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REVIEW

Signal molecule-mediated hepatic cell communication during liver regeneration

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Abstract

Liver regeneration is a complex and well-orchestrated process, during which hepatic cells are activated to produce large signal molecules in response to liver injury or mass reduction. These signal molecules, in turn, set up the connections and cross-talk among liver cells to promote hepatic recovery. In this review, we endeavor to summarize the network of signal molecules that mediates hepatic cell communication in the regulation of liver regeneration.

Key words: Signal molecule; Hepatic cells; Cellular cross-talk; Signal communication; Liver regeneration

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INTRODUCTION

The liver is a vital organ. It has a wide range of functions, which include synthesis, metabolism, storage and redistribution of amino acids, proteins (e.g. albumin and acute-phase proteins, enzymes and cofactors), carbohydrates, fats and vitamins. It also is involved in detoxification through: (1) removal of waste and xenobiotics, by breakdown of insulin and other hormones, hemoglobin, toxic substances, and medical products of drugs; (2) conversion of ammonia to urea; and (3) production and excretion of bile. The parenchymal cells (hepatocytes), which make up 70%-80% of hepatic cells, carry out most of these functions. The other 20% comprise the non-parenchymal cells, which include Kupffer cells, stellate cells, sinusoidal endothelial cells (SECs), biliary epithelial cells, lymphocytes, and oval cells[1].

The liver is the only organ that can regenerate fully after injury in mammals[2]. When the liver is subjected to surgery, toxic substances, or viral infection, it has an amazing regenerative ability to restore functional hepatic mass. Injury induces the priming of recovery mechanisms with large changes in hepatic composition, such as activation of non-parenchymal cells, production and activation of multiple factors. These factors further lead to proliferation of hepatocytes and non-parenchymal cells, recovery and re-establishment of tissue architecture. When the liver recovers its normal volume and function, the regenerative response is terminated. Liver regeneration is actually compensatory hyperplasia, which is mediated typically by the proliferation of surviving hepatocytes[3]. When hepatocyte proliferation is inhibited, oval cell proliferation occurs[4]. This review endeavors to summarize the roles of hepatocytes and non-parenchymal cells, as well as signal communication among the hepatic cells during liver regeneration.

FUNCTION OF VARIOUS TYPES OF LIVER CELL REGULATED BY DIFFERENT SIGNAL MOLECULES

Hepatocytes

Hepatocytes are organized into single-cell plates in mammals separated by vascular channels (sinusoids). Hepatocytes perform most liver functions such as synthesis, storage, metabolism and transformation of carbohydrates, amino acids, proteins, fats and vitamins, and detoxification, modification and excretion of exogenous and endogenous substances. The hepatocyte also initiates the formation and secretion of bile. During

liver regeneration, hepatocytes are primed to proliferate, maintain metabolic function, secrete interleukin (IL)-6, proteases and protease inhibitors, and hepatocyte growth factor (HGF)[5].

Kupffer cells

Kupffer cells are liver-resident macrophages with a pronounced phagocytic and endocytic capacity mainly located within the sinusoids[6]. Kupffer cells play an important role in the clearance of senescent and damaged erythrocytes. Kupffer cells also secrete potent mediators of the inflammatory response[7]. During the priming phase of liver regeneration, Kupffer cells are activated and secrete pro-inflammatory cytokines, most prominently tumor necrosis factor (TNF)-α, IL-6, and IL-1β, which can initiate the acute phase response in hepatocytes[8]. Small-for-size orthotopic liver transplantation may often cause graft failure. In a mouse partial 30% liver transplantation model, interruption of TNF-α signaling by Kupffer cell inactivation through administration of pentoxifylline and $GdCl_3$, or the use of *Tnfr-1*$^{-/-}$ mice improves animal survival and enhances liver regeneration. These results are partly due to the fact that inhibition of TNF-α reduces leukocyte adherence, and improves portal flow and microcirculation[9].

