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TOPIC HIGHLIGHT

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Hedgehog signaling pathway as a new therapeutic target in pancreatic cancer

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Abstract

Pancreatic cancer is one of the most aggressive and difficult cancers to treat. Despite numerous research efforts, limited success has been achieved in the therapeutic management of patients with this disease. In the current review, we focus on one component of morphogenesis signaling, Hedgehog (Hh), with the aim of developing novel, effective therapies for the treatment of pancreatic cancer. Hh signaling contributes to the induction of a malignant phenotype in pancreatic cancer and is responsible for maintaining pancreatic cancer stem cells. In addition, we propose a novel concept linking Hh signaling and tumor hypoxic conditions, and discuss the effects of Hh inhibitors in clinical trials. The Hh signaling pathway may represent a potential therapeutic target for patients with refractory pancreatic cancer.

Key words: Hedgehog signaling pathway; Pancreatic cancer; Cancer stem cells; Hypoxic condition; Therapeutic target

Core tip: Hedgehog (Hh) signaling is involved in the induction of malignant potential in pancreatic cancer, controlling processes of proliferation, invasiveness and tumorigenesis. This phenotypic change is closely associated with the nuclear factor kappa-light-chain-enhancer of activated B cells transcription factor, both in an autocrine and paracrine manner. Hh signaling is also capable of maintaining pancreatic cancer stem cells, and may be activated under conditions of tumor hypoxia. Thus, the Hh signaling pathway may represent a potential therapeutic target for patients with refractory pancreatic cancer and the use of Hh inhibitors will likely play an important role in future therapeutic strategies.

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INTRODUCTION

Pancreatic cancer remains one of the deadliest cancers, with an overall survival rate of < 5%[1]. An underlying reason for this may be that few patients undergo curative, surgical operations because of the advanced stage of the cancer at the time of diagnosis. Furthermore, apart from chemotherapy and radiation therapy, there are no effective, alternative therapies for the treatment of refractory pancreatic cancer, and as such, the development of novel therapeutic strategies is urgently required. Recently, it was shown that the Hedgehog (Hh) signaling pathway, which plays a key role in morphogenesis signaling, is re-activated in pancreatic cancer[2]. Hh signaling contributes to tumor aggressiveness, affecting key tumorigenic processes such as proliferation, invasion

