Title
Therapeutic targets for liver regeneration after acute severe injury: a preclinical overview

Journal
Expert Opinion on Therapeutic Targets, 24(1)

ISSN
1472-8222

Authors
Kojima, Hidenobu
Nakamura, Kojiro
Kupiec-Weglinski, Jerzy W

Publication Date
2020-01-02

DOI
10.1080/14728222.2020.1712361

Peer reviewed

HHS Public Access

Author manuscript

Expert Opin Ther Targets. Author manuscript; available in PMC 2021 January 10.

Published in final edited form as:

Expert Opin Ther Targets. 2020 January ; 24(1): 13–24. doi:10.1080/14728222.2020.1712361.

Therapeutic targets for liver regeneration after acute severe injury: a preclinical overview

Hidenobu Kojima[1], **Kojiro Nakamura**[2], **Jerzy W. Kupiec-Weglinski**[1]

[1]The Dumont-UCLA Transplantation Center, Department of Surgery, Division of Liver and Pancreas Transplantation, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, U.S.A.

[2]Department of Surgery, Kyoto University, 54 Shogoin, Kawaracho, Sakyo-ku, Kyoto, 606-8507, Japan; Department of Surgery, Nishi-Kobe Medical Center, 5-7-1 Koji-dai, Nishi-ku, Kobe, Hyogo, 651-2273, Japan

Abstract

Introduction: Liver transplantation is the only viable treatment with a proven survival benefit for acute liver failure (ALF). Donor organ shortage is however a major hurdle, hence alternative approaches that enable liver regeneration and target acute severe hepatocellular damage are necessary.

Areas covered: This article sheds light on therapeutic targets for liver regeneration and considers their therapeutic potential. ALF following extensive hepatocyte damage and small-for-size syndrome (SFSS) are illuminated for the reader while the molecular mechanisms of liver regeneration are assessed in accordance with relevant therapeutic strategies. Furthermore, liver background parameters and predictive biomarkers that might associate with liver regeneration are reviewed.

Expert opinion: There are established and novel experimental strategies for liver regeneration to prevent ALF resulting from SFSS. Granulocyte-colony stimulating factor (G-CSF) is a promising agent targeting liver regeneration after acute severe injury. Autophagy and hepatocyte senescence represent attractive new targets for liver regeneration in acute severe hepatic injury. Liver support strategies, including tissue engineering, constitute novel regenerative means; the success of this is dependent on stem cell research advances. However, there is no firm clinical evidence that these supportive strategies may alleviate hepatocellular damage until liver transplantation becomes available or successful self-liver regeneration occurs.

Corresponding author: Jerzy W. Kupiec-Weglinski, M.D., Ph. D. Dumont-UCLA Transplant Center, 77-120 CHS, 10833 Le Conte Ave, Los Angeles, CA 90095. Tel.: (310) 825-4196; Fax: (310) 267-2358. jkupiec@mednet.ucla.edu.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

Keywords

Acute Liver Failure; Liver Regeneration; Liver Transplantation; Hepatocyte Senescence; Autophagy

1. Introduction

Liver inherently has high regeneration capacity to confront a wide variety of xenobiotics and non-native toxins that enter the organ from the intestinal tract through the portal vein. Even under the man-made acute severe injury, such as hepatic resection or transplantation, liver regenerates to restore its function or mass with proliferation and hypertrophy of native hepatocytes. The mechanisms for liver regeneration have been extensively studied over the last decades using animal models of drug toxicity, partial hepatectomy (PHx), and liver transplantation. Many genes and signaling pathways are involved in liver regeneration pathophysiology along with inter-cellular communication, including hepatocytes and non-parenchymal cells (NPCs). These molecular mechanisms are discussed in previous reviews.[1-3]

Acute severe liver injury disturbs effective regeneration process in liver cells, resulting in acute liver failure (ALF). Therapeutic strategies targeting liver regeneration in ALF could enhance liver regeneration while reducing regeneration inhibitory factors. In clinical practice, well-established regenerative strategies to increase liver volume and function, include portal vein embolization (PVE), two-stage hepatectomy, and associating liver partition and portal vein ligation for staged hepatectomy (ALPPS).[4-6] Liver support strategies aim to alleviate hepatocellular injury and/or substitute liver function until donor liver transplantation or successful self-liver regeneration. Plasma exchange and extracorporeal liver support for ALF can also be categorized as support strategies. Besides these treatments, quite a few studies have reported that liver regeneration therapies activating or inhibiting specific pathways improved acute liver injury outcomes in animal models. However, no study has achieved the standard application of these treatments to the human. Prostaglandin E1, matrix metalloproteinases (MMP) inhibitor, and sevoflurane failed in clinical trials because of insufficient therapeutic effects or unfavorable adverse effects.[7-10] Further, therapeutic targets that promote liver regeneration are often connected to carcinogenesis due to massive non-specific cell proliferation.[11] In this review, we discuss preclinical targets for liver regeneration, mainly focusing on their putative therapeutic applications in humans. In addition, the conceivable mechanisms in liver regeneration are assessed in accordance with those strategies. Liver background clinical parameters and predictive biomarkers that might associate with liver regeneration are also discussed. Strategies targeting and augmenting liver protection, such as machine perfusion can maintain or improve hepatocellular function.[11-13] Modulation of portal blood flow might affect liver regeneration through activation or inhibition of liver sinusoidal endothelial cells (LSECs) activity [14-17]. Reduction of ischemia-reperfusion injury (IRI) may well improve liver transplant outcomes by minimizing the incidence of early acute and late chronic rejection episodes.[18] Although supporting liver regeneration, the discussion of anti-IRI

regimens in the inflamed liver, machine perfusion, or surgical intervention remains beyond the scope of this review.

2. Acute Liver Failure (ALF)

Although normally nonproliferative, hepatocytes regain proliferative activity triggered by local liver insult, such as hepatic resection, liver transplantation, inflammation or chemical stress.[19] The liver mass is restored within 5-7 days even after two-thirds partial hepatectomy (PHx) in the experimental murine model.[2] In the human partial liver transplantation, the liver graft increases its size up to approximately 80-100% of the recipient standard liver volume during the first week.[20] Adult hepatocytes play the key role in liver regeneration as ‘compensating’ hepatic mass and function. However, hepatocyte injury beyond their ability to regenerate, and failure to compensate their function due to severe and wide injury, results in liver failure, often accompanied by encephalopathy, coagulopathy, ascites, prolonged hyperbilirubinemia, and hypoalbunemia.[21] Liver failure can lead to remote organ dysfunction, renal or pulmonary failure, and ultimately death. There are various etiologies for ALF; drugs (commonly acetaminophen), toxins, viral infection, metabolic etc.[22] In addition, small-for-size syndrome (SFSS) after liver surgery, such as hepatic resection and liver transplantation, also causes ALF. The small or poor quality liver after surgery does not have enough capacity for sufficient regeneration to maintain its function, leading to SFSS.[23] A previous survey demonstrated that the minimal remnant liver volume for acceptable hepatic resection was 25% and the minimal graft-recipient weight ratio (GRWR) for safe living donor liver transplantation was 0.8 (40% of the total liver volume).[24] Besides liver size, age, steatosis, hepatitis, ischemia, obstructive cholestasis, fibrosis are the risk factors for SFSS.[23] The main difference between extensive hepatocyte injury and SFSS is whether the normal liver remains functional or not.[25] Therefore, the processes for liver regeneration are different between these etiologies, indicating the therapeutic targets for liver regeneration may differ as well.

3. Preclinical Targets for Liver Regeneration

Multidisciplinary therapies, including specific treatments in accordance with the disease etiology and non-specific treatments such as plasma exchange and albumin dialysis are being performed for ALF, while liver transplantation is the only treatment with a proven survival benefit. Although successful liver regeneration in acute severe injury can lead to recovery from ALF, regenerative strategies against the existing ALF have not been established in humans to date. PVE, two-stage hepatectomy, or ALPPS can increase the feasibility of curative resection for large and multiple tumors with enlargement of future remnant liver volume, leading to prevention of postoperative liver failure.[26] Besides these treatments, there are several clinical relevant strategies targeting liver regeneration, including ALF prevention (Figure 1A, Table 1).

The Hippo/YAP pathway is a newly recognized regulator of the liver size. Activation of the Hippo signaling results in phosphorylation and inhibition of the transcriptional coactivator YAP, leading to its cytoplasm localization [27]. By translocating into the nucleus, activated YAP exerts transcriptional activity in cell proliferation/regeneration, and may mitigate

hepatic damage and fibrosis during liver ischemia-reperfusion injury in mouse and human.[28] Moreover, Hippo/YAP pathway may control liver cell fate as YAP activation dedifferentiated adult hepatocytes into progenitor characteristics [29]. Wnts, extracellular ligands that bind to frizzled receptors, induce nuclear translocation and activation of β-catenin, which in turn initiates transcription of genes involved in cell proliferation [30]. In addition, β-catenin might directly up-regulate epidermal growth factor receptor (EGFR) and contribute to the mitogenic response [31]. Although both Hippo/YAP and Wnt/β-catenin pathways are crucial in experimental liver regeneration/hepatocyte proliferation settings, their clinical relevance awaits to be confirmed in ALF human patients.