Hepatic stellate cells (HSCs)

HSCs, also known as Ito cells, fat-storing cells or liver fibroblasts, are located in the space of Disse between the hepatocytes and the hepatic sinusoidal endothelial lining. In normal liver, HSCs sustain a quiescent state. Quiescent HSCs represent 5%-8% of liver cells, store 80% of total body retinol (vitamin A) as cytoplasmic lipid droplets, control turnover of extracellular matrix (ECM), and regulate the contractility of sinusoids[5-7]. Following hepatic damage, HSCs trans-differentiate into ECM-secreting myofibroblasts (activated HSCs)[10], with the loss of retinol-rich droplets[11]. HSCs can secrete ECM proteins, including laminins, collagens and proteoglycans, growth factors such as HGF, fibroblast growth factor (FGF), transforming growth factor (TGF)-β and cytokines such as IL-6, and produce some matrix metalloproteinases and tissue inhibitors of metalloproteinases[5]. The HSC is the major cell type involved in liver fibrosis. HSCs also behave as professional liver-resident antigen-presenting cells (APCs)[6,11]. A recent study has suggested that quiescent HSCs of rats retained within the space of Disse express stem/progenitor cell markers (e.g. CD133 and Oct4) and possess a differentiation potential. The space of Disse acts as a HSC niche, which is similar to the stem cell niche. These characteristics of quiescent HSCs are determined by the special microenvironment in the space of Disse, which is composed of basal lamina proteins (laminin and collagen type Ⅳ), sympathetic innervation and the adjacent cells. The adjacent cells include SECs, which release stromal cell-derived factor-1 to attract HSCs *via* the cysteine-X-cysteine receptor 4, and hepatocytes, which synthesize β-catenin-dependent Wnt ligands and Jagged-1 to attract HSCs through Wnt receptor frizzled, and to have direct physical interactions with HSCs through Jagged-1 receptor notch[11-13].

Neurotropin receptor $p75^{NTR}$ is a low-affinity pan-neurotropin receptor that belongs to the TNF receptor superfamily. In the diseased liver, $p75^{NTR}$ is expressed in HSCs. When $p75^{NTR}$ is activated, it activates the Rho/Rho kinase pathway, which enhances actin filament formation and phosphorylation of cofilin in a ligand-independent manner. This pathway contributes to the transition of quiescent stellate cells into myofibroblast-like cells that are important cellular sources of HGF. HGF drives healthy hepatocytes into proliferation. During perpetuation of trans-differentiation, secretion of pro-nerve growth factor (NGF) or NGF by neighboring regenerating hepatocytes leads to ligand-induced activation of apoptotic pathways, including the neurotrophin receptor interacting factor/TNF receptor-associated factor/Ras-related C3 botulinum toxin substrate/c-Jun N-terminal kinase/caspase pathway in stellate cells. In mice, depletion of $p75^{NTR}$ exacerbates liver pathology and inhibits hepatocyte proliferation *in vivo*. $p75^{NTR-/-}$ HSCs fail to differentiate into myofibroblasts and do not support hepatocyte proliferation[14,15].

SECs and biliary epithelial cells

Liver SECs constitute the wall of the hepatic sinusoid and separate hepatocytes from the sinusoidal blood. They perform an important filtration function due to the presence of open fenestrations, with an average diameter of 120 nm, which allow free diffusion of many substances, but not of particles of the size of chylomicrons, between the blood and the hepatocyte surface. SECs have large endocytic and metabolic capacity for many ligands including glycoproteins, lipoproteins, ECM components (e.g. hyaluronate, collagen fragments, fibronectin, or chondroitin sulfate proteoglycan), immune complexes, transferrin and ceruloplasmin. SECs may function as APCs in the context of both major histocompatibility complex (MHC)- Ⅰ and MHC-Ⅱ restriction, with the resulting development of antigen-specific T cell tolerance. They are also active in the secretion of cytokines (IL-6), HGF, TGF-β, eicosanoids (i.e. prostanoids and leukotrienes), endothelin-1, nitric oxide, and some ECM components[5,7].

Biliary epithelial cells (cholangiocytes) constitute the bile ducts in hepatic portal triads. Cholangiocytes may transport water, ions and solutes; secrete growth factors and peptides that mediate cross-talk with other cells of the liver in a paracrine mode; secrete chemokines (e.g. monocyte chemotactic protein-1) and cytokines (e.g. IL-6) and express adhesion molecules that attract effector leukocytes and promote the clearance of infected cells; and promote fibrogenesis by attraction of HSCs[5,16].

