The Adventures of Sonic Hedgehog in Development and Repair.

II. Sonic hedgehog and liver development, inflammation, and cancer

Alessia Omenetti and Anna Mae Diehl

1Division of Gastroenterology, Department of Medicine, Duke University Medical Center, Durham, North Carolina

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Omenetti A, Diehl AM. The Adventures of Sonic Hedgehog in Development and Repair. II. Sonic hedgehog and liver development, inflammation, and cancer. Am J Physiol Gastrointest Liver Physiol 294: G595–G598, 2008. First published January 24, 2008; doi:10.1152/ajpgi.00543.2007.—Hedgehog (Hh) signaling modulates tissue remodeling by controlling the fate of Hh-responsive cells. Healthy adult livers exhibit little Hh activity. However, cells involved in adult liver repair, including myofibroblasts and progenitors, are capable of producing and responding to Hh ligands. During adult liver injury, Hh ligand production increases and populations of Hh-responsive cells expand. This process is accompanied by fibrosis. Ligand production and Hh-responsive cells diminish as fibrosis resolves and normal hepatic architecture is restored, but Hh signaling persists in hepatocellular carcinomas. These findings suggest that the Hh pathway mediates remodeling responses that are triggered by adult liver damage.

Hedgehog signaling; liver repair; stellate cells; liver fibrosis

In general, the Hedgehog (Hh) signaling pathway regulates tissue construction (during development) and reconstruction (in adults). This is accomplished via Hh-dependent autocrine/paracrine mechanisms that control the size and localization of Hh-responsive cell populations (2, 9). Interestingly, many types of stromal cells and progenitors are Hh-responsive, whereas more mature epithelial cells generally are not. Ligand-producing cells (which may or may not be Hh-responsive themselves) synthesize and release soluble Hh ligands that interact with the Hh receptor Patched (Ptc) on Hh-responsive target cells. Binding of Hh ligands to Ptc liberates the coreceptor Smoothened (Smo) from Ptc-mediated inhibition and permits the propagation of Hh-initiated signals that culminate in the nuclear localization and binding of Glioblastoma (Gli), family transcription factors to Hh-target genes. The latter include several components of the Hh signaling pathway itself (e.g., Ptc, Gli-1, Gli-2), permitting the pathway to both positively and negatively autoregulate its activity.

Hh pathway activation typically enhances the growth and viability of Hh-responsive cells, whereas abrogating Hh signaling in such cells generally triggers apoptosis unless other factors are available to expedite cellular differentiation to a more mature phenotype that no longer requires Hh-induced viability signals. Thus up- or downregulation of Hh signaling respectively expands or contracts various populations of Hh-responsive progenitor cells, thereby controlling the ultimate cellular composition of many tissues. Because cells with Hh pathway activity also tend to have a relatively mesenchymal phenotype, induction of Hh signaling modulates cell migration and influences tissue architecture by controlling the localization of Hh-responsive cells and their progeny (2, 9).

At present, there is little direct proof that Hh signaling plays an important role in liver development (25), although considerable indirect evidence supports this concept. On the other hand, two of the predominant cell populations that regulate adult liver repair, i.e., hepatic stellate cells (HSC) and liver epithelial progenitors, are capable both of producing and responding to Hh ligands (20, 22, 24). Moreover, Hh signaling controls the growth and viability of these cells, at least in vitro (20, 22, 24). Progenitors for several other cell types in adult livers (e.g., endothelial cells and cells of the immune system) are also Hh-responsive (9). Emerging evidence demonstrates that Hh-responsive cell populations expand significantly in many types of adult liver injury (7, 10, 14) and regress when recovery is completed (16). Constitutive activation of Hh signaling has also been demonstrated in both hepatocellular carcinoma (HCC) (2, 21) and cholangiocarcinoma (2). Together, these findings suggest a previously unsuspected role for the Hh pathway in reconstruction of damaged adult livers, identify Hh pathway components as potential therapeutic targets, and justify additional research to clarify the outcomes of Hh pathway induction and the mechanisms that regulate Hh responses in adult liver cells.

