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Hedgehog Signaling in Pancreatic Fibrosis and Cancer

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Abstract: The hedgehog signaling pathway was first discovered in the 1980s. It is a stem cell-related pathway that plays a crucial role in embryonic development, tissue regeneration, and organogenesis. Aberrant activation of hedgehog signaling leads to pathological consequences, including a variety of human tumors such as pancreatic cancer. Multiple lines of evidence indicate that blockade of this pathway with several small-molecule inhibitors can inhibit the development of pancreatic neoplasm. In addition, activated hedgehog signaling has been reported to be involved in fibrogenesis in many tissues, including the pancreas. Therefore, new therapeutic targets based on hedgehog signaling have attracted a great deal of attention to alleviate pancreatic diseases. In this review, we briefly discuss the recent advances in hedgehog signaling in pancreatic fibrogenesis and carcinogenesis and highlight new insights on their potential relationship with respect to the development of novel targeted therapies.

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Abbreviations: DFS = disease free survival, Dhh = desert hedgehog, ECM = extracellular matrix, EDC = epidermal differentiation complex, EMT = epithelial-to-mesenchymal transition, HS = heparan sulfate, Ihh = Indian hedgehog, Ipf1/Pdx1 = insulin promoter factor 1/pancreatic and duodenal homeobox 1, LATS = large tumor suppressor, miRNAs = microRNAs, MMPs = matrix metalloproteinases, OS = overall survival, PARs = protease-activated receptors, PDAC = pancreatic ductal adenocarcinoma, PSCs = pancreatic stellate cells, Shh = Sonic hedgehog, Smo = smoothened, YAP = yes-associated protein.

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The ethical approval is not necessary for the reasons that this is a review of current knowledge of hedgehog signaling in pancreas and this paper has no relation with animal experiment or patient.
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INTRODUCTION

In 1980, hedgehog signaling was first discovered in the fruit fly by Nusslein-Volhard,[1] and has since been found in vertebrates within various organs. Hedgehog signaling, a pathway characterized by being conserved but considerably multifunctional,[2,3] is involved in a variety of developmental and physiological processes, such as body axis formation, angiogenesis, and stem cell homeostasis. As a result, the developing tissues grow into the correct size with the appropriate cell types, orientation, and vascularization.[4,5]

According to the World Cancer Report in 2012, pancreatic cancer was ranked as the seventh most common cause of cancer deaths, with 330,000 deaths globally and a 5-year survival of less than 5%.[6] Pancreatic cancer cells exhibit tenacious growth, early dissemination, metastatic ability, and resistance to radiotherapy and chemotherapy, all of which contribute to high mortality. Without proper and early diagnosis, delayed detection is common. In this case, most patients are diagnosed with end-stage pancreatic carcinoma. Thus, only 10%–15% patients are able to receive surgery, even though an operation is still the most valid therapeutic method; the 5-year survival of these patients is approximately 10%. The patients who are unable to undergo surgery will inevitably suffer through chemotherapy and radiotherapy. The standard remedy for pancreatic cancer established by Burris et al[7] has been updated to include gemcitabine with erlotinib.[8] When compared with gemcitabine alone, the significantly improved 0.3-month survival advantage seems to have no obvious effect on clinical treatment. Hedgehog boosts the initiation and development of pancreatic cancers.[9] Studies indicate that the inhibition of hedgehog can cure malignant diseases.[10–12] Currently, the underlying mechanism of hedgehog signaling in carcinoma is being increasingly studied, as such a somber condition as pancreatic cancer warrants the development of novel and effective methods.

Pancreatic tissue fibrosis is a terminal and distinguishing feature of pathological changes with diverse means of inflicting harm. The formation of pancreatic fibrosis is a complicated and long-term process in which multiple factors interact with each other. Injuries (apoptosis and necrosis) of the pancreas can induce the synthesis and release of proinflammatory factors, chemokines and growth factors such as PDGF, TGF-β1, and angiotensin II,[13–15] resulting in the activation of pancreatic stellate cells (PSCs) and the accumulation of myofibroblasts. Myofibroblasts are terminally differentiated cells that are responsible for the synthesis and deposition of extracellular matrix (ECM) components such as type I and III collagens.[16,17] If repair mechanisms are disrupted or ineffective, excessive deposition of ECM components will form a barrier around the original pathological injury, leading to the intensive resistance to radiotherapy and chemotherapy.[18–20] Hedgehog signaling is an important pathway involved in the activation of PSCs. Inhibition of hedgehog signaling can reduce or even reverse PSCs activation, leading to improved outcomes in chronic pancreatitis. For example, resveratrol, a botanical compound derived mainly from the skins of red grapes, may have

antifibrotic effects on the pancreas by antagonizing the hedgehog pathway.[21] Therefore, screening of highly effective pharmaceutical agents to inhibit the activation of hedgehog signaling provides a great opportunity for the development of antifibrotic drugs.

