Hepatic Regeneration—Revisiting the Myth of Prometheus

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Myriad signals such as growth factors, cytokines, growth inhibitors, hormones, ions, extracellular matrix, and the resident hepatic cells are involved in the regulation of hepatic regeneration. These regulatory factors ultimately mediate changes in gene expression, a critical step in this well-orchestrated restorative process.

The liver is a remarkable organ, given its inherent capacity to fully restore itself after significant hepatic tissue loss either from partial hepatectomy (PHx) or acute liver injury. The tremendous regenerative potential of the liver has been recognized since ancient times. In classical Greek mythology, Prometheus, after stealing the secret of fire and introducing it to earthlings, was punished by having an eagle of Zeus feast daily on his liver. His punishment was the ultimate torture, as his liver regenerated eternally while the eagle continued his perpetual daily feeding sessions from a constantly replenished source.

The classical model of hepatic regeneration is that of partial hepatectomy in which ~70% of the liver is resected. The remaining lobes enlarge and reconstitute the original size of the liver. Hepatic regeneration after PHx in the rat takes 5-7 days.

“...having an eagle of Zeus feast daily on his liver.”

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References

The regenerative potential of the liver, in which there is eventual full restoration of the full size of the liver after hepatic resection, has also been demonstrated in larger animals and humans. From these observations, it is generally accepted that hepatic regeneration is a well-orchestrated phenomenon, remarkably regulated with signals from the organism that exert modulatory effects (positive or negative) on the liver until the optimum size is achieved. In this review, the cells that play an important role in this regenerative process in the liver, cell cycle progression, regulatory growth factors/cytokines (both stimulatory or inhibitory), transcriptional control of genes that govern hepatic regeneration, and the influence of the hepatic extracellular matrix (ECM) are discussed.

Cell replication after PHx

Liver regeneration after PHx is mediated by proliferation of the mature resident liver cells to restore lost hepatic tissue. These liver cells include hepatocytes, endothelial cells, biliary epithelial cells, hepatic stellate cells, and Kupffer cells. In sharp contrast to other regenerating tissues such the skin or bone marrow, in the liver, progenitor cells or stem cells do not account for much of the regenerative capacity. Of the resident liver cells, the hepatocytes are the first to proliferate and are the major target for parenchymal regeneration. The adult liver is usually quiescent, exhibiting minimal replicative ability, with mitosis observed in approximately 1 in every 20,000 hepatocytes. After tissue loss or injury, hepatocytes enter the cell cycle from a quiescent state (G0) to a prereplicative state (G1), which is followed by DNA synthesis (S) and mitosis (M), with cell division completing the sequence. The prereplicative phase of the cell cycle can be divided into two components, a “priming” stage (G1→G1) and a “progression” stage (G1→S). In response to partial hepatectomy, the low rate of DNA synthesis in resting hepatocytes continues for ~12 h; subsequently, some of the hepatocytes begin to enter the S phase. Mitosis then follows DNA synthesis 6–8 h later. During hepatic regeneration, most hepatocytes are estimated to replicate once or twice. Replication of the nonhepatocyte resident liver cells (nonparenchymal cells) is generally delayed 24 h but demonstrates a similar synchronous pattern of DNA synthesis and mitosis as seen in hepatocytes (11). Once the original size and volume of the liver is achieved, hepatocytes revert to their nonreplicative, quiescent, but functional state.

There are multiple pieces of evidence that suggest that the clonogenic potential of the hepatocyte itself appears to be boundless. In an animal model of hereditary tyrosinemia type I, a recessive liver disease characterized by fumarylacetacetate hydrolase deficiency in which there is massive, potentially lethal liver tissue destruction, ~1,000 transplanted hepatocytes are sufficient to rescue the entire liver. A normal rat liver has, on average, 3 x 10^6 hepatocytes; thus one could predict that a single rat hepatocyte has clonogenic potential to generate ~50 rat livers (11).

