Hallmarks of postoperative liver regeneration: An updated insight on the regulatory mechanisms

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Abstract
Performance and advances in liver surgery makes remarkable progress of the understanding of liver regeneration. Liver regeneration after liver resection has been widely researched, and the underlying mechanism mostly concerns proliferation of hepatocytes and the influence by inflammation through activation of Kupffer cells and the other parenchymal cells, the second regenerative pathway by hepatic progenitor cells (HPCs), inducing angiogenesis, remodeling of a extracellular matrix (ECM), and termination mechanisms. New clinical surgeries and the updated multomics analysis are exploiting the remarkable progress, especially in immune regulation and metabolic process of two emerging hallmarks. This review briefly represents a systemic outline of eight hallmarks, including hepatocyte proliferation, contribution of hepatic progenitor cells, inducing angiogenesis, reprogramming of the extracellular matrix, apoptosis and termination of proliferation, inflammation, immune and metabolic regulation, which are set as organizing characteristics of postoperative liver regeneration and future directions of refining treatment targets.

Introduction
Liver regeneration is a reparative process following damage to the liver or the partial removal of liver tissue, which has been well known for the punishment of Prometheus in the Greek mythology who was sent to feed an eagle with his liver, and the remnant liver would grow back overnight to be eaten again.1 The regenerative capability of liver enables the remnant liver or liver graft to grow in size to completely restore the liver mass and thus makes it feasible to perform liver surgery, including partial hepatectomy (PHx), living donor liver transplantation (LDLTx), and reduced-size liver transplantation. The application of liver surgery in turn makes the further investigation on liver biology.

The majority of the literature studying parenchymal restoration in the context of liver resection or partial transplantation, have focused on the regulation of hepatocyte proliferation and mechanism of portal hyperperfusion injury, and maintenance of the liver function in the remnant liver.1,2 Empirical evidences indicated that the size of the remnant liver or the graft size should be at least 25–30% of standard liver volume or a ratio above 0.5–0.8% between the liver graft mass and recipient body weight.3 Recent preclinical studies and clinic experience indicate that size of future liver remnant (FLR) and degree of portal hyperperfusion are not the only determining factors involving liver regeneration.5 New emerging evidences have suggested that modulation of immune microenvironment through the innate immune system may accelerate liver regeneration after liver surgery.6

The aim of this paper is to review the scientific literature on liver regeneration after liver surgery, with a particular emphasis on regulatory mechanisms, to provide more insight into possible future treatment strategies.

Novel mechanism of liver regeneration after liver resection
Most of the novel knowledge of liver regeneration was based on the research after PHx. PHx is a widely used experimental model for liver regeneration. The performance of 70% PHx in rodents7,8 has been the most popular model for the study of liver regeneration.9 Because of the multilobe structure of the rodent liver, both the median lobe and left lobe (representing 70% of the liver mass) of the four lobes can be removed by ligation of lobe pedicle and lobectomy. The remaining two lobes (right lobe and caudate lobe) grow in size to restore the mass of the original intact lobes.
**Proliferation of hepatocytes.** Liver regeneration after PHx can be divided into an initiation stage, proliferation stage, and termination stage, with highly coordinated molecular and cellular events that work in concert to restore the original mass, structure and functions of the liver through signal communication between activation of nonparenchymal cells and proliferation of the remnant mature hepatocytes.9,10

In the initiation stage, the regenerative progression is marked with acute inflammation and is mediated by proinflammatory cytokines including tumor necrosis factor α (TNF-α) and interleukin 6.10 Downstream of receptor engagement and the activation of signal transducer and activator of transcription 3 and nuclear factor-κB drive G0 to G1 transition of hepatocytes.11,12 Hepatocyte proliferation is stimulated by growth factors that promote G1 to S and G2 to M transitions, including hepatocyte growth factors (HGFs), epidermal growth factors (EGFs), transforming growth factor-α, growth hormones, heparin-binding EGFs, amphiregulin, and fibroblast growth factors, and their interactions with activation of HGF receptors (c-Met), EGF receptors, and growth hormone receptors.13,14 Transforming growth factor β (TGF-β) may participate in mitosis termination through interaction with the TGF-β receptor, eliciting apoptosis and causing most hepatocytes to return to a quiescent state.15