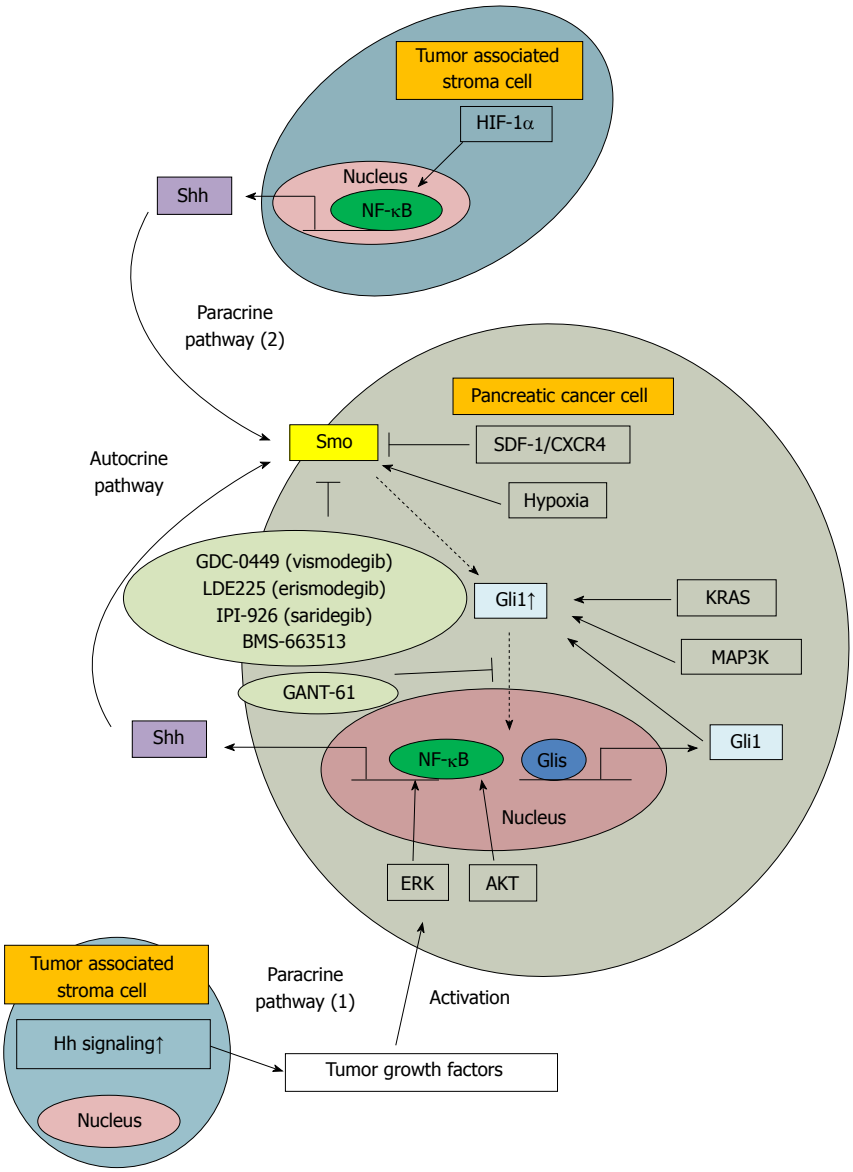

Figure 1 Schematic review. Hedgehog (Hh) signaling is activated in both autocrine and paracrine pathways. Tumor associated stroma cells play a pivotal role in tumor progression related to activation of Hh signaling [paracrine pathways (1) and (2)]. Induction of sonic Hh (Shh) is closely associated with activation of the nuclear factor kappa-light-chain-enhancer of activated B cells transcription factor in pancreatic cancer. Shh is produced by NF-κB activation in pancreatic cancer cells and tumor associated stroma cells. Pathways contributing to Smo and Gli1 activation include SDF-1/CXCR4, hypoxia, KRAS and MAP3K. The effects of Hh inhibitors including GDC-0449 (vismodegib), LDE225 (erismodegib), IPI-926 (saridegib), BMS-663513 and GANT-61 in clinical trials are under investigation. Dotted arrows show components of the Hh signaling pathway in tumor cells focused on in this review.

and progression of cancer cells. Therefore, inhibitors targeting Hh signaling have drawn significant attention as novel, molecularly targeted drugs. Hh signaling components including Patched and Smoothened (Smo) have been detected in almost 70% of human pancreatic cancer specimens and consequently, Hh signaling may play a critical role in the genesis of pancreatic cancer cells[2]. In this review, we summarize recent efforts in the development of new, therapeutic strategies to treat pancreatic cancer, targeting the Hh signaling pathway.

HH SIGNALING PATHWAY

The Hh signaling pathway plays a pivotal role in embryonic patterning and growth control, acting as a morphogen, mitogen and inducing factor of developing organs[3-7]. Hh signaling normally ceases after embryogenesis, however in various cancers, including pancreatic cancer, Hh signaling is re-activated[8]. Therefore, the regulation of Hh signaling in pancreatic cancer likely plays important role in tumorigenesis. The Hh signaling pathway is composed of Hh proteins (sonic Hh; Shh, Indian Hh and Desert Hh), the 12-transmembrane Patched proteins (Patched 1 and Patched 2), the 7-transmembrane protein, Smo and the 5-zinc-finger transcription factors, Gli1, Gli2 and Gli3[9-11]. In the absence of Hh ligand, Patched suppresses Smo, which is the driving protein for Hh signaling, and Gli2 and Gli3 are cleaved

by ubiquitin ligases to generate transcriptional repressor isoforms[12-14]. In contrast, in the presence of Hh ligand, inhibition of Smo by Patched is released, Smo is activated, and Gli2 and Gli3 are transmitted to the nucleus as full-length activators leading to the transcription of target genes such as Patched and Gli1[12-14]. Recent studies demonstrated the existence of primary cilia on the cell surface and showed that Smo moves from the cytoplasm to primary cilia in the process of activation[15]. One of the target genes of Hh signaling; Ptch and Gli1 regulate the transcription of the Hh responsive genes by themselves[16]. Other target genes of Hh signaling are the cell cycle regulator Cyclin D1, p21 and N-Myc which plays important role for carcinogenesis and is also typically dysregulated in the cancer cells[7,17,18]. The Hh signaling pathway is unique because several components of this pathway consist of both oncogenes and cancer suppressor genes.