Hedgehog Signaling in Liver Development

Hedgehog signaling plays a critical role in construction of multiple tissue types during development, including the nervous system, heart, lungs, proximal gastrointestinal tract, limbs, teeth, and skin (9). In all of those tissues, Hh pathway activation results from epithelial-mesenchymal exchange of paracrine signals, such as bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), and generally promotes the survival of progenitor cells. Both FGFs and BMPs are also essential for early stages of liver organogenesis, and there is at least one report that these factors induce ventral endodermal expression of Sonic hedgehog (Shh) prior to hepatic specification (3). Hh pathway activity has been demonstrated in fetal liver cells that were isolated later during development at 11.5 days postconception (22). However, liver phenotypes in mice with targeted disruption of various Hh pathway components are unknown. Recent reports of liver pathology in some patients with congenital defects in the Hh pathway, as well as growing evidence that this pathway is strongly activated in various types of adult liver disease and liver cancer (see Hedgehog Signaling and Liver Repair and Hedgehog Signaling...
and Hepatocarcinogenesis), will likely renew interest in the role of Hh during liver development.

**Hedgehog Signaling and Liver Repair**

Adult liver damage evokes a complex, wound-healing response. Repair of damaged livers in postnatal life involves a complex wound-healing response that variably engages all of the cell types that reside in adult livers. Hepatocytes repopulate healthy livers after partial liver resection and myofibroblastic cells play a role in the pathogenesis of cirrhosis during chronic liver injury. For this reason, most attention has focused on mechanisms that regulate the proliferation of mature hepatocytes (6) and/or the accumulation of myofibroblastic cells (1). Recently, it has become apparent that replication of mature hepatocytes is inhibited in most types of chronic liver injury (13, 17). Therefore, appropriate expansion and differentiation of progenitor cells is necessary for recovery. This has stimulated research to improve understanding about mechanisms that regulate progenitor populations.

Progenitor populations play crucial roles in adult liver repair. Populations of liver epithelial progenitors in adults are heterogeneous and include small cells with scant cytoplasm and oval-shaped nuclei (oval cells), as well as ductular-type cells and small hepatocytes (5). All of these cell types exhibit considerable plasticity. For example, certain oval cells appear capable of generating either hepatocytes or cholangiocytes (5). Some ductular cells may give rise to either fibroblastic cells or hepatocytes, and some hepatocytes may also become fibroblastic (11) or generate cholangiocytes (12). Controversial evidence suggests that liver epithelial progenitors may also be derived from infiltrating multipotent progenitors from bone marrow or other extrahepatic tissues, such as the pancreas (4, 23). Liver epithelial progenitors and myofibroblasts localize in areas of fibrosis during chronic liver injury (19). In addition to their potential epithelial origin, these myofibroblasts may derive from resident HSC dispersed in sinusoids throughout the liver parenchyma, portal tract fibroblasts, and/or bone marrow-derived fibrocytes (1). In healthy adult livers, canals of Hering are thought to comprise the stem cell “niche” and house the most primitive epithelial progenitors until injury-related signals entice these cells to migrate and differentiate to replace dead hepatocytes (4, 5). Thus adult liver repair requires expansion, differentiation, and migration of stromal and progenitor cells, and this process continues until healthy hepatic architecture is reconstituted.