3.1 Pharmacological Interventions

3.1.1 Pentoxifylline—The effects of pentoxifylline, which enhances production of interleukin (IL)-6 while inhibiting the TNFα, were evaluated in 101 non-cirrhotic patients undergoing major hepatectomy.[32] Continuous intravenous administration of pentoxifylline, starting 12 hours before and ending 72 hours after the surgery, resulted in significantly higher regeneration volume for small liver (remnant liver to body weight ratio ≤ 1.2%) and stronger induction of interleukin-6 (IL-6) mRNA levels. This study suggested the beneficial effects of pentoxifylline on liver regeneration in small remnant livers, mediated by IL-6 production. Indeed, perioperative treatment with pentoxifylline might enhance liver regeneration following major hepatic resection.

3.1.2 Omega-3 fatty acids—Omega-3 fatty acids nutrition for 7 days after the surgery was reported to mitigate liver injury, reduce infection morbidities, and shorten the post-hospital stay in patients receiving liver transplantation.[33] Another clinical study showed that liver regeneration calculated with volume factors was significantly increased in patients receiving omega-3 fatty acids enriched lipid emulsion for 3 days (2 days before surgery and postoperative day 0) compared with controls after hepatic resection.[34] Experimentally, polyunsaturated fatty acids administration enhanced expression of the liver kinase B1-adenosine monophosphate-activated protein kinase (LKB1-AMPK) signaling pathway after 70% PHx in rats, leading to improved integrity of tight junctions and hepatocellular function.[35] LKB1 was necessary in the phosphorylation of Akt downstream targets, including FoxO3a and glycogen synthase kinase 3β (GSK3β).[36] As AMPK activation stimulates the transport from nucleus to cytoplasm of Hu antigen R (HuR), which is an RNA-binding protein that increases the half-life of target mRNA involved in cell cycle progression,[37] the deletion of AMPKα1 was reported to delay liver regeneration.[38] Clinical and experimental studies suggest that omega-3 fatty acids might promote liver regeneration through the LKB1 and AMPK activation even despite a short term administration.

3.1.3 Platelets and serotonin—A recent study evaluated the effects of intra-platelet serotonin on liver regeneration and oncologic outcome in 96 patients.[39] This study demonstrated that patients with preoperative high intra-platelet serotonin levels exhibited a significant reduction in liver dysfunction after hepatic resection for malignant tumors. On the other hand, these patients suffered from a high incidence of postoperative tumor recurrence. In addition, patients receiving perioperative selective serotonin reuptake inhibitor

(SSRI) treatment, which effectively reduced the intra-platelet serotonin levels, displayed a substantial increase in postoperative morbidities and had no tumor recurrence within 12 months after the surgery. This study suggested the potential of serotonin in both liver regeneration and tumor growth. Induction of thrombocytosis drives liver regeneration, while inhibition of platelet aggregation or reduction of platelet number impairs liver regeneration in mice after PHx.[40,41] It has been commonly conceived that release of platelet granule contents including serotonin and other growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1) may enhance liver regeneration. A recent study demonstrated that pro- and anti-regenerative proteins in the granules of platelet were selectively released upon activation and were involved in liver regeneration.[42] However, the role of platelet in liver regeneration has not been fully elucidated. It is also controversial whether serotonin may directly exert mitogenic activity on hepatocytes or indirectly influences liver regeneration through platelet activation. It has been suggested that the role of serotonin on tumor growth is concentration-dependent; while high serotonin dose stimulates tumor growth, low doses of serotonin can inhibit tumor growth by decreasing blood supply.[43] Although it is unclear which factors contribute to liver regeneration, the increase of platelet and serotonin levels might promote liver regeneration. Of note, mitogenic activity of serotonin should be carefully considered in patients with carcinoma.[39]

3.1.4 Granulocyte colony-stimulating factor (G-CSF)—A randomized controlled clinical trial evaluated the impact of granulocyte colony-stimulating factor (G-CSF) on liver regeneration in 47 consecutive patients with acute-on-chronic liver failure.[44] Twelve doses of subcutaneous G-CSF, starting within 48 hours of admission, significantly improved patient survival in addition to Child-Pugh score, Model for End-Stage Liver Disease (MELD), and Sequential Organ Failure Assessment (SOFA), which all relate to severity (acuity) of the liver disease. As a matter of course, neutrophil counts were higher in G-CSF treated patient cohort. A recent experimental study in mice with acetaminophen-induced acute liver injury demonstrated that neutrophil infiltration was involved not only in injury amplification at the early phase, but also during liver-tissue repair at the later phase.[45] The administration of MMP inhibitor in those mice successfully ameliorated hepatic damage and mitigated local inflammation along with reduction of neutrophil migration into the liver. However, MMP inhibitor impaired liver tissue repair and function restoration at later time points. Neutrophils seem to contribute to tissue regeneration through the various mechanisms, such as MMP delivery.[46] By regulating neutrophil function, G-CSF is a promising new therapeutic agent for hepatocellular regeneration after severe liver injury.

3.1.5 Farnesoid X nuclear receptor (FXR) agonist and FGF19 analogue—Although there is no evidence of FXR agonist or FGF19 analogue to affect acute liver failure in humans, these are attractive therapeutic targets for liver regeneration (Figure 1B). Patients without biliary drainage had significantly higher serum bile acids levels and regenerated liver volumes than patients with biliary drainage after major hepatectomy.[47] Bile acids drive liver growth by activating their receptor FXR, and are involved in the maintenance of normal liver size through the regulation of fibroblast growth factor (FGF)15 in mice and FGF19 in humans. FGF15/19 binding to FGF receptor (FGFR) 4 on hepatocytes negatively

regulates bile acids synthesis with suppression of CYP7A1.[48] Mice with humanized livers, without normal FGF15/19 regulation, demonstrate increased bile acids levels, ultimately leading to hepatocyte proliferation and enlarged liver size.[49] Inhibition of FXR or FGFR4 signaling was reported to impair hepatocyte proliferation due to reduction of the downstream forkhead box protein M1 (FoxM1) and signal transducer and activator of transcription 3 (STAT3) after PHx in mice.[50,51] In marked contrast, activating FXR enhances hepatocyte proliferation. Oral administration of obeticholic acid, a potent FXR activator, promoted liver regeneration after PVE in a rabbit model.[52] FGF19 conjugated to apolipoprotein A-I to increase half-life of FGF19 in circulation, successfully reduced liver steatosis and improved the liver regeneration after PHx in obese mice.[53] Thus, FXR and FGF19 analogue have been reported to promote liver regeneration in animal models. The therapeutic effects of FXR agonist and FGF19 analogue in humans have been studied for nonalcoholic steatohepatitis, primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC).[54,55] Obviously it might be worth investigating whether FXR agonist or FGF19 analogue impacts on liver regeneration in humans, with obeticholic acid being a good candidate to support liver enlargement after PVE.

3.1.6. Receptor for advanced glycation end products (RAGE)—The levels of soluble receptor for advanced glycation end products (sRAGE) and RAGE ligands including high mobility group box-1 (HMGB1) were significantly higher in 60 patients with acetaminophen-related ALF as compared with 30 normal controls [56]. In those 60 patients with acetaminophen-related ALF, sRAGE levels were significantly higher in 30 patients who underwent liver transplantation and/or died as compared with 30 spontaneous survivors. In mouse studies, blockage of RAGE signaling using sRAGE reduced hepatocyte apoptosis and increased liver regeneration in both hepatic ischemia and PHx models [57,58]. RAGE in liver remnants was up-regulated in mice with 85% PHx as compared with 70% PHx, and sRAGE therapy increased the survival of 85% PHx mice from 30% to 90% [58]. While sRAGE suppresses ligand-induced stimulation of RAGE serving as a decoy receptor, which binds ligands of RAGE competitively, sRAGE seems to be associated with severity of liver injury in humans. Another experimental study using mice demonstrated that angiotensin II receptor antagonist, losartan mitigated IRI through peroxisome proliferator-activated receptor γ (PPAR-γ) activation, which inhibited RAGE signaling [59]. Further studies are required to make the RAGE signaling clinically relevant in liver regeneration.