Hedgehog Signaling: Structure and Function

The hedgehog signaling pathway is classified into 2 modalities: canonical and noncanonical. Noncanonical hedgehog signaling refers to hedgehog signaling receptor dependent signals that do not operate via Gli or Smo. Noncanonical hedgehog signaling is divided into 2 types: Type I acts through Ptch,[22–24] while type II acts through Smo without being regulated by Gli.[23,25] In total, hedgehog signaling molecules include 3 ligands (Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh)),[26–28] 2 receptors (Ptch1 and Ptch2),[29,30] a signal transducer Smoothened (Smo),[4,31] and 3 transcription factors (Gli1, Gli2, and Gli3).[32–34] Each ligand functions in various ways.

As a secretory signal protein, the hedgehog ligand acts as the initiating event in a signaling cascade. When the signaling pathway is activated, hedgehog ligand undergoes autoprocessing and lipid modification reactions to generate the hedgehog signaling peptide with N-terminal palmitoylation and C-terminal cholesterylation, and thereby activates itself.[35–38] Mature hedgehog peptide with lipid modifications will be released from secreting cells by multitransmembrane transporter-like proteins that are dispatched[39] and transduced to target cells through the multiple-transmembrane receptor Ptch.[40]

Ptch is encoded by the *Patched* gene and is a 12-transmembrane protein that acts as the receptor for hedgehog signaling.[35] *Patched* is a tumor suppressor gene and mutations in this gene can lead to Gorlin syndrome, medulloblastoma, and esophageal squamous cancer.[41,42] Ptch1, which is confined to target cells, is upregulated in response to hedgehog-signaling proteins.[43] It is the subtype that is definitively involved in the activation of hedgehog signaling. The transcription of Ptch2 is independent of pathway activation and is coexpressed with hedgehog proteins.[44] Goodrich et al[45] found in Ptch1 knockout mice that the inhibition of Smo activity was abolished. Further studies confirmed that Ptch inhibits Smo activity when hedgehog ligands are absent.[22,23]

When hedgehog peptides are released and activate the hedgehog receptors of target cells, Ptch activity is suppressed, leading to Smo translocation to the plasma membrane, interaction with Cos2, and increased autophosphorylation. Smo is encoded by a protooncogene (Smo gene). It is a 7-transmembrane protein that is coupled with a heterotrimeric G-protein.[46] As previously reported, the small molecule cyclopamine targets the Smo protein to inhibit hedgehog signaling.[47] In Drosophilae, Smo directly recruits the cytoplasmic complex Cos2/Ci/Fu through Cos2 and then influences their activity.[48] In the absence of Smo, the complex Cos2/Ci/Fu phosphorylates and binds to the zinc finger-like transcription factor Gli.

In summary, the hedgehog ligand protein spindles Ptch, which is bound to the ciliary, thereby inducing the release of Smo; then, the Gli protein is released from Smo and is translocated to the nucleus where it functions as a transcriptional activator.[49,50] Only the repressed form of Gli can enter the nucleus and induce the transcription of target genes.[51] The aberrant expression of Gli1 or the dominant activating mutant Gli2 in keratinocytes regulates overlapping transcriptional processes of these 2 proteins (Figure 1).[52]

In contrast to the canonical pathway referred to above, studies have demonstrated that not all hedgehog signaling proceeds through Gli activation; these subtypes of Gli-independent pathways were named noncanonical hedgehog signaling.[52] The binding of a hedgehog isoform to Ptch is required in type I noncanonical hedgehog signaling, which is mediated by the novel functions of Ptch that are unrelated to Smo repression and are thus insensitive to modulators of Smo. In the absence of a hedgehog ligand, the ectopic expression of Ptch1 induces apoptosis.[53] Several studies corroborate the hypothesis that the proapoptotic effect of Ptch1 is exerted through a type I noncanonical pathway. For example, the in vitro or in vivo disruption of detectable canonical hedgehog signaling,[20,21] the absence of an effect of Smo antagonists to prevent the Shh-dependent reduction in caspase-3 activity and the inability of the Smo agonist to mimic the antiapoptotic effect of Shh,[25] and Ptch1 regulation of the cell cycle through cyclin B1 in a Smo- and Gli-independent manner.[52,54]

