expression of collagen-1 or alpha-smooth muscle actin or on proliferation of PSCs, but Ihh modulates the migration potency by changing the amount of membrane-type 1 matrix metalloproteinase and its localization on the plasma membrane leading to a pro-migration status of PSCs. Although the Ihh effects are mediated by Gli1, experimental overexpression of Gli1 using an adenovirus-mediated or RNA interference techniques revealed a negatively regulation by Gli1 to Ihh effects in vitro.

But the question remains: Why is Hh pathway up-
Table 1  Summary of the role of Hedgehog signaling in pancreatitis, indicating the protective role in acute pancreatitis vs disease-progressive function in chronic pancreatitis as well the possible association to pancreatic cancerogenesis (32-38, 42, 43)

| Pathogenetic effect of Hh | Protective | Chronic pancreatitis |
|--------------------------|-----------|---------------------|
| Detected members of Hh   | ↓ Shh (Ihh, Dhh), Gli1 | ↑ Ihh (Shh), Ptc, Hip, SMO, Gli1, Gli2 |
| Interactive cells (auto-and paracrine effects) | Acinar/ductal cells with; acute inflammatory cells | Acinar/ductal cells with; PSC |
| (Immune) mediators of inflammation | IL-10, IL-6, mIL-8, Mcp-1, and M-CSF (Csf1) | MTI-MMP, MMP9, TGF-β1, smooth muscle actin, fibronectin 1, type 1 collagen |
| Association to cancerogenesis | No | Yes, possibly via ADM and Panin |

Hh: Hedgehog; Shh: Sonic Hh; Ihh: Indian Hh; Dhh: Desert hedgehog; IL: Interleukin; mIL-8: Murine homolog of IL-8; Mcp-1: Monocyte chemoattractant protein-1; M-csf: Macrophage colony-stimulating factor; Ptc: Patched; Hip: Hh-interacting protein; SMO: Smoothened; MT1-MMP: Membrane-type 1 matrix metalloproteinase; MMP-9: Matrix metalloproteinase 9; TGF-β: Transforming growth factor beta; ADM: Acino-ductal metaplasia; Panin: Pancreatic intraductal neoplasia; PSC: Pancreatic stellate cells.

regulated within the fibrogenic process of CP? Based on in vitro and in situ studies with xenografts as well as in humans with pancreatitis, it is postulated that par- and partially autocrine activation of stromal cells by Hh ligands from epithelial components and vice versa are responsible.[29-41] The experimental data of Jung et al.[42] are based on transgenic phenotypes in zebrafish, with over-expression of either Inh or Shh along with green fluorescence protein. Consecutive analysis of these transgenic phenotypes using quantitative and qualitative investigations of mRNA and protein levels including PCR, in situ hybridization, and immunohistochemistry revealed that myofibroblasts and ductal cells are activated and proliferate which is triggered by paracrine Hh signaling in a restricted expression of Ptc1, Smo and Gli1/2. Additionally, Hh ligands could induce matrix metalloproteinase 9 and TGF-β1 in this animal model.[42]

Recent investigations by Tsang et al.[43] could support the published findings of pro-fibrogenic effects of Hh in CP by using an in vivo model. The application of Rhein, a natural anthraquinone derivative, reduces the activation of PSCs in mice with experimental induced CP. The morphological effect of Rhein in reduced pancreatic fibrosis was paralleled by reduced molecular expression of fibrogenic markers including alpha-smooth muscle actin, fibronectin 1, type I collagen as well as the members of the Hh pathway Shh and Gli1.

Interestingly, the promoting fibrotic effect of Hh signaling is not only existent in pancreas, but also could be observed in other organs like lung, bile duct and liver implicating a tissue independent overriding principle of the Hh pathway in this pathogenesis.[44-46].

CP and pancreatic carcinogenesis
Since chronic recurrent inflammation has been linked to carcinogenesis, especially in pancreas, some findings of Hh in AP/CP and pancreatic carcinogenesis are presented in the following for supporting this already emphasized linkage.[47,48] First of all, Hh modulates the axis between inflammation and cancerogenesis via activation and production of cytokines by human peripheral CD4+ T cells.[49] Furthermore, experimental studies of Hh in AP and CP revealed morphological changes like ductal metaplasia promoted by Shh, which are per se no pre-tumorous conditions.[33,50]. Nevertheless, during progression of CP, morphological changes of the ducal pancreatic tissue like papillary lesions with nuclear atypia resulting in PanIn lesions could be observed which have a high association to aberrant Hh expression and pancreatic cancer.[31,50]

In conclusion (summarized in Table 1), members of the Hh pathway have protective properties in case of AP, whereby the face of Hh changes to a progressive and disease-promoting function in CP. Especially in CP, the negative effects of Hh on tissue remodeling and repair favored the possibility of cancerogenesis via de- and trans-differentiation.[51-54].

