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

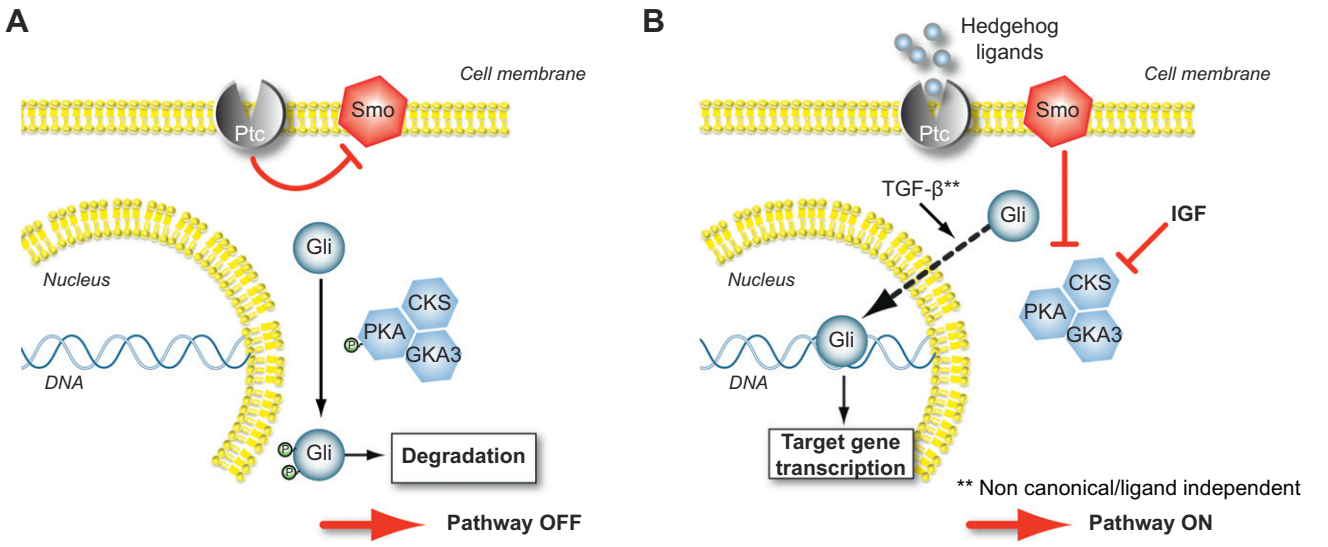

Fig. 1. Hedgehog signaling. (A) Hh pathway is silent in Hh-responsive cells when Hh ligands are absent. Cells which are capable of responding to Hh ligands (i.e., Hh-responsive cells) express Hh receptors. Patched (Ptc) is the receptor that physically interacts with Hh ligands. In the absence of Hh ligands, 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].