HH SIGNALING AND THE INDUCTION OF MALIGNANT POTENTIAL IN PANCREATIC CANCER

Originally, the relationship between Hh signaling and tumorigenesis was reported following the association of mutations in genes such as *Gli1*, *Patch* and *Smo* in glioblastoma, basal cell carcinoma and rhabdomyosarcoma[19-21]. In pancreatic cancer, ligand-dependent activation of Hh signaling, but not genomic mutation, was first reported[2]. Previous studies have also shown that Shh overexpression is sufficient to initiate pancreatic intraepithelial neoplasia (PanIN)-like precursor lesions[2,22]. At present, this ligand-dependent pathway is thought to be the major mechanism underlying Hh signaling activation. Two distinct ligand-dependent activation pathways exist; autocrine and paracrine. In addition, association between chronic inflammation and the development of cancer has been recognized for several years[23-27]. In both autocrine and paracrine pathways, NF-κB plays a pivotal role. NF-κB is a transcription factor that controls expression of numerous genes involved in inflammation and immune response processes, including proliferation, invasion, adhesion, angiogenesis and apoptosis[28]. In the autocrine pathway, Shh is a direct transcriptional target of NF-κB, and proliferation of pancreatic cancer cells is accelerated *via* overexpression of Shh[29,30]. In the paracrine paradigm, tumor-associated stroma is important as a microenvironmental factor[31,32]. In one paracrine pathway, stroma cells surrounding pancreatic ductal adenocarcinoma cells, secrete tumor-growth factors through stromal Hh signaling activation[31]. This may explain why low concentrations of Hh signaling antagonist are sufficient to inhibit tumor growth [paracrine pathway (1), Figure 1][31]. In an alternative paracrine pathway, NF-κB-activated monocytes located in the tumor stromal area produce Shh, which stimulates the Hh signaling pathway in pancreatic cancer [paracrine pathway (2), Figure 1][33]. Inhibition of Hh signaling targets pancreatic stellate cells in the tumor-associated stroma, specifically reducing pancreatic tumor growth and metastasis[34,35]. In addition, Singh *et al*[36] showed that CXCL12/CXCR4 protein signaling induces Shh expression in pancreatic cancer *via* extracellular regulated kinase (ERK) and Akt kinase-mediated activation of NF-κB. Some other molecules affected by the activation of Hh signaling may also contribute to the induction of malignant potential in pancreatic cancer. Decrease in Cyclin D1by the inhibition of Hh signaling induces the G_0/G_1 arrest and inhibits cell proliferation[37]. Matrix metalloproteinase (MMP)-9 and MMP-2 locate the downstream of Gli1 and are involved with the invasiveness in pancreatic cancer[38,39].

HH SIGNALING AND PANCREATIC CANCER STEM CELLS

Solid tumor cancer stem cells were first identified in breast cancer as $CD24^{-/low}CD44^{+}$ cells[40]. $CD44^{+}CD24^{+}$epithelial-specific antigen $(ESA)^{+}$ pancreatic cancer cells are reported to exhibit the stem cell characteristics of self-renewal and the ability to produce differentiated progeny[41]. Most importantly, cancer stem cells (CSCs) are characterized by features of resistance towards conventional chemotherapy and radiotherapy[42-45]. Pancreatic CSCs exhibit upregulation of Shh[46]. Recently, inhibition of Hh signaling was reported to inhibit the self-renewal of pancreatic CSCs and reverse chemoresistance[47]. Subsequent studies demonstrated that various agents were capable of inhibiting pancreatic CSCs *via* suppression of Hh signaling. For example, Tang *et al*[48] revealed that epigallocatechin-3-gallate, an active compound in green tea, inhibits the self-renewal capacity of pancreatic CSCs *via* inhibition of Hh signaling components including Smo, Ptch, Gli1 and Gli2. Other groups demonstrated that sulforaphane, a component of dietary cruciferous vegetables, decreases pancreatic CSC self-renewal *via* inhibition of Hh signaling components, Smo, Gli1 and Gli2[49,50]. Han *et al*[51] has revealed that suppression of Hh signaling by arsenic trioxide leads to the inhibition of the viability of pancreatic CSCs using animal models. A better understanding of the molecular pathways driving CSCs will lead to the development of effective, new therapeutic approaches for the treatment of pancreatic cancer.

