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# Underlying potential: cellular and molecular determinants of adult liver repair

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**Review Series**

The liver has a unique and extraordinary capacity for regeneration, even in adult organisms. This regenerative potential has traditionally been attributed to the replicative capabilities of mature hepatocytes and cholangiocytes, though emerging evidence suggests that other resident liver cell types such as progenitors, liver sinusoidal endothelial cells, and hepatic stellate cells respond to liver injury and contribute to repair. These other cells types are also associated with liver scarring, dysfunction, and carcinogenesis, which suggests that appropriate regulation of these cells is a major determinant of response to liver injury. The Reviews in this series explore possible contributions of liver progenitor cells, liver sinusoidal endothelial cells, and hepatic stellate cells to liver homeostasis and repair and highlight how these processes can go awry in chronic liver injury, fibrosis, and liver cancer.



# Underlying potential: cellular and molecular determinants of adult liver repair

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**The liver has a unique and extraordinary capacity for regeneration, even in adult organisms. This regenerative potential has traditionally been attributed to the replicative capabilities of mature hepatocytes and cholangiocytes, though emerging evidence suggests that other resident liver cell types such as progenitors, liver sinusoidal endothelial cells, and hepatic stellate cells respond to liver injury and contribute to repair. These other cells types are also associated with liver scarring, dysfunction, and carcinogenesis, which suggests that appropriate regulation of these cells is a major determinant of response to liver injury. The Reviews in this series explore possible contributions of liver progenitor cells, liver sinusoidal endothelial cells, and hepatic stellate cells to liver homeostasis and repair and highlight how these processes can go awry in chronic liver injury, fibrosis, and liver cancer.**

## Adult liver tissue has exceptional regenerative potential

Adult organisms require a wound-healing response and mechanisms of repairing sublethal injury in all tissues. In most adult organs, cell replacement is inefficient and injury tends to result in scarring and functional impairment rather than regeneration and recovery. The adult liver, similar to adult bone marrow, is a notable exception to this general rule, and liver has tremendous regenerative capabilities, as illustrated by the ability to completely reconstitute functional liver mass within days (in rodents and fish) to weeks (in humans) following acute 70% partial hepatectomy (1, 2). In addition, a more gradual regeneration and complete recovery are also observed after massive ischemic, toxic, and infectious types of acute liver injury.

Historically, the liver's unique regenerative potential has been attributed to the proliferative capabilities of mature hepatocytes, the major type of liver epithelial cell. Although mature hepatocytes are typically polyploid and rarely proliferate in healthy adult livers (3), hepatocytes harvested from healthy adult donor rats were shown to repopulate the livers of recipients after serial partial hepatectomies, leading the authors of those studies to estimate that a single adult hepatocyte is capable of replicating at least 69 times (4, 5). This discovery, in turn, is the basis for current dogma that mature hepatocytes are facultative liver stem-like cells (6). Unlike other stem/progenitor cells, however, replication-quiescent mature hepatocytes are quite metabolically active and appear to retain highly differentiated functions even when proliferating (1). In addition, hepatocytes are not known to express high levels of telomerase (7, 8) or to dedifferentiate into resident liver stromal cell types such as hepatic stellate cells, portal fibroblasts, or liver sinusoidal endothelial cells (9). Whether or not mature hepatocytes can transdifferentiate into benign cholangiocytes is debated, although recent reports suggest that hepatocytes can give rise to cholangiocarcinomas (10). Recovery of normal liver function after 70% resection, however, requires regrowth of all of these cell types as well as of hepatocytes. Re-establishment of normal cell-cell interactions is also necessary. The mechanisms that acutely coordinate these processes with mature hepatocyte repopulation are not well understood but must be highly effective because partial hepatectomy and other acute causes of massive hepatocyte loss trigger global liver repair responses that efficiently reconstruct completely functional liver tissue.

## Liver regeneration occurs in a context-specific manner

Curiously, despite the liver's remarkable ability to regenerate after acute injury, many types of much more indolent, chronic liver injury result in some degree of scarring. As in other tissues, progressive replacement of functional hepatic parenchyma with scar (dubbed cirrhosis) disfigures the tissue and results in organ dysfunction that is ultimately fatal (11). Cirrhosis is also the major risk factor for primary neoplasms of liver epithelial cells (hepatocytes and cholangiocytes) (12). Because repetitive toxic, metabolic, and infectious liver injuries are highly prevalent, cirrhosis and liver cancer are major causes of death worldwide (13). Hence, defective regeneration is the root cause of most liver failure and liver-related mortality, supporting concepts that scarring is the "end stage" of various chronic diseases and that scarred organs are irreparable.

## Lessons can be gleaned from defective liver repair

Recent breakthroughs in the treatment of chronic viral hepatitis have resulted in growing evidence that challenge the dogma that scarring is irreversible. In humans with chronic viral hepatitis–related cirrhosis, cirrhosis is now known to gradually resolve once the viral infection is cured (14). Earlier work in rodents with noninfectious types of liver injury and cirrhosis also demonstrated that liver scarring regresses when injury is alleviated (15). These discoveries affirm the extraordinary regenerative powers of adult livers and identify key factors that gate effective repair. Evidence that ongoing injury easily derails regeneration, even in a tissue with tremendous regenerative prowess, demonstrates that injury-related factors play pivotal roles in modulating repair. This observation in turn focuses attention on cell types that typically accumulate during scarring, prompting questions about their roles in wound healing and the mechanisms that modulate such responses. The latter issues seem particularly important because the incidence and prevalence of primary liver cancers are increasing worldwide, despite recent declines in the prevalence of cirrhosis (13). Clarification of the cell types and molecular mechanisms that are necessary for reconstruction of scarred livers might also have implications for scarring in other tissues as well.