The balance between stimulatory and inhibitory factors during liver regeneration is necessary to process hepatocyte proliferation and restore the organ size. It should be noted that these intracellular signaling in liver regeneration are mainly regulated by tyrosine phosphorylation.16 Recent studies showed that microRNAs can post-transcriptionally modify gene expression and cell proliferation during liver regeneration in both animals and humans.17 Via ubiquitination, Nedd4-1 and SOCS2 may inhibit the stimulatory factor-mediated activity and terminate liver regeneration.18,19

**Activation and proliferation of nonparenchymal cells.** Hepatic nonparenchymal cells and their functions during liver inflammation and regeneration are illustrated in Table 1.14,20–24

Kupffer cells (KCs) make up the largest population of macrophages in the liver. Communication between KCs, macrophage, and monocytes is necessary for regeneration and secretes TNF-α and interleukin 6 that directly induce hepatic expression of multiple genes associated with acute phase proteins to facilitate the proliferation of hepatocytes.1 Hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells also play crucial roles in the proliferation of hepatocyte and sinusoidal remodeling after liver injury. The HSCs stimulated by inflammation contribute to the initiation of liver regeneration by secreting HGF. In addition, the activated HSCs also produce a extracellular matrix (ECM) that serves as a scaffold for the proliferation of hepatocytes and maintains the mechanical stability in the damaged region.25 The liver sinusoidal endothelial cells activated by acute inflammation also secrete HGFs through a direct cellular contact in liver sinusoids to promote liver regeneration.26

Replication of hepatocytes generally starts within 1 day after major resection in both rodents and humans. Nonparenchymal cells usually replicate in a delayed fashion.9,27 The original size and weight of the liver is reestablished within 7 days after PHx in rodents.9 Afterward, the lobes are slowly reorganized, and hepatic histology is completely restored after 3 weeks in rodents and 3–6 months in humans.3,28 The above described physiological process is dependent on a normal liver tissue. Clinical data have shown that liver regeneration is significantly impaired in damaged livers because of a reduced response of injured hepatocytes to proliferative stimulation in severely injured livers, including viral hepatitis, alcoholic liver disease and non-alcoholic fatty liver disease, and intrahepatic cholestasis.1,28

**Activation and differentiation of hepatic progenitor cells.** In the chronic liver injury induced by chemicals or toxic drugs combined with PHx in rodents and in liver diseases such as chronic hepatitis B virus and hepatitis C virus infection, alcoholic fatty liver disease, hemochromatosis, and other conditions, where the ability of hepatocytes to replicate is severely impaired; a second regenerative mechanism marked by activation of hepatic progenitor cells (HPCs) can be actuated.9 Following severe and prolonged liver injury, HPCs are capable of proliferation and differentiation into both hepatocytes and cholangiocytes and typically form the hepatoblast, comprising small ductules and strings of cholangiocytes, termed the ductular reaction.29 Activation of HPCs after injury can be of significance to maintain and restore liver homeostasis after stress. Hippo/Yes-associated protein signaling plays an essential role in determining the fate relationship

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<th>Table 1</th>
<th>Anatomy of hepatic cells and their function in liver inflammation and regeneration</th>
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<td><strong>Cell subsets</strong></td>
<td><strong>Percentage</strong></td>
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<td>Parenchymal cells</td>
<td>Hepatocyte</td>
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<td>Nonparenchymal cells</td>
<td>Cholangiocyte</td>
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<td>Liver sinusoidal endothelial cell</td>
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between putative hepatic progenitors and hepatocytes. Elevated Yes-associated protein activity defines identity of HPCs, and its ectopic activation in hepatocytes results in their dedifferentiation, driving HPC appearance. New cell therapy approaches and new insights are being investigated in human subjects and animal models, which may represent a novel way to restore tissue function for cell-based therapy of chronic liver diseases.

