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*MINIREVIEW*

# An Overview of Hedgehog Signaling in Fibrosis

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**ABSTRACT**

The Hedgehog (Hh) signaling pathway plays a key role during embryogenesis and tissue regeneration. Recently, studies revealed that overactivated Hh signaling leads to fibrogenesis in many types of tissues. The activation of Hh signaling is involved in the epithelial-mesenchymal transition and excessive extracellular matrix deposition. Blockade of Hh signaling abolishes the induction of the epithelial-mesenchymal transition and ameliorates tissue fibrosis. Therefore, new therapeutic targets to alleviate fibrosis based on the Hh signaling have attracted a great deal of attention. This is a new strategy for treating fibrosis and other related diseases. In this review, we discuss the crucial role of Hh signaling in fibrogenesis to provide a better understanding of their relationship and to encourage the study of novel targeted therapies.

## Introduction

In fibrosis, tissue parenchyma cell necrosis is caused by sustained inflammatory stimulation and is a pathologic process with enhanced production and excessive deposition of extracellular matrix (ECM). Fibrosis is a repairing process and also induces sclerosis and tissue hypofunction when the injury is persistent or the repair process is not sufficient. Tissue fibrosis is the common final outcome of a wide variety of chronic diseases, regardless of the initial causes (Liu, 2006; Boor et al., 2010; Zeisberg and Neilson, 2010). It is known that some signaling pathways have an important role in the occurrence and development of tissue fibrosis, such as Wnt/β-catenin (He et al., 2009), Notch (Bielesz et al., 2010), Ras-Raf-Mek (Grande et al., 2010), and the PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) pathway (Niu et al., 2007). Recently, evidence has suggested that the Hedgehog (Hh) signaling pathway may be involved in fibrogenesis in multiple tissues (Omenetti et al., 2007; Jung et al., 2011; Fabian et al., 2012).

Hh signaling was first discovered in the *Drosophila* fruit fly (Nusslein-Volhard and Wieschaus, 1980). In mammals, Hh signaling plays a crucial role in embryonic development and in differentiation and proliferation in brains and spinal cords as well as in the pattern of internal organs and limbs so that the developing tissues are the correct size with the appropriate cell types and degrees of innervation and vascularization. Additionally, evidence also has shown that Hh signaling has a pivotal role in maintaining the number of tissue stem cells (Beachy et al., 2004). Furthermore, Hh signaling regulates body height and aging and associated inflammatory and chronic degenerative diseases (Weedon et al., 2008; Dashti et al., 2012; Neureiter, 2012). Deactivation of this signaling may result in hereditary developmental defects such as holoprosencephaly, whereas overactivation of this signaling by mutations may lead to many tumors such as prostate cancer (Xie, 2005), pancreatic cancer (Hidalgo and Maitra, 2009), and basal cell carcinoma (Xie, 2008). Moreover, activated Hh signaling is also involved in the fibrogenesis of many tissues, such as liver fibrosis, pulmonary fibrosis, and renal fibrosis (Sicklick et al., 2005; Bolanos et al., 2012; Fabian et al., 2012). In this study, we provide an overview of the Hh signaling and discuss its role in the occurrence and development of tissue fibrosis.

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## Hh Signaling Pathway

In mammals, there are three Hh homologs with different spatial and temporal distribution patterns: Desert hedgehog,

**ABBREVIATIONS:** Akt, protein kinase B; BDL, bile duct-ligated; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; GANT61, 2,2′-[[dihydro-2-(4-pyridinyl)-1,3(2*H*,4*H*)pyrimidinediyl]bis(methylene)]bis[*N*,*N*-dimethyl]-benzenamine; GDC-0449, vismodegib; Hh, Hedgehog; HSC, hepatic stellate cell; Ihh, Indian hedgehog; IL, interleukin; IPF, idiopathic pulmonary fibrosis; IPI-926, saridegib; MMP, matrix metalloproteinase; NASH, nonalcoholic steatohepatitis; NKT, natural killer T; OPN, osteopontin; PDGF, platelet derived growth factor; PI3K, phosphoinositide 3-kinase; Ptch, Patched; SANT-1, *N*-[(1*E*)-(3,5-dimethyl-1-phenyl-1*H*-pyrazol-4-yl)methylidene]-4-(phenylmethyl)-1-piperazinamine; Shh, Sonic hedgehog; Smo, Smoothened; TGF-β, transforming growth factor-β.

Indian hedgehog (Ihh), and Sonic hedgehog (Shh) (Krauss et al., 1993; Riddle et al., 1993; Roelink et al., 1994). These palmitoyl- and cholesterol-modified proteins are expressed by a number of different types of cells and have functional specificity that is governed in part by their regulatory mechanisms and the expression patterns in a given cell type (Pathi et al., 2001).