Notch-1 and Jagged-1 are expressed in bile duct cells and hepatocytes in normal rat liver. Moreover, Notch-1 is also expressed in endothelial cells of the sinusoids and small vessels. After partial hepatectomy (PH) in rats, both Notch-1 and Jagged-1 proteins are upregulated and mainly exist in periportal hepatocytes. Notch receptor expressed in endothelial cells may be stimulated by its ligand Jagged, which is highly expressed in proliferating hepatocytes. Such interactions between ligands/receptors cause a decrease in endothelial cell proliferation and

promote formation of mature sinusoids. The Jagged/Notch signal is required to maintain a differentiated phenotype of bile duct cells, but more functions of Jagged/Notch signaling in bile duct cell proliferation and duct assembly remain vague[17].

Dendritic cells (DCs), natural killer (NK) cells and NKT cells

Interstitial liver DCs play important roles in innate and adaptive immunity. The outcome of liver DCs interacting with the antigen-specific T cells determines the balance between tolerance and immunity. Systemic and local environmental factors influence hepatic DC migration, maturation, and function[18].

After 75% PH in male C57BL/6 mice, $CD11c^+$ (DC marker) liver (L)DCs increased significantly within 6 h and maintained an inherent, immature phenotype in both PBS- and Flt3L (fms-like tyrosine-3 ligand, a hematopoietic growth factor that expands dramatically the number of DCs in lymphoid and non-lymphoid tissues, including the liver, without changing their maturation state)-pretreated mice[19]. The increase was more notable in mice pretreated with Flt3L compared with PBS. The numbers of $CD11c^+$ LDCs returned to pre-hepatectomy levels by 24 h. The expanded LDC population showed increased IL-10 and reduced interferon (IFN)-γ gene transcription 6 h after PH. The concomitant increase in expression of the anti-inflammatory cytokine IL-10 suggests that LDCs are involved actively in promoting a state of local immunosuppression. The decrease in IFN-γ is associated with inhibition of hepatic NK cell lytic activity. LDCs isolated from the liver 6 h after 75% PH exhibit enhanced estrogen receptor expression, concomitant with increased serum 17-β-estradiol levels. Flt3L-treated mice showed a significant increase in proliferating cell nuclear antigen labeling index compared with PBS-treated mice at 12, 24, 48 and 72 h after 40% PH, with a peak at 48 h. These results indicate that the increased numbers of estrogen-exposed DCs may play a key role in local immune suppression and promote progression of liver regeneration, by altering the balance toward a Th2-like microenvironment[19].

NK cells are cytotoxic large granular lymphocytes derived from $CD34^+$ hematopoietic stem cells. NKT cells have three categories: I type (classical, Vα14-Jα18$^+$TCR/CD1-dependent); II type (non-classical, all other CD1d-dependent T cells); and NKT-like cells (CD1d-independent $NK1.1^+$ T cells). NK and NKT cells are components of the innate immune system and participate in the inflammatory processes during hepatic injury. NK and NKT cells also contribute to adaptive immune responses by interacting with APCs[20]. NK and NKT cells accelerate liver injury through production of pro-inflammatory cytokines and killing hepatocytes. NKs inhibit liver fibrosis *via* killing early-activated and senescent-activated stellate cells and producing IFN-γ. For the regulation of liver fibrosis, NKT cells appear to be less important than NK cells as a result of hepatic NKT cell tolerance[21].

Treatment of mice with murine cytomegalovirus (MCMV) infection and toll-like receptor (TLR)3 ligand poly I:C results in the activation of NK cells to produce IFN-γ and attenuates liver regeneration after PH. NKT cells may only play a minor role in the negative suppressive effects of MCMV and poly I:C on liver regeneration[22]. However, in HBV transgenic (HBV-tg) mice, PH-induced liver regeneration is delayed. The impaired liver regeneration is related to the increased activation and number of NKT cells and their enhanced IFN-γ production. NKT cells usually are activated by antigen-loading CD1d on APCs and soluble cytokines, such as IL-12 that is produced by Kupffer cells. Elevated CD1d on hepatocytes contributes to NKT cell activation and subsequent impairment of liver regeneration in HBV-tg mice. The impairment of liver regeneration in HBV-tg mice is largely ameliorated by NKT cell depletion, but not by NK cell depletion[23].