3.1.7 Hepatocyte senescence—The cellular senescence of hepatocytes has been described in chronic liver disease (Figure 1C).[60] A recent study suggested that hepatocyte senescence might be involved in acute severe liver injury, in which viable hepatocytes fail to proliferate, leading to impairment of liver regeneration.[61] Senescence-related markers, such as p21 and senescence-associated β-galactosidase (SA-βGal) were expressed in human livers resected from patients receiving transplantation for hyper-acute fulminant hepatic failure. These patients had no prior liver disease history and their liver specimens were obtained less than a week from the onset of liver failure symptoms, nevertheless, hepatocyte senescence could be observed. This study demonstrated that administration of transforming growth factor beta receptor 1 (TGFβR1) inhibitor after 12 hours of acetaminophen-induced acute liver injury mitigated senescence induction, increased hepatocyte proliferation, and

reduced jaundice in mice. As TGFβR1 inhibitor has been already evaluated for hepatocellular carcinoma in humans [62], one may envision its application as a novel therapeutic strategy to stimulate liver regeneration and reduce hepatocyte senescence in acute severe injury.

3.1.8 Autophagy—Autophagy is a homeostatic mechanism that prevents accumulation of damaged intracellular proteins and organelles. Liver regeneration after PHx in liver-specific knockout of autophagy-related gene 5 (L-Atg5) mice was significantly impaired, accompanied by cell-cycle arrest and compensating hepatocyte hypertrophy.[63] Cell senescence was suggested with high p21 and SA-βGal in those mice. The study concluded autophagy played a critical role in liver regeneration by preserving cellular quality and preventing hepatocyte senescence. With these evidences taken into consideration, an autophagy inducer, such as mammalian target of rapamycin complex 1 (mTORC1) inhibitor, may serve as a therapeutic target for liver regeneration.

4 Liver Background Parameters and Regeneration

4.1 Steatosis

Obese patients are characterized by significantly more advanced steatotic liver phenotype and slower regenerative response in liver volume, as compared with non-obese individuals.[64] Liver regeneration indices calculated with liver volume were significantly lower in patients with moderate-to-severe steatosis at 6 months after partial hepatectomy.[65] Experimental studies confirmed impairment of steatotic liver regeneration in rats after 70% PHx.[66] Susceptibility to IRI, deteriorated sinusoidal blood flow, and mitochondrial hypo-oxidation resulting in low production of adenosine triphosphate (ATP) might all lead to hepatocellular injury and poor regeneration in steatotic liver.[67,68] A close correlation between hepatic fat accumulation and markers of hepatocyte senescence suggests impaired hepatocyte regeneration in steatotic livers [69]. However, some discrepancies regarding negative effects of steatotic livers in other studies might result from differences in assessing hepatic steatosis both quantitatively and qualitatively, even in the very same section among expert pathologists.[23] Obviously, precise assessment for steatotic livers is required to accurately elucidate the influence of steatosis pathology on hepatic regeneration.

4.2 Fibrosis

Liver fibrosis impairs liver regeneration with poor hepatocyte proliferation. Hepatocyte senescence in chronic liver disease is one of the mechanisms for decreased regeneration capacity.[60] As described above, considering the role of TGFβR1 inhibition to reduce senescence or G-CSF to enhance MMP production, further studies are expected to investigate as to whether and how these strategies may affect fibrotic livers.[46,61] Although therapeutic targets for liver fibrosis are beyond the threshold of this review, improvement of fibrosis should restore liver regeneration and expand the donor pool available for life saving transplantation.

Although donor liver with fibrosis stage 2 or greater in the Ishak/Knodell classification is generally considered unsuitable for transplantation due to primary or early allograft

dysfunction, there is no clear evidence to support this suggestion.[70,71] A recent report demonstrated that there was no significant difference in 5-year graft survival and overall survival after liver transplantation between 101 patients with stage 1 or 2 fibrosis liver and 208 patients with no fibrotic allografts.[72] These studies highlight limitations in evaluating donor liver fibrosis, based on H&E staining of pre-transplant frozen sections rather than specific staining for hepatic fibrosis (e.g., Masson's trichrome or Sirius Red). As with steatotic livers, the impact of fibrosis on liver regeneration often remains unclear due to the inaccurate diagnosis.

4.3 Aged liver

Various clinical and basic studies imply the relationships between liver age and liver tissue regeneration. Younger donor clinical cohort (< 30 years) showed significantly higher liver regeneration rates in volume than the older donor group (> 50 years) at 1 week after living donor partial liver transplantation (LDLT), although there was no difference in the liver regeneration rate between those groups at 1 month after LDLT.[73,74] Cumulative recipient survival rate was also significantly higher in younger donor group (<20 years) compared with older donor group (20 years or above) after LDLT, despite no difference in recipient age.[75] Donor age was an independent prognostic factor in LDLT. An experimental study in mouse model showed an age-related loss of fenestration of LSECs together with thickening of the endothelium, basal lamina formation, and collagen deposition in the Space of Disse.[76] These age-related structural changes of LSECs and Space of Disse ultimately decrease sinusoidal perfusion. The impairment of regeneration in aged liver is due, in part, to such a dysfunction of LSEC in addition to reduction of sinusoidal blood flow. In addition, aged liver is associated with decreased intrahepatic energy source, ATP levels, caused by mitochondrial dysfunction.[77] In a recent study, mitochondrial dysfunction-associated senescence was reported.[78] Autophagy increases in accordance with accumulation of damaged mitochondria, and leads to cell death.[79] These mechanisms might be also partially related to impaired regeneration in aged livers.

4.4 Chemotherapy

Neoadjuvant chemotherapy is performed aiming curative resection of colorectal cancer liver metastasis (CRCLM), which are initially unresectable. On the other hand, oxaliplatin or irinotecan commonly used for colorectal cancer can cause steatosis or sinusoidal obstruction syndrome, respectively. In a recent retrospective study, the impact of neoadjuvant chemotherapy including oxaliplatin and irinotecan on liver regeneration was evaluated in 226 patients (85 major hepatecomy, 141 PVE) with CRCLM between 2003 and 2013.[80] The number of cycles, interval from chemotherapy to intervention, or chemotherapy agents was not associated with liver regeneration in both hepatic resection and PVE. Sufficient increases of liver volume could be observed after both interventions, and estimated liver regeneration was similar with and without chemotherapy. Although liver regeneration after neoadjuvant chemotherapy for CRCLM seems to be expected for the following interventions in most previous studies, the combined risk factors, such as steatosis and aged liver should be noted.

4.5 Biliary disease

The impact of cholangitis on liver regeneration and postoperative outcomes was evaluated in 450 patients who underwent preoperative PVE and major hepatectomies.[81] The daily increase rate of non-embolized lobe was significantly lower in patients with cholangitis. There were also significant differences in post-hepatectomy liver failure, prothrombin time, total bilirubin levels, and infectious complications. This study suggested cholangitis might delay and aggravate liver regeneration. In the early phase after 70% PHx, the mRNA levels coding for HGF and EGF were significantly lower while those for IL-6, TNF-α, and toll-like receptor (TLR) 4 were all higher in rats with choledochojejunostomy, resulting in delayed restoration of the liver weight.[82] Cholangitis, common complication with choledochojejunostomy due to intestinal contents reflux, might impair liver regeneration. A recent study showed that cholangiocyte senescence impaired the regenerative response of the liver parenchyma with induction of hepatocyte senescence [83]. This might account for the loss of hepatocyte function in human PBC/PSC patients. Thus, biliary tree pathologies, such as cholangitis, PBC, and PSC might impair parenchymal regeneration.

5. Biomarkers for Liver Regeneration

Colony stimulating factor 1 (CSF1) was measured in serum samples from 55 patients after hepatic resection and 78 patients with acetaminophen overdose acute liver failure.[84] Serum CSF1 levels increased in patients with hepatic resection in proportion to the resected liver volume, and a low serum CSF1 level was associated with worse prognosis (King's College criteria) and increased mortality/transplantation in patients with acetaminophen overdose acute liver failure. Serum CSF1 level was a better predictor than HMGB1 released by necrotic cells and proposed as a prognostic marker for ALF.[85] Furthermore, as subcutaneous CSF1-Fc administration promoted the macrophage accumulation and liver regeneration in mice with acetaminophen acute liver injury [84], CSF1 could be not only a prognostic marker, but also a therapeutic target for liver regeneration in ALF. Indeed, recombinant human macrophage-CSF was apparently well tolerated in humans [86].

The microRNAs mediate both activation and inhibition of gene expression at the posttranscriptional or translational levels, and affect biological pathways including cellular differentiation, proliferation, apoptosis, and tissue remodeling [87]. Recently, microRNAs have been demonstrated to regulate cell proliferation during liver regeneration, and to serve as putative therapeutic targets for liver regeneration [88-90]. Patients with spontaneous recovery from ALF showed significantly higher serum levels of microRNA-122, microRNA-21, and microRNA-221, compared to non-recovered patient cohort.[91] The elevated microRNAs serum levels were accompanied with down-regulation of growth inhibitory targets in the liver tissue, such as heme oxygenase-1, programmed cell death 4, and the cyclin-dependent kinase inhibitors p21, p27, and p57, as well as increased cyclin D1 expression and hepatocyte proliferation. Thus, microRNA-122, microRNA-21, and microRNA-221 involved in liver regeneration might also serve as a biomarker to predict ALF.