The Hh signaling pathway includes two transmembrane proteins: Patched (Ptch), a 12-transmembrane protein, and Smoothened (Smo), a 7-transmembrane protein with a topology reminiscent of G-protein–coupled receptors, which acts as a signal transducer (Murone et al., 1999). In mammals, two isoforms of Ptch are encoded by Ptch1 and Ptch2. Ptch1 is the only isoform definitively involved in the activation of Hh signaling, which is confined to target cells and is up-regulated in response to Hh proteins. Ptch2 is coexpressed with Hh proteins, but its transcription is independent of pathway activation (St-Jacques et al., 1998). Goodrich et al. (1997) found that the inhibition of Smo activity was abolished in Ptch1 knockout mice. In vivo, the Smo protein exists in either an inactive or active state that appears to be defined, in addition to other modifications, by its location within the cells, either inside or outside the primary cilium (Corbit et al., 2005). The primary cilium has a microtubule-based antenna-like structure which originates from the surface of most of mammalian cells (Drummond, 2012).

The Ptch protein localizes to the primary cilium and suppresses Smo activity when the Hh ligands are absent. Here, only Gli repressor forms are able to enter the nucleus. Binding of Hh ligands to the Ptch protein shuttles Ptch out of the cilium, resulting in the release and activation of Smo; then the Gli protein can translocate the nucleus and function as a transcriptional activator (Gill and Rosenblum, 2006; Varjosalo and Taipale, 2008; Choy and Cheng, 2012) (Fig. 1).

Gli proteins belong to the Kruppel-like family of transcription factors with highly conserved zinc finger DNA-binding domains. There are three Gli proteins in mammals, Gli1, Gli2, and Gli3, and each is encoded by distinct genes. By contrast, their homolog Cubitus interruptus in *Drosophila* is unique (Kasper et al., 2006; Hui and Angers, 2011). In vivo, different types of Gli proteins exhibit distinct regulation, biochemical properties, and target genes. Abnormal expression of Gli1 or a dominant-active mutant form of Gli2 in keratinocytes regulates both the overlapping and distinct transcriptional programs for these two proteins (Eichberger et al., 2006). For example, whereas both Gli1 and Gli2 induce Ptch1 and Tenascin-C, Gli1 exclusively regulates vestigial-like 4, and lipopolysaccharide-induced tumor necrosis factor is a specific target of Gli2. Other studies have identified MUC5A (Inaguma et al., 2011) and osteopontin (OPN) (Das et al., 2009) as Gli1 targets, whereas functional binding sites of Gli2 have been characterized on Bcl-2 (Regl et al., 2004), follistatin (Eichberger et al., 2008), PTHrP (Sterling et al., 2006), and BMP-2 (Zhao et al., 2006) promoters.

In addition to the previously described "canonical" Hh pathway, there are several Hh signaling pathways that do not signal through Gli or Smo. These are named the "non-canonical" Hh signaling pathways (Jenkins, 2009). Current evidence suggests that there are at least two distinct classes of noncanonical Hh signaling (Fig. 2), and these two noncanonical Hh signaling pathways may lead to various physiologic functions in different types of tissues.

Type I noncanonical signaling works through functions of Ptch1 that are unrelated to its inhibitory activity on Smo (Pola et al., 2001; Lavine et al., 2008; Chinchilla et al., 2010; Brennan et al., 2012). Knockdown of Ptch1 gene by small-interfering RNA (siRNA) in endothelial cells enhances cell survival but lacks detectable canonical signaling in endothelial cells both in vitro and in vivo (Lavine et al., 2008; Chinchilla et al., 2010). In addition, the Smo antagonists cyclopamine and SANT-1 (*N*-[(1*E*)-(3,5-dimethyl-1-phenyl-1*H*-