**Regulating angiogenesis and remodelling of the extracellular matrix.** Major surgery results in a rapid influx of angiogenic factors that could alter the intrahepatic microenvironment and initiate remodelling of the ECM after the priming of liver regeneration. Angiogenesis involves upregulation of proangiogenic growth factors such as hypoxia-induced factor-1α, vascular endothelial growth factors, platelet-derived growth factors and metallopeptidases, and the ECM remodelling. During the proliferation phase and the later stages, with the reorganization of sinusoids, new vessels are formed from proliferation and migration of hepatocytes and endothelial cells, and the recruitment of HPC from bone marrow. HSCs function as fibroblastic-like cells and secrete ECM components under the stimulation of TGF-β that ultimately leads to establishment of a microcirculatory network. A liver ECM provides a structural framework of hepatic cells and maintains hepatic homeostasis. During regeneration, changes in protein synthesis patterns, pericellular proteolytic activity of hepatic cells, and invading inflammation could result in the ECM remodelling, which could alter cell signaling and facilitate liver regeneration. After mouse liver injury, MMP-10 is induced as hepatoprotective role and may participate in the liver regenerative response.

**New insights of postoperative liver regeneration**

**Liver regeneration characteristics after living donor liver transplantation, two-stage hepatectomy, and associating liver partition and portal vein ligation for staged hepatectomy.** Liver regeneration after LDLTx is an important and essential clinical field of liver research, because graft size and postoperative regenerative ability is closely related to donor safety and recipient prognosis. After LDLTx, the rapid graft restoration may be associated with the degree of graft and body weight mismatch and whether small-for-size syndrome can be avoided. A recent clinical study showed that kinetic growth rate of the FLR in patients after LDLTx negatively correlated with the size of FLR. In addition, graft regeneration is influenced by ischemia reperfusion injury, portal inflow, and immune-related responses. A shorter cold ischemia time, higher graft-recipient bodyweight ratio, and an immediate and remarkable increase in graft portal vein flow predicted a better prognosis after LDLTx. Few studies based on clinical practice have however looked at whether there is a distinct pattern and/or particular mechanisms involved in liver regeneration after LDLTx.

Two-stage hepatectomy with or without portal vein embolization has been regarded as an effective way to completely remove multiple bilobar colorectal cancer metastases. Liver regeneration after two-stage hepatectomy was found similar to PHx and different from repeat hepatectomy, the later which was characterized by diminished hepatic regenerative capacity and functional restoration.

Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has been developed to increase FLR and enables surgical therapy in patients otherwise deemed unresectable. The principal advantage of this approach can induce a greater volume increase of the FLR in shorter time and in terms of kinetic growth rate yielded superior results to two-stage hepatectomy, portal vein embolization, or portal vein ligation alone. This could imply that a greater proportion of patients might then be offered a curative resection. The clinical series published have also demonstrated a high perioperative morbidity and mortality, possibly attributable to a mismatch between volume of the FLR and actual functional reserve.

There is a rising in the particular mechanism involved in ALPPS-associated liver regeneration. Schlegel et al developed the first preclinical ALPPS model to study hypertrophy of FLR, and concluded that the circulating inflammation factors, induced by parenchyma transection, mediated the enhanced liver regeneration. We utilized a rat model of ALPPS to study the relationship between the FLR size, liver regeneration, and restoration of function. The results verified that the kinetic growth rate of the FLR in patients after ALPPS correlates directly with the size of the FLR; the smaller the initial FLR, the greater was the increase in FLR weight and maximal kinetic growth rate at the end of the experiment, which is different from the regeneration pattern after PHx hepatic hypertrophy of the FLR after ALPPS is associated with hypoxia, increased Hippo/Yes-associated protein signaling, and HPC activation. Further studies of the particular pattern of regeneration in the ALPPS model might elucidate the mechanisms behind this extraordinary capability of the liver for regeneration. This could potentially be utilized to develop new therapeutic options that can stimulate and enhance the regenerative potential in difficult clinical scenarios such as acute liver failure and chronic liver diseases.