Above all, most simple and available marker suggesting liver regeneration is alpha-fetoprotein (AFP). AFP is also known as a hepatic progenitor-associated marker besides a

representative marker of hepatocellular carcinoma [92]. In a prospective study of 206 patients, the AFP ratio (AFP concentration at day 3 of admission divided by that at day 1) ≥1 predicted the survival of ALF patients [93]. Rising AFP levels over the first 3 hospital stay days might indicate a better survival rate. AFP levels increase during embryonic development,

6. Liver Support Strategies

6.1 Extracorporeal liver support

Plasma exchange and albumin dialysis have been clinically used in ALF. Albumin dialysis improved encephalopathy, however, no significant survival benefit has been reported for a randomized, prospective cohort with either form of albumin dialysis.[94] While plasma exchange or albumin dialysis support liver regeneration with alleviation of acute liver injury, bioartificial liver (BAL) containing liver cells aim to substitute for liver function. In a prospective, randomized controlled study of BAL using porcine hepatocytes, the survival benefit was not significant in 171 patients with fulminant/sub-fulminant hepatic failure and primary non-function after liver transplantation.[95] In a recent prospective, randomized controlled study in 203 patients, no significant difference in survival was recorded between BAL using hepatoblastoma-derived cells vs. controls.[96] There is no published data documenting the clinical efficacy of BAL.

6.2 Hepatocyte transplantation

Transplantation of liver cells has been studied as an alternative strategy for liver transplantation. A recent clinical series reported fresh allogenic hepatocyte transplantation in three patients with liver-based metabolic deficiencies.[97] After a total of 2.0×10^8 viable hepatocytes/kg were infused into the liver through the portal vein, the hepatocellular function improved for a few months to one year. However, satisfactory outcomes over the long term have not yet been achieved with hepatocyte transplantation. Mesenchymal stem cells (MSCs) that can differentiate to hepatocyte-like cells have been also used as the cell source in clinical studies. Compared with native hepatocytes, MSCs have advantages in terms of high proliferative ability in addition to availability with relative ease from bone marrow, umbilical cord blood, peripheral blood, and adipose tissue.[98] Histological analysis of donor liver in human recipients after partial liver transplantation showed that a substantial fraction (>35%) of cells in the sinusoid and periportal areas was of recipient origin.[99] The study also demonstrated that human bone marrow MSCs transplanted into mice after PHx express liver progenitor cell (LPCs) markers at 1 week, and major liver cell markers such as hepatocytes, cholangiocytes, LSECs, and Kupffer cells at 2 weeks after transplantation with decreasing LPCs marker, suggesting MSCs shifted in their differentiation from LPCs toward various mature liver cells. MSCs have also been demonstrated to attenuate ALF by modulating production of inflammatory cytokines as well as immunomodulatory molecules [100]. Thus, MSCs that differentiate into both parenchymal and NPCs, or modulate inflammatory cytokines and immune response might be a promising cell source. However, the evidence of clinical benefits for liver regeneration has not been yet achieved [101].

Along with the progress of stem cell research, various cell types have been reported to support liver function and promote liver regeneration. Human-induced pluripotent stem cells (iPSCs) have been demonstrated to differentiate to hepatocyte-like cells.[102] As somatic cells can be directly induced to hepatic lineage with direct reprograming, hepatocyte derived from human fibroblast with direct reprograming restored the liver function and prolonged survival in mouse ALF model.[103] As these cells show poor function, incomplete gene expression, and risk of oncogenesis as compared to mature hepatocytes, further advances are needed for clinical use of iPSCs in liver regeneration.

Another promising cell source for liver regeneration is LPCs. When regeneration of hepatocytes is disturbed due to chronic or acute severe injury, LPCs contribute to liver regeneration with activation as the second-line defense to restore liver homeostasis.[104] Although LPCs express biliary and stem cell markers and have bi-lineage differentiation potential to both hepatocytes and cholangiocytes, they account for exceedingly small number in the liver.[105] As the origin of LPCs is controversial, hepatocytes and cholangiocytes may function as facultative stem cells for each other. However, there exist liver cells that possess stem cell feature. Recent studies demonstrated that cholangiocytes acted as LPCs and formed functional hepatocytes during impaired hepatocyte regeneration with wide-spread injury or senescene [92,106]. Adult bile duct-derived bipotent progenitor cells from human liver can be cultured for a long term with stable genome and differentiate into functional hepatocytes.[107] A rat experimental study demonstrated that transplanted LPCs derived from fetal liver could engraft, differentiate into hepatocytic and biliary epithetial cells, and generate new liver mass in the host liver tissue.[108] Enough and stable supply of the human cell source of LPCs might enable the clinical application of LPC transplantation in acute severe liver injury.

6.3 Organ engineering

6.3.1 Decellularized liver—Decellularized liver scaffold was reported as an approach for whole-liver engineering.[109] Decellularization removes cells from native liver, and produces liver specific extracellular matrix (ECM), maintaining molecules in ECM and 3-dimensional (3D) microstructure, including vasculature and biliary duct. Engineered functional liver can be reconstructed with seeding hepatocytes and NPCs into the decellularized liver scaffold. However, no clinically relevant engineered liver graft has yet been achieved. In addition to reconstruction of biliary components, the important task for successful transplantation is complete vascularization, including sinusoidal space, which enables the continuous blood supply. The usage of decellularized liver in extracorporeal blood perfusion system might be a potential approach for pre-transplantation.[110]

6.3.2 Liver bud—iPSCs-derived immature endodermal cells co-cultured with human mesenchymal stem cells and human umbilical vein cells self-organized into 3D liver bud, which possessed liver specific protein production and drug metabolism.[111] Further, vasculature in liver bud connected to the host vessels after transplantation into cranial window. Liver bud implanted on mesentery was able to rescue the drug-induced lethal liver failure in a mouse model. Although the site for transplantation of liver bud might be controversial as vasculature connection after mesentery transplantation cannot be figured in

the study and the blood supply from mesentery is unclear, the self-organization strategy using immature cells derived from iPSCs is a promising approach for tissue engineering to reconstruct complex liver structure.

7. Conclusion

Pentoxifylline, omega-3 fatty acids, and portal flow modulation might prevent ALF following SFSS by enhancing liver regeneration other than PVE, two-stage hepatectomy, and ALPPS. While platelet and serotonin might promote liver regeneration, further studies are required to focus on factors with mitogenic responses. The impact of FXR agonist and FGF19 analogue on liver regeneration is worth investigating in humans. G-CSF is a promising agent for liver regeneration after acute severe injury, in which hepatocytes are extensively injured. Although there is no clinical data, TGFβR1 inhibitor and rapamycin can be good candidates for targeting liver regeneration based on the recent knowledge that cell senescence and autophagy might be involved in acute severe injury. CSF1 and microRNA could serve not only as novel predictive biomarkers for ALF, but also therapeutic targets for liver regeneration. Biliobiliary anastomosis might be better for reconstruction of biliary duct than choledochojejunostomy as preventing cholangitis, which might impair liver regeneration. Progressive steatosis, fibrosis, and aged liver might also impair liver regeneration, whereas accurate and firm diagnosis is required to clarify the impact of degree of steatosis or fibrosis on liver regeneration. Although liver support strategies for regeneration as substitutes for liver function are appealing, the research progress on stem cells including iPSCs is required to supply human cell sources for clinical applications for these refined therapies.

8. Expert Opinion

Currently used nonspecific plasma exchange, albumin dialysis or hemodiafiltration may alleviate severe hepatocellular injury in ALF patients until liver transplant becomes available or successful self-liver regeneration occurs. While liver transplantation remains the only treatment option with a proven survival benefit for ALF, the acute donor organ shortage restricts patients receiving the life-saving procedure. Clearly, new therapeutic strategies targeting liver regeneration are urgently needed to rescue ALF patients. As the regenerative strategy to prevent ALF following SFSS, PVE, two-stage hepatectomy, and ALPPS have been probed in the clinical practice. Modulation of portal flow, treatment with pentoxifylline, and omega-3 fatty acids are also being tested. On the other hand, only a few studies to date have demonstrated the clinically relevant therapeutic targets for liver regeneration in ongoing ALF with extensive hepatocyte injury and no viable or functional hepatocytes. G-CSF is one of the few promising preclinical agents targeting liver regeneration against ongoing ALF pathology. However, a randomized controlled study is needed to document the survival benefit of G-CSF seen in a small patient cohort with acute-on-chronic liver failure.[44] It has not been elucidated as to whether and which effects of G-CSF, i.e., mobilization of bone marrow-derived stem cells, CD34+ cells, or neutrophils impacted liver regeneration in the acute phase. MMP delivered by neutrophils has been also reported to degrade extracellular matrix, and potentially improve fibrosis in chronic liver injury.[46] There is no doubt G-CSF is worth investigating as to its role in liver regeneration

against ongoing ALF and chronic liver disease, along with molecular mechanisms of hepatoprotection.