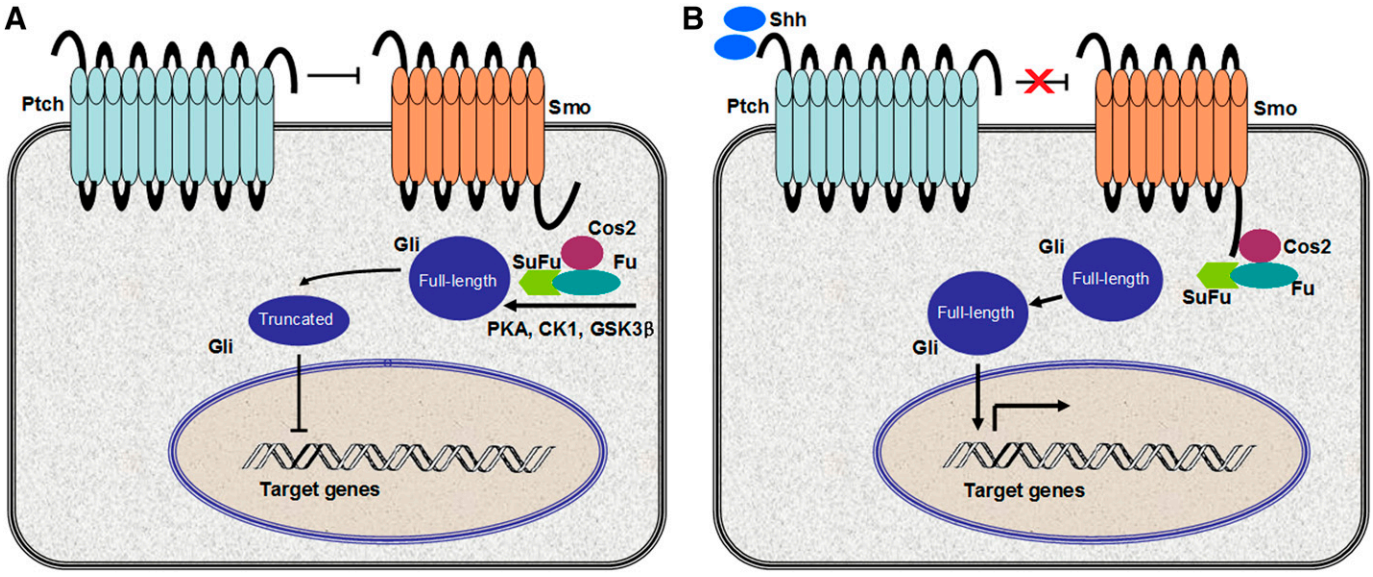


**Fig. 1.** Hh signaling in vertebrates. (A) In the absence of Hh ligand (e.g., Shh), Ptch inhibits Smo from reaching the plasma membrane, and thus the microtubule-associated Cos2-Fu-SuFu complex can bind full-length Gli, which can be phosphorylated by glycogen synthase kinase 3$\beta$ (GSK3$\beta$), protein kinase A (PKA), and casein kinase 1 (CK1). Phosphorylated Gli is cleaved to an N-terminal form and then translocates the nucleus to suppress transcription. (B) In the presence of Hh 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. This figure is based on previously published illustrations (Gill and Rosenblum, 2006).

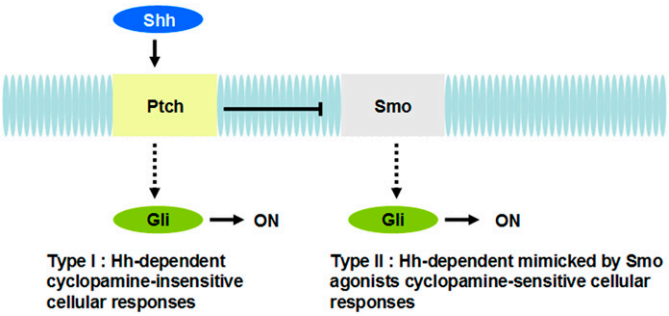


**Fig. 2.** Two types of noncanonical Hh signaling. Type I requires only binding of an Hh isoform to Ptch and is mediated by novel functions of Ptch unrelated to Smo repression, and it is by definition insensitive to Smo modulators. Type II is dependent on Smo and in some cases it has been shown to rely on signaling through Gli proteins, and it is both mimicked by Smo agonists and inhibited by Smo antagonists. This figure is based on previously published illustrations (Brennan et al., 2012).

pyrazol-4-yl)methylidene]-4-(phenylmethyl)-1-piperazinamine) cannot mimic the Shh-mediated antiapoptotic effect (Chinchilla et al., 2010). Furthermore, Ptch1 regulates cell cycle through cyclin B1 in a Smo- and Gli-independent manner (Barnes et al., 2001; Jenkins, 2009). Thus, these findings support that notion that type I noncanonical signaling is a pathway engaged exclusively by Ptch1.

Type II noncanonical signaling operates via Smo functions beyond Gli regulation (Chinchilla et al., 2010; Polizio et al., 2011; Brennan et al., 2012). Recent studies have shown that Smo-dependent signaling is mediated through the activation of small GTPases (Polizio et al., 2011). Another recent study by Bijlsma et al. (2007) suggested that Shh-induced fibroblast migration is Smo-dependent but Gli-independent. Thus, type II noncanonical signaling is a pathway engaged by Smo.

## Hh Signaling and Liver Fibrosis

Cirrhosis is regarded as a lethal end point of a large number of chronic liver diseases, such as obesity-related liver disease and chronic viral hepatitis (Pinzani et al., 2011). When regenerative processes fail to keep pace with hepatic cell death, cirrhosis of the liver develops and results in the progressive replacement of functional epithelial cells with scar tissue (Wells, 2008; Lee and Friedman, 2011). As a hallmark of cirrhosis, liver fibrosis is hypothesized to drive the changes in liver function and blood flow that cause liver-related morbidity and mortality (Wells, 2008; Hernandez-Gea and Friedman, 2011).