**Immune regulation in liver regeneration.** The process of inflammation following liver surgery and the innate immune response are critical factors for liver regeneration. The innate immune system consists of an intricate network of interacting immune cells and cytokines. Liver resident KCs, natural killer cells (NK), and natural killer T (NKT) cells are the most abundant effectors of the intrahepatic innate immune system, which can exert significant effects. The available knowledge tended to believe that innate immune cells take part in downregulation of liver regeneration except for KC’s priming regeneration at the initiation stage by secreting TNF-α and interleukin 6, and further investigation on immune regulation of liver regeneration is needed.

Recently, macrophage M1/M2 polarization paradigm has been introduced as a mechanism that may promote hepatocyte proliferation through modulation of inflammatory factors. CD11b+ macrophages (M1 phenotype) have been demonstrated to be involved in liver regeneration via TNF-α signaling after PHx. Interestingly, our unpublished data showed that blocking of PD-1 and PD-L1 interaction could boost in vivo liver regeneration through induction of M2 to M1 polarization.
After PHx, the number of NKT cells increases in the remnant liver, and NKT cells may be involved in the process of liver regeneration.\textsuperscript{56} Large amounts of cytokines produced by NKT cells have multiple functions in liver regeneration and immune responses.\textsuperscript{57} The depletion of both NKT and NK cells enhances liver regeneration, whereas the depletion of NK cells alone has no effect, indicating the negative regulation of activated NKT cells in liver regeneration.\textsuperscript{58} Interleukin-4 knockout mice exhibit restored liver regeneration by interleukin-4\textsuperscript{-}mediated NKT cell expansion and increase of interferon gamma expression,\textsuperscript{58} suggesting that NKT cells affect liver regeneration by influencing the inflammatory hepatic microenvironment.

The activation of HSCs is critical for liver regeneration and fibrosis related to immune responses. HSCs directly interact with NK cells, NKT cells, and T cells, which is mediated by Toll-like receptors to trigger proinflammatory responses.\textsuperscript{59}

As part of the innate immune system, the complement system has recently been revealed as key regulator of both ischemia reperfusion injury and liver regeneration.\textsuperscript{60} The central components of the complement cascade, complement component 3 and complement component 5, were confirmed to play a central role in liver regeneration.\textsuperscript{51} Plasmin may contribute to nontraditional complement activation in liver regeneration complement activation in liver regeneration.\textsuperscript{62} Complement component 3/complement component 5-deficient mice showed increased parenchymal damage and impaired liver regeneration after carbon tetrachloride injury compared to wild-type mice.\textsuperscript{63,64} An integrated model for analysis of the complement-induced priming phase of liver regeneration, activates c-Fos and promotes the TNF-\(\alpha\) signaling pathway, regulates the efflux and the metabolism of cholesterol, contributing for cell cycle and proliferation.\textsuperscript{65}

Compared with the recent knowledge about the involvement of the innate immune response, few studies have demonstrated any significant involvement of the adaptive immune system the regulation of liver regeneration. The transfer of splenocytes from wild-type mice but not lymphotoxin-deficient mice improved liver regeneration in T cell-deficient mice.\textsuperscript{66} Further research is needed to identify the immune molecules involved and whether this could constitute potential therapeutic approaches with a clinical applicable effect on liver regeneration.