Recent reports on cell senescence and autophagy in ALF settings provide rationale for novel therapeutic targets in liver regeneration. As a fundamental mechanism to protect against malignant pathology, cellular hepatocyte senescence, cholangiocytes, stellate cells and immune cells have been described in chronic liver disease.[60] However, acute liver injury was associated with a suite of senescence markers in previously uninjured hepatocytes.[61] Indeed, severe acute hepatic necrosis might induce the spread of senescence to remaining viable hepatocytes, which in turn might severely impair hepatocyte-mediated regeneration. Administration of TGFβR1 inhibitor successfully reduced such hepatocyte senescence in mice, leading to enhanced liver regeneration and improved survival even after 12 hours of acetaminophen-induced acute hepatocellular damage [61]. Of note, TGFβR1 inhibitor might be effective in delayed treatment, a common clinical situation for the patients with acetaminophen-induced liver injury. Another study suggested that autophagy might prevent hepatocyte senescence by maintaining intracellular energy production.[63] If hepatocyte senescence occurs in the acute phase of severe liver injury, mTORC1 inhibitor, an autophagy inducer, such as rapamycin, could also serve as a therapeutic candidate for liver regeneration besides TGFβR1 inhibitor. Rapamycin protected against hepatic IRI by inhibiting mTORC1 and inducing autophagy while activating Akt by enhancing mTORC2 signaling pathway [112]. Although TGFβR1 inhibitor or rapamycin has been evaluated clinically for hepatocellular carcinoma, there are few experimental data using these agents in models of liver regeneration. Further studies are required to elucidate therapeutic impacts and mechanisms of cell senescence and autophagy on liver regeneration.

Fabrication of a ‘new liver’ with tissue engineering approach is an ultimate regenerative strategy to resolve donor organ shortage and could be applied to both acute and chronic liver failures. Cell-based therapies, including BAL and cell transplantation, have been studied both in animals and humans. Although there are clinical benefits to some extent with those treatments, satisfactory outcomes over a long term have not yet been achieved. An essential issue for the successful cell-based therapies is a current lack of abundant cell sources with enough qualities as primary hepatocytes rapidly lose their original morphology and function after isolation.[113] Clinical applications of BAL, such as bridge to liver transplantation or bridge to successful self-liver regeneration, can be expected with plenty of human cell sources following the research progress of iPSCs. Recent advances in the 3D culture methods, which maintain the character of mouse and human primary hepatocytes for a long-term might help the future hepatocyte transplantation or BAL therapy, as providing a plenty of high quality hepatocytes [114,115]. Likewise, successful cell transplantation requires the supply of abundant cell sources to control host immune rejection response. Obviously, optimized seeding routes and improved engraftment capabilities are essential for the efficient cell transplantation.

A decellularized liver scaffold is a promising biomaterial for tissue engineering as maintaining molecules that promote cell attachment and cell growth in addition to organ specific microstructure.[116] Although regeneration of 3D transplantable liver using decellularized scaffold has been studied for over 10 years by now, blood clotting in the

scaffold disturbs its function while reconstruction of bile system in the liver tissue is another important barrier for successful transplantation. With various strategies reported to improve hemocompatibility of decellularized liver, there is a major limitation to cover the microvasculature, including sinusoid with endothelial cells in addition to reconstruction of well-organized biliary system in an acellular liver scaffold by engineering manner. The approach in fabrication of liver bud, such as self-organization of stem cells might be a new and promising strategy to reconstruct 3D organ. Seeding human iPSCs or iPSCs-derived LPCs into the decellularized liver scaffold might enable a long culture of engineered liver and self-reconstruction of well-organized microvasculature and biliary system as well as parenchyma. Fabrication of transplantable liver graft with human iPSCs can resolve serious donor organ shortage and be an ideal treatment option without mounting the host immune rejection response. Further advances in stem cell research are needed to successfully apply these regenerative strategies in bench-to-bedside translational studies.

Acknowledgments

Funding

The work of JW Kupiec-Weglinski has been supported by National Institute of Health Grants P01 AI120944, R01 DK062357, DK107533, DK102110.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers

[1]. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006;43:S45–53. [PubMed: 16447274]

[2]. Michalopoulos GK. Liver regeneration. J Cell Physiol. 2007;213:286–300. [PubMed: 17559071]

[3]. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. Compr Physiol. 2013;3:485–513. [PubMed: 23720294]

[4]. Imamura H, Shimada R, Kubota M, et al. Preoperative portal vein embolization: an audit of 84 patients. Hepatology. 1999;29:1099–1105. [PubMed: 10094953]

[5]. Adam R, Laurent A, Azoulay D, et al. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. Ann Surg. 2000;232:777–785. [PubMed: 11088072]

[6]. Schnitzbauer AA, Lang SA, Goessmann H, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg. 2012;255:405–414. [PubMed: 22330038]

[7]. Henley KS, Lucey MR, Normolle DP, et al. A double-blind, randomized, placebo-controlled trial of prostaglandin E1 in liver transplantation. Hepatology. 1995;21:366–372. [PubMed: 7843707]

[8]. Klein AS, Cofer JB, Pruett TL, et al. Prostaglandin E1 administration following orthotopic liver transplantation: a randomized prospective multicenter trial. Gastroenterology. 1996;111:710–715. [PubMed: 8780576]

[9]. Nelson AR, Fingleton B, Rothenberg ML, et al. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol. 2000;18:1135–1149. [PubMed: 10694567]

[10]. Beck-Schimmer B, Bonvini JM, Schadde E, et al. Conditioning With Sevoflurane in Liver Transplantation: Results of a Multicenter Randomized Controlled Trial. Transplantation. 2015;99:1606–1612. [PubMed: 25769076]

[11]. Selzner N, Rudiger H, Graf R, et al. Protective strategies against ischemic injury of the liver. Gastroenterology. 2003;125:917–936. [PubMed: 12949736]

[12]. Nickkholgh A, Maluf D. Emerging graft protective strategies in clinical liver transplantation. Expert Rev Gastroenterol Hepatol. 2017;11:623–631. [PubMed: 28438069]

[13]. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. Nature. 2018;557:50–56. [PubMed: 29670285]

[14]. Kelly DM, Demetris AJ, Fung JJ, et al. Porcine partial liver transplantation: a novel model of the "small-for-size" liver graft. Liver Transpl. 2004;10:253–263. [PubMed: 14762864]

[15]. Eguchi S, Yanaga K, Sugiyama N, et al. Relationship between portal venous flow and liver regeneration in patients after living donor right-lobe liver transplantation. Liver Transpl. 2003;9:547–551. [PubMed: 12783393]

[16]. Hessheimer AJ, Fondevila C, Taura P, et al. Decompression of the portal bed and twice-baseline portal inflow are necessary for the functional recovery of a "small-for-size" graft. Ann Surg. 2011;253:1201–1210. [PubMed: 21587116]

[17]. Lorenz L, Axnick J, Buschmann T, et al. Mechanosensing by beta1 integrin induces angiocrine signals for liver growth and survival. Nature. 2018;562:128–132. [PubMed: 30258227]

[18]. Zhai Y, Petrowsky H, Hong JC, et al. Ischaemia-reperfusion injury in liver transplantation--from bench to bedside. Nat Rev Gastroenterol Hepatol. 2013;10:79–89. [PubMed: 23229329]

[19]. Fausto N Liver regeneration: from laboratory to clinic. Liver Transpl. 2001;7:835–844. [PubMed: 11679980]

[20]. Yagi S, Iida T, Taniguchi K, et al. Impact of portal venous pressure on regeneration and graft damage after living-donor liver transplantation. Liver Transpl. 2005;11:68–75. [PubMed: 15690538]

[21]. Clavien PA, Petrowsky H, DeOliveira ML, et al. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med. 2007;356:1545–1559. [PubMed: 17429086]

[22]. Lee WM, Squires RH Jr., Nyberg SL, et al. Acute liver failure: Summary of a workshop. Hepatology. 2008;47:1401–1415. [PubMed: 18318440]

[23]. Clavien PA, Oberkofler CE, Raptis DA, et al. What is critical for liver surgery and partial liver transplantation: size or quality? Hepatology. 2010;52:715–729. [PubMed: 20683967]

[24]. Breitenstein S, Apestegui C, Petrowsky H, et al. "State of the art" in liver resection and living donor liver transplantation: a worldwide survey of 100 liver centers. World J Surg. 2009;33:797–803. [PubMed: 19172348]

[25]. Forbes SJ, Newsome PN. Liver regeneration - mechanisms and models to clinical application. Nat Rev Gastroenterol Hepatol. 2016;13:473–485. [PubMed: 27353402]

[26]. de Santibanes E, Clavien PA. Playing Play-Doh to prevent postoperative liver failure: the "ALPPS" approach. Ann Surg. 2012;255:415–417. [PubMed: 22330039]

[27]. Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol. 2011;13:877–883. [PubMed: 21808241]