Pretreatment of Vα14 NKT/Jα281$^{+/+}$ mice with IL-12 or α-galactosylceramide (α-GalCer) 5 d before PH induced activation of NKT cells. The activated NKT cells expressed increased mRNA levels for TNF-α and IFN-γ and enhanced liver injury at 24 h after PH. Hepatic NKT cells rather than Kupffer cells might produce TNF-α after the administration of IL-12 or α-GalCer. TNF-α produced by activated Vα14 NKT cells was more than sufficient to enhance liver damage during the early phase of liver regeneration. The regenerating hepatocytes were destroyed specifically through the TNF receptor 1 (TNFR1) mediated TNF-α/TNFR1 pathway, which led eventually to impaired liver regeneration[24]. However, in another study, in mice injected with α-GalCer 36 h after 70% PH, the induced activation of NKT cells greatly enhanced hepatocyte mitosis 44 h after surgery, *via* the TNF-α/TNFR1 and Fas/FasL-mediated pathways, accompanied by increased expression of TNFR1 and FasL in liver NKT cells[25].

Stem cells and progenitor cells

After injury, liver regeneration occurs typically through replication of existing hepatocytes, however, when hepatocyte proliferation is attenuated or blocked, the liver is repopulated by induction, proliferation, and differentiation of the progenitor cell compartment. Hepatic progenitor cells are rare quiescent cells that are thought to reside in the canals of Hering. The oval cells are a type of liver progenitor cells that express markers in common with cholangiocytes and embryonic hepatocytes in rodents[4,26-28]. Oval cells are characterized by expressing phenotypic markers such as A6[27,28] and thymus cell antigen 1, and α-fetoprotein (AFP)[29].

Oval cells are much less sensitive to TGF-β-induced growth inhibition than hepatocytes *in vivo* and *in vitro*. These results are partly due to Mothers against decapentaplegic homolog (Smad)6 intervention. Smad6 is present in much higher amounts in oval cells (LE-2 and LE-6 cells) compared with hepatocytes (AML-12 cells or primary hepatocytes). The significant levels of Smad6 in oval cells inhibit TGF-β signaling by associating with the type I receptor, thereby interfering with Smad2 phosphorylation by the activated receptor complex, which

prevents translocation of Smad2 to the nucleus, and subsequent target gene transcription[4]. The combination of IFN-γ with lipopolysaccharide (LPS) or TNF-α causes a reversible cell cycle arrest in cultured hepatocytes (AML-12 cells), but stimulates DNA replication in oval cells (LE-6 cells). Hepatocyte cell cycle arrest is caused, at least in part, through NO release produced by inducible NO synthase after IFN-γ/LPS or IFN-γ/TNF-α administration[26].

The hepatic expression of *lymphotoxin-β* (*Lt-β* or *Tnf-β*) and *Ifn-γ* produced by oval cells is upregulated and promotes oval cell-mediated liver regeneration in a choline-deficient, ethionine-supplemented (CDE) diet/PH mouse model. In *Lt-βr* knock-out (KO), *Lt-β* KO, and *Ifn-γ* KO mice, oval cell-mediated liver regeneration is impaired, which confirms a role for LT-β/LT-βR and IFN-γ in oval cell-mediated liver regeneration[27].

