Liver regeneration

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The liver is unique in its ability to regenerate in response to injury. A number of evolutionary safeguards have allowed the liver to continue to perform its complex functions despite significant injury. Increased understanding of the regenerative process has significant benefit in the treatment of liver failure. Furthermore, understanding of liver regeneration may shed light on the development of cancer within the cirrhotic liver. This review provides an overview of the models of study currently used in liver regeneration, the molecular basis of liver regeneration, and the role of liver progenitor cells in regeneration of the liver. Specific focus is placed on clinical applications of current knowledge in liver regeneration, including small-for-size liver transplant. Furthermore, cutting-edge topics in liver regeneration, including *in vivo* animal models for xenogeneic human hepatocyte expansion and the use of decellularized liver matrices as a 3-dimensional scaffold for liver repopulation, are proposed. Unfortunately, despite 50 years of intense study, many gaps remain in the scientific understanding of liver regeneration. (Translational Research 2014;163:352–362)

Abbreviations: DNA = deoxyribonucleic acid; EGF = epidermal growth factor; ERK1/2 = extracellular signal-related kinase 1 and 2; FAH = fumarylacetoacetate hydrolase; FGF = fibroblast growth factor; HBEGF = heparin-binding EGF-like growth factor; HGF = hepatocyte growth factor; IGFBP = insulinlike growth factor binding protein; IL = interleukin; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; miRNA = microRNA; MKK4 = mitogen-activated protein kinase kinase 4; mTOR = mammalian target of rapamycin; NFκB = nuclear factor κB; SFSS = small-for-size-syndrome; SOCS = suppressors of cytokine signaling; STAT3 = signal transducer and activator of transcription 3; TGF = transforming growth factor; TLR = toll-like receptor; TNF = tumor necrosis factor

Regeneration of the liver can be defined more correctly as compensatory hyperplasia, during which the remaining liver tissue expands to meet the metabolic needs of the organism. Unlike anatomic true regeneration, the expanding liver does not regain its original gross anatomic structure.[1] It is also important to note the origin of cells used to replace the missing hepatocytes. Contrary to true regeneration, in the case of partial hepatectomy and some chemical liver injuries, the liver mass is replaced by replication of existing hepatocytes without activation of progenitor cells. In other cases of chemical liver injury, including galactosamine toxicity, activation and replication of progenitor cells does occur.[2]

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TIMING OF REGENERATION

Certain aspects of liver regeneration vary according to circadian rhythms. Matsuo et al[3] demonstrated that, after partial hepatectomy in mice, the transition from G2 to mitosis occurred at the same time of day despite variability in the time of day the partial hepatectomy was performed. DNA synthesis, however, peaked at 36 hours after surgical intervention, regardless of the light/dark cycle used. These data strongly support that the transition from G2 to mitosis is controlled, at least in part, by circadian-dependent cell cycle-related genes. Specifically, these genes modulate the expression of cyclin B1-Cdc2 kinase, an important regulator of mitosis. Matsuo et al[3] further presented *Wee1* as a candidate for

the circadian regulator of hepatocyte division. At high levels, *Wee 1* phosphorylates Cdc2 kinase, disrupting the activity of the cyclin B1-Cdc2 kinase complex.[4] Therefore, the progression of hepatocytes into mitosis is postponed until levels of *Wee 1* are low.

In contrast to the circadian rhythm-regulated hepatocyte mitosis, DNA replication is independent of circadian rhythm but appears to be an intrinsic property of hepatocytes. There is species variation in peak DNA synthesis after partial hepatectomy, with rat DNA synthesis peaking 12–16 hours earlier compared with mice. Weglarz and Sandgren[5] demonstrated the timing of hepatocyte entry into DNA synthesis after partial hepatectomy is cell autonomous. They transplanted rat hepatocytes into the livers of mice after partial hepatectomy and found the rat hepatocytes replicated earlier than mouse hepatocytes in the chimeric liver. These results defined DNA synthesis as cell autonomous and suggest that cytokines or growth factors may have a permissive but not an instructive role in hepatocyte progression to the S phase.