In general, Hh ligands are not expressed in healthy liver tissue, and Hh signaling is not activated either in mature cholangiocytes or in hepatocytes (Omenetti et al., 2007; Yang et al., 2008; Choi et al., 2009). However, these two types of mature epithelial cells start to secrete Hh ligands when subjected to certain injury-associated cytokines or lethal stresses (Jung et al., 2010; Omenetti and Diehl, 2011). The Hh ligands diffuse away from the wounded epithelial cells and enter the bile canaliculi and hepatic sinusoids to stimulate the viable Hh-responsive cells that line these structures. Hepatic stellate cell (HSC) is a kind of Hh-responsive cell in the space of Disse and progenitors along the canals of Hering. Activated Hh signaling induces the HSCs to differentiate into fibrogenic myofibroblasts. In turn, both liver progenitors and myofibroblasts derived from HSC can secrete Hh ligands and then further enrich the injured microenvironment with these factors (Omenetti and Diehl, 2011). Not all Hh-pathway activation promotes cirrhosis, but sustained or excessive Hh signaling does (Ochoa et al., 2010).

Recently, it has been demonstrated that the activation of Hh signaling can promote liver fibrosis. In mice (Fleig et al., 2007; Syn et al., 2009a) and humans (Syn et al., 2009a), hepatic activation of Hh signaling strongly correlates with the fibrogenic progress and the severity of liver injury. Furthermore, activated Hh signaling can promote liver fibrosis by combining with other cells and factors. In rodents and humans with nonalcoholic steatohepatitis (NASH), Hh signaling activation leads to the recruitment, retention, and viability of natural killer T (NKT) cells. In turn, NKT cells induce the production of Hh ligands that trigger liver fibrosis (Syn et al., 2009b, 2010, 2012). For example, more NKT cells accumulated in Ptch knockout mice, which thereby developed worse hepatic fibrosis. CD1d-deficient mice, which lack NKT cells, were protected from fibrogenesis (Syn et al., 2010), and NASH-related cirrhosis was prevented by NKT cell depletion in rodents (Syn et al., 2010). Tissue expression of the NKT cell chemoattractant CXCL16, an Hh-inducible gene, and hepatic expression of interleukin-15 (IL-15) and Cd1d, which are the three factors that increase NKT cell viability, are significantly up-regulated in mice with enhanced Hh signaling activity and NASH-related fibrosis (Omenetti et al., 2009; Tajiri et al., 2009). Moreover, Shh protein induces NKT cells to release profibrogenic cytokines such as IL-13 and IL-4, which play pivotal roles in liver fibrosis (Chiaramonte et al., 1999; Fichtner-Feigl et al., 2006; Syn et al., 2009b). Additionally, a number of researchers have demonstrated that liver NKT cells can produce OPN, a Hh-regulated cytokine, which acts in both an autocrine and paracrine manner to induce HSC activation and liver fibrosis, suggesting that OPN directly mediates the fibrogenic actions of NKT cells (Diao et al., 2004; Syn et al., 2009; Choi et al., 2009). Similarly, high plasma levels of OPN may be predictive of cirrhosis in patients with chronic hepatitis B and C (Zhao et al., 2008; Huang et al., 2010).

Leptin is another factor that activates Hh signaling to regulate gene expression programs that control cell fate, with important implications for hepatic fibrosis (Choi et al., 2010). First, leptin increases the expression of snail mRNA in HSCs, and this response is blocked by inhibitors of PI3K and Akt and cyclopamine (Saxena et al., 2004; Niu et al., 2007; De Minicis et al., 2008). Snail is a transcription factor that exhibits a major role in the epithelial-mesenchymal transition (EMT), and many conditions that promote the EMT induce the expression of snail mRNAs (Li et al., 2006). Second, the interaction between leptin and ObRb (a leptin receptor) activates Hh signaling, resulting in the mesenchymal transition in HSCs. Hh signaling activation is required for the transition of epithelioid quiescent HSCs into HSC-derived myofibroblasts and liver fibrosis (Yang et al., 2008; Choi et al., 2010; Michelotti et al., 2013). Other factors that regulate the transdifferentiation and growth of HSC-derived myofibroblasts, such as transforming growth factor-$\beta$ (TGF-$\beta$) and platelet derived growth factor (PDGF), also activate and depend upon Hh signaling for their fibrogenic actions (Jung et al., 2008; Yang et al., 2008; Philips et al., 2011).

Chronic infection with hepatitis B virus and hepatitis C virus is a major cause of cirrhosis and liver cancer worldwide

(Caldwell and Park, 2009; Fung et al., 2009). Pereira et al. (2010) showed that virus hepatitis significantly increased the expression of the Hh-ligands Ihh and Shh and target genes Ptch and Gli2. They also showed that patients with more advanced stages of liver diseases (i.e., bridging fibrosis to cirrhosis) expressed higher levels of Shh, Ptch, and Gli2 than those with little or no fibrosis (Pereira et al., 2010). These findings explain why many individuals who are infected with hepatitis B virus or hepatitis C virus do not develop significant liver fibrosis or neoplasia.