As previously discussed, there are numerous reports describing $CD44^{+}CD24^{+}$ double positive cells in pancreatic CSCs. However to date, there have been relatively few studies investigating CD24 or CD44 molecules alone as therapeutic targets in pancreatic CSCs. CD24 is a unique molecule because it is described as a marker of pancreatic CSCs, whereas it is expressed at low levels or is absent in breast CSCs. CD24 is thought to act as an adhesion molecule[52,53]. Recently, truncated Gli1 was shown to induce clinically more aggressive cancer *via* the increased expression of CD24[54]. Ringel *et al*[55] showed that constitutive expression of CD44 variants may also be associated with

the malignant state of invasive pancreatic carcinoma. However the precise roles CD24 and CD44 in pancreatic CSCs remain unclear.

HH SIGNALING AND HYPOXIA

Pancreatic cancer is thought to occur under high levels of hypoxia[56]. Therefore, a detailed understanding of the hypoxic microenvironment is crucial for developing effective therapeutic approaches to treat this malignancy. Previous studies have shown that the oxygen concentration in venous blood and deep tumor environments is 5.3% and 1.3%, respectively[57,58]. Thus, to accurately analyze the molecular mechanisms underlying pancreatic cancer, experiments performed under hypoxic conditions are required. The relationship between hypoxia and Hh signaling activation was first reported in 2011, with a study showing that hypoxia activates Hh signaling pathway by upregulating Smo transcription[38]. Thereafter, it was reported that hypoxia induces epithelial to mesenchymal transition (EMT) *via* activation of Hh signaling[59]. Interestingly, under hypoxic conditions, activation of Hh signaling is independent of hypoxia inducible factor (HIF)-1α and is also ligand-independent, with no observable increase in Shh[38,59]. Conversely, Spivak-Kroizman *et al*[60] showed that hypoxia and desmoplasia led to more aggressive and therapy-resistant tumors *via* activation of Hh signaling by Shh, due to HIF-1α activation in the stroma. The mechanisms underlying activation of Hh signaling under hypoxic conditions remains unclear. However, given that Hh signaling is activated under tumor hypoxic conditions, this pathway may represent an important therapeutic target. Indeed, protein-bound polysaccharide decreases invasiveness and proliferation in pancreatic cancer by inhibition of Hh signaling, especially under hypoxia[39].

HH SIGNALING AND THERAPEUTIC APPROACHES IN PANCREATIC CANCER

Pancreatic cancer is often refractory to standard treatments, and many patients are unable to undergo surgery because of the advanced stage of disease at the time of diagnosis. Chemotherapy using gemcitabine and 5-FU derivatives, Tegafur-Gimeracil-Oteracil Potassium (S-1), are often used in Japan. However, combined use of Hh inhibitors with gemcitabine or 5-FU may induce chemoresistance[37]. One reason may be that gemcitabine and 5-FU are sensitive to S-phase and that Hh inhibitor often induces G_1 arrest in cancer cells[37]. Conversely, several groups have shown that combined treatment with Hh inhibitors and gemcitabine has a synergistic effect on tumor growth in a xenograft model[61]. Combined use of Hh inhibitors and cisplatin, a cell cycle independent drug, may also have a synergistic effect[37]. Molecular targeting drug is now well established and the combined use of Hh inhibitors and other targeted drugs is currently being studied and utilized. For example, there is a possible synergistic relationship between Hh and epidermal growth factor receptor (EGFR) signaling pathways in pancreatic cancer[62-64]. Although combination therapy with Hh inhibitors remains controversial, these findings will be essential for developing new effective therapeutic strategies. Radiation is considered the third therapeutic strategy for the treatment of pancreatic cancer. Recently, focal radiation in combination with Hh inhibitors exhibited synergistic effects on reducing lymph node metastasis in pancreatic cancer[65]. Immunotherapy is anticipated as the fourth line of therapy after surgery, chemotherapy and radiation. In this approach, activated lymphocytes and dendritic cells (DCs) derived from patients with advanced cancer are often used. Recently, it was reported that Hh signaling is revitalized in activated lymphocytes and DCs derived from patients with advanced cancer and used for immunotherapy, and that this plays a pivotal role in the maintenance of their functions[66,67]. Therefore, Hh inhibitors may not have a synergistic effect when combined with immunotherapy.