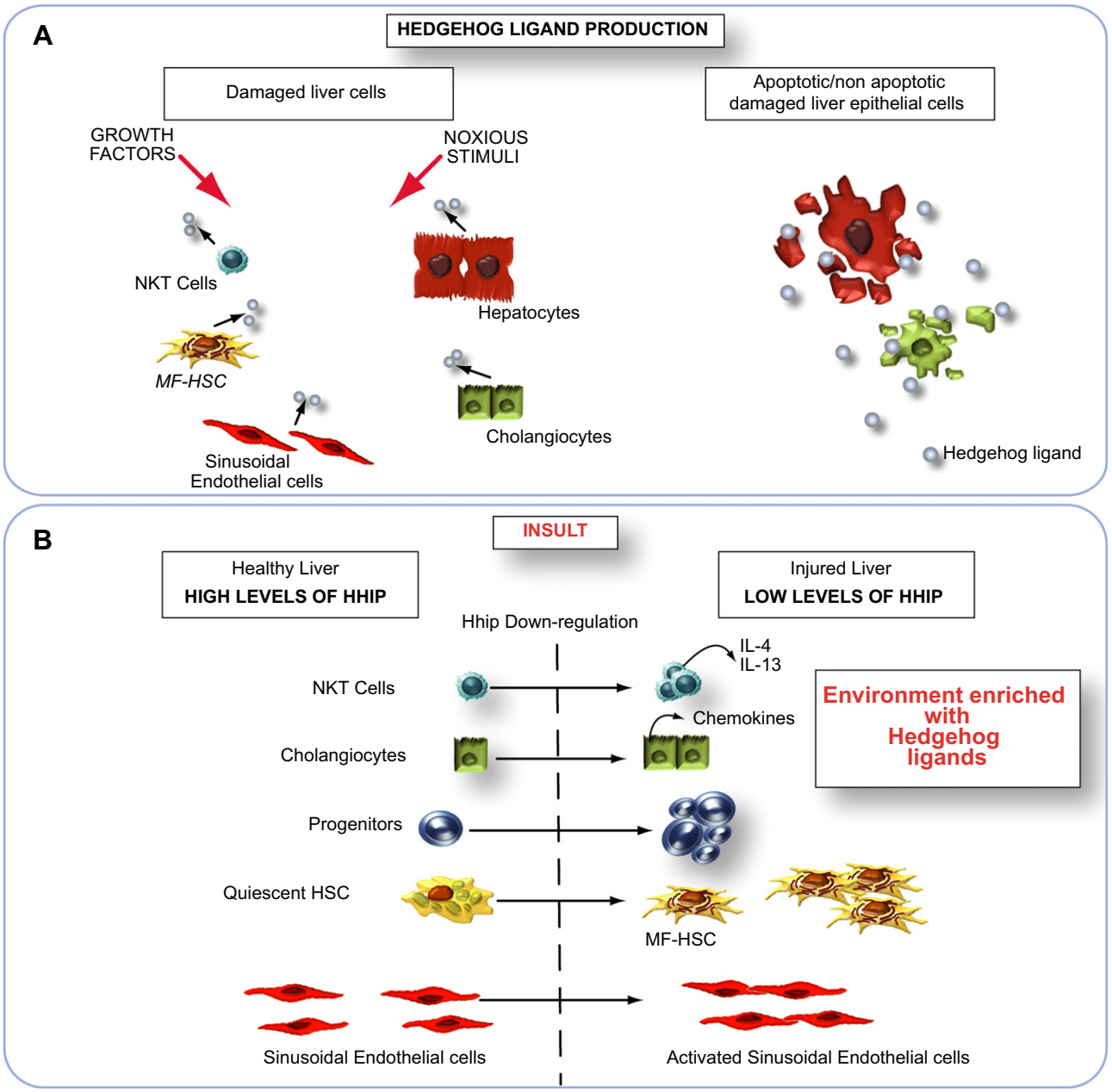

Fig. 2. Differential activity of the Hedgehog pathway in healthy and injured livers. (A) Healthy livers express low levels of Hedgehog (Hh) ligands. Several types of resident liver cells are capable of producing Hh ligands, including hepatocytes, cholangiocytes, hepatic stellate cells (HSC), natural killer T (NKT) cells, and sinusoidal endothelial cells. Ligand production can be stimulated by growth factors/cytokines, as well as by cytotoxic/apoptotic stress. Thus, diverse stimuli that promote liver regeneration/remodeling induce hepatic production of Hh ligands. (B) Healthy livers express low levels of Hh ligands (a) and relatively high levels of Hh interacting protein (Hhip) (b), which binds to Hh ligands, preventing them from engaging receptors on Hh-responsive target cells. During liver injury, production of Hh ligands increases (Fig. 2A) and Hhip is repressed, permitting ligand-receptor interaction and activation of the Hh signaling pathway in Hh-responsive cells. The latter includes several types of resident liver cells, including NKT cells, cholangiocytes, progenitors and quiescent hepatic stellate cells (Q-HSC). Activation of Hh signaling in each of these cell types induces responses that contribute to fibrogenic repair. For example, Hh pathway activation stimulates growth of NKT cell populations and induces their production of pro-fibrogenic factors, such as IL4 and IL13. It also stimulates cholangiocyte growth and production of chemokines, including chemokines that recruit NKT cells and other inflammatory/immune cells to the liver. In addition, Hh ligands promote growth of liver progenitors and stimulate Q-HSC to transition to become myofibroblastic (MF)-HSC. The growth of MF-HSC is further stimulated by Hh pathway activity. Coupled with expansion of Hh-responsive ductular and progenitor populations, this contributes to the fibroductular reaction that often accompanies liver injury. Finally, Hh ligands activate liver sinusoidal endothelial cells, causing them to express adhesion factors and other mediators that contribute to vascular remodeling.