Hh IN PANCREATIC CANCER: FROM IN VITRO TO IN SITU

In vitro: Findings in cell culture experiments and xenografts
Hh signaling in the normal pancreas and in pancreatic ductal adenocarcinoma is exclusively paracrine with expression of Shh (tumor cell and stroma signal circle as shown in Figure 2)[30]. The silencing of Smo in pancreatic cancer epithelium in mice showed no altered tumor spread or development, so the Hh signaling does not occur in an autocrine way.[55]

In paracrine signaling, the Hh ligand sends signals directly to the stroma and provides a selective tumor growth advantage. This was established through a pancreatic cancer model where Hh signal was needed for overall tumor growth while the particular tumor cells themselves did not respond to Hh ligand.[56]

The existence of cancer stem cells (CSC) in different tumors, including pancreatic cancer, offers an explanation why some therapy assessments are ineffective.[57,58] Therefore, a good knowledge base for new therapies, which target pancreatic CSCs, is very important. The Hh signaling pathway plays a vital role in pancreatic and embryonic development; autocrine or paracrine secreted Shh activates a signal transduction cascade that includes other Hh members like Ptc and Smo, which then activates the canonical Hh pathway through Gli.

This leads to transcription of multiple targets like...
Nanog, Cyclin D1, Ptc, Gli1 and Gli2. Activation of Shh signaling seems to precede the transformation of pancreatic tissue stem cells to cancerous stem cells. This was shown in mice, which were treated with sulforaphane to inhibit the growth of these stem cells. Sulforaphane is a natural compound found in cruciferous vegetables like broccoli that as an inhibitor acts on various receptors and pathways with anti-cancerous properties like apoptosis induction and cell proliferation[59]. This experimental study showed that human pancreatic stem cells need the activity of the Hh-Gli pathway for proliferation, survival, self-renewal and tumorigenicity[60].

In 2002, Chen et al[61] modulated mammalian embryonic pancreas development in vitro using cyclopamine treated pancreatic explants. A recombinant form of Shh was added to pancreatic buds to activate the Hh signaling pathway. The fluorescently labeled epithelium of the pancreatic explants underwent extensive growth and branching when treated by cyclopamine, which indicates that Hh inhibition did not block branching in the epithelium[61].

Walter et al[62] isolated pancreatic fibroblasts from benign and malignant primary pancreatic resection specimens by immunohistochemistry marker selection through vimentin. Together with two fibroblast cell lines, SC2 and SC3 (from non-neoplastic pancreas), the cancer-associated fibroblasts (CAF) were characterized for Hh activity. The fibroblast cell lines and the isolated CAFs where treated with Shh ligands to observe any expression changes on Gli mRNA. As a result they detected overexpression of Smo in pancreatic CAFs, which could transduce the Shh signal followed by Gli1 activation. The Hh pathway has been identified as activated in cancer associated stromal fibroblasts in mouse models of pancreatic cancer. CAFs can actively transduce the Hh signal to induce Gli expression. CAFs expressing Gli1 respond to exogenous Shh ligand, whereas control fibroblasts lacking Smo expression are unresponsive to Hh ligand, and downregulation of Smo in CAFs inhibits transduction of the Hh signal[62].

In human tumor xenografts, expression of Shh by tumor cells correlated with increased expression of Gli1 and Ptch1 in the stromal compartment. Pathway inhibition affected only stromal Gli1 and Ptch1 expression and resulted in decreased tumor growth exclusively in Hh ligand-expressing tumors[63].

Tian et al[64] demonstrated that the expression of an oncogenic allele of Smo (SmoM2) in mouse pancreas neither activated Hh signaling in epithelial cells nor promoted their neoplastic transformation. In murine pancreatic cancer models as well as in human pancreatic cancer specimens, activation of the Hh pathway was observed only in stromal cells surrounding Hh ligand-expressing tumor cells[64].

**In-situ: Findings in human specimen of pancreas**

Tumors of the pancreas can develop either from ductal, neuroendocrine or acinar cell populations. Due to a lack of information about the role of the Hh signaling pathway in acinar and neuroendocrine tumors of the pancreas, the following paragraphs will concentrate on DPC.

Among all cancers, DPC has one of the worst pro-
gnoses among all cancers with an overall 5-year survival rate of less than 5%[65]. Chemo- and radiotherapy are largely ineffective; furthermore, metastatic spread frequently occurs even after complete surgical resection[66]. The Hh pathway is one highly promising signaling transduction pathway for a better understanding of the origin of DPC. 