EMT is necessary for tissue fibrosis, and TGF-$\beta$ is the most established mediator of EMT (Zeisberg et al., 2003; Thiery et al., 2009). TGF-$\beta$–induced hepatocyte EMT has been confirmed in mouse experiments (Kaimori et al., 2007). The ability of Hh-ligands to promote EMT has been demonstrated in various tissues, including the liver (Choi et al., 2009; Syn et al., 2009), bile duct (Omenetti et al., 2008b), and others. It has been verified that Hh-signaling regulates EMT during development (Hay, 1995; Bailey et al., 2007). These results suggest that Hh-related EMT may depend, at least partially, on the induction of TGF-$\beta$ (Wang et al., 2013).

Recently, several small-molecule inhibitors of Hh pathway have been used in treating liver fibrosis. The Smo antagonist cyclopamine attenuated EMT-associated fibrogenesis in rats with nonalcoholic fatty liver disease (Syn et al., 2009a) and reverted the myofibroblastic transition in vitro (Choi et al., 2009). Another study, by Philips et al. (2011), has suggested that treatment with GDC-0449 (vismodegib), another small-molecule inhibitor that binds to Smo, significantly decreases liver myofibroblasts and progenitors and reduces liver fibrosis without increasing mortality. In addition, GDC-0449 also can effectively attenuate early liver fibrosis by suppressing Hh signaling (Pratap et al., 2012). This evidence supports that pharmacologic inhibition of hedgehog signaling may have therapeutic potential for liver fibrosis.

## Hh Signaling and Bile Duct Fibrosis

Fibrosis is the common pathologic process of chronic biliary injury in both rodents and humans (Lazaridis et al., 2004). Rygiel et al. (2008) showed that EMT might have an important role in the pathogenesis of biliary fibrosis because there is high expression of vimentin and other mesenchymal markers in proliferating bile ductules within fibrotic portal tracts in liver tissues from patients with primary biliary cirrhosis, as demonstrated by immunostaining. Omenetti et al. (2008b) showed that activated Hh signaling induced EMT in adult bile ductular cells in their study of liver tissues from rats and patients with biliary fibrosis and manipulated Hh signaling activity in primary cholangiocyte cells, cholangiocytes, and mice subjected to biliary injury. They also demonstrated Hh ligand accumulation and Hh-signaling activation in the liver tissues of patients with primary biliary cirrhosis (Jung et al., 2007) and bile duct-ligated (BDL) rodents (Omenetti et al., 2007). In vivo, a similar process likely regulates EMT because changes in gene expression associated with EMT and liver fibrogenesis were amplified after BDL in Ptch knockout mice, which have an impaired ability to turn off Hh signaling after biliary injury (Omenetti et al., 2008b). In addition to these observations, Hh may promote EMT crosstalk with TGF-$\beta$ via both the canonical Hh pathway (Huber et al., 2005) and noncanonical Hh pathway (Lauth and Toftgard, 2007).

The types of cells in the biliary tract that produce Hh and other ligands remain unknown. Immunohistochemical analysis demonstrated that ductular cells can induce the production of Hh ligands Shh and Ihh in diseased and healthy liver samples from adults and children. The production of Hh ligands is induced by various biliary stressors, including viral infection, immune-mediated biliary attack, mechanical obstruction of BDL, and various genetic disorders that interfere with biliary transport (e.g., progressive familial-intrahepatic cholestasis types 1 and 2) (Jung et al., 2007; Omenetti et al., 2007). Hh ligands can also be produced by ductular-appearing cells when hepatocyte injury is induced by viral infection (Pereira et al., 2010), nutritional factors (Syn et al., 2009a, 2010, 2011; Jung and Diehl, 2010), or toxins (Jung et al., 2008, 2010). Mature cholangiocytes, such as murine 603B and normal rat cholangiocytes (NRC1), and primary cholangiocytes isolated from healthy adult rodents have been identified to express mRNA and proteins of Hh ligands indicated by real-time polymerase chain reaction and Western blot/immunocytochemistry, respectively (Omenetti et al., 2007, 2008a). Moreover, these cells release active Hh ligands that can activate Hh signaling in some neighboring cells that have been transfected stably with Gli-luciferase reporter constructs (Witek et al., 2009).

The mechanisms regulating Hh ligand production by these cells are not clear yet. In vitro, activation of cholangiocyte induced by exogenous platelet-derived growth factor isoform BB (PDGF-BB) promotes exosomes production. These exosomes contain biologically active Shh and Ihh ligands, and stimulate biliary cells to start Hh signaling in adjacent hepatocytes through paracrine pathway (Witek et al., 2009). The significant enrichment of membrane-associated Hh ligands in the bile occurs after an injury such as BDL that activates cholangiocytes in situ (Witek et al., 2009), increasing the possibility that such liver-derived Hh ligands may also activate Hh signaling in Hh-responsive cells that reside in other tissues immediately "downstream" of the biliary or hepatic venous outflow, such as the heart or intestine. In activated hepatic stellate cells and cholangiocytes (Yang et al., 2008), PDGF-BB is produced and then induces the expression of Shh and inhibitors of AKT and/or PI3K that act downstream of PDGF-BB to suppress this process (Omenetti et al., 2008a). In some types of liver injury, myofibroblasts localize near ductular type cells and release some soluble factors that trigger Hh signaling in ductular cells. Thus, paracrine mechanisms may also increase the expression of Hh target genes in cholangiocytes. Evidence from antibody neutralization experiments indicates that some factors from myofibroblasts including Shh and TGF-$\beta$ may also be involved (Omenetti et al., 2007, 2008a).