Compared with type I noncanonical hedgehog signaling, type II signaling is defined by Smo-dependent and Gli-independent cascades.[23,55] Hedgehog signaling responds to Smo agonists that are mediated through the activation of small GTPases to elicit cellular responses. Bijlsma et al[56] found that Shh-induced fibroblast migration was Smo-dependent but Gli-independent. In Chinchilla study,[23] hedgehog proteins in endothelial cells promoted actin stress fiber formation and endothelial cell tubulogenesis in a Smo-dependent manner.[57] In axonal development, Shh signals stimulated the activity of Src family kinase members in a Smo-dependent manner. In a later study,[58] a novel signaling cascade operating through SH3 domain-containing proteins was found to be directly stimulated by hedgehog ligands (Figure 1).

Hedgehog Signaling in the Pancreas: Development to Adult Tissue Remodeling

In humans, the pancreas is located in the abdominal cavity behind the stomach and acts as an endocrine and exocrine gland for the digestive and endocrine systems. Hedgehog signaling influences the development of both endocrine and exocrine functions from the newborn to the mature adult. Staining of embryos reveals that Shh signaling is expressed throughout the gut endoderm,[59,60] with the exception of the pancreatic bud endoderm. During embryogenesis, the pancreas develops from the pancreas bud of the end endodermal foregut and its surrounding mesoderm.[61–63] The function of pancreatic cells emerges from common precursors present in the early gut endoderm.[64,65] Pancreatic bud endoderm expresses high levels of the homeodomain protein Ipf1/Pdx1 (insulin promoter factor 1/pancreatic and duodenal homeobox 1), an essential regulator of early pancreatic development.[66–69] When pancreatic explants were exposed to Shh, Ipf1/Pdx1 induced constitutive Shh overexpression in the pancreatic bud, thus promoting pancreatic mesoderm differentiation into smooth muscle and interstitial Cajal cells, which are typical of the developing intestine rather than the pancreatic mesenchyme and spleen.[70]

Inhibition of Hedgehog signaling with the specific inhibitor cyclopamine would induce a variety of changes in the pancreas,[9] for example, gastric explants expressing endocrine pancreatic markers such as insulin and glucagon,[71] an increase in islet cell number and size and the high level expression of Ipf1. The experiment conducted by diIorio in zebrafish clarified that gene mutation of Shh and the relative signaling pathway had the specific dependence with endocrine function. These

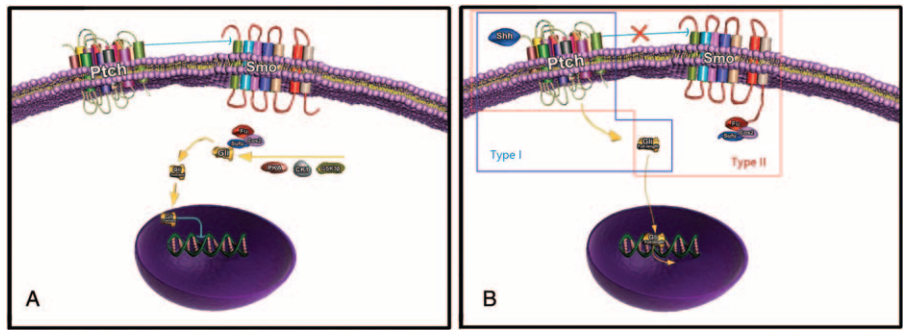

FIGURE 1. Hedgehog signaling in vertebrates. A, In the absence of hedgehog ligand (e.g., Shh), Ptch inhibits Smo from reaching the plasma membrane. In this case, the microtubule-associated Cos2–Fu–SuFu complex can bind full-length Gli, which can be phosphorylated by glycogen synthase kinase-3β (GSK-3β), protein kinase A (PKA), and casein kinase 1 (CK1). Phosphorylated Gli is cleaved to an N-terminal form and then will translocate the nucleus to suppress transcription. B, In the presence of hedgehog ligand, Ptch activity is suppressed, and thereby Smo translocates to the plasma membrane and interacts with Cos2. In this state, the Cos2–Fu–SuFu complex cannot bind Gli, and Gli is able to enter the nucleus and induce transcription of target genes. In the blue and orange frames there are 2 types of noncanonical hedgehog signaling. Blue frame: Type I requires only binding of a hedgehog isoform to Ptch and is mediated by novel functions of Ptch unrelated to Smo repression, and it is by definition insensitive to Smo modulators. Orange frame: Type II is dependent on Smo and in some cases it has been shown to rely on signaling through Gli proteins, and it is both mimicked by Smo agonists and inhibited by Smo antagonists.

