Review

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Hedgehog signaling in the liver

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Reactivation of Hedgehog (Hh), a morphogenic signaling pathway that controls progenitor cell fate and tissue construction during embryogenesis occurs during many types of liver injury in adult. The net effects of activating the Hedgehog pathway include expansion of liver progenitor populations to promote liver regeneration, but also hepatic accumulation of inflammatory cells, liver fibrogenesis, and vascular remodeling. All of these latter responses are known to be involved in the pathogenesis of cirrhosis. In addition, Hh signaling may play a role in primary liver cancers, such as cholangiocarcinoma and hepatocellular carcinoma. Study of Hedgehog signaling in liver cells is in its infancy. Additional research in this area is justified given growing experimental and clinical data supporting a role for the pathway in regulating outcomes of liver injury.

General significance of the hedgehog pathway

Hedgehog (Hh) is a signaling pathway that regulates critical cell fate decisions, including proliferation, apoptosis, migration, and differentiation. The pathway plays vital roles in tissue morphogenesis during fetal development. It also modulates wound healing responses in a number of adult tissues, including the liver [24,84]. The key events involved in Hh signaling are depicted in Fig. 1. Hh signaling is initiated by a family of ligands (Sonic hedgehog – Shh, Indian hedgehog – Ihh, and Desert hedgehog – Dhh) which interact with a cell surface receptor (Patched – Ptc) that is expressed on Hh-responsive target cells. This interaction de-represses activity of another molecule, Smoothened (Smo), and permits the propagation of intracellular signals that culminate in the nuclear localization of Glioblastoma (Gli) family transcription factors (Gli1, Gli2, Gli3) that regulate the expression of Gli-target genes (Fig. 1A and B). Pertinent details about the Hh signaling pathway are summarized in the next section in order to highlight the general implications of pathway activation, as well as the inherent complexity of its regulation. The remainder of the review focuses on the role of Hh signaling in adult liver repair.

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Details about the Hh signaling pathway

Hh signaling may be initiated via autocrine, paracrine or endocrine mechanisms depending on whether the source of Hh ligands is the Hh-responsive cell itself, neighboring cells, or cells in distant tissues that release Hh ligands in membrane-associated particles with features of exosomes. Hh ligands are synthesized as propeptides and undergo auto-catalyzed cleavage to generate an N-terminal fragment that is further lipid-modified by cholesterol and prenylation before moving to the plasma membrane and being released into the extracellular space. Lipid modification limits the local diffusion of Hh ligands within tissues, but is not required for the ligands to engage Ptc, the trans-membrane spanning receptor on the surface of Hh-responsive cells [24,63,64]. Also, membranous particles that contain biologically-active Hh ligands have been purified from blood and bile, permitting Hh ligands that are produced in one locale to initiate signaling in distant sites [87]. Release of Hh ligands from Hh ligand-producing cells is facilitated by the membrane-associated molecule, Dispatched, but the precise mechanisms involved remain somewhat obscure [24]. Maturation of Hh propeptides can also occur extracellularly. In the proximal GI tract, for example, digestive enzymes appear to catalyze cleavage of Hh ligands to generate biologically-active amino-terminal fragments [92].

Various growth factors, cytokines, and certain types of cellular stress stimulate ligand-producing cells to express Hh ligands (Fig. 2A). For example, epidermal growth factor (EGF) has been shown to induce gastric parietal cells to express Shh [76]; hepatic stellate cell expression of Shh was demonstrated to occur after treatment with platelet-derived growth factor (PDGF) [90] or leptin [8]. In each case, induction of Shh was demonstrated to depend upon growth factor activation of PI3K/Akt signaling. Induction of Ihh expression was reported to occur in hepatocytes that were exposed to TGFβ in concentrations that were sufficient to provoke eventual apoptosis [30]. Other stimuli that result in caspase 3 activation and eventual hepatocyte apoptosis also upregulate expression of Shh and Ihh [33]. It remains to be determined if pro-apoptotic stimuli, like growth factors, engage PI3K/Akt to affect Hh ligand induction. However, the aggregate findings suggest that Hh ligand expression generally increases in response to various stimuli that promote tissue construction/remodeling. At present, the biological implications of producing distinct Hh ligands remain poorly understood. It appears that different Hh ligands are synthesized by different cell types/tissues (e.g., production of Dhh is particularly robust in ovary, testes, and peripheral nerves) [29,61,86]; Shh is generated by intestinal crypt cells, while Ihh is expressed by intestinal cells near the tips