## Hh Signaling and Renal Fibrosis

Renal tubulointerstitial fibrosis, a process often considered to be a result of failed wound repair after injury (Zeisberg and Neilson, 2010), is the common outcome of an wide variety of progressive chronic kidney diseases (Liu, 2006; Boor et al., 2010; Zeisberg and Neilson, 2010). There is growing evidence that the aberrant activation and dysregulation of key development-associated signaling may play an important role in the pathogenesis of chronic tissue destruction and impaired renal function (Surendran et al., 2005; He et al., 2009). Because of the importance of Hh signaling in nephron formation and kidney development

(Yu et al., 2002; Cain and Rosenblum, 2011), some researchers hypothesize that the abnormal activation of this signaling pathway most likely results in renal fibrosis (Ding et al., 2012; Fabian et al., 2012).

Fabian et al. (2012) confirmed that during renal fibrosis Ihh induction promotes Ptch1 and Gli1 expression in the kidney cortex and medulla, particularly in the adjacent tubular epithelium, and that the induction of Gli1 was completely suppressed by the Smo antagonist IPI-926 (saridegib). The epithelial localization of both Ihh and Shh in the kidney, combined with the stromal expression of Gli1 and Gli2 in the renal interstitium, suggest that Hh acts in a paracrine manner during renal fibrosis, similar to its role during renal development (Yu et al., 2002; Cain et al., 2009). Hh signaling also induces the activation of myofibroblast and production of ECM by directly regulating the expression of a series of fibrogenic genes such as Gli1, Snail1 (Thiery, 2003; Rowe et al., 2009), type I collagen, fibronectin, desmin, and $\alpha$-smooth muscle actin, leading to increased ECM deposition and scar formation (Ding et al., 2012).

In addition, the Hh pathway may promote renal fibrosis by acting in combination with other signaling pathways. For example, Hh signaling up-regulates Wnt-2b and Wnt-5a and the Notch ligand Jaggad-2 (Katoh and Katoh, 2008, 2009), and both Hh signaling and Wnt/$\beta$-catenin can increase Snail1 expression (Dai et al., 2009), the combination of which can accelerate the progression of several diseases, including tissue fibrosis and cancer. Activated Hh signaling may trigger renal fibrosis by regulating other fibrogenic signaling such as the PI3K/Akt pathway. In turn, both the RAS-RAF-MEK and PI3K/AKT pathways can potentiate Gli1 function or activate Gli signaling independent of Smo (Pasca di Magliano et al., 2006; Ji et al., 2007; Stecca and Ruiz I Altaba, 2010), and both pathways are implicated in renal myofibroblast activation (Rodriguez-Pena et al., 2008; Bechtel et al., 2010; Grande et al., 2010). TGF-$\beta$, which has a critical role in renal fibrosis (Böttinger and Bitzer, 2002), can also activate Gli2 expression independent of Ptch1/Smo in human fibroblasts (Dennler et al., 2007).

The important role of Hh signaling in renal fibrosis is also demonstrated by the therapeutic efficacy of its inhibitors. Cyclopamine, a small molecule inhibitor of Smo, inhibits the activation of fibroblast and production of ECM in vitro and attenuates renal fibrosis in vivo in an obstructive nephropathy model. Intriguingly, the expression level of Shh is not affected, but the induction of Gli1 and Snail1 downstream is largely inhibited by cyclopamine. These results are consistent with the ability of Shh to antagonize Smo activity. Notably, the ameliorative effect of cyclopamine appears greater than that of Gli1 deficiency, and this indicates that Gli1 may not be the only mediator of Hh signaling during obstruction-induced renal fibrogenesis (Ding et al., 2012). Another finding was that the Smo inhibitor suppresses Gli1 induction and does not inhibit Gli2 induction, indicating that Gli2 may be the more important effector in renal fibrosis (Fabian et al., 2012). Additional studies are necessary to understand the signaling pathway.

## Hh Signaling and Pulmonary Fibrosis

Pulmonary fibrosis is a pathologic condition associated with chronic airway inflammation. Architectural remodeling and the fibrosis of tissues can severely damage lung function, resulting in the worst outcomes. Several cell types interact during this remodeling, including endothelial cells, epithelial cells, fibroblasts, and both recruited and resident cells of the immune system (Stewart et al., 2003). When the remodeling process fails to repair the tissue, fibrosis develops, with the formation of scar tissue (Kasper and Haroske, 1996). Several airway structural cells, including epithelial cells, endothelial cells, and pericytes, contribute to pulmonary fibrosis through a process of molecular reprogramming, mediated by proteins such as Shh (Stewart et al., 2003) and TGF-$\beta$ (Khalil et al., 1996; Levine et al., 2000).