mutants confirmed that during gastrulation, the transient Shh signaling induced the pancreatic endoderm to differentiate into the islet tissue subsequently. In the later development, a second hedgehog-dependent activity appeared which was similar to role of Shh in the foregut endoderm assisting the localization of pancreas.[72]

The biological function of hedgehog signaling in the pancreatic epithelium remains controversial, while activation or inhibition of hedgehog signaling pathway only has slight effect on epithelial development. It suggests that hedgehog pathway might mainly focus in pancreatic mesenchyme.[73–76] During embryo development, beta-cell was transiently delayed due to the Pdx1-driven loss of Smo that Lau and Hebrok[73] discovered in pancreatic epithelial progenitor cells of $Pdx1^{-}$/$Smo^{-/-}$ mice. After given birth, beta-cell numbers would restore; however they were dysfunctional. In this case, the phenomenon (reduced insulin secretion, slight insulin-dependent diabetes, and enhanced insulin sensitivity) occurred. The primary cilium that was structurally required as signaling downstream of Smo had close relationship with the mild responsiveness to the activation of hedgehog signaling in pancreatic epithelial cells. Overactivation of hedgehog pathway in mature epithelial cell specifically with resection of primary cilium caused the following results that pancreas tissue dedifferentiated, markers of progenitor cells reexpressed, and endocrine area decreased.[77,78]

Hedgehog Signaling: A Role in PSCs

PSCs are myofibroblast-like cells that can switch between quiescent and activated phenotypes, such as hepatic stellate cells, and reside in exocrine areas of the pancreas.[16,17] They are also the effector cells that respond to pathogens. Hedgehog signaling influences PSCs to a certain degree. In the pancreas, hedgehog signaling is rigorously regulated. Quiescent hedgehog signaling is vitally important for proper differentiation and development of the pancreas. When pathogens lead to pancreatic fibrosis or carcinoma, the sensitive signaling of the hedgehog pathway is easily activated.

Activated PSCs will migrate to the injured location and participate in tissue repair activities by secreting ECM components.[79,80] Research by Shinozaki et al[81] revealed in an *in vitro* study that exogenous Ihh protein could enhance the migrational ability of PSCs by increasing membrane type-1 matrix metalloproteinase on the plasma membrane, while Gli1 negatively regulated Ihh stimulated PSCs migration by inhibiting active MT1-MMP. They also found that Ihh signaling did not modulate PSC activation or proliferation. However, the data from Sicklick et al[82] were not consistent with this study. This could be because in different organs, hedgehog signaling may function differently.

Myofibroblasts were detected in capan-2 tumors, thus supporting the hypothesis that these represented pancreatic stellate cells within the tumor and proliferated in response to Shh stimulation. Shh can promote the differentiation and proliferation of PSCs. Jennifer and colleagues stimulated PSCs with recombinant Shh for 24 hours at 1 and 10 μg/mL, and observed an increase in the mesenchymal markers Sma, vimentin and desmin and a decrease in the epithelial marker CK19. This was the first study to show that Shh induces the differentiation of PSCs into myofibroblasts.[83]

However, in the stroma, tumor cells overexpressing Shh led to the consequences that activated the hedgehog signaling pathway in PSCs instead of themselves. Activated PSCs were crucial in enhancing neural invasion of pancreatic cancer along axons and nerve growth of cancer cell colonies that assisted pancreatic cancer cell migration. In vivo pancreatic cancer model, paracrine Shh activated the PSCs and finally resulted in the tumor cell invasion of the trunk, sciatic nerve dysfunction, and the growth and metastasis of the orthotopic xenograft tumor, while the inhibitor cyclopamine could impede these pathological changes.[84]

Expression of hedgehog signaling pathway components revealed that Smo and Gli were primarily observed in stromal-derived PSCs, whereas the ligands Shh and Ihh were limited to pancreatic tumor cells.[85] The development of PDAC tumors was not affected when Smo was genetically ablated in the pancreatic epithelium of PDAC-susceptible mice.[86] This suggested that there might be a paracrine signaling mechanism involved. Treatment of PSCs with Shh and Ihh activated the hedgehog signaling pathway (the upregulation of Gli mRNA supports this finding), and stimulated the proliferation of PSCs.