MODELS FOR LIVER REGENERATION

A number of models have been proposed for the study of liver regeneration. The most completely studied model is that of liver regeneration after partial hepatectomy. A rodent model of two-thirds hepatectomy was first proposed by Higgins and Anderson[6] in 1931. The rodent liver is multilobar, allowing for the removal of 3 of 5 liver lobes (two-thirds of the liver mass). Within 5–7 days of surgical removal, the remaining liver has regenerated to a size equivalent to the original mass. This model has remained a popular model of study because there is no injury to the residual liver. The resultant sequence of events can be delineated clearly without histologic evidence of damage to the residual liver tissue.

Zebrafish have been recognized as an exceedingly important model of developmental biology because of their prolific production of offspring and transparent embryos, offering constant visualization and experimental manipulation. Furthermore, organogenesis occurs rapidly, with presence of nearly all major organ systems by 2 days postfertilization; a mature liver is visualized under standard light microscope by 5 days postfertilization.[7] Forward genetic screening, the technique of targeting embryonic mutants defective in a particular process, has allowed researchers to identify essential genes for various processes of hepatogenesis within this vertebrate model.[8]

Chemical-mediated hepatotoxic injury, including carbon tetrachloride (CCL_4), has also served as a common model of liver injury. The challenge of CCL_4-mediated injury is that it triggers necrosis of lobular zones of the liver, leading to an acute inflammatory response. The inflammatory response is dominated by polymorphonuclear leukocytes and macrophages infiltrating the liver to remove necrotic hepatocytes. The intense inflammatory response is thought to affect both the onset and duration of the liver regenerative response.[9]

D-galactosamine is known to cause acute liver damage in animal models. The mechanism of D-galactosamine hepatotoxicity is not understood completely, but D-galactosamine is believed to cause an intracellular deficiency of uridine metabolites, leading to acute liver failure.[10] As illustrated in Fig 1, acute liver injury by D-galactosamine is associated with waste accumulation, systemic inflammation, and impaired regeneration. These 3 problems are also seen in humans and often contribute to death after drug-induced acute liver injury, which makes the porcine model of D-galactosamine acute liver failure an appropriate large-animal model for testing extracorporeal liver assist devices.

Acetaminophen intoxication is a common clinical cause of acute liver failure. After an overdose of acetaminophen, the liver cannot perform the necessary breakdown steps of glucuronidation and sulfation, and the P450 system takes over. Subsequently, a toxic accumulation of N-acetyl-benzoquinoneimine occurs, leading to the formation of radicals and Kupffer cell activation.[11] The systemic manifestation of acetaminophen hepatotoxicity is believed to be mediated by proinflammatory cytokines and the innate immune system (Fig 1). For example, mice with mutant Toll-like receptor (TLR) 4 had improved survival significantly after acetaminophen overdose compared with normal wild-type mice. Furthermore, survival of wild-type mice was improved significantly both by depletion of Kupffer cells or pretreatment with a TLR4 antagonist. Kupffer cells express high levels of TLR4.[12] These studies show that reduction of TLR4 activity through clinical treatment is associated with mitigation of systemic inflammation and improved survival in a mouse model of acetaminophen-induced liver failure. They also show that the TLR4 activity of Kupffer cells is a main contributor to the systemic inflammatory response of acute liver failure, and that modulation of the TLR4 pathway by depletion of Kupffer cells or direct antagonism of TLR4 receptor leads to improved survival after acetaminophen-induced acute liver failure. Future studies should address whether improved survival is also the result of enhanced liver regeneration.

Genetically modified animals with inborn errors of metabolism have also been proposed to serve as models of liver regeneration. Most impressive may be the immunodeficient, fumarylacetoacetate hydrolase (FAH)-deficient mouse model developed by Azuma et al.[13] The livers of these triple knockout mouse are capable of engraftment and significant repopulation with mature