Shh, which is critical for the normal development of the lungs via its interactions with its receptor Ptch1, activates the Gli family of transcription factors (Motoyama et al., 1998; Pepicelli et al., 1998). Moreover, the paracrine signaling of Shh specifically contributes to branch morphogenesis in the embryonic lung (Bellusci et al., 1997). During the branching morphogenesis of the lung, Shh is produced by the endoderm and stimulates mesenchymal cellular proliferation and differentiation, as evidenced by the observation that overexpression of Shh leads to an aberrant increase of the lung mesenchyme (Weaver et al., 2003). These observations raise the possibility that the Hh signaling pathway participates in pulmonary fibrosis. In a model of bleomycin-induced adult lung injury, there are abundant Gli1-positive cells in the preserved alveolar septa and an increased number of Gli1-positive mesenchymal cells in fibrotic lesions, and adenovirus-mediated overexpression of Shh enhances ECM production (Liu et al., 2013). In another study, Stewart et al. (2003) identified overexpression of Shh in the lung epithelium in human idiopathic pulmonary fibrosis (IPF) and murine lung inflammation and fluorescein isothiocyanate-induced fibrosis. Using in situ hybridization, Coon and colleagues showed that Shh is highly expressed in the epithelium of cysts within the IPF lung (Stewart et al., 2003; Coon et al., 2006). Recently, Bolanos et al. (2012) found that in human IPF the Hh pathway is activated. They also provided extensive in vitro data indicating that Shh increases the migration, proliferation, and survival of fibroblasts, and the production of ECM, which is also demonstrated by the observation that Hh signaling increases ECM production and triggers the fibroblast-to-myofibroblast transformation (Horn et al., 2012b).

Furthermore, Stewart et al. (2003) detected the Shh receptor Ptch in normal resting peripheral blood T lymphocytes and infiltrating mononuclear cells and alveolar macrophages. In patients with interstitial lung disease, this remodeling is continuous and results in lung fibrosis accompanied by a predominantly mononuclear lymphoid infiltrate in which both B and T lymphocytes are present (Tuder, 1996; Lympany and du Bois, 1997). Katoh and Katoh (2004) and Tseng et al. (2004) found that FOXF1 is a downstream target of Shh in the lung, a target for Gli2/3 proteins activated by Shh (Motoyama et al., 1998). However, the expression level of Shh and FOXF1 in lungs with usual interstitial pneumonitis and nonspecific interstitial pneumonitis was differential. It is postulated that the pathogenic pathways of interstitial pneumonitis may include a defect in Hh signaling, thereby activating FOXF1 (Coon et al., 2006). These findings demonstrate that Hh signaling promotes pulmonary fibrosis through contact with other various types of factors. Therefore, it is useful to thoroughly study the pathway in pulmonary fibrosis and other tissue fibrosis.

## Hh Signaling and Other Tissue Fibrosis

In addition to the types of fibrosis already described, there are other types of fibrosis, such as pancreatic fibrosis (Jung et al., 2011) and cardiac fibrosis (Bijlsma et al., 2008), which are also closely related to Hh signaling pathway.

In a study by Bijlsma et al. (2008), endogenous Shh protein was shown to contribute to ischemia-reperfusion–induced injury; cyclopamine treatment reduced this myocardial ischemia-reperfusion–induced injury. However, several studies have suggested that the Shh protein may exert beneficial effects. As described by Kusano et al. (2005), intramyocardial gene transfer of naked DNA encoding human Shh promotes the recovery and preservation of left ventricular function in both acute and chronic myocardial ischemia models. Shh has considerable therapeutic potential in patients with acute and chronic myocardial ischemia by enhancing neovascularization, recruiting bone marrow-derived progenitor cells, and reducing cardiac apoptosis and fibrosis (Kusano et al., 2005). Hh signaling also exhibits an important role in adult cardiovascular pathophysiology. The Shh protein up-regulates markedly the expression of Hh target genes, such as vascular endothelial growth factor and the angiopoietins Ang-1 and Ang-2, which can induce neovascularization (Pola et al., 2001). Based on these observations, we speculate that the Hh signaling may play a discriminatory role in different types of tissues or during different phases of disease development; the latter could be assessed at different time points. Therefore, the activation of Hh signaling appears to exert a dualistic effect in cardiac ischemia in which high exogenous levels of Shh can foster tissue repair and endogenous Hh protein may aggravate ischemic diseases.