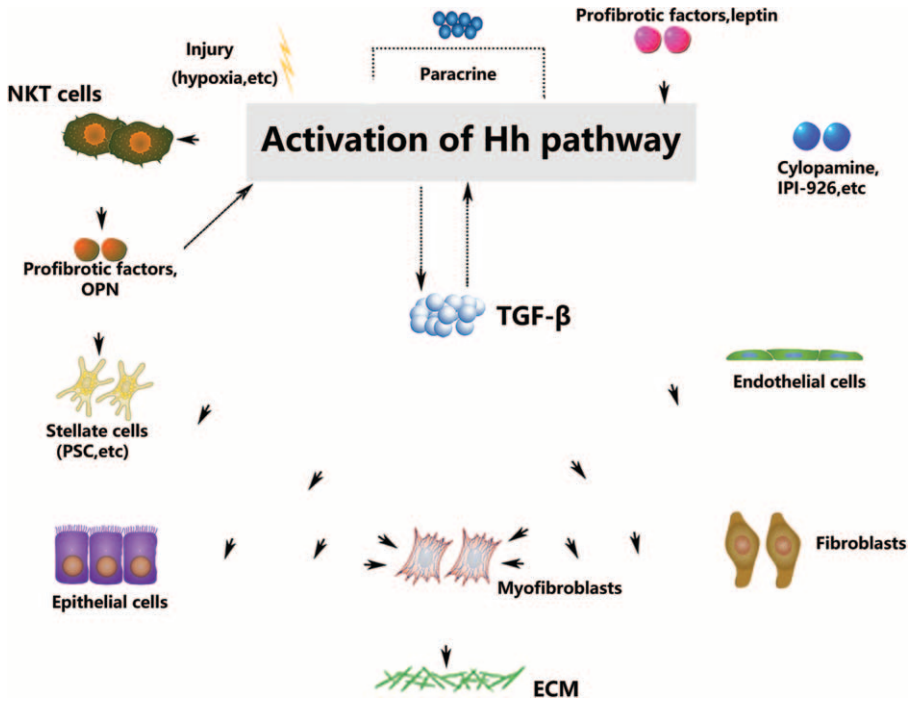

FIGURE 2. Activation of hedgehog signaling promotes the myofibroblast phenotypes in pancreas.

Hedgehog signaling ligands secreted from neighboring cancer cells stimulated PSCs to produce soluble factors that then fed back on cancer cells to promote their activity.[87]

Hedgehog Signaling: A Role in Pancreatic Fibrosis

When the pancreas suffers injuries, PSCs undergo morphological and functional changes to become activated to express α-Smooth muscle actin.[88,89] They will proliferate, migrate, and secrete ECM proteins, thus leading to pancreatic fibrosis.[90,91] Fibrogenesis will appear approximately during the onset of a lesion. Hedgehog signaling induces progressive pancreatic fibrosis intermingled with proliferating ductal structures that are accompanied by the destruction of acinar structures. Paracrine hedgehog signaling activates myofibroblasts and leads to their proliferation, and it shows the restricted expression of hedgehog signaling downstream components including Ptch, Smo, and Gli1/2 in hedgehog-responsive cells, while hedgehog signaling ligands induce matrix metalloproteinases (MMPs) in all hedgehog-responsive cells, as shown in Figure 2.[92]

The hedgehog signaling pathway can be expressed in the embryonic mouse pancreas and is also observed in the adult mouse pancreas.[93] Ptch and Smo are also expressed in islet β cells and activated PSCs. Because Ihh expression is elevated in the pancreas of patients with chronic pancreatitis, Ihh is thought to participate in chronic pancreatic injury, especially pancreatic fibrosis.[82,94] Islet cells of tissues undergoing chronic pancreatitis exhibit an abnormal localization pattern for Ihh. As a signal transducer, Smo is upregulated in cancer-associated stromal fibroblasts, and the expressed Smo could transduce the Shh to activate Gli1 expression.[95]

However, Ihh does not directly activate PSCs nor stimulate PSC proliferation; in other words, the parameter of PSCs transformation (expression of collagen-1 or alpha-smooth muscle actin) stays unresponsive to Ihh.[82] Ihh has a powerful effect on PSC migration not only in chemotactic manner but also in chemokinetic way. When target cells get stimulated and need to migrate, Ihh raises the level of membrane-type 1 matrix metalloproteinase (MT1-MMP) and shifts them onto the plasma membrane from original location. According to the result from Satoshi Shinazaki and his colleagues, we could conclude that Ihh promote PSCs migration via Gli1-dependent signaling pathway.[81] They applied adenovirus to cause Gli1 overexpression and RNA interference technique to reduce Gli1 expression.[81] The results that overexpression of Gli1 nevertheless blocks MT1-MMP localization on the plasma membrane and PSCs migration while RNA interference-mediated knockdown of Gli1 augments PSCs migration indicated that there was a negative feedback loop in the relationship between Ihh and Gli1.[81]