Growth factors/cytokines—initiators/activators of hepatic regeneration

The trigger of hepatic regeneration has been the subject of intense investigation. Complete mitogens are defined as substances that stimulate DNA synthesis and mitosis of cultured hepatocytes in serum-free media. Comitogens have no direct proliferative effect on hepatocytes in culture but augment the stimulatory effect of complete mitogens and decrease the inhibitory effect of other factors. See Table 1 for a representative list of factors implicated in hepatic regeneration.

Hepatocyte growth factor. Hepatocyte growth factor (HGF), the most potent of liver mitogens, is a heterodimeric glycoprotein, also known as scatter factor (SF), which mediates its key biological role during hepatic growth via its receptor, c-Met, a member of the receptor tyrosine kinase superfamily. HGF is produced by mesenchymal cells (both intrahepatic and extrahepatic) but not by hepatocytes or other epithelial cells of the body. The plasma levels of HGF rise substantially (>20-fold) within 1 h after PHx in both rats and humans. Expression of HGF mRNA increases in hepatic stellate cells 3–6 h after PHx and reaches a peak in 18-24 h (14). An increase in HGF mRNA is also seen in other mesenchymal cells of other tissues such as lung and spleen. The mechanism for this extrahepatic induction of HGF mRNA after PHx is unclear. HGF exerts its mitogenic effect on hepatocytes by both a paracrine and an endocrine manner. The unanswered question is, Does HGF trigger hepatic regeneration? Despite the marked induction of HGF in various tissues after PHx and unilateral nephrectomy, mitogenic responses are found only in the liver or kidney, respectively, after these operations, although HGF is an active mitogen for many different cell types. This supports the argument that HGF activation occurs in the affected organ, perhaps as a local event at the cell surface or as a consequence of c-Met regulation.

Tumor necrosis factor-α and interleukin-6. There is an assortment of evidence implicating tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 in hepatic regeneration. Pretreatment with anti-TNF-α antibodies results in diminished DNA synthesis and attenuates the increases in Jun kinase, c-jun mRNA, and nuclear adaptor protein...
complex-1 (AP1) activity after PHx (5). DNA synthesis after PHx is markedly impaired in mice deficient in TNF-α type 1 receptor (15). In these mice, exogenous IL-6, a proinflammatory cytokine secreted by Kupffer cells, restored the abrogated elevation of signal transducer and transcriptional activator 3 (STAT-3) and nuclear factor-κB (NF-κB), thus supporting a regulatory role of TNF-α in IL-6 secretion during PHx (15).

IL-6 is a crucial cytokine in liver regeneration whose secretion is enhanced in response to TNF-α. Plasma levels of IL-6 increase rapidly in the first 24 h after PHx, whereas hepatocyte DNA synthesis during liver regeneration is inhibited in IL-6 knockout (KO) mice, with marked reduction of STAT-3 activation [a known consequence of epidermal growth factor (EGF) and IL-6], AP1, Myc, and cyclin D1 (3). These changes in DNA synthesis and cell cycle gene expression are restored in these IL-6 KO mice (IL-6 –/–) by exogenous IL-6 (3).

EGF and transforming growth factor-α. EGF, produced mainly in the salivary glands of rats and mice, is a primary mitogen for hepatocytes. In rats, sialadenectomy, which causes a major decline in plasma EGF, abrogates the hepatic regenerative response. Paradoxically, post-PHx EGF levels rise only minimally (~30%). Norepinephrine, which increases dramatically after PHx, also augments the production of EGF from Brunner’s glands, which further amplifies the amount of EGF made available after PHx.

In contrast to EGF, transforming growth factor (TGF-α) appears to be involved in the later stages of regeneration and functions through an autocrine loop. TGF-α produced by hepatocytes acts as a mitogenic stimulus to hepatocytes by ligating its cognate receptor, EGF, found on hepatocytes (TGF-α has 35% homology to EGF and shares the same receptor, EGFRI). Despite an appreciable increase in TGF-α mRNA, TGF-α protein in regenerating liver increases only twofold (12). Overexpression of TGF-α in hepatocytes of transgenic mice leads to a high rate of DNA synthesis, which eventually leads to tumor formation (8). Thus the existing evidence supports a proliferative role for TGF-α in hepatic regeneration; however, the exact mechanism is not entirely clear.