[28]. Liu Y, Lu T, Zhang C, et al. Activation of YAP attenuates hepatic damage and fibrosis in liver ischemia-reperfusion injury. J Hepatol. 2019;71:719–730. [PubMed: 31201834]

[29]. Yimlamai D, Christodoulou C, Galli GG, et al. Hippo pathway activity influences liver cell fate. Cell. 2014;157:1324–1338. [PubMed: 24906150]

[30]. Thompson MD, Monga SP. WNT/beta-catenin signaling in liver health and disease. Hepatology. 2007;45:1298–1305. [PubMed: 17464972]

[31]. Tan X, Apte U, Micsenyi A, et al. Epidermal growth factor receptor: a novel target of the Wnt/beta-catenin pathway in liver. Gastroenterology. 2005;129:285–302. [PubMed: 16012954]

[32]. Petrowsky H, Breitenstein S, Slankamenac K, et al. Effects of pentoxifylline on liver regeneration: a double-blinded, randomized, controlled trial in 101 patients undergoing major liver resection. Ann Surg. 2010;252:813–822. [PubMed: 21037437]

[33]. Zhu XH, Wu YF, Qiu YD, et al. Liver-protecting effects of omega-3 fish oil lipid emulsion in liver transplantation. World J Gastroenterol. 2012;18:6141–6147. [PubMed: 23155344]

[34]. Ibrahim ES, Saleh SM, El Hoseeny M, et al. Effect of omega-3 on hepatic regeneration in adult living donors undergoing hepatic resections for liver transplantation: A randomized controlled trial. J Crit Care. 2016;31:157–162. [PubMed: 26518479]

[35]. Yan XP, Wang S, Yang Y, et al. Effects of n-3 polyunsaturated fatty acids on rat livers after partial hepatectomy via LKB1-AMPK signaling pathway. Transplant Proc. 2011;43:3604–3612. [PubMed: 22172813]

[36]. Zhong D, Liu X, Khuri FR, et al. LKB1 is necessary for Akt-mediated phosphorylation of proapoptotic proteins. Cancer Res. 2008;68:7270–7277. [PubMed: 18794113]

[37]. Martinez-Chantar ML, Vazquez-Chantada M, Garnacho M, et al. S-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. Gastroenterology. 2006;131:223–232. [PubMed: 16831604]

[38]. Merlen G, Gentric G, Celton-Morizur S, et al. AMPKalpha1 controls hepatocyte proliferation independently of energy balance by regulating Cyclin A2 expression. J Hepatol. 2014;60:152–159. [PubMed: 24012615]

[39]. Padickakudy R, Pereyra D, Offensperger F, et al. Bivalent role of intra-platelet serotonin in liver regeneration and tumor recurrence in humans. J Hepatol. 2017;67:1243–1252. [PubMed: 28842294]

[40]. Myronovych A, Murata S, Chiba M, et al. Role of platelets on liver regeneration after 90% hepatectomy in mice. J Hepatol. 2008;49:363–372. [PubMed: 18602717]

[41]. Lesurtel M, Graf R, Aleil B, et al. Platelet-derived serotonin mediates liver regeneration. Science. 2006;312:104–107. [PubMed: 16601191]

[42]. Starlinger P, Haegele S, Offensperger F, et al. The profile of platelet alpha-granule released molecules affects postoperative liver regeneration. Hepatology. 2016;63:1675–1688. [PubMed: 26528955]

[43]. Sarrouilhe D, Clarhaut J, Defamie N, et al. Serotonin and cancer: what is the link? Curr Mol Med. 2015;15:62–77. [PubMed: 25601469]

[44]. Garg V, Garg H, Khan A, et al. Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival of patients with acute-on-chronic liver failure. Gastroenterology. 2012;142:505–512 e501. [PubMed: 22119930] •• This study is the randomized clinical trial in which clinical benefit of G-CSF has been demonstrated in patients with acute severe liver injury.

[45]. Alvarenga DM, Mattos MS, Lopes ME, et al. Paradoxical Role of Matrix Metalloproteinases in Liver Injury and Regeneration after Sterile Acute Hepatic Failure. Cells. 2018;7.• This study has demonstrated a dual role of neutrophils, such as aggravation of liver injury at early phase and protective effects with tissue regeneration at later phase in acetaminophen-induced acute liver injury.

[46]. Peiseler M, Kubes P. More friend than foe: the emerging role of neutrophils in tissue repair. J Clin Invest. 2019;129:2629–2639. [PubMed: 31205028]

[47]. Otao R, Beppu T, Isiko T, et al. External biliary drainage and liver regeneration after major hepatectomy. Br J Surg. 2012;99:1569–1574. [PubMed: 23027074]

[48]. Alvarez-Sola G, Uriarte I, Latasa MU, et al. Bile acids, FGF15/19 and liver regeneration: From mechanisms to clinical applications. Biochim Biophys Acta Mol Basis Dis. 2018;1864:1326–1334. [PubMed: 28709961]

[49]. Naugler WE, Tarlow BD, Fedorov LM, et al. Fibroblast Growth Factor Signaling Controls Liver Size in Mice With Humanized Livers. Gastroenterology. 2015;149:728–740 e715. [PubMed: 26028580] • This study showed that FGF19 regulation was essential for the maintenance of normal liver size in mice with humanized livers.

[50]. Chen WD, Wang YD, Zhang L, et al. Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead box m1b transcription. Hepatology. 2010;51:953–962. [PubMed: 19998409]

[51]. Padrissa-Altes S, Bachofner M, Bogorad RL, et al. Control of hepatocyte proliferation and survival by Fgf receptors is essential for liver regeneration in mice. Gut. 2015;64:1444–1453. [PubMed: 25416068]

[52]. Olthof PB, Huisman F, Schaap FG, et al. Effect of obeticholic acid on liver regeneration following portal vein embolization in an experimental model. Br J Surg. 2017;104:590–599. [PubMed: 28195307]

[53]. Alvarez-Sola G, Uriarte I, Latasa MU, et al. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. Gut. 2017;66:1818–1828. [PubMed: 28119353]

[54]. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2015;385:956–965. [PubMed: 25468160]

[55]. Hirschfield GM, Chazouilleres O, Drenth JP, et al. Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: A multicenter, randomized, double-blind, placebo-controlled phase II trial. J Hepatol. 2019;70:483–493. [PubMed: 30414864]

[56]. Basta G, Del Turco S, Navarra T, et al. Circulating levels of soluble receptor for advanced glycation end products and ligands of the receptor for advanced glycation end products in patients with acute liver failure. Liver Transpl. 2015;21:847–854. [PubMed: 25825217]

[57]. Zeng S, Feirt N, Goldstein M, et al. Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia and reperfusion injury to the liver in mice. Hepatology. 2004;39:422–432. [PubMed: 14767995]

[58]. Cataldegirmen G, Zeng S, Feirt N, et al. RAGE limits regeneration after massive liver injury by coordinated suppression of TNF-alpha and NF-kappaB. J Exp Med. 2005;201:473–484. [PubMed: 15699076]

[59]. Koh EJ, Yoon SJ, Lee SM. Losartan protects liver against ischaemia/reperfusion injury through PPAR-gamma activation and receptor for advanced glycation end-products down-regulation. Br J Pharmacol. 2013;169:1404–1416. [PubMed: 23647130]

[60]. Aravinthan AD, Alexander GJM. Senescence in chronic liver disease: Is the future in aging? J Hepatol. 2016;65:825–834. [PubMed: 27245432]

[61]. Bird TG, Muller M, Boulter L, et al. TGFbeta inhibition restores a regenerative response in acute liver injury by suppressing paracrine senescence. Sci Transl Med. 2018;10.•• This study has shown in acute severe liver injury that hepatocyte senescence was observed, and TGFβ signaling might be a therapeutic target for liver regeneration with reduction of senescence.

[62]. Faivre S, Santoro A, Kelley RK, et al. Novel transforming growth factor beta receptor I kinase inhibitor galunisertib (LY2157299) in advanced hepatocellular carcinoma. Liver Int. 2019;39:1468–1477. [PubMed: 30963691]

[63]. Toshima T, Shirabe K, Fukuhara T, et al. Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice. Hepatology. 2014;60:290–300. [PubMed: 24668739] •• This study has demonstrated that autophagy played a critical role in liver regeneration preventing hepatocytes from cell senescence in mice after PHx.