Expression of Hh pathway members is usually not present in healthy adult pancreatic tissue[67]. In 2008, a global sequencing analysis revealed that the Hh pathway is one of the core signaling pathways that undergoes somatic alterations in nearly all pancreatic cancers[68]. Kayed et al[37] showed an aberrant activity of the Hh pathway in chronic pancreatitis and pancreatic cancer. Later on, it was recognized that Shh expression enhances the proliferation of pancreatic duct epithelial cells[69] and is not only up-regulated in the setting of pancreatic injury, but also in noninvasive precursor lesions of DPC: (1) PanIn; and (2) intraductal papillary mucinous neoplasia (IPMN) starting with rising expression values up to Hh pathway persistence in metastatic state[67,70,71]. Additionally, it was stated that up-regulation of the Shh ligand is sufficient to misdirect the pancreatic ductal epithelium towards a gastrointestinal metaplastic phenotype, which explains the involvement in IPMN formation[50,63].

However, dysfunction, or rather re-activation of the Hh pathway is not the only reason for the development of PanIn and DPC. Lauth et al[72] described a synergistic molecular crosstalk between Hh pathway and activated V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (K-RAS) signaling pathway[72]. Over 90% of patients suffering from DPC showed a K-RAS mutation, thus identifying the K-RAS pathway as another key mediator of pancreatic carcinogenesis[68,73]. Patients with a K-RAS mutation developed PanIn; and an additional P53 loss of function leads to subsequent development of the lesion towards DPC[74]. According to various studies, the crosstalk between Hh and K-RAS takes place via the RAF/MEK/MAPK pathway[75,76].

In 2013, Mills et al[77] were able to identify Gli1 as an effector of K-RAS at early stages of pancreatic carcinogenesis. They showed in a mouse model that loss of Gli1 impairs K-RAS-induced carcinogenesis. Although the mice still developed PanIn, the incidence of PanIn decreased and as a result, no mice suffered from DPC[77].

In recent studies, another central role in pre-neoplastic lesions of the pancreas is awarded to the signal transducer and activator of transcription 3 (STAT3) and its upstream cytokine IL-6. It is supposed that STAT3 activation is involved in driving early changes in the microenvironment promoting PanIn formation in the presence of oncogenic K-RAS[78,79]. Mills et al[77] stated that Gli1 also acts on CAF by paracrine regulation of the IL-6/STAT3 pathway in stromal cells of the tumor microenvironment (TME) and, thus, regulating the progression of precursor lesions and tumor formation (Figure 2).

DPC pathogenesis is characterized by a desmoplastic reaction to invading tumor cells, including a dense extracellular matrix that was recently shown to be the result of epithelial to mesenchymal transition (EMT)[80,81]. The epithelial-mesenchymal interaction, especially in the paracrine model of the Hh pathway, plays a distinctive role in different carcinoma entities as well as in DPC. Deregulated Hh pathway in PanIn and DPC leads to the secretion of Hh ligands Shh and Ihh, followed by a paracrine activation of CAFs in the surrounding stroma leading to expansion and desmoplasia[40,82,83]. In detail, neoplastic epithelium secretes Shh, which binds to the cognate Ptc-receptor on stromal cells, followed by desmoplastic stromal expansion and microenvironment remodeling. Moreover, supporting the paracrine action model of Hh pathway in DPC, Yauch et al[83] showed that treatment with Hh pathway antagonist results in downregulation of Hh target genes only in the tumor stroma but not in the epithelial cancer cell. In the same way, Smo expression decreases in mesenchymal cells in the pancreas resulting in Hh pathway activation. However, Lee et al[80] described that Hh pathway activity controls the balance between epithelial and stromal elements: Pathway activation causes stromal hyperplasia and reduced epithelial growth, whereas Hh inhibition causes accelerated growth of epithelial elements and suppression of desmoplasia.

It is suggested that the TME and extensive desmoplasia are partly responsible for chemoresistance in DPC by creating a “fence” around the tumor cells, which protects them against therapeutic compounds[84]. Therefore, tearing down this barrier could be a promising strategy to improve therapeutic approaches. Singh et al[85] already showed that inhibition of Hh pathway depleted tumor-associated stromal tissue.