During in vitro wound healing, Shh increases the ability of myofibroblasts to migrate into a wounded area as a monolayer. Shh significantly increases the ability of myofibroblasts to recover from the wound and promotes the migration and invasion of human pancreatic myofibroblasts in vitro.[85]

The receptor Ptch also influences this process substantially. There are 3 hedgehog signaling coreceptors, GAS1, BOC, and CDON, which are cell-surface-associated proteins that act as pathway activators[96–98] and are expressed in cancer-associated fibroblasts.[96,99] The deletion of 2 coreceptors (Gas1 and Boc) in fibroblasts leads to a reduction in hedgehog signaling responsiveness. Additionally, these fibroblasts promote greater tumor growth and tumor-associated vascularity. The deletion of all 3 coreceptors (Gas1, Boc, and Cdon) results in the almost complete abrogation of hedgehog signaling and a corresponding failure to promote tumorigenesis and angiogenesis. In general, the study by Mathew et al[100] identified a role for hedgehog dosage in the promotion of pancreatic

cancer and interpreted the clinical failure of blocking the hedgehog signaling pathway as a therapeutic approach in pancreatic cancer.

Hedgehog Signaling: A Role in Pancreatic Cancer and Crosstalk With Other Factors

Referring to tumorigenesis, hedegehog signaling has the nonnegligible relationship. The role of hedgehog signaling relating to carcinoma was first identified in patients with Gorlin syndrome caused by mutation of Ptch.[101] Once loss-of-function mutations in Ptch or mutations in Smo, it leads to sustained activation of hedgehog pathway. Overexpression of Gli1 and Hedgehog proteins is associated with a variety of cancers and implicated in the onset of pancreatic ductal neoplasia and the maintenance of advanced cancers.[102,103] Not as we expected, in pancreatic cancer, ligand-dependent activation of hedgehog signaling instead of genomic mutation was reported and overexpression of Shh was considered adequate to trigger the initiation of pancreatic cancer.[104,105] As the vital effect of hedgehog signaling on pancreatic cancer, many other factors may interact with it to influence the development of the malignant disease.

Perineural invasion is a common pathologic feature in pancreatic cancer by which cancer cells invade the peripheral nerves and are disseminated.[106] Shh overexpression is involved in perineural invasion in PSCs. Several studies showed that stromal PSCs may play a regulatory role in the interaction between cancer cells and nerves.[107–109] Specific cell types and tissues that release bioactive Shh from pancreatic cancer cells can produce heparan sulfate (HS). According to in vivo knockdown and in vitro cell culture studies, glypican HS proteoglycans release bioactive Shh morphogens from the surface of transfected Bosc23 cells through HS chains in a cell autonomous manner. HS specifically modifies Shh processing on the cell surface, and purified glycosaminoglycans enhance the proteolytic removal of N- and C-terminal Shh peptides under cell-free conditions.[110] The overexpression of Shh in tumor cells activates the hedgehog signaling pathway in PSCs in the stroma rather than activating tumor cells directly to augment the output of MMP2, MMP9, and NGF. These activated PSCs and associated molecules promote pancreatic cancer cell migration along nerve axons and nerve outgrowth to pancreatic cancer cell colonies. Coimplantation of PSCs activated by paracrine Shh induces tumor cell invasion of the trunk, stimulates nerve dysfunction, and promotes orthotropic xenograft tumor growth, metastasis, and perineural invasion in vivo.[84]

In recent years, several experiments proved that in both vivo and vitro, the hippo pathway exerted regulator ability of cell density.[111–114] When the cell density exceeded the threshold, the hippo cascade would be turned on and lead to the activation of large tumor suppressor (LATS) kinases, finally causing the phosphorylation of Yes-associated protein and its paralog TAZ (YAP/TAZ). In cell culture, the activity of hedgehog signaling is dependent on cell-to-cell contact. When the cell intimately contacted with each other or the intensity reached high enough, hedgehog pathway activity would be regulated to higher level.[115] It seems contact inhibition has no effect on hedgehog signaling in normal fibroblasts.[116,117] Adenovirus-mediated YAP overexpression nevertheless prevents hedgehog signaling while the knockdown of YAP expression RNA interference augments hedgehog/Gli activity.[118] However, hedgehog signaling enhances the post-transcription of YAP to promote its activity. It indicates that there is a negative feedback loop. However, in human and mouse pancreatic cancers, low hedgehog signaling pathway activity accompanies strong nuclear YAP immunoreactivity. But tumor could utilize both ocogenic pathway simultaneously on the condition there exist protease-activated receptors (PARs) which has the potential ability to override the Hippo/hedgehog pathway.[118]