Progenitor response to adult liver injury is regulated by mechanisms that activate production of Hh morphogens. Expansion of epithelial progenitor and myofibroblast populations is orchestrated by a complex (and incompletely understood) network of paracrine and autocrine signals that are initiated when liver cell survival is threatened by various hepatotoxic agents. Some of the earliest signals appear to include various injury-related proinflammatory cytokines (e.g., tumor necrosis factor-α, lymphotixin-β, interferon-γ) and growth factors [e.g., hepatocyte growth factor, insulin-like growth factor, epidermal growth factor, platelet-derived growth factor-BB (PDGF-BB), and transforming growth factor-β (TGF-β1)] released by the dying cells themselves, resident and infiltrating immune cells, myofibroblasts, and surviving liver epithelial cells. Our group recently demonstrated that at least some of these factors (e.g., epidermal growth factor, PDGF-BB, and TGF-β1) induce certain types of adult liver cells to produce Hh ligands (14, 16, 24). We also proved that optimal viability and growth of certain types of liver myofibroblasts and epithelial progenitors requires Hh pathway activation (14, 20, 22, 24). These exciting findings identify a mechanism that preferentially enhances the outgrowth of cell types, such as myofibroblasts and liver epithelial progenitors that are normally not abundant in healthy livers but that progressively accumulate during chronic liver injury.

Adult HSC produce and respond to Hh ligands. Rat, mouse, and human HSC express proteins that regulate Hh signaling, such as Shh, Hh-interacting protein (HIP, the Hh pathway inhibitor), Ptc, and Smo (14, 16, 20, 24). When freshly isolated from adult livers, HSC produce some Shh but exhibit relatively little Hh signaling activity, due to relatively high levels of HIP. During “spontaneous” culture-induced activation to the myofibroblastic (MF) HSC phenotype, HIP is downregulated, production of Shh increases, and expression of Gli-2, a Hh target gene, is induced (24). Antibodies that neutralize Hh significantly reduce the viability of MF-HSC, demonstrating that Shh is an autocrine viability factor for these cells. MF-HSC also release Shh into the culture medium, raising the possibility that they may regulate Hh activity in adjacent Hh-responsive cells, including sinusoidal endothelial cells and epithelial progenitors. Transfer of HSC-conditioned medium to cells that were transfected with Hh reporter constructs (24) and studies of HSC cocultures (14) support the latter concept. Hh signaling is also required for HSC mitogens, such as PDGF-BB, to elicit their full mitogenic activity. Neutralizing Shh with anti-Shh antibodies or inhibiting Hh signaling downstream of Ptc by treatment with the Smo antagonist, cyclopamine, virtually abolishes PDGF-induced proliferation (24). These results suggest the following model: injury-related factors, such as PDGF-BB, activate Hh morphogenesis of Hh ligands, and the latter induces Hh signaling that promotes the growth of MF-HSC populations, as well as neighboring Hh-responsive cells, including certain types of epithelial progenitors.

**Adult liver epithelial progenitors are Hh responsive.** Various types of liver epithelial progenitors from fetal and adult rodents, as well as human fetuses, produce and respond to Hh ligands (22). Immunochemistry of liver sections from patients with chronic liver damage revealed coexpression of Ov-6 (progenitor marker) and Gli-2, and thus demonstrated that human adult liver epithelial progenitors are also Hh responsive (10). Cultures of human or rodent liver progenitor cells undergo apoptosis when treated with Hh pathway inhibitors (22). Hence, like MF-HSC, some liver epithelial progenitors require Hh pathway activity for survival. Interestingly, sensitivity to Hh ligands declines dramatically during the differentiation process. Hence, the most primitive liver progenitor cells exhibit the highest Hh activity; more differentiated progenitors remain somewhat Hh responsive, but healthy, mature hepatocytes lack Hh signaling entirely (22). Liver progenitors are also capable of producing Hh ligands. The type of ligand that is expressed also varies with cellular differentiation status: more primitive cells produce predominately Shh, whereas more differentiated cells produce Indian hedgehog (Ihh) (22). As with MF-HSC, treatment of immature cholangiocytes with PDGF-BB elicits production of Shh (A. Omenetti, Y. Popov, Y. Jung, S. S. Choi, R. P. Witek, L. Yang, K. D. Brown, D.
when such cells are exposed to TGF-β1 (Y. Jung, K. D. Brown, R. P. Witek, A. Omenetti, L. Yang, M. Vandenberg, R. J. Milton, I. Hines, R. A. Rippe, L. Spahr, L. Rubbia-Brandt, and A. M. Diehl, unpublished observations). Together, these findings predict that the growth of certain types of liver epithelial progenitors will be favored by circumstances, such as liver injury, that increase local production of Hh ligands.