Gp130-mediated IL-6 signaling may play a role in oval cell proliferation *in vivo*. In livers of *IL-6*$^{-/-}$ mice fed with a CDE diet, the numbers of oval cells were reduced compared with *IL-6*$^{+/+}$ control mice. The hyperactive signal transducer and activator of transcription (STAT)3 signaling *Gp130*^{Y757F} mouse model was derived when the tyrosine 757 residue of gp130 was mutated to a phenylalanine that prevented suppressor of cytokine signaling proteins (SOCS)3 binding and Src homology 2 (SH2) domain-containing protein-tyrosine phosphatase (SHP)2/RAS/extracellular signal-regulated kinases (ERK) signaling upon gp130 activation. Hyperactive STAT3 signaling in *Gp130*^{Y757F} and *Socs3*$^{-/-\Delta Alb}$ (*Socs3*$^{-/-}$) mice fed a CDE diet results in increased oval cell proliferation compared with wild-type and gp130-mediated hyperactive ERK1/2 signaling (*Gp130*$^{\Delta STAT}$) mice. However, SOCS3 overexpression or ERK1/2 activation inhibits oval cell proliferation in oval cell lines[28]. Proliferation of oval cells is associated with activation of nuclear factor (NF)-κB and STAT3 during oval cell-mediated liver regeneration in a 2-acetylaminofluorene (AAF)/PH rat model. Sustained NF-κB signaling has a critical role in protecting oval cells against apoptosis during stem cell-mediated liver regeneration. STAT3 plays a important role in driving proliferation and regulating differentiation of the hepatic stem cell progenies[30].

Connective tissue growth factor (CTGF or CCN2) is a secreted matricellular protein that belongs to the CCN family. This protein comprises four mosaic conserved modules. CTGF normally is expressed at a very low level in the liver. However, when the liver suffers from chronic or acute injury, CTGF level is upregulated in diverse repair processes[29]. Recruitment and proliferation of Thy-1^{+} oval cells is a hallmark of liver regeneration in rats after 2-AAF/PH. Sorted Thy-1^{+} oval cells in rats after 2-AAF/PH express a high level of *Ctgf* gene accompanied by upregulated CTGF protein expression. Blocking CTGF induction by iloprost (a known inhibitor of CTGF synthesis) significantly decreased the oval cell proliferation and lowered the level of AFP expression as compared with control animals. These results suggest that CTGF induction is important for robust oval cell proliferation after 2-AAF/PH treatment in rats[31]. Yeast two hybrid experiments have identified that fibronectin (FN) is a CTGF-binding protein. FN that binds to modules I and Ⅳ of CTGF and co-localizes with CTGF on the provisional hepatic ECM around the periportal regions promotes oval cell adhesion and migration, thereby facilitating oval cell activation[29].

In 2-AAF/PH-treated rats, hepatocytes strongly express multidrug resistance protein 1b, but oval cells express high levels of active multidrug resistance associated protein (Mrp)1 and Mrp3. Mrp1 functions mainly as a cellular efflux pump of cysteinyl-leukotrienes and glutathione-S-conjugates, and Mrp3 as a pump for glucuronides and mono- and divalent bile salts, thus Mrp1 and Mrp3 may have a role in removing exogenous and endogenous toxic drugs/metabolites from oval cells, and facilitate oval cell proliferation in conditions of severe hepatotoxicity[32].

Sympathetic nervous system inhibition using prazosin (PRZ, an α-1 adrenoceptor antagonist) or 6-hydroxydopamine (6-OHDA, an agent that induces chemical sympathectomy) significantly enhanced hepatic accumulation of oval cells and reduced liver damage in mice fed antioxidant-depleted diets to induce liver injury. Neither PRZ nor 6-OHDA affects the expression of cytokines, growth factors, or growth factor receptors that are known to regulate progenitor cells[33].

The plant lectin concanavalin A (Con A) may induce T cell-mediated hepatitis in mice. Following PH, ConA-treated mice show significantly impaired early regenerative responses, such as decreased cyclin D1 and E expression and STAT3 activation within hepatocytes, in conjunction with reduced IL-6 production, increased IFN-γ, TGF-β and p21^{waf} expression, and increased TGF-β-induced Smad2 phosphorylation. However, Con A may induce an increase in the number of NK cell-sensitive oval cells (CD117, AFP, albumin, and cytokeratin-positive cells) and hematopoietic-like cells (Sca-1^{+} cells) in these mice[34]. Furthermore, much more accumulated lipid was seen in the liver of mice with Con A-induced hepatitis, by Oil Red O staining[35].

The IL-6/gp80/gp130 signaling system contributed to rapid expansion of the progenitor cell populations including liver hematopoietic progenitor cells and liver epithelial progenitor cells in a Con A/PH-mediated mouse liver injury model[36].