Hh helps to orchestrate liver growth and repair

Although there is little argument that TGF beta and Wnt are important regulators of growth and repair responses in adult livers [18,48], the possibility that Hh was involved in these processes was not considered until relatively recently [52], and initial evidence supporting the concept was met with considerable skepticism. The latter likely reflected three main facts: (i) liver

phenotypes had not been reported as major outcomes when various Hh signaling components were knocked-down experimentally in developing embryos, (ii) Hh pathway activity had not been noted in healthy livers of adult rodents of humans, and thus, (iii) there seemed to be little rationale for investigating Hh signaling in injured adult livers, so this had not been done systematically.

It is now evident, however, that complete silencing of Hh signaling in embryos interrupts the formation of the nervous and cardiovascular systems, causing lethality before liver bud formation. Also, redundancies in the mechanisms that assure Hh pathway activation permit partial compensation for incomplete or the later disruption of Hh signaling during liver development, and result in relatively subtle hepatic defects that are often overlooked in the context of devastating neurological, cardiovascular, and/or musculoskeletal deformities [27]. It has also become apparent that Hh ligands are expressed by the primitive ventral endoderm that ultimately gives rise to hepatic progenitors [20], and that transcriptional activation of foxa2 (a transcription factor that is required for hepatic specification of this endoderm[43]) is directly regulated by Gli factors [70]. More recent data further support the molecular evidence for Hh pathway involvement in liver development: (i) transcriptional activation of ptc has been demonstrated in embryonic livers of day 11.5 ptc-LacZ reporter mice [75], (ii) fetal liver cells that were harvested from d11.5 WT mouse embryos and purified by flow cytometry proliferated in response to Hh ligands and Hh pathway activity was required for optimal viability in hepatoblasts, but negatively regulated differentiation of such liver progenitors at later developmental stages [23], (iii) cells that produce and respond to Hh ligands were localized to the ductal plates of developing human livers and Smoothened inhibitors dramatically reduced the viability of clonally-derived human fetal hepatoblasts in culture [75], (iv) various ciliopathies that disrupt Hh signaling exhibit a significant hepatic phenotype (cystic malformations of the intrahepatic biliary tree and liver fibrosis) [12,59,66,85], and (v) the Hh pathway regulates the growth of hepatoblastomas, a progenitor-derived tumor that is the most common type of primary liver cancer in very young children [57].

An explanation for the general lack of Hh pathway activity in healthy adult livers has also emerged. First, little, if any, production of Hh ligands is demonstrable in healthy adult liver cells [32,53,56]. Second, liver sinusoidal cells (e.g., endothelial cells and quiescent hepatic stellate cells) strongly express Hhip, which interacts with soluble Hh ligands and prevents them from engaging Ptc [9,10,73,90]}. Third, Hh pathway activity is progressively silenced during the process of liver epithelial cell maturation, such that expression of ptc1 is exponentially lower in healthy mature hepatocytes than in bipotent hepatic progenitors, and the latter is likewise reduced compared to that of multipotent endodermal progenitors [75]. Healthy adult livers harbor relatively small progenitor populations, and these tend to localize along canals of Hering [93], where immunohistochemical analysis has now demonstrated expression of Hh ligands, Hh-regulated transcription factors, and other Hh-responsive genes, in healthy adult human and rodent livers [17,32,53,56].