As a converse effect of YAP, BRD4, a regulator of epigenetic proteins that can activate Shh members in a ligand-independent manner in PDAC cells, has recently appeared as an alternative therapeutic strategy. BRD4 induces PDAC cell proliferation and chemoresistance. In vitro, suppression of BRD4 damaged PDAC cell viability and proliferation and negatively influenced the tumor growth rate.[119] Gemcitabine can increase the expression of BRD4. Combination treatment of gemcitabine and BRD4 silencing has a synergistic effect on chemotherapeutic efficacy and significantly promoted apoptosis in the PANC-1 and MIAPaCa-2 cell lines. This combination can overcome the side effects of gemcitabine as the single medication. This suggests that BRD4 is a promising target of the transcriptional program of PDACs.[120]

Not surprisingly, tissue repair occurs in pancreatic carcinoma. Gli1 was found as a central player in pancreatic tissue repair upon Kras inactivation. Its activity in pancreatic fibroblasts leads to the expression of IL-6, an inflammatory cytokine that activates Stat3 in pancreatic cancer cells.[121] Improper stromal remodeling and persistence of the inflammatory infiltrate in pancreatic tumorigenesis is a consequence of the deletion of Gli1, while partial loss of Gli1 affects fibrogenesis and the recruitment of immune cells. IL-6, mIL-8, Mcp-1, and M-csf (Csf1), as a subset of cytokines, potentially direct Gli1 target genes to mediate this phenomenon.[122]

In recent years, noncoding RNA has become a popular in many fields. microRNAs (miRNAs) are a class of small noncoding RNAs that play important roles in carcinogenesis.[123] Smo is a direct target of miR-125b, miR-193b, miR-324-5p miR-326,[124] and miR-338-3p.[125] Downregulation of miR-125b, miR-193b, miR-324-5p, miR-326, and miR-338-3p in human cancers derepress Smo and promote tumor proliferation and invasion through aberrant hedgehog signaling. miR-324-5p downregulation is caused by deletion of the miR-324-5p gene in a high percentage of MBs as a consequence of the loss of chromosome 17p. Deletion of chromosome 17p leads to the loss of certain genes, thus contributing to hedgehog induced tumorigenesis.[126,127]

The levels of tumor suppressor miR-let7b, which targets several genes (K-RAS, MUC4, NCOA3, HMGA2, TGFβR1, and STAT3 phosphorylation)[128–130] involved in PDAC pathogenesis, are down-regulated.[131,132] Kumar et al[133] found that the combination therapy of miR-let7b and GDC-0449 effectively inhibited tumor growth when injected to athymic nude mice bearing ectopic tumors generated using MIA PaCa-2 cells compared with micelles carrying GDC-0449 or miR-let7b alone.

MiR-212 is upregulated in PDAC tissues and cells.[134] Gain-of-function and loss-of-function experiments show that a pro-oncogenic function of miR-212 exists in PDAC. Ptch1 is a direct target of miR-212 in nonsmall lung cancer.[135] A new study shows that this also applies in PDAC.[136] Upregulation of Ptch1 can attenuate the effect induced by miR-212. This suggests that miR-212 could facilitate PDAC progression and metastasis by targeting Ptch1, implicating a novel mechanism for the progression of PDAC.[136]

miRNA is not the only relevant nucleic acid, as some discoveries in DNA have been made as well. Twenty-five

members have been reported to belong to the S100 family in humans. Twenty-one of them are known as the epidermal differentiation complex (EDC) involved in epithelial-derived cell differentiation, which are coded by genes clustered at chromosome locus 1q21.[137] S100A2, S100A4, S100A6, S100A11, and S100A14 from the S100 gene family are found to be significantly down-regulated due to Gli1 knockdown. Gli1 primarily regulates S100A family members via cis-acting elements. S100A4 and vimentin genes are up-regulated significantly by increased Shh/Gli1 expression, while E-cadherin is significantly reduced at the same time. Migration of pancreatic cancer cells is increased significantly with dose-dependent increases in Gli1 expression. Silencing of S100A4 significantly reversed the response of pancreatic cancer cells induced by L-Shh transduction.[138]