SIGNAL COMMUNICATION THAT OCCURS IN THE PROGRESSIVE PHASES OF LIVER REGENERATION

Liver injury causes significant changes in the expression and activity of a variety of signal mediators produced by hepatic cells, endocrine glands and platelets. These molecules include complement components C3 and C5; cytokines (TNF-α and ILs); growth factors [TGF, epidermal growth factor (EGF), platelet-derived growth factor, vascular endothelial growth factor (VEGF), FGF,

insulin-like growth factor (IGF)-I, and HGF]; hepatic ECM; extracellular proteases and protease inhibitors; hormones [insulin, growth hormone (GH), thyroid hormone, vasopressin, prostanoids, and endothelin-1] and neurotransmitters (serotonin); metabolites [bile acids, reactive oxygen species (ROS), NO, lipids, glutathione, S-adenosylmethionine, and sphingosine-1-phosphate]; and chemokines[1,5,7,37-42]. Liver regeneration progression is highly coordinated by the signal communication between hepatocytes and non-parenchymal cells, and is also influenced by endocrine glands, sympathetic innervation, and blood circulation. The progression of liver regeneration is segmented into several phases. Here, we describe the mechanisms of liver regeneration in each phase.

Priming phase

In models of liver injury induced by toxins, such as CCl_4, or Fas ligand, the hepatocytes are damaged and undergo necrosis, for which, the growth factor- and cytokine-mediated pathways are similar to those in PH models[1]. Liver injury causes the release of ROS and LPS, which trigger the activation of the complement system. After complement activation, cleavage of C3 or C5 leads to the generation of the potent anaphylatoxins C3a and C5a. LPS, C3a and C5a in turn activate the non-parenchymal cells such as Kupffer cells, through the cell surface receptor TLR4 and G protein-coupled receptors C3aR and C5aR, which causes activation of the NF-κB signaling pathway and the production of cytokines such as TNF-α and IL-6. TNF-α then interacts with TNFR on Kupffer cells, which stimulates intensive synthesis of TNF-α and IL-6. Furthermore, SECs, HSCs, biliary epithelial cells and hepatocytes may also produce IL-6[5,43,44]. The cytokines TNF-α and IL-6 are responsible for priming the quiescent hepatocytes into the cell cycle (G0 to G1) through binding to their receptors TNFR1 and IL-6R; activating the NF-κB, JAK/STAT3 and MAPK signal pathway; initiating the transcription of immediate early genes; and sensitizing hepatocytes to the proliferative effects of growth factors[37].

Proliferative phase

In rat liver PH models, the rate of DNA synthesis in hepatocytes begins to increase after about 12 h and peaks around 24 h. However, induction of DNA synthesis occurs later in the non-parenchymal cells (at about 48 h for Kupffer, biliary epithelial and stellate cells, and at about 96 h for endothelial cells). Subsequent levels of DNA synthesis in hepatocytes are lower, as complete restoration of liver mass requires an average of about 1.66 cycles of replication in all cells. By comparison, the peak in DNA synthesis in mice occurs later (36-40 h after PH) and varies between strains[1,45]. Many growth factors and growth factor-binding proteins are produced to promote the progression of liver regeneration.

The bulk of IGF-I is synthesized by hepatocytes, but is also produced by other types of non-parenchymal liver cells. Hepatic IGF-I synthesis is not only regulated by growth hormone, insulin, and IGF-I, but also by cytokines released from activated Kupffer (IL-1, TNF-α and TGF-β) or stellate (TGF-α and TGF-β) cells[7]. The biological actions of IGF-I are mediated through its physiologic receptor IGF-1R and insulin receptor. The activity of IGFs is modulated by a family of high-affinity binding proteins (IGFBP-1-6) and IGFBP proteases[46]. HGF is produced mainly by HSCs, but also by hepatocytes, SECs, and performs its functions through interacting with its receptor c-met[5]. The EGF family consists of several members, including EGF, TGF-α, heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AR), β cellulin, and epiregulin[47]. HB-EGF is expressed mainly in Kupffer and endothelial cells[48]; TGF-α is synthesized mainly by hepatocytes[49]; expression of AR is induced by hepatocytes, Kupffer cells and HSCs[50,51]; and these factors transmit their signal through EGF receptor.