[64]. Truant S, Bouras AF, Petrovai G, et al. Volumetric gain of the liver after major hepatectomy in obese patients: a case-matched study in 84 patients. Ann Surg. 2013;258:696–702; discussion 702-694. [PubMed: 23979277]

[65]. Kele PG, van der Jagt EJ, Gouw AS, et al. The impact of hepatic steatosis on liver regeneration after partial hepatectomy. Liver Int. 2013;33:469–475. [PubMed: 23311417]

[66]. Vetelainen R, van Vliet AK, van Gulik TM. Severe steatosis increases hepatocellular injury and impairs liver regeneration in a rat model of partial hepatectomy. Ann Surg. 2007;245:44–50. [PubMed: 17197964]

[67]. Selzner M, Rudiger HA, Sindram D, et al. Mechanisms of ischemic injury are different in the steatotic and normal rat liver. Hepatology. 2000;32:1280–1288. [PubMed: 11093735]

[68]. Selzner M, Clavien PA. Failure of regeneration of the steatotic rat liver: disruption at two different levels in the regeneration pathway. Hepatology. 2000;31:35–42. [PubMed: 10613725]

[69]. Ogrodnik M, Miwa S, Tchkonia T, et al. Cellular senescence drives age-dependent hepatic steatosis. Nat Commun. 2017;8:15691. [PubMed: 28608850]

[70]. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–699. [PubMed: 7560864]

[71]. Melin C, Miick R, Young NA, et al. Approach to intraoperative consultation for donor liver biopsies. Arch Pathol Lab Med. 2013;137:270–274. [PubMed: 23368870]

[72]. Wadhera V, Harimoto N, Lubezky N, et al. The impact of donor liver allograft fibrosis on patients undergoing liver transplantation. Clin Transplant. 2018;32:e13187. [PubMed: 29314293]

[73]. Ikegami T, Nishizaki T, Yanaga K, et al. The impact of donor age on living donor liver transplantation. Transplantation. 2000;70:1703–1707. [PubMed: 11152100]

[74]. Ono Y, Kawachi S, Hayashida T, et al. The influence of donor age on liver regeneration and hepatic progenitor cell populations. Surgery. 2011;150:154–161. [PubMed: 21719061]

[75]. Kubota T, Hata K, Sozu T, et al. Impact of Donor Age on Recipient Survival in Adult-to-Adult Living-donor Liver Transplantation. Ann Surg. 2018;267:1126–1133. [PubMed: 28288061]

[76]. Ito Y, Sorensen KK, Bethea NW, et al. Age-related changes in the hepatic microcirculation in mice. Exp Gerontol. 2007;42:789–797. [PubMed: 17582718]

[77]. Selzner M, Selzner N, Jochum W, et al. Increased ischemic injury in old mouse liver: an ATP-dependent mechanism. Liver Transpl. 2007;13:382–390. [PubMed: 17318856]

[78]. Wiley CD, Velarde MC, Lecot P, et al. Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. Cell Metab. 2016;23:303–314. [PubMed: 26686024]

[79]. Enkhbold C, Morine Y, Utsunomiya T, et al. Dysfunction of liver regeneration in aged liver after partial hepatectomy. J Gastroenterol Hepatol. 2015;30:1217–1224. [PubMed: 25682855]

[80]. Simoneau E, Alanazi R, Alshenaifi J, et al. Neoadjuvant chemotherapy does not impair liver regeneration following hepatectomy or portal vein embolization for colorectal cancer liver metastases. J Surg Oncol. 2016;113:449–455. [PubMed: 26955907]

[81]. Yokoyama Y, Ebata T, Igami T, et al. The adverse effects of preoperative cholangitis on the outcome of portal vein embolization and subsequent major hepatectomies. Surgery. 2014;156:1190–1196. [PubMed: 25017134]

[82]. Takagi T, Yokoyama Y, Kokuryo T, et al. Liver regeneration following experimental major hepatectomy with choledochojejunostomy. Br J Surg. 2015;102:1410–1417. [PubMed: 26312457]

[83]. Ferreira-Gonzalez S, Lu WY, Raven A, et al. Paracrine cellular senescence exacerbates biliary injury and impairs regeneration. Nat Commun. 2018;9:1020. [PubMed: 29523787]

[84]. Stutchfield BM, Antoine DJ, Mackinnon AC, et al. CSF1 Restores Innate Immunity After Liver Injury in Mice and Serum Levels Indicate Outcomes of Patients With Acute Liver Failure. Gastroenterology. 2015;149:1896–1909 e1814. [PubMed: 26344055]

[85]. Antoine DJ, Jenkins RE, Dear JW, et al. Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity. J Hepatol. 2012;56:1070–1079. [PubMed: 22266604]

[86]. VandePol CJ, Garnick MB. Clinical applications of recombinant macrophage-colony stimulating factor (rhM-CSF). Biotechnol Ther. 1991;2:231–239. [PubMed: 1845128]

[87]. Catalanotto C, Cogoni C, Zardo G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. Int J Mol Sci. 2016;17.

[88]. Ng R, Song G, Roll GR, et al. A microRNA-21 surge facilitates rapid cyclin D1 translation and cell cycle progression in mouse liver regeneration. J Clin Invest. 2012;122:1097–1108. [PubMed: 22326957]

[89]. Yang D, Yuan Q, Balakrishnan A, et al. MicroRNA-125b-5p mimic inhibits acute liver failure. Nat Commun. 2016;7:11916. [PubMed: 27336362]

[90]. Lv T, Kong L, Jiang L, et al. Dicer1 facilitates liver regeneration in a manner dependent on the inhibitory effect of miR-21 on Pten and Rhob expression. Life Sci. 2019;232:116656. [PubMed: 31306658]

[91]. John K, Hadem J, Krech T, et al. MicroRNAs play a role in spontaneous recovery from acute liver failure. Hepatology. 2014;60:1346–1355. [PubMed: 24913549]

[92]. Lu WY, Bird TG, Boulter L, et al. Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nat Cell Biol. 2015;17:971–983. [PubMed: 26192438]

[93]. Schiodt FV, Ostapowicz G, Murray N, et al. Alpha-fetoprotein and prognosis in acute liver failure. Liver Transpl. 2006;12:1776–1781. [PubMed: 17133565]

[94]. Nyberg SL. Bridging the gap: advances in artificial liver support. Liver Transpl. 2012;18 Suppl 2:S10–14. [PubMed: 22767444]

[95]. Demetriou AA, Brown RS Jr., Busuttil RW, et al. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann Surg. 2004;239:660–667; discussion 667-670. [PubMed: 15082970]

[96]. Thompson J, Jones N, Al-Khafaji A, et al. Extracorporeal cellular therapy (ELAD) in severe alcoholic hepatitis: A multinational, prospective, controlled, randomized trial. Liver Transpl. 2018;24:380–393. [PubMed: 29171941]

[97]. Soltys KA, Setoyama K, Tafaleng EN, et al. Host conditioning and rejection monitoring in hepatocyte transplantation in humans. J Hepatol. 2017;66:987–1000. [PubMed: 28027971]

[98]. Liu WH, Song FQ, Ren LN, et al. The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases. J Cell Mol Med. 2015;19:511–520. [PubMed: 25534251]

[99]. Katagiri H, Kushida Y, Nojima M, et al. A Distinct Subpopulation of Bone Marrow Mesenchymal Stem Cells, Muse Cells, Directly Commit to the Replacement of Liver Components. Am J Transplant. 2016;16:468–483. [PubMed: 26663569]

[100]. Gazdic M, Arsenijevic A, Markovic BS, et al. Mesenchymal Stem Cell-Dependent Modulation of Liver Diseases. Int J Biol Sci. 2017;13:1109–1117. [PubMed: 29104502]

[101]. Mohamadnejad M, Alimoghaddam K, Bagheri M, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. Liver Int. 2013;33:1490–1496. [PubMed: 23763455]

[102]. Kajiwara M, Aoi T, Okita K, et al. Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2012;109:12538–12543. [PubMed: 22802639]

[103]. Huang P, Zhang L, Gao Y, et al. Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell. 2014;14:370–384. [PubMed: 24582927]

[104]. Greenbaum LE, Wells RG. The role of stem cells in liver repair and fibrosis. Int J Biochem Cell Biol. 2011;43:222–229. [PubMed: 19914396]

[105]. Michalopoulos GK, Khan Z. Liver Stem Cells: Experimental Findings and Implications for Human Liver Disease. Gastroenterology. 2015;149:876–882. [PubMed: 26278502]

[106]. Raven A, Lu WY, Man TY, et al. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature. 2017;547:350–354. [PubMed: 28700576]

[107]. Huch M, Gehart H, van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015;160:299–312. [PubMed: 25533785]

[108]. Yovchev MI, Xue Y, Shafritz DA, et al. Repopulation of the fibrotic/cirrhotic rat liver by transplanted hepatic stem/progenitor cells and mature hepatocytes. Hepatology. 2014;59:284–295. [PubMed: 23840008]

[109]. Uygun BE, Soto-Gutierrez A, Yagi H, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med. 2010;16:814–820. [PubMed: 20543851]

[110]. Kojima H, Yasuchika K, Fukumitsu K, et al. Establishment of practical recellularized liver graft for blood perfusion using primary rat hepatocytes and liver sinusoidal endothelial cells. Am J Transplant. 2018;18:1351–1359. [PubMed: 29338127]

[111]. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013;499:481–484. [PubMed: 23823721]

[112]. Zhu J, Lu T, Yue S, et al. Rapamycin protection of livers from ischemia and reperfusion injury is dependent on both autophagy induction and mammalian target of rapamycin complex 2-Akt activation. Transplantation. 2015;99:48–55. [PubMed: 25340604]