Finally, improved understanding of the mechanisms that regulate Hh ligand production predicts that Hh pathway activation would likely occur when major re-construction of the liver is required in adulthood. First, various growth factors for hepatocytes and liver nonparenchymal cells induce expression of Shh and Ihh (Fig. 2A). Consistent with these data, hepatic expression of Shh and Ihh increases significantly after 70% partial hepatectomy (PH) which provides a tremendous stimulus for liver regeneration [51]. Noxious stimuli that provoke compensatory hepatic repair also stimulate liver cells to produce Hh ligands. For example, Shh expression has been localized to ballooned hepatocytes in patients with NASH [77], while Ihh expression has been demonstrated in bile ductular epithelial cells of patients with destructive cholangiopathies, such as primary biliary cirrhosis [32,53,56]. Second, it has been shown that membrane fragments released from apoptotic and non-apoptotic liver epithelial cells harbor biologically-active Hh ligands [87]. This finding, coupled with a third line of evidence demonstrating dramatic down-regulation of Hhip expression at early stages of myofibroblastic trans-differentiation of hepatic stellate cells (HSC) and during activation of sinusoidal endothelial cells (SEC) [9,87,90], predicts that Hh ligands derived from liver epithelial cells would be capable of activating Hh signaling in neighboring stromal cells via paracrine mechanisms. Consistent with this concept, certain types of Hh-responsive cells that rely upon Hh signaling for optimal viability and growth (e.g., myofibroblastic (MF)-HSC, activated SEC, and immature liver epithelial cells, including bipotent liver progenitors) are relatively inconspicuous in healthy adult livers, but accumulate in livers that are producing high levels of Hh ligands, but relatively little Hhip [17,53,77] (Fig. 2B). Immunohistochemistry of diseased human livers, such as those with chronic viral hepatitis [60], alcoholic liver disease, or NAFLD [17,30,31,77], confirms that Hh-regulated transcription factors (e.g., Gli2) co-localize with markers of activated SEC (e.g., CD31), MF (alpha-smooth muscle actin), and immature liver epithelial cells (e.g., Ker7), and reveals that numbers of Hh-responsive cells closely parallel the level of Hh ligand production. Moreover, both hepatic production of Hh ligands and accumulation of Hh-responsive cells generally increase with the severity of liver damage and fibrosis [17,31,53].

Accumulating evidence also demonstrates that hepatic accumulation of Hh-target cells is not merely an epi-phenomenon that accompanies liver re-construction. Rather, such cells actively contribute to regenerative/remodeling processes in adult livers. Treating healthy mice with Smoothened antagonists to inhibit Hh signaling after PH, for example, significantly reduced hepatic accumulation of progenitors and MF, inhibited proliferation of hepatocytes and cholangiocytes, blocked liver regeneration, and resulted in death of most mice by 72 h post-PH [51]. Conversely, mice with haploinsufficiency of ptc exhibited sustained overactivation of the Hh pathway, accumulated greater numbers of MF and immature liver cells, and developed much worse fibrosis during liver injury [56,77]. Together, these findings suggest that transient activation of the Hh pathway is necessary for adult livers to regenerate after an acute injury, but that sustained increases in Hh signaling (as occurs when injury is persistent) perpetuate the expansion of cell types, such as MF, activated SEC, and immature liver epithelial cells, which are involved in the pathogenesis of cirrhosis. This logic helps to explain why chronic liver injury is a much greater risk factor for cirrhosis than acute liver injury.

Determining the relative significance of Hh as a regulator of adult liver growth and repair

As discussed earlier, Hh interacts with several other key signaling pathways to modulate cell fate decisions. Thus, it is clearly not