Hedgehog signaling pathway inhibitors accelerate rather than delay the progression of oncogenic Kras-driven disease. This finding was substantiated in 3 distinct genetically engineered mouse models used to study the effects of genetic or pharmacologic inhibition of the hedgehog signaling pathway activity. The balance between epithelial and stromal elements is notably influenced by pharmacologic inhibition of hedgehog, thus suppressing stromal desmoplasia. This inhibition also accelerated the growth of the PanIN epithelium. In contrast, the activation of this pathway using a small molecule agonist causes stromal hyperplasia and reduces epithelial proliferation. The stromal response to hedgehog signaling is protective against PDAC, and the pharmacologic activation of the pathway can slow tumorigenesis. Thus, it may offer an explanation for the failure of hedgehog inhibitors in clinical trials and may offer new insights into novel therapeutic interventions.[139]

Hedgehog Signaling: A Diagnostic Tool in Pancreatic Cancer

Pancreatic cancer is a highly aggressive carcinoma with an ultrahigh case fatality rate due to poor diagnosis and the lack of proper therapies. Considering the unique role hedgehog signaling plays in pancreatic cancer, many strategies are focused on utilizing this pathway as a diagnostic tool for pancreatic carcinoma. Clinical studies showed that the levels of Shh in human blood were lower in patients with pancreatitis and pancreatic cancer compared with healthy individuals. However, hematopoietic cells do not express Shh. This finding suggests that hedgehog is secreted into the bloodstream. However, hedgehog activity is blocked by plasma proteins. Reduced plasma levels of hedgehog were found in pancreatic cancer patients, but they were insufficient alone to predict pancreatic cancer.[140]

The epithelial-to-mesenchymal transition (EMT) is a common phenomenon in various cancers. Hedgehog signaling controls EMT, enhances cell proliferation in an MAPK- and PI3-kinase-dependent manner, decreases apoptosis through the regulation of Bcl-2 and Bcl-X, and induces the proliferation of cancer stem cells. Gli1 is positively associated with MMP9. Patients with Gli1 and MMP9 coexpression have poor overall survival. Silencing of Gli1 alone without external stimulus had no effect on EMT, but inhibited TGF-β1 and EGF-induced EMT. It performs a protumor role in the aggressive invasion of PSCs by promoting TGF-β1 and EGF-induced EMT.[141]

Shh and the clinical outcome in esophageal cancer demonstrated that Gli1 was a strong and independent prognostic factor for a poor outcome.[142] Furthermore, Gli1 and Shh are independent of the major clinical features known to influence prognosis and could serve as an adjunct to current staging systems. Importantly, no interaction was observed among Gli1, Shh, and adjuvant treatment activity, ensuring that this confounding factor will not affect the relationship between Gli1 and Shh. In resected pancreatic ductal adenocarcinoma (PDAC), the protein abundance of Shh and Gli1 was an independent prognostic factor. Gli1 expression is essential for PDAC cell survival by facilitating the migration and invasion of cells by promoting EMT.[143–145] Lower expression of Gli1 or Shh in tumor cells contributed to longer disease free survival (DFS) and overall survival (OS) times. The combination of Shh and Gli1 levels is thought to be the most significant predictor for overall survival of OS.[146]

Previous studies demonstrated that activation of both the NF-κB and hedgehog signaling pathways played prominent roles in the initiation and progression of pancreatic cancer by acting not only in the cancer cell itself (autocrine) but also in stromal cells (paracrine),[11,86,104,147] while the expression of either nuclear or cytoplasmic NF-κB was a poor prognostic factor for pancreatic cancer.[148] Nuclear expression of either Gli1 or NF-κB (RelA/p65) is associated with poor overall survival in pancreatic cancer patients, and nuclear expression of NF-κB was the only significant prognostic factor according to multivariate analysis.[149]

In conclusion, NF-κB expression, MMP9 expression, and Gli1 expression may have prognostic significance in advanced pancreatic cancer and could be used as biomarkers to guide therapies for pancreatic adenocarcinoma in the future.

PROSPECTIVE

Most pancreatic diseases have poor outcomes such as pancreatic fibrosis and carcinoma. As a physiologic and pathologic process, fibrosis in the pancreas has a firm relationship with pancreatic cancer. Due to considerable roles in 2 lethal pathologies, the hedgehog signaling pathway has become a focus of new treatment strategies. With an increased understanding of this pathway in relevant fields, more useful and accurate methods to detect and treat these terrible diseases may improve the 5-year survival rate. Hedgehog signaling transduction and its regulatory factors are the basis of this promising field. Currently, the mechanism underlying the failures in the clinical application of this pathway is being discovered, and these findings will go a long way toward assisting doctors in the development of new therapies.

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