**Metabolism profile in liver regeneration.** The liver harbors the comprehensive collection of metabolic processes throughout the body, regulating the metabolisms of carbohydrates, amino acids, fatty acids, and the detoxification of xenobiotics to maintain homeostasis. After surgical injury, the liver has to maintain metabolic homeostasis and at the same time support regeneration. Detailed investigations of the metabolic adaptation during liver regeneration would help to understand the interaction between cell division and proliferation and metabolism and post-translational regulation during tissue repair. Identification of novel metabolic pathways that promote restoration of homeostasis after damage or tissue loss could have a distinct therapeutic potential. The key liver-specific metabolic mediator, bile acid, and its receptors, farnesoid X receptors, and G-protein-coupled bile acid receptors 1, as well as fibroblast growth factor 15, the key regulator of bile acid metabolism, were reported to be essential for liver metabolism.

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Hallmarks of postoperative liver regeneration and the potential therapeutic targets. Characteristics and hallmarks as emerging therapeutic targets of postoperative liver regeneration are summarized [Color figure can be viewed at wileyonlinelibrary.com]
regeneration. The role of bile acid and its regulation during hepatic injury and liver regeneration has received more attention recently.

Comprehensive analyses of the epigenome, transcriptome, proteome, metabolism, and a combination of multomic studies have provided a better description and understanding of the molecular landscape and metabolic profile during liver regeneration. The results indicated that at the early phase of liver regeneration, lipid and carbohydrate metabolism is enhanced and leads to the elevated triglycerides and cholesterol levels throughout the regeneration process. Ingenuity Pathway Analysis indicated that 14-3-3 protein epsilon is located at the center of pathway networks after PHx, suggesting the critical role of 14-3-3 protein epsilon in regulating liver regeneration. Additionally, the role of Cdc42 was revealed in the termination of liver regeneration. Metabolic remodeling in liver regeneration is accompanied by reduced cellular oxidation and reduction of mitochondrial respiration, which results in increased metabolic flux through alanine transaminase. When nicotinamide adenine dinucleotide H levels were modulated by using nicotinamide adenine dinucleotide+ repletion, a promotion of hepatocyte division was observed after liver damage. Microbiota and the liver-microbiome crosstalk may be critical in various liver diseases. The emerging impact of the gut microbiota on liver regeneration has been under investigation. Metabolomics analysis revealed certain bile acids and retinoic acid, which are produced by specific bacteria and regulates hepatic lipid, accelerated PHx-induced liver regeneration.

Hallmarks of liver regeneration and the therapeutic perspective

Cell proliferation, activation and differentiation of HPCs, inducing angiogenesis, remodelling of ECM, apoptosis and termination of proliferation, inflammation, immunoregulation and metabolism, these eight hallmarks constitute the logical framework that reflect the current knowledge of liver regeneration and may provide potential therapeutic targets that can be utilized to accelerate liver regeneration and improve function after surgery (Fig. 1).

Current potential therapeutic strategies focus on administration of exogeneous hormones, cytokines, growth factors, and chemokines, including EGF, HGF, and vascular endothelial growth factor, and fibroblast growth factor. Preclinical animal experiments suggest that this may stimulate liver regeneration, prevent hepatic failure, and increase postoperative survival. TGF-β could contribute to remodeling of the liver microenvironment through mediation of ECM proteins and myofibroblast differentiation, and control the organ size through antiproliferative response. In addition, administration of mesenchymal stem cells and derived stem cell are other possible emerging options that could constitute a potent regenerative therapeutic opportunities. Nevertheless, there is still a lack of solid evidence for the clinical safety and efficacy of the mentioned strategies, and further preclinical studies are needed to complete our knowledge in the field before clinical trials can realistically be conducted.

In summary, although research on liver regeneration has increased in recent decades, the knowledge of liver regenerative processes is still far from complete. Greater understanding will lead to safer operations on living donors and on patients undergoing major liver surgery or liver transplantation with partial grafts.

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