the sole pathway that dictates how adult livers respond to situations that provoke growth and/or repair. Delineating the hierarchy of signal transduction that drives the construction of liver tissue is also challenging because the relative importance of any given pathway might differ according to cell type and/or differentiation status, and would also be expected to vary with moment-to-moment changes in levels of factors that promote, as opposed to inhibit, each pathway. Nevertheless, experimental evidence suggests that once activated in liver tissue, the Hh pathway generally tends to auto-amplify as long as Hh ligands persist, despite "built-in" mechanisms in individual cells which would be predicted to constrain further Hh pathway activation (i.e., Hh-driven induction of Ptc and Hhip). This finding may be explained, in part, by the fact that Hh pathway activation in resident Hh-responsive liver cells gradually increases the net number of Hh ligand-producing cells and Hh-responsive cells in the liver. For example, Hh ligands stimulate trans-differentiation of resident quiescent HSC into MF-HSC, as well as the growth of MF-HSC populations that produce and respond to Hh ligands [73,90]. Similarly, Hh ligands promote biliary epithelial cell expression of chemokines (e.g., CXCL16) that recruit subpopulations of Hh ligand producing- and Hh-responsive immune cells (e.g., NKT cells) into liver [55,78,79]. It has also been suggested that expanding populations of Hh-responsive cells enrich the hepatic micro-environment with other factors that potentiate Hh pathway activity by stimulating further production of Hh ligands (e.g., PDGF and TGF beta) [52], or by acting down-stream of Smoothened to stabilize the activity of Hh-responsive transcription factors, such as Gli1 and Gli2 (e.g., IGF-1, TGF beta), as discussed earlier. At some point, regenerative/repair responses that were initially triggered by Hh signaling might also proceed independently of further Hh pathway activity. This possibility is supported by evidence that Hh pathway activation stimulates certain types of lymphocytes to produce other fibrogenic factors (e.g., IL4, IL13) [78,79].

More research is needed to characterize the types of cells, cell-type specific responses, and particular aspects of liver reconstruction that are most dependent upon Hh signaling in order to judge the potential merits of manipulating Hh pathway activity to improve adult liver repair. In liver, such research is in its infancy. Nonetheless, progress has been made regarding apoptosis regulation by Hh signaling, and Hh pathway control of epithelial-to-mesenchymal transitions, in certain liver cell types. Hence, the final two sections will summarize existing information about those topics.

Hh activation regulates pro-survival pathways in several types of liver cells

Growing evidence reveals that Hh signaling plays a key and conserved role across multiple liver cell types to inhibit hepatic apoptosis. The signaling mechanisms involved have been best delineated in bile ductular cells (cholangiocytes). Cholangiocytes display differential sensitivity to apoptosis depending on their pathophysiologic state. While apoptosis increases in various cholangiopathies, subpopulations of ductular cells exhibit reduced apoptotic activity in liver diseases that are accompanied by fibroductular reactions [4,46]. Malignant cholangiocytes also tend to be relatively protected from apoptosis [21,65,82]. Studies of apoptotic signaling in cholangiocytes have identified TRAIL and its death receptors, DR4 and DR5, as major initiators of apoptosis [41,81], and the Bcl-2 family member, Mcl-1, as a key anti-apoptotic factor [49]. Cholangiocytes are Hh-responsive cells and it was demonstrated that Hh ligands reduce cholangiocyte apoptosis [56]. Interestingly, this may reflect the ability of Hh-regulated transcription factors to modulate expression of DR4 and Mcl-1. The Hh-inducible transcription factor, Gli3, binds to the DR4 promoter and represses DR4 transcription in an Hh-dependent manner [41]. The Hh pathway also regulates expression of Mcl-1 but the mechanism involved is somewhat complex. In cholangiocarcinoma cells, translation of Mcl-1 is repressed by miR-29b binding to the 3′UTR of mcl-1 mRNA [50]. Hence, factors that reduce miR-29b expression lead to increased synthesis of Mcl-1 protein. Functional Gli binding sites have been demonstrated in the miR-29b promoter and Gli represses transcriptional activity of miR-29b [50]. Thus repressive actions of Hh-induced transcription factors result in ultimate increases in cellular content of Mcl-1 protein. The combined effects of Hh pathway activation on DR4 and Mcl-1 protect cholangiocytes from apoptosis by reducing expression of the death receptor, DR4 while increasing expression of the anti-apoptotic factor, Mcl-1.

In addition to cholangiocytes, Hh signaling has been shown to inhibit apoptosis in HSC [56]. Further research is needed to determine if this occurs via mechanisms that involve death receptor signaling and Mcl-1, as occurs in cholangiocytes.