Norepinephrine. Plasma levels of norepinephrine, a comitogen, increase rapidly within an hour of PHx, augmenting secretion of EGF by Brunner’s glands of the duodenum while countering the mitoinhibitory effects of TGF-β on cultured hepatocytes isolated from the early stages of regeneration. The net effect of norepinephrine is to augment mitogenic signals of EGF and HGF in regenerating hepatocytes by its actions via the α1-adrenergic receptor.

Insulin. Insulin by itself does not have mitogenic effects on hepatocytes; rather, it acts as a comitogen for hepatocytes in the presence of other growth factors. In the presence of relative hepatic insulin deficiency (as occurs after portacaval shunt surgery, in which portal circulation is diverted from the liver), the liver atrophies. Insulin injections have been shown to reverse or prevent hepatic atrophy under these conditions.

**Inhibitors of hepatic regeneration**

The termination of hepatic regeneration still remains an enigma. A variety of factors have
been touted as growth inhibitors/terminators during the regenerative response once recovery of the liver mass has been achieved. TGF-β, a fibrogenic cytokine secreted by hepatic stellate cells, is a potent inhibitor of hepatocyte proliferation and has been suggested as the main terminator of hepatic regeneration. TGF-β increases immediately after PHx with kinetics similar to that of HGF. TGF-β mRNA, almost undetectable in normal liver, increases within 3-4 h after PHx and attains a plateau after 48-72 h (1). A potential inhibitory role of TGF-β during hepatic regeneration is further supported by evidence in transgenic mice in which there is enhanced expression of TGF-β1 in the liver and which exhibit defective hepatic regeneration (13).

Although TGF-β is the only mito-inhibitor that has been noted to play a crucial role in terminating hepatic regeneration, other candidates include metabolites, growth factors, cytokines, and the ECM. These factors may either exert their mito-inhibitory activities singularly or constitute an aggregate of signals for terminating hepatic regeneration. Activin, a potent inhibitor of hepatocyte proliferation, is undetectable in normal liver but increases exponentially after PHx.

Multicellular organisms eliminate redundant or injured cells by a program of cell suicide, termed apoptosis. Apoptosis-associated gene expression during hepatic regeneration is the subject of intense investigation and has not been completely defined. Apoptosis, although rare in the normal liver, is prominent in the liver in which there is a deficiency of growth stimuli such as mitogens, hormones, xenobiotics, and tumor promoters. TGF-β and activin may be important mediators of apoptosis under these circumstances.

Regulation of priming and progression through the cell cycle during hepatic regeneration

The growth response after PHx is governed by priming and progression through the cell cycle. The priming phase coincides with loss of growth inhibition and represents the G₀ to G₁ transition, whereas the progression phase acts to promote cell replication and represents the G₁ to S transition. It has been proposed that growth factors/cytokines induce priming events on G₀ hepatocytes, thus enabling hepatocytes to acquire proliferative competence for progression through the cell cycle. Alternatively, cytokines induce priming events while other growth factors activate progression through the cell cycle. Thus the initiation of the growth response depends on complex interactions among hepatocytes and nonparenchymal cells, the ECM, endocrine, autocrine, paracrine, and neuroregulatory factors, oxygen free radicals, metabolites, and nutrients. There appears to be a redundancy of “early priming” signals. “Priming” stimuli include EGF, TNF-α, IL-6, insulin, matrix changes, etc. Progression signals include HGF, TGF-α, EGF, insulin, etc. (see Fig. 1). The regulation of the hepatic regenerative process is dependent on a number of myriad factors that ultimately modulate immediate-early, delayed-early, and liver-specific gene expression (see Fig. 1 and Table 2).

Immediate-early gene response. Hepatic regeneration after PHx is a exceptional in vivo model in which to study gene expression, cell cycle, and growth regulation. Within minutes after PHx, hepatocytes exhibit changes in membrane hyperpolarization...
Immediate-early genes provide a framework for understanding the cell cycle-dependent response to PHx because these genes are important regulators of cell growth and proliferation.