Within the class of Hh inhibitors, recent drug development has focused on Smo inhibitors. Although exact patients' outcome has not been reported yet, Sekulic *et al*[68] has shown that the independently assessed response rate was 30% and 43%, and the median duration of response was 7.6 mo using two-cohort study with GDC-0449 (vismodegib) in metastatic and locally advanced basal-cell carcinoma. GDC-0449 and IPI-926 (saridegib) are currently under phase Ⅱ clinical trials in metastatic, advanced and recurrent pancreatic cancer[69] and BMS-663513 is under phase I clinical trial[70]. A recent study demonstrated that LDE225 (erismodegib), a Smo antagonist, suppresses tumor growth and prolongs survival in a murine model of islet cell neoplasms[71]. Furthermore, GANT-61, a Gli transcription factor inhibitor, has been shown to inhibit pancreatic cancer stem cell growth[72]. An overview of Hh signaling inhibitors is shown in Figure 1. More recently, inhibition of Hh signaling has received significant attention as an anti-tumor strategy. Based on this, the relationship between Hh signaling and various materials has been reported. For instance, resveratrol, 3,4',5-trihydroxystilbene inhibits proliferation and induces apoptosis *via* Hh signaling in pancreatic cancer[73]. Curcumin, a phenolic compound extracted from Zigiberaceae turmeric, reverses EMT of pancreatic cancer by inhibiting Hh signaling[74]. Triparanol, a known cholesterol biosynthesis inhibitor blocking the 24-dehydrocholesterol reductase, suppresses pancreatic cancer tumor growth by deregulation of Hh signaling[75].

Gli1 is both a transcription factor and a target gene, as shown in previous reviews, and crosstalk between Hh signaling and other pathways has been demonstrated[8]. Gli1 is activated *via* several kinds of signaling pathways. In pancreatic cancer, various signaling pathways including KRAS[76], ERK[36], AKT[36], MAP3K[77] and SDF-1/CXCR4[78] are associated with Hh signaling (Figure 1). Because Gli1 is located downstream in many of these

pathways, it may represent a better therapeutic target.

CONCLUSION

In this review, we have summarized the development of pancreatic cancer treatment, with specific focus on the Hh signaling pathway. The Hh signaling pathway may represent an important therapeutic target in pancreatic cancer because this pathway is activated in the majority of pancreatic cancers and both ligand-dependent and independent inhibitors are effective. Hh inhibitor can successfully inhibit tumor growth and invasiveness *in vitro* and can be a promising drug, however, in clinical trial, it is not easy to verify the effectiveness of Hh signaling inhibitor. This reason may be that the actual function of Hh signaling molecules are not fully understood[79,80].

Hh signaling inhibitors should be effective in cancers in which Hh components are mutated such as basal cell carcinoma, basal cell nevus syndrome and medulloblastoma because Hh signaling is constitutively activated[81]. And in these cancers, Hh signaling inhibitors may become the first use drug in future clinical life. However, for other tumors, appropriate combination therapy may be required for the effective therapy. In January 2012, the Smo inhibitor, vismodegib, was clinically approved for the first time by the US Food and Drug Administration, for the treatment of unresectable or metastatic basal cell carcinomas of the skin[82]. Hh signaling inhibitors will now be used in pancreatic cancer as a monotherapy and in combination therapy with other chemodrugs, molecularly targeted drugs or radiation therapy.

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