The Hh pathway also promotes the viability of healthy liver epithelial progenitors [75] and certain types of malignant hepatocytes. Investigators studying hepatocellular carcinomas (HCCs; which represent the majority of hepatic cancers) found that inhibition of hedgehog signaling (using Shh neutralizing antibodies) in HCC cell lines with detectable endogenous Hh signaling (Hep3B, Huh7 and PLC/PRF/5), decreases expression of hedgehog target genes, and induces apoptosis [25]. Consistent with this, SMO antagonism using KAAD-cyclopamine recapitulates this effect [25,74]. As expected, modulation of Hh signaling in HCC cells that display no endogenous Hh signaling (HCC36 and HepG2) has no effect on cellular viability, showing results are specific for the Hh pathway. Similarly, recent studies reveal that Hh signaling is activated in hepatoblastoma (HB), the most common liver tumor in childhood [16]. Deregulation of the Hh pathway appears to be caused by methylation of the inhibitory Hhip locus in a large number of HB patients [16]. Pharmacologic inhibition of Hh signaling with cyclopamine had a strong inhibitory effect on cell proliferation of HB cell lines and caused a massive induction of apoptosis [16].

Hh pathway activation promotes epithelial-to-mesenchymal transitions in biliary epithelial cells and hepatic stellate cells

Many types of cells are capable of considerable plasticity, particularly when immature. For example, gastrulation (one of the earliest events in embryogenesis) involves disaggregation of epiblast epithelial cells and their invasion into adjacent stroma. Subsequent construction of various tissues requires carefully orchestrated waves of epithelial-to-mesenchymal transition (EMT) and reciprocal mesenchymal-to-epithelial transition (MET). Cells derived from each of the three germ layers (i.e., endoderm, mesoderm, and ectoderm) are capable of undergoing EMT/MET during embryogenesis, reversibly acquiring an epithelial phenotype (defined as polarized and adherent to adjacent cells) or a mesenchymal

phenotype (defined as migratory and invasive) [1]. Recently published studies in mouse embryonic fibroblasts demonstrate that the reversibility of such transitions changes over time because they trigger progressive cascades of gene expression. Hence, cells which are early in the process are most plastic. Nevertheless, fibroblastic cells that are no longer transitional can be coaxed to acquire epithelial characteristics transiently and eventually become pluripotent progenitors that generate all three germ layers by enforcing over-expression of a small group of transcription factors. Notably, however, exposing mesenchymal cells that harbored all of the reprogramming transcription factors to exogenous stimuli that activated TGF beta signaling prevented them from undergoing MET and aborted their reprogramming to pluripotent progenitor cells, thus proving that extrinsic factors strictly gate cell fate decisions even in cells that are intrinsically capable of enormous plasticity [44,62,69].

These exciting findings suggest mechanisms that permit multipotent progenitors to persist in selected "niches" in various adult tissues, and may be particularly relevant to regenerative/repair responses in adult livers because the latter typically harbor small numbers of (at least) bipotent progenitors [93]. There is conflicting evidence that such bipotent liver progenitor cells exhibit characteristics of transitional cells [7,13,40,71,91] However, it has already been demonstrated that Hh and TGF beta (two of the main signal transduction pathways that modulate EMT/MET in developing embryos [5]) influence EMT/MET in cells that are involved in adult liver repair, including immature ductular cells [54] and hepatic stellate cells [9,10].