**Delayed-early gene response.** Delayed-early genes are induced within a few hours after PHx and are expressed during the G0 to G 1 phase transition (progression phase) but are dependent on protein synthesis. They are critical modulators of hepatocyte regeneration and include p53, H-ras, and K-ras genes, which regulate delayed gene expression during G1 phase of the regenerating liver.

Specific hepatic nuclear factors (HNF) such as HNF-1, HNF-3, and HNF-4 are basically unchanged after PHx. The intricate interplay of these transcription factors and growth factors contributes to the progression of hepatocytes through the cell cycle (see Fig. 1).

**Transcriptional and posttranscriptional regulation after PHx**

The priming step that confers a state of replicative competence of hepatocytes is an initiating event in liver regeneration that involves the activation and DNA binding of transcription factors such as nuclear factor-κB (NF-κB), STAT proteins, and CCAAT/enhancer binding protein (C/EBP) isoforms to promoter elements of immediate-early genes. The transcriptional regulation of gene expression of immediate-early genes is critical to the initiation and termination of hepatic regeneration. Because immediate-early genes are induced in a protein synthesis-independent fashion, the transcriptional activation of these genes occurs by induction of preexisting transcription factors. The key transcription factors during hepatic regeneration appear to be NF-κB, STAT-3, and the C/EBP isoforms.

**NF-κB.** NF-κB is a pivotal transcription factor during liver regeneration. NF-κB activation is a rapid process that involves posttranslational modifications of proteins and is triggered by a variety of stimuli such as endotoxins (lipopolysaccharide), TNF-α, IL-1, IL-2, ultraviolet light, and oxidants. Many of the immediate-early genes contain sequences in their promoter region that are responsive to NF-κB. NF-κB preexists in the normal liver in an inactive form and is rapidly activated in hepatocytes within 30 min after PHx, an important component of the regenerative response (4).

Recent work suggests that induction of NF-κB during liver regeneration after PHx appears to be a required event to prevent apoptosis and allows for normal cell cycle progression. Further evidence for an antiapoptotic role of NF-κB is suggested by the embryonic death from massive hepatic apoptosis of NF-κB p65 KO mice at day 15 of embryogenesis. Subsequent in vitro studies, following the demonstration of massive apoptosis in developing hepatocytes in p65 KO mice, have shown that NF-κB is an important antiapoptotic factor in hepatocytes. The mechanism of NF-κB-mediated protection has not been completely clarified. TGF-β-mediated apoptosis of hepatocytes is prevented by NF-κB-dependent inhibition of the expression of the proapoptotic genes cIAP-1 and cIAP-2, and the proinflammatory genes TNF-α and IL-1β.

### Table 2. A representative sample of the >70 genes implicated in hepatic regeneration

<table>
<thead>
<tr>
<th>Immediate-Early Genes</th>
<th>Late-Early Genes</th>
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<tbody>
<tr>
<td>κB-α</td>
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<td>NF-κB</td>
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<td>c-fos</td>
<td>α-FNR</td>
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<td>β-FNR</td>
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<td>H-ras</td>
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<td>C/EBP-β</td>
<td>K-ras</td>
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<td>MKP-1</td>
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κB-α, inhibitory κB-α; NF-κB, nuclear factor-κB; PEPCK, phosphoenolpyruvate carboxykinase; C/EBP, CCAAT/enhancer binding protein; EGF, epidermal growth factor; G6Pase, glucose-6-phosphatase; MKP-1, mitogen-activated protein kinase phosphatase-1; PRL-1, phosphotyrosine phosphatase-related gene-1; MHC, major histocompatibility complex; FNR, fumurate and nitrate reduction regulator.

"NF-κB is a pivotal transcription factor during liver regeneration."
cytes may result partially from TGF-β-mediated inhibitory IκB-α (IκB-α) stabilization and inactivation of NF-κB. Iimuro et al. (7) have shown that blocking NF-κB, with a super-repressor, IκB-α, results in enhanced apoptosis, reduced mitosis, and increased liver dysfunction after PHx.