**Hh mediates signaling in adult liver epithelial and mesenchymal cells.** In injured livers, oval cells and ductular cells typically localize close to myofibroblastic cells within fibrous stroma (19). The proximity of these cell types in damaged livers suggests that they may be exchanging signals that promote their growth (19). To evaluate this possibility, we placed immature cholangiocytes in transwell cocultures with MF-HSC and then compared the phenotypes of the cocultured cells to that of monocultures of each cell type (14). Coculture significantly increased the proliferative activity and decreased the apoptotic activity of both cholangiocytes and MF-HSC. Coculture also upregulated production of Hh ligands and increased expression of Hh target genes in both cell types. Antibody neutralization studies demonstrated that the growth-stimulatory and prosurvival effects of coculture were predominately mediated by Hh ligands. Ongoing analysis of microarray data suggests that coculture also evoked epithelial mesenchymal transition (EMT) in the cholangiocytes (15). This result was not unexpected given strong experimental evidence that Hh signaling represses mechanisms that maintain epithelial integrity and thereby promotes EMT in other systems (9). Given this background and growing evidence for EMT in certain types of adult liver injury in humans (18) and rodents, our findings suggest that Hh pathway activation may be an important trigger for this type of repair response during liver damage.

**Hh pathway activation occurs in rodent models of chronic liver injury.** To obtain a better understanding of the significance of Hh pathway activation in adult livers, we evaluated several rodent models of chronic liver injury [e.g., bile duct ligation (BDL) before and after biliary decompression via Roux-en-Y biliary-enteric anastomosis, nonalcoholic and alcoholic fatty liver disease, and ethanol-induced toxic liver injury] (7, 14, 16). Hh pathway activation was observed in all models, and the degree of induction tightly paralleled the severity of liver injury and thus the intensity of the resultant repair response. Regardless of the cause of liver injury, Hh-responsive cells (as demonstrated by expression of Hh-target genes) predominately localized within populations of liver epithelial progenitors and myofibroblasts as long as the injury-provoking agent was present. After the cause of liver injury was removed, these cell populations gradually dwindled as normal liver architecture was restored over a period of weeks to months. Within days of removing the cause of liver injury, Hh target gene expression also consistently emerged in more mature-appearing hepatocytic cells. This too subsided with recovery of liver health. These findings demonstrate that various types of liver injury activate Hh signaling and suggest that Hh-responsive cells play an active role in repair of damaged adult livers. The latter concept is supported by studies of genetically altered mice with haplo insufficiency of Ptc (14). The partial deficiency of Ptc impairs downregulation of Hh pathway activity (8) and is associated with a more exuberant bile ductular reaction (i.e., expansion of myofibroblasts and ductular cells) following BDL (14).