There are many other different tumor specific characteristics that are influenced by the interrelation of Hh pathway and the TME. Bailey et al[86] identified paracrine Shh-mediated fibroblasts within the TME as source of Hypoxia-inducible factor 1 alpha (HIF-1α), which is known to be a regulator of angiogenesis and metastasis in cancer. Another example is the CXC-motif-chemokine 12/CXCR4 pathway, which is on the hand critical for normal cellular processes, but on the other hand contributes to metastasis, growth, survival and stem cell characteristics of cancer cells[87-89]. CXCL12, the sole ligand for CXCR4, is produced by tumor-associated stromal cells, is increased in DPC; and after binding to its receptor CXCR4, leads to activation of extracellular signal-regulated kinases resulting in release and nuclear translocation of NF-κB, which then directly binds the Shh promotor[86,90,91]. In summary, Hh pathway acts in a predominantly paracrine manner, thereby influencing and being influenced by the TME (for an overview of Hh-dependent interactions between tumor and stroma cells in DPC, Figure 2).

CSC, also called tumor initiating cells are suggested to be responsible for cancer initiation, progression and chemo-resistance in several malignancies including DPC[92]. The transcription factors Nanog, octamer-binding transcription factor 4 and BMI1 Proto-Oncogene, Polycomb Ring Finger (BMI-1) are essential for the
“stemness”, including characteristics like self-renewal of CSC\(^9^{3}\)-\(^9^{5}\). The Hh pathway is implicated in the maintenance of pancreatic CSCs: For example, Li et al\(^9^{6}\) stated that Shh expression was 46-fold greater in pancreatic CSCs (CD24\(^{+}\)/CD44\(^{+}\)/ESA\(^{+}\)) as in other DPC cells (CD24\(^{-}\)/CD44\(^{-}\)/ESA\(^{-}\)). Additionally, Gli1 is known to up-regulate genes that are crucial for many properties for stemness of CSC - like Nanog and BMI-1\(^9^{7}\)-\(^9^{9}\).

Recapitulating this chapter, Hh pathway plays an important role in DPC, beginning from PanIn precursors to progressed metastatic disease. Hh signaling cross talks with a variety of other signaling pathways, like K-RAS, requires the interaction with the EMT in particular via paracrine pathway stimulation in order to contribute to the development of DPC (Figure 1).

### Hh-BASED CLINICAL TRIALS FOR PANCREATIC CANCER

At present, clinical trials using Hh inhibitors enroll patients with pancreatic malignancies including advanced, metastatic, recurrent or resectable pancreatic cancer. Currently, no trials are listed within the United States National Institutes of Health database (www.clinicaltrials.gov) which target pancreatitis or other pancreatic non-neoplastic conditions. As summarized in Table 2, most trials in the phase 1 or 2 setting use GDC-0449 (vismodegib) which is a small molecular weight inhibitor of Smo\(^10^{0}\) thereby interfering with Hh signaling at the plasma membrane level similarly to cyclopamine, a naturally occurring Smo antagonist\(^10^{1}\). Other Hh-targeting drugs in current clinical trials on pancreatic cancer are the Smo-inhibitors LDE-225 (Sonidegib)\(^10^{2}\) and IPI-926 (Saridegib)\(^10^{3}\).

For the latter, a preclinical study on pancreatic cancer in mice demonstrated that IPI-926 depletes tumor-associated stromal tissue and facilitated the delivery and increased the intratumoral concentration of gemcitabine\(^8^{8}\). In line with these results, all currently ongoing clinical trials combine selective Hh antagonists with established chemotherapies (gemcitabine, paclitaxel) or other targeted drugs (erlotinib epidermal growth factor receptor inhibitor) or BKM120 (Phosphatidylinositol-4,5- bisphosphate 3-kinase inhibitor) to investigate possible therapeutic benefits of these drug combinations. Taken together, current clinical studies employ inhibitors of the Smo co-receptor in combination with established chemotherapeutic drugs. Novel experimental inhibitors targeting the Hh pathway at the level of the transcriptional regulation (e.g., Gant-61, Gant-58) have not yet entered the stage of clinical evaluation\(^10^{4}\).

### CONCLUSION

Besides its physiologic functions in human pancreatic development, the Hh pathway is activated in numerous pathological conditions, including carcinogenesis. However, the data on its functional aspects currently available draw a more nuanced picture. Progression from pancreatic cancer precursors lesions (PanIn) to DPC and metastatic disease is strongly influenced by a paracrine Hh signal modulating the interaction between DPC cells and CAFs. This Hh driven signaling predominantly includes the IL-6/STAT3 and the CXCL12/CXCR4 pathways resulting in disease progression by invasion, angiogenesis, metastasis formation and chemoresistance as well as gaining of stem cell like characteristics. Therefore, therapeutic targeting of the Hh pathway may provide new therapeutic approaches to improved disease control and prognosis for both, chronic pancreatitis and pancreatic carcinogenesis.

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P- Reviewer: Boros M, Morini S S- Editor: Kong JX L- Editor: A E- Editor: Li D