The signaling mechanisms by which Hh induces EMT in adult liver cells have been studied most systematically in HSC. Freshly isolated, quiescent (Q)-HSC have some characteristics of mesenchymal cells, i.e., they express desmin and certain mesenchyme-associated transcription factors. However, expression of most other typical myofibroblast-associated genes, including alpha-smooth muscle actin (α-sma) and collagen 1α1, is conspicuously absent. Rather, gene expression profiles in Q-HSC are more consistent with those of adipocytic/neuroepithelioid cells, characterized by easily demonstrated expression of peroxisome proliferator activating receptor (PPAR)-γ, Glial fibrillary acidic protein (GFAP), E-cadherin, desmoplakin, and certain epithelial cytokeratins that are also expressed by liver progenitors and biliary epithelial cells (Ker7 and Ker19). Q-HSC also express high levels of Hhip, but mRNAs for Hh ligands and other Hh target genes (e.g., Glis) are barely detectable. Within 24 h of culture in serum-containing medium, Hhip expression falls by 90%, followed by HSC production of Shh and Hh pathway activation (evidenced by accumulation of Gli mRNAs). As Hh pathway activation occurs, the cells down-regulate expression of all quiescence/epithelial markers and gradually up-regulate expression of myofibroblast-associated genes, including α-sma, coll1α1, vimentin, fibronectin, S100A4, and TGFbeta1, as well as snail, a Gli-responsive transcription factor that is known to mediate TGFbeta-initiated EMT [9]. Transition of Q-HSC to MF-HSC is also accompanied by reduced expression of bmp7 and its target, id2. Down-regulation of bmp7 and id2 has been shown to permit repression of E-cadherin expression and occurs in other cells as they undergo EMT [19,83]. Conversely, induction of bmp7 mediates up-regulation of E-cadherin during reprogramming of MEFs to induced pluripotent stem cells [44,62,69]. Days after primary HSC acquiring a fully myofibroblastic phenotype (or years after this phenotype was acquired in clonal HSC lines), mesenchymal gene expression can be silenced and quiescence/epithelial gene expression restored by treating cells with a Smoothened inhibitor, cyclopamine, to abrogate Hh signaling. Hh pathway inhibition restores expression of bmp7, id2, E-cadherin, desmoplakin, and other epithelial/quiescence markers, represses expression of the mesenchymal gene program, and causes loss of the typical migratory/invasive phenotype of MF-HSC [9]. EMT/MET in HSC involves cytoskeletal reorganization and is accompanied by changes in activity of Rac1, a small cytoskeletal-associated GTPase. Manipulating Rac1 activity in cultured HSC with adenoviral vectors also dramatically influences Hh signaling and EMT/MET, with increased Rac1 stimulating EMT and Rac1 repression promoting MET [10]. Manipulating Rac1 activity in rodents evoked similar responses and resulted in significant alterations in liver fibrosis when the animals were challenged with either bile duct ligation or carbon tetrachloride [10]. Emerging evidence suggests that Hh-dependent alterations in epithelial/mesenchymal gene expression may be a conserved response to other fibrogenic stimuli. For example, it occurs when HSC are treated with leptin and is necessary for leptin to repress HSC quiescence and promote acquisition/maintenance of the MF-HSC phenotype [8].

Summary

Emerging data indicate that hedgehog signaling mediates both adaptive and maladaptive responses to liver injury, depending upon the balance between its actions as a regulator of progenitor cell growth and its ability to promote liver inflammation and fibrogenic repair. Synthesis of hedgehog ligands is stimulated by diverse factors that trigger liver regeneration, including both liver cell mitogens and liver cell stressors. These Hh ligands, in turn, are released from ligand-producing cells into the local environment where they engage receptors on Hh-responsive cells. The latter include progenitor cells, hepatic stellate cells, sinusoidal endothelial cells, and certain types of resident hepatic immune cells. In general, Hh ligands function as trophic factors and promote the viability of Hh-target cells. This enhances the outgrowth of liver progenitor populations, triggers tissue remodeling, and promotes liver regeneration. However, Hh ligands also stimulate certain cell types (e.g., hepatic stellate cells, immature liver epithelial cells) to acquire a less epithelial and more mesenchymal state during which such cells generate inflammatory mediators and scar tissue. By promoting EMT (while inhibiting MET), Hh pathway activation, therefore, induces liver fibrogenesis. Hence, excessive or persistent Hh pathway activity actually aborts successful regeneration of damaged liver tissue and contributes to the pathogenesis of liver fibrosis. Clearly, further studies are required to elucidate the mechanisms of hedgehog-mediated hepatic repair to preferentially activate therapeutically beneficial pathways to treat chronic liver diseases.

Disclosures

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