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## Review objectives — profiling liver repair's supporting cast

The overall objective of this Review series is to summarize emerging information about resident liver cell types that are involved in hepatic wound healing during chronic injury. Because recent liver regeneration reviews have emphasized mature liver epithelial cells (i.e., hepatocytes and cholangiocytes) and the factors that regulate their proliferation (summarized in ref. 1), here we focus on other resident liver cell types that undergo dramatic phenotypic changes and outgrow in chronically injured livers, namely progenitors, liver sinusoidal endothelial cells, and hepatic stellate cells. Unlike hepatocytes, which are widely regarded as regenerative "stars," these other cells are often blamed for scarring, liver dysfunction, and carcinogenesis. Ironically, growing evidence indicates that regeneration from both acute and chronic liver injury depends upon appropriate regulation of the fates of these cells. Therefore, in the subsequent Reviews we highlight data that provide new insight into the identities of additional cell types influencing liver repair and the factors that control their destiny during liver injury.

*Liver progenitors and their microenvironment*. Several Reviews in the series focus on the role of liver progenitors because an emerging consensus suggests that these cells play pivotal roles in both adult liver regeneration and carcinogenesis. Yoshiya Kawaguchi summarizes evidence that Sox9, a transcription factor that programs pancreatic and liver progenitors during development, also regulates the fates of adult progenitors in these tissues (16). He also describes the technical challenges of mapping the fates of adult progenitors and the controversies that have resulted, as well as the immediate opportunities to advance mechanistic understanding of adult regeneration by targeting pathways that are known to control lineage decisions during embryogenesis. In their Review, Boulter, Lu, and Forbes confirm and extend the wisdom of the latter approach (17). They detail recent data that show how the microenvironment in injured livers differentially modulates Wnt and Notch signaling to specify the appropriate differentiation of adult bipotent liver progenitors along either the hepatocyte or cholangiocyte lineage. Yamashita and Wang's Review summarizes information on cancer stem cells, progenitors gone awry (18). They also compare and contrast liver cancer stem cells with extensively studied stem cells in other epithelial malignancies, such as breast cancer. The Review by Kordes and Häussinger completes the progenitor component of the series by characterizing the putative stem cell niches in adult livers (19). The authors emphasize how various cells that comprise these niches, particularly stellate cells and endothelial cells, play pivotal roles in adult liver repair.

*Liver sinusoidal endothelial cells and hepatic stellate cells*. Two Reviews provide more in-depth discussions of these cell types within the liver microenvironment. Laurie DeLeve's Review on liver sinusoidal endothelial cells draws attention to the liver's ability to recruit bone marrow–derived cells to help replenish this unique liver cell population when necessary (20). Stainier and colleagues's Review on the hepatic stellate cell emphasizes the inherent plasticity of this cell type, which appears to assume many identities (21). Fueling controversy about their origins, hepatic stellate cells bear markers of all three germ layers. Furthermore, although stellate cells are required for effective regeneration and control liver perfusion by functioning as pericytes, they also drive scarring by becoming liver myofibroblasts. Hence, hepatic stellate cells may be a "lynchpin" for regenerative success. Knowledge is growing about the factors that control their fate.

*Liver scar as a therapeutic target*. Finally, Schuppan and Kim describe why an improved understanding of fibrosis and other aspects of liver wound healing is critical (22). Patient data demonstrating that recovery from liver cirrhosis is feasible have spawned a new era of therapeutics that aim to prevent and reverse fibrosis. In the liver, as in other vital organs, fibrosis is the face of failed regeneration. Historically, attention has focused on the mechanical consequences of scarring (e.g., stiffness, increased resistance to blood flow) as well as the fact that a scar replaces normal parenchyma with matrix. However, growing evidence indicates that a scar is much more than matrix: it encompasses various types of wound-healing cells whose behaviors are actively orchestrated by the scar-related matrix. These wound-healing cells in turn actively remodel the matrix. During successful regeneration, this bidirectional exchange eventually replaces scarred tissue with healthy parenchyma. Adult livers appear to have perfected the technique of temporary scarring because most liver injuries, even when massive or chronic, do not result in permanent fibrosis. Rather, adult livers typically regenerate after injury. When the wound-healing process becomes deregulated or derailed, however, defective repair ensues and bad outcomes, including cirrhosis and/or liver cancers, result. Clarification of the mechanisms that underlie liver regeneration may suggest targets to ameliorate scarring and optimize regeneration of injured livers, and could have implications for other organs with less robust regenerative capabilities.

## Summary and conclusions

Wound-healing responses generally aim to replace dead cells while compensating for their loss. Adult livers demonstrate extraordinary regenerative capabilities but are still subject to scarring under conditions of prolonged injury or impaired regeneration. While the amazing proliferative range of its regenerative stars (i.e., mature liver epithelial cells) is well known, recent evidence that recovery from cirrhosis is feasible suggests that additional cell types (i.e., progenitor cells and stromal cells) may be the unsung heroes that are key to the regenerative success of liver. Such cells interact with each other, surviving mature liver epithelia, and bone marrow–derived cells to mold the regenerative milieu. They also retain considerable plasticity in adulthood, and hence can be mobilized and directed down various pro-regenerative paths, even in livers that have been badly scarred. Improved understanding of the mechanisms that facilitate (or obstruct) these processes might help to perfect repair, offering the hope that scarring may eventually be eliminated as a cause of organ failure.

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