Most growth factors are usually formed in an inactive precursor bound with ECM or integral to membrane. During liver regeneration, extracellular protease activation and ECM degradation occur in the first few hours before hepatocyte DNA synthesis and division, and growth factors are released and activated from the bound ECM or cell membrane by the extracellular protease[5]. These activated growth factors binding to their corresponding receptors drive hepatic cells into DNA replication and mitosis. Furthermore, factors such as insulin from the pancreas, EGF from the duodenum or salivary gland, norepinephrine from the adrenal gland, triiodothyronine from the thyroid gland, GH from the anterior pituitary gland, arginine vasopressin (AVP) from the posterior pituitary, serotonin from platelets, and prostaglandins (PGs) from Kupffer cells and hepatocytes are also involved in the process of liver regeneration[1,38,52-54].

Remodeling phase

After cell division, hepatocytes are formed in clusters that no longer associate with sinusoids. The nascent endothelial cells travel among cell clusters to form sinusoids that will line hepatocyte plates. VEGF, angiopoietin and their receptors Flt-1 (VEGF-R1), Flk-1/KDR (VEGF-R2), Flt-4 (VEGF-R3), and Flt3/Flk2, as well as tyrosine kinase containing immunoglobulin and epidermal growth factor homology domain (Tie)-1 and Tie-2 might be involved in this process[5,55]. Meanwhile, biliary epithelial cells rebuild the biliary tree in hepatic portal triads. Synthesis of new ECM, vasculature and biliary tree then reestablishes tissue architecture.

Terminating phase

TGF-β and activin A belong to the TGF-β superfamily of cytokines. TGF-β is produced mainly in HSCs, but is also expressed in SECs and Kupffer cells[4,5]. In the liver, activin A is synthesized predominantly in hepatocytes, but is also expressed in non-parenchymal cells under pathological conditions. Activin A is an autocrine growth inhibitor that is produced in hepatocytes, and is involved in inhibiting the proliferation of hepatocytes,