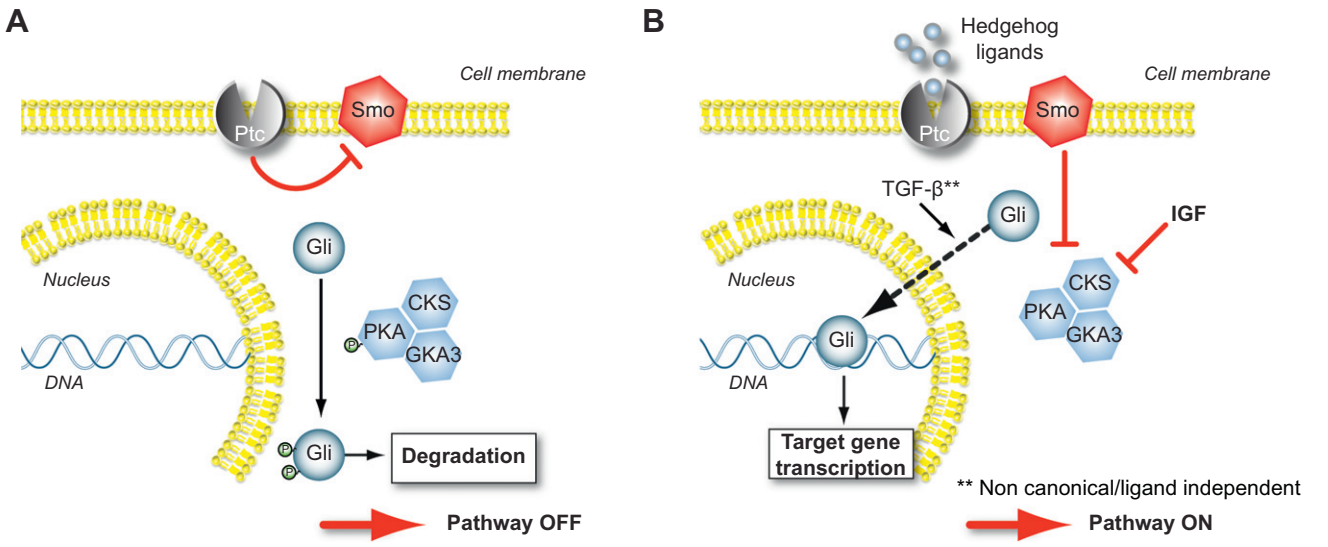

Fig. 1. Hedgehog signaling. (A) Hh pathway is silent in Hh-responsive cells when Hh ligands are absent. Cells which are capable of responding to Hh ligands (i.e., Hh-responsive cells) express Hh receptors. Patched (Ptc) is the receptor that physically interacts with Hh ligands. In the absence of Hh ligands, Ptc represses the activation of a co-receptor-like molecule, Smoothened (Smo). This repression prevents Smo from interacting with other intracellular factors that permit the stabilization and accumulation of Glioblastoma (Gli) transcription factors. Thus, Gli proteins undergo phosphorylation by various intracellular kinases (PKA, GSK3β, CSK), become ubiquitinated, move to proteasomes, and are degraded. Reduced availability of Gli factors influences the transcription of their target genes. Lack of Gli1 and Gli2 generally reduces target gene transcription, while lack of Gli3 can either stimulate or inhibit transcriptional activity. (B) Hh ligands activate Hh pathway signaling. Interaction between Hh ligands and Ptc liberates Smoothened from the normal repressive actions of Ptc. This results in eventual inhibition of factors that promote Gli phosphorylation/degradation, and permits cellular accumulation of Gli. Other factors that inhibit Gli-phosphorylation, such as insulin-like growth factor-1 (IGF), have also been shown to facilitate stabilization of Gli1 in cells that are otherwise capable of producing this protein. There is also a report that Transforming Growth Factor beta (TGFβ) can stimulate Gli accumulation via mechanisms that may operate independently of Smoothened. Nuclear accumulation of Gli factors, in turn, influences transcriptional activity of Gli-target genes. Gli1 and Gli2 generally increase gene transcription, while Gli3 can either increase or decrease gene transcription depending on its post-translational modification.