Signal transducers and activators of transcription. The STAT family of transcription factors are preexisting transcription factors that are activated by phosphorylation on tyrosine residues that results in STAT activation.

IL-6- and EGF-induced activation of STAT-3 is a critical step in liver regeneration. STAT-3 binding activity is increased in the remnant liver within 30 min of partial hepatectomy and peaks to >30-fold at 3 h (2). The cytokines TNF-α, IL-1, and IL-6 induce both NF-kB and STAT pathways, suggesting a possible redundancy in signaling after PHx.

CCAAT/enhancer binding protein. C/EBP is a family of transcription factors that play a critical role in liver regeneration and development. The antiproliferative transcription factor, C/EBP-α, which has been associated with hepatocyte differentiation and growth arrest, is suppressed during compensatory regeneration. In contrast, C/EBP-β, another member of the C/EBP family of transcription factors that is involved in hepatocyte-specific gene expression, is increased during hepatic regeneration and is a vital component of the hepatic regenerative response (6). Restoration of normal architecture—the ECM during hepatic regeneration

The ECM is a complex, dynamic, non-inert structure that not only affords physical scaffolding but also modulates biological function of adjoining cells. In the liver, the ECM has been shown to modulate cell adhesion, migration, and development, maintenance of a differentiated state, normal architecture, and hepatic regeneration. ECM exerts its biological function by acting as a solid ligand interacting by ligating cell surface receptors or by the sequestering and subsequent release of cytokines such as occurs with platelet-derived growth factor (PDGF), TGF-β, basic fibroblast growth factor, and vascular endothelial growth factor. Additionally, these cytokines have profound effects on the synthesis, deposition, and catabolism of the ECM.

The ECM is of vital importance in regulating liver regeneration, promoted by cell-ECM interactions. There is no appreciable change in the ECM in the first 24 h after PHx. Mitotic activity declines by post-PHX day 4, which coincides with deposition of laminin, an ECM component, by hepatic stellate cells. During PHx, the hepatic matrix composition evolves from one of high laminin content to that characteristic of the mature liver, such that by day 10, the normal distribution of ECM has been restored and new sinusoids formed (for review, see Ref. 10).

The components of the ECM during liver regeneration are distinct from those seen during the fibrotic response of the liver to chronic injury. During hepatic regeneration there is no basement membrane formation in the hepatic sinusoids, unlike the situation during hepatic fibrosis. Additionally, enactin, a major basement membrane component, is not detectable during hepatic regeneration but is characteristically seen during hepatic fibrosis.

ECM degradation is a critical component of remodeling seen after PHx. Urokinase-type plasminogen activator, a major initiator of the matrix proteolysis cascade and an activator of plasminogen and HGF, increases very rapidly (within a minute) after PHx (9). Rapid reorganization of selected ECM components appears to be a critical event during hepatic regeneration.

Unanswered questions and future perspectives

What initiates and terminates hepatic regeneration? What is the role of apoptosis during hepatic regeneration? Are hepatic stem cells critical in the regenerative response in vivo? Answers to these tantalizing questions are being addressed, and knowledge of the exact regulatory pathways during hepatic regeneration could be further clarified in the near future.

A better understanding of the hepatic regenerative process could be of potential clinical benefit. For example, the ability to modulate the regenerative response in the liver offers the potential development of novel therapeutic options in a number of liver diseases. In fulminant liver failure, the possibility of enhanced replication of the remaining viable hepatocytes or stem cells has the potential of preventing death in a disease in which medical therapy alone (in the absence of transplantation) has a 80% mortality. New information about growth factors/cytokines that regulate the regenerative ability of liver cells in vivo will be important in the design of liver assist devices and will be a useful adjunct in hepatocyte transplantation. Presently, such treatments remain experimental. One could envision boosting the regenerative potential of hepatocytes in patients with end-stage liver disease to improve the hepatic synthetic function of these patients and possibly avoid liver transplantation. Before such treatment options become available, there is the need for

"ECM exerts its biological function by acting as a solid ligand..."
better delineation of the key molecular mechanisms underlying hepatic regeneration.

References