[113]. Sellaro TL, Ranade A, Faulk DM, et al. Maintenance of human hepatocyte function in vitro by liver-derived extracellular matrix gels. Tissue Eng Part A. 2010;16:1075–1082. [PubMed: 19845461]

[114]. Hu H, Gehart H, Artegiani B, et al. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell. 2018;175:1591–1606 e1519. [PubMed: 30500538]

[115]. Peng WC, Logan CY, Fish M, et al. Inflammatory Cytokine TNFalpha Promotes the Long-Term Expansion of Primary Hepatocytes in 3D Culture. Cell. 2018;175:1607–1619 e1615. [PubMed: 30500539]

[116]. Soto-Gutierrez A, Wertheim JA, Ott HC, et al. Perspectives on whole-organ assembly: moving toward transplantation on demand. J Clin Invest. 2012;122:3817–3823. [PubMed: 23114604]

Article Highlights

- Hepatocytes fail to proliferate after severe liver injury, and this results in liver failure
- Liver transplantation remains the only treatment option with a proven survival benefit for ALF, but the acute donor organ shortage restricts patients receiving the life-saving procedure
- Successful liver regeneration can lead to recovery from acute liver failure
- There are established and preclinical strategies for liver regeneration to prevent acute liver failure caused by small-for-size syndrome. G-CSF is a promising therapeutic agent for liver regeneration after acute severe liver injury
- Recent reports on cell senescence and autophagy in ALF settings provide rationale for novel therapeutic targets in liver regeneration.
- Progress of stem cell research has the potential to enable the clinically relevant cell based therapy, including liver engineering as supplying abundant human cell sources

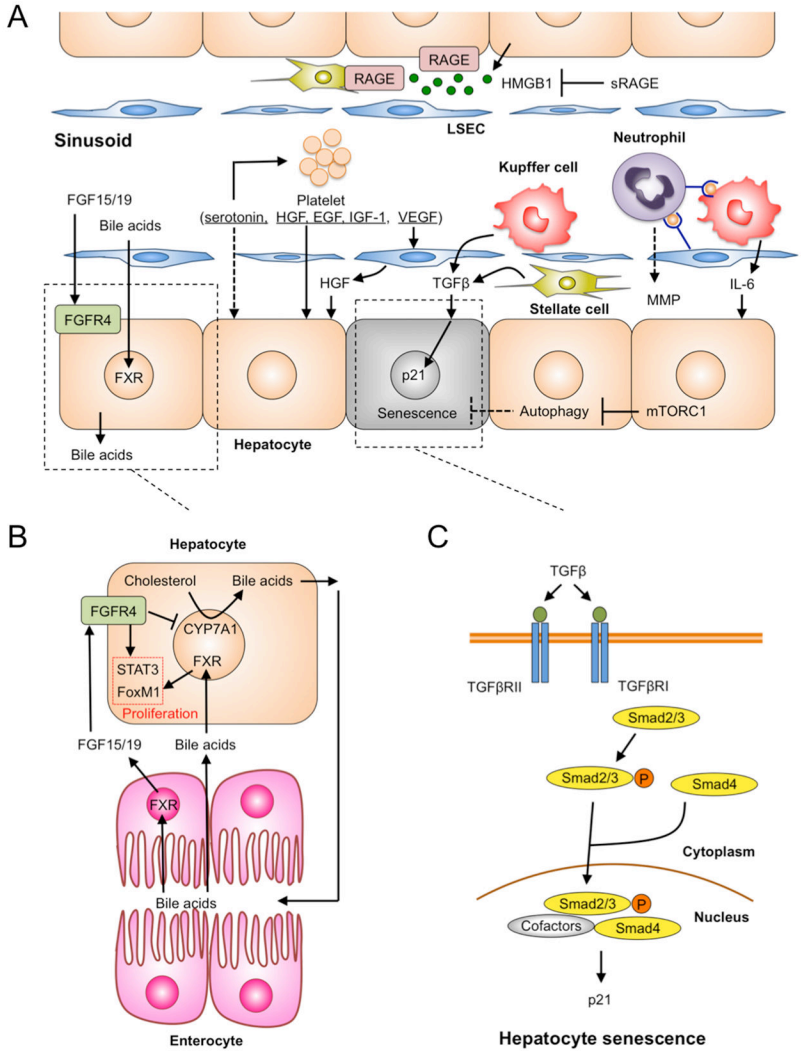

Figure 1. Preclinical targets for liver regeneration in acute severe injury

(A) Principal molecules and cell communication networks disussed in this review. Solid line arrows indicate established mechanisms. Dotted line arrow indicate possible mechanisms.

(B) In enterocytes, farnesoid X nuclear receptor (FXR) activated by bile acids promote the secretion of fibroblast growth factor (FGF)15 in mice and FGF19 in humans. FGF15/19 and absorbed bile acids are transported to hepatocytes via portal flow. In hepatocytes, bile acids binding to FXR results in hepatocyte proliferation through the induction of forkhead box protein M1 (FoxM1). fibroblast growth factor receptor 4 (FGFR4) bound with FGF15/19 also promote hepatocyte proliferation through the activation of mitogenic targets, such as signal transducer and activator of transcription 3 (STAT3), FoxM1, and others. In addition, FGFR4 inhibits bile acids synthesis through the suppression of CYP7A1.

(C) Activated transforming growth factor beta receptor 1 (TGFβR1) promotes phosphorylation of Smad2/3, and forms a trimer complex with Smad4. Thereafter, the Smad composites translocate into nucleus and interact with other cofactors, leading to induction of cell senescence through cell-cycle arrest gene, such as p21.

Abbreviations: EGF: epidermal growth factor, FGF: fibroblast growth factor, FGFR4: fibroblast growth factor receptor 4, FoxM1: forkhead box protein M1, FXR: farnesoid X nuclear receptor, HGF: hepatocyte growth factor, HMGB1: high mobility group box-1, IGF-1: insulin-like growth factor 1, IL-6: interleukin-6, LSEC: liver sinusoidal endothelial cell, MMP: matrix metalloproteinases, mTORC1: mammalian target of rapamycin complex 1, RAGE: receptor for advanced glycation end products, sRAGE: soluble receptor for advanced glycation end products, STAT3: signal transducer and activator of transcription 3, TGFβR: transforming growth factor beta receptor, VEGF: vascular endothelial growth factor.

Table 1

Possible targets and roles in liver regeneration for acute severe injury

Role in liver regeneration	Targets	Animal study	Human study (evidence in liver regeneration)	Application to severe liver injury		References
				Prevention of ALF following SFSS	Ongoing ALF or extensive hepatocyte injury	
Promotion	PVE, ALPPS, two-stage hepatectomy	Yes	Yes (Clinically standard technique)	Yes	N/A	[4-6]
	Modulation of portal flow	Yes	Yes (Retospective study, beneficial)	Yes	N/A	[14-17]
	Pentoxifylline	Yes	Yes (RCT, beneficial)	Yes	N/A	[32]
	Omega-3 fatty acid	Yes	Yes (RCT, beneficial)	Yes	N/A	[33-35]
	Platelet, serotonin	Yes	Yes (Prospective observational study, beneficial)	Yes	N/A	[39-42]
	G-CSF	Yes	Yes (RCT, beneficial)	N/A	Yes	[44,45]
	FXR agonist, FGF19 analogue	Yes	No	Yes	N/A	[52,53]
	RAGE inhibitor	Yes	No	Yes	N/A	[56-59]
	TGFβR1 inhibitor	Yes	No	N/A	Yes	[61]
	mTORC1 inhibitor (Autophagy inducer)	No	No	N/A	N/A	[63]
Inhibition	Steatosis	Yes	Yes (Equivocal due to inaccuracy of diagnosis)	Risk factors		[23, 64-69]
	Fibrosis	Yes	Yes (Equivocal due to inaccuracy of diagnosis)			[60,70-72
	Aged liver	Yes	Yes (Retrospective study, injurious)			[73-75]
	Cholangitis	Yes	Yes (Retrospective study, injurious)			[81,32]
Support (Alternation)	BAL	Yes	Yes (RCT, no beneficial)	N/A	Yes	[95,96]
	Cell transplantation	Yes	Yes (Case series, limited beneficial)	N/A	Yes	[97,99,101]
	Organ engineering	Yes	No	N/A	Yes	[109-111]

ALF: acute liver failure, ALPPS: associating liver partition and portal vein ligation for staged hepatectomy, BAL: bioartificial liver. FGF: fibroblast growth factor, FXR: farnesoid X nuclear receptor, G-CSF: granulocyte colony-stimulating factor, mTORC1: mammalian target of rapamycin complex 1, N/A: not available, PVE: portal vein embolization, RAGE: receptor for advanced glycation end products, RCT: randomized controlled clinical trial. SFSS: small-for-size syndrome, TGFβR1: transforming growth factor beta receptor 1