of villi) [3], but some cells are clearly capable of producing more than one type of ligand (e.g., hepatocytes, bile ductular cells, and hepatic stellate cells can each express both Shh and Ihh) [33,56,90]. Few head-to-head comparisons of different ligands have been reported. Although many similarities have been demonstrated [6,39], different ligands exhibit variable potency for activating Hh signaling [45,58], and one study reported that all of the effects of Shh and Ihh are not identical, even in a given type of Hh-responsive cell [2].

When Hh ligands engage Ptc, this inhibits its normal function, which is to repress Hh signaling by preventing activation of Smoothened (Fig. 1). Emerging evidence suggests that Smoothened becomes localized to primary cilium during its activation, and that Ptc represses this process when Hh ligands are absent [11,68]. The fact that certain inherited ciliary defects disrupt Hh signaling supports this concept [59,66]. Other Hh signaling components, such as Gli3, are also deregulated in some ciliopathies. Because Gli3 normally represses transcriptional activation of certain Hh-regulated genes, ciliary dysfunction can also result in aberrant activation of various Hh targets [22]. Additional research is needed to clarify the mechanisms by which various ciliary structural components interact with components of the Hh pathway to modulate the propagation of Hh ligand-initiated signaling. At this point, however, it seems that ciliary dysfunction can both inhibit and activate Hh signaling [12,85].

Efforts to map Hh pathway activity are further confounded by the fact that some of the components of the pathway, including Ptc (which is necessary to engage Hh ligands and activate signaling, but which also silences pathway activity when it is present in excess of Hh ligands), Gli1 (which generally activates transcription of Hh target genes), and Hh interacting protein (Hhip, a soluble antagonist of Hh ligands) are themselves the products of genes that are transcriptionally activated by Gli-family factors. Although Gli1 and Gli2 generally function as transcriptional activators, their actions are not fully redundant, suggesting that the two factors differ somewhat in their DNA binding affinities and/or ability to recruit transcriptional co-activators or repressors. The final Gli family member, Gli3, often represses gene transcription, but may also activate transcription depending upon its post-translational modification [84].

Further complexity is introduced by the fact that nuclear accumulation of Gli transcription factors is influenced by factors other than Hh ligands [28,42]. For example, insulin-like growth factor has been shown to inhibit protein kinase A (PKA)-dependent phosphorylation of Gli1 in certain Hh-responsive cells. This inhibits subsequent Gli phosphorylation by GSK-3 beta and prevents its proteosomal degradation. The resultant stabilization of Gli-1 protein enhances Hh pathway activation [67]. TGF beta was recently reported to promote transcription of Gli2 without activating Smoothened, suggesting a "non-canonical" mechanism for modulating expression of Hh-regulated genes [14,15]. Hh signaling components are also targets for epigenetic regulation, and appear to be particularly sensitive to changes in methylation status [47,72,80,88,89]. Conversely, Hh-sensitive transcription factors (i.e., Gli family members) also regulate transcription of pleiotropic TGF beta-target genes, such as snail [26,36], and influence expression of factors that modulate Wnt signaling, including Wnt5a (a Wnt pathway activator) and soluble frizzled receptor-1 (sFRP1, an inhibitor of Wnt signaling) [37]. Suffice it to say, the Hh pathway is part of a complex signaling network that engages other fundamental cell fate regulators, such as TGFβ and Wnt, to orchestrate global changes in the phenotypes of Hh-responsive cells [34,35,37,38].