**Hh pathway activation also occurs in human adult liver injury.** We are examining liver specimens from patients with various types of liver damage to determine if Hh pathway activation is a conserved response to liver injury in adult mammals. Immunohistochemistry of liver sections from three patients with primary biliary cirrhosis (PBC) demonstrated consistent and highly significant increases in hepatic expression of Hh ligand (Ihh), receptor (Ptc), and target genes (Gli-2) compared with healthy liver controls. Costaining of the PBC samples with established markers for oval cells, bile ductular cells, and myofibroblasts indicated that bile ductular cells were the predominant source of Ihh. Populations of oval cells, immature ductular cells, and myofibroblasts were particularly enriched with Hh-responsive cells (10). More recently, we analyzed liver samples from nine patients who were hospitalized with clinically severe alcoholic liver disease (Y. Jung, K. D. Brown, R. P. Witek, A. Omenetti, L. Yang, M. Vandenberg, R. J. Milton, I. Hines, R. A. Rippe, L. Spahr, L. Rubbia-Brandt, and A. M. Diehl, unpublished observations). All subjects had steatohepatitis superimposed upon cirrhosis. Because only formalin-fixed sections were available, Hh ligand expression could not be assessed, but we were able to examine expression of Ptc and Gli-2 and to colocalize these factors with other liver cell markers. Numerous Ptc- and Gli-positive cells were noted in all patients. In addition to myofibroblastic, oval, and ductular cells, more mature hepatocytic cells that reacted with a panectokeratin antibody that marks liver progenitors were also Hh responsive in many patients. Moreover, quantitative analysis of these hepatocytic Hh-responsive cells demonstrated a striking correlation with the Maddrey discriminant function (DF), a formula that uses serum bilirubin and prothrombin time to predict clinical outcome. A DF > 32 is a highly reliable predictor of short-term mortality. In our analysis, patients who had a higher risk of short-term mortality (i.e., those with a DF > 32) exhibited significantly greater accumulation of Hh-responsive immature hepatocytes than patients with a lower mortality risk (i.e., DF < 32). This finding suggests that repopulation of the liver by immature hepatocytes impairs hepatic-specific functions (i.e., clearance of bilirubin and synthesis of clotting factors) and may promote mortality in the short term, despite contributing to eventual liver repair. If verified by further research, this insight has important clinical implications because it suggests that Hh pathway activation may be a “double-edged sword,” with either insufficient or excessive Hh activity being potentially problematic. This concept is supported by evidence that some HCC and cholangiocarcinomas exhibit constitutive Hh pathway activity.

**Hedgehog Signaling and Hepatocarcinogenesis**

Dysregulation of the Hedgehog pathway has been implicated in the genesis of malignancies derived from various tissues (2). Hh pathway activity has been reported in some cholangiocarcinoma and hepatoblastoma cell lines (2) and subgroups of patients with HCC (21). Hh pathway inhibitors were also shown to block the growth of hepatoblastoma cells in culture (21), raising the possibility that inappropriate activation of Hh signaling plays a role in hepatocarcinogenesis. Indeed, a novel
Smo mutation has been reported in at least one patient with HCC (21). A procarcinogenic role for the Hh pathway is further supported by other evidence that HCC typically arises in the context of cirrhosis, a condition that favors the outgrowth of Hh-responsive cells.

Conclusion
To repair damage, many adult tissues reactivate mechanisms, including Hh pathway activation, that regulate tissue construction during development. Although the role of Hh signaling in liver development remains unproven, there is growing evidence that the Hh pathway is activated during various types of liver injury in adults. The resident populations of adult liver cells that are most actively engaged in remodeling damaged adult livers (e.g., myofibroblastic hepatic stellate cells and liver epithelial progenitors) are capable both of producing and responding to Hh ligands. When exposed to injury-related cytokines, such as PDGF-BB, these cells increase their production of Hh ligands, and the latter enhance their viability.

These findings suggest a novel model for adult liver repair. Specifically, because mature hepatocytes are not Hh-responsive, progressive enrichment of the liver microenvironment with Hh ligands during liver injury provides a selective survival advantage for the Hh-responsive cells, which include myofibroblastic and progenitor cells. This encourages the outgrowth of these cell populations as long as the injury-related cytokines persist. However, as the factors that incited liver injury subside, the stimuli for Hh activation wane, Hh signaling gradually abates, other (as yet poorly understood) factors promote the differentiation of Hh-responsive cells, and the liver is repopulated by the progeny of the original Hh-responsive populations. Such progeny include mature hepatocytes and bile duct cells. Failure to appropriately downregulate Hh signaling permits the survival of Hh-responsive cells and may thereby contribute to hepatic fibrosis and neoplasia.

REFERENCES