In pancreatic tissues, Hh signaling is strictly controlled. Quiescent Hh signaling is a key event for proper pancreatic differentiation and development. However, in fibrogenic pancreatic diseases the signaling is frequently reactivated. An in vitro study revealed that exogenous Ihh protein enhanced the migrational ability of pancreatic stellate cells (Shinozaki et al., 2008). These stellate cells located in the vicinity of the acini are the main source of proliferating fibroblasts in human diseases. Another recent observation showed that overexpression of Smo in pancreatic cancer-associated fibroblasts is a potential determinant for Hh-responsiveness (Walter et al., 2010). Jung et al. (2011) have provided in vivo evidence that secreted Hh ligands induce pancreatic fibrosis by activating responsive cells in a paracrine fashion. They identified TGF-$\beta$ and matrix metalloproteinases (MMPs) as important mediators of Hh signaling, which is consistent with the observations that TGF-$\beta$ cooperates with canonical Hh signaling to regulate the expression of Gli proteins and Hh target gene (Karhadkar et al., 2004; Dennler et al., 2007) and that, in cultured pancreatic cells, exogenous Hh molecules or ectopic expression of Gli1 or Hh molecules promote the expression of MT1-MMP and MMP9 (Nagai et al., 2008; Liao et al., 2009).

## Perspectives

Tissue fibrosis is a physiologic and pathologic process in many diseases. In fibrogenesis, Hh signaling plays a crucial role, and its targeted interference exerts antifibrotic effects to some extent. Therefore, Hh signaling transduction and its regulatory factors are the basis of a promising field because Hh signaling may be a therapeutic target for disease treatment (Fig. 3).

Cyclopamine is an alkaloid that specifically inhibits the activity of the Hh receptor Smo (Incardona et al., 2000; Chen et al., 2002). It has been reported that in the liver (Pratap et al., 2011) and in cardiac diseases (Bijlsma et al., 2008) cyclopamine can reduce injury and fibrosis. An in vivo study demonstrated that inhibition of Smo by LDE223 (Peukert et al., 2009), or transfection with small-interfering RNAs against Smo attenuates experimental fibrosis and induces the regression of established fibrosis (Horn et al., 2012a). GANT61 (2,2′-[[dihydro-2-(4-pyridinyl)-1,3(2*H*,4*H*)pyrimidinediyl]bis(methylene)]bis[*N*,*N*-dimethyl]-benzenamine), an inhibitor of Gli transcription factors in the nucleus, can decrease pulmonary fibrosis and collagen

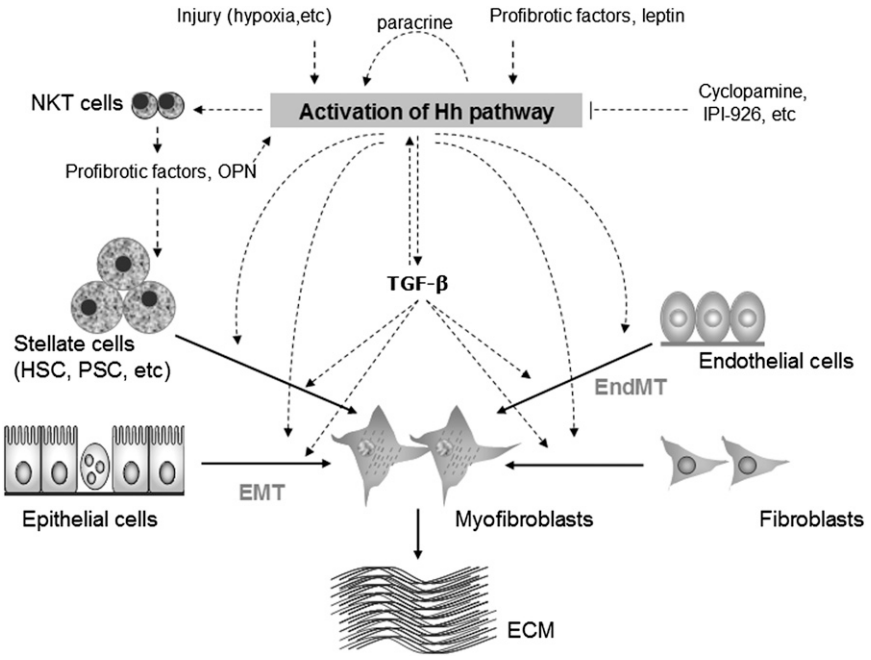


**Fig. 3.** Activation of Hh signaling promotes the myofibroblast phenotypes in many types of tissues.

accumulation and promote an antifibrotic and anti-inflammatory environment in a bleomycin-induced lung injury model in mice (Moshai et al., 2014). Thus, new therapeutic targets must be identified in the Hh signaling pathway to treat tissue fibrosis. This will lead to new strategies for treating tissue fibrosis and other related diseases. However, in some types of tissues, the blockade of Hh signaling does not reduce fibrosis. A study by Kusano et al. (2005) suggests that activated Hh signaling by exogenous Hh also exerts a beneficial effect by increasing neovascularization, recruiting bone marrow-derived progenitor cells, and reducing cardiac apoptosis and fibrosis. Under such circumstances, inhibiting the Hh pathway may not be ideal for attenuating fibrosis. Therefore, the different roles of Hh signaling in different types of tissue fibrosis should be ascertained. Similarly, appropriate and effective therapies based on Hh signaling are also necessary.

#### Authorship Contributions

*Participated in research design:* Hu, Lin, Bai.

*Wrote or contributed to the writing of the manuscript:* Hu, Lu, Chen, Bai.

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