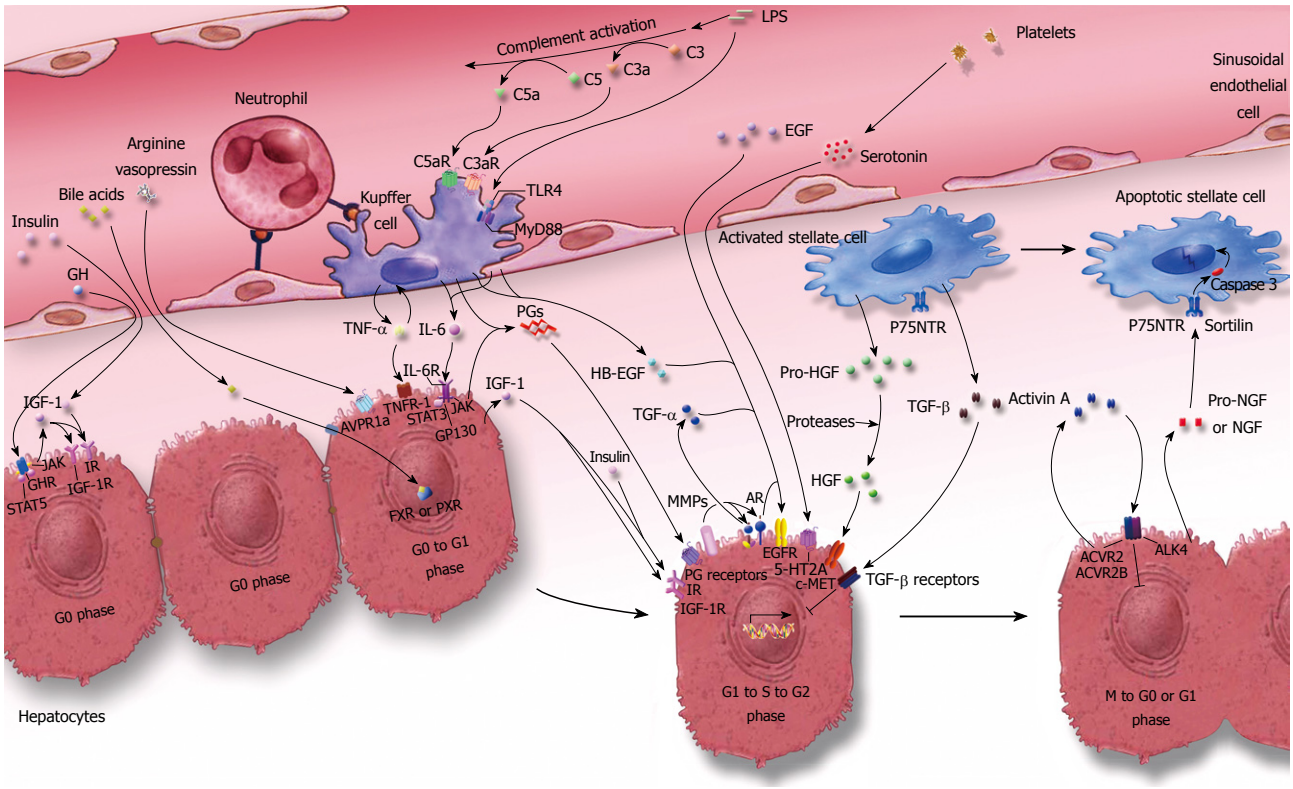

Figure 1 Scheme depicting the network of signaling among hepatic cells and blood during liver regeneration. After liver injury such as PH, gut-derived factors such as LPS reach the liver through the portal blood supply. LPS activate the complement system, which releases the anaphylatoxins C3a and C5a. LPS, C3a and C5a activate Kupffer cells through TLR4, C3aR and C5aR, and further lead to production of TNF-α and IL-6. These factors are involved in priming the hepatocytes from G0 to G1 phase. Insulin, GH, bile acids, AVP, platelet-derived serotonin, and EGF from the blood, cooperating with PGs, HB-EGF, HGF, as well as IGF-1, TGF-α and AR from different hepatic cells, promote hepatocyte transition from G1, through S and G2, to the M phase of the cell cycle. TGF-β produced mainly by HSCs inhibits G1 to S phase transition of hepatocytes, and TGF-β signalling is blocked during the proliferative phase. Pro-NGF or NGF produced by the neighboring regenerating hepatocytes promotes the termination of the activated state of HSCs by pro-NGF- or NGF-induced apoptotic pathways. When the liver mass is restored to its normal volume, the increased signaling by activin A, apoptosis and other factors may promote termination of liver regeneration. The prototype of this figure is originated from reference[43].

inducing the differentiation of hepatocytes, augmenting the tubulogenesis of SECs, and stimulating collagen production in HSCs[56].

TGF-β and activin A may bind to their high-affinity cell surface type Ⅱ receptor TGFBR2/TβRⅡ and ACVR2/ActRⅡ or ACVR2B/ActRⅡb, respectively, either directly or *via* co-receptors, and recruit and activate their cell surface type I receptors TGFBR1/ALK5 (TβRI), ACVRL1/ALK1, ACVR1/ALK2 and ACVRL1/ALK1, ACVR1/ALK2, ACVR1B/ALK4, respectively, which leads to activation of their downstream Smad signal pathway[57,58]. TGF-β inhibits G1 to S phase transition in hepatocytes, but TGF-β signaling is blocked during the proliferative phase; furthermore, intact TGF-β signaling is not required for the termination of liver regeneration[1,59]. When the liver mass restores its normal volume, the increased signaling by activin A, apoptosis and other factors[59], and the decreased expression and activation of promoting proliferation factors due to the restored ECM and tissue architecture may promote termination of liver regeneration. Figure 1 depicts the main signal communication network that occurs in the progression of liver regeneration.

Following liver injury, the expression and activity of signal molecules produced by activated hepatic cells are controlled in a time- and micromilieu-dependent mode. The direct interactions among hepatic cells and the indirect interactions mediated by secreted signal molecules with hepatic cells constitute the dynamic recovery process of liver regeneration. Many more signal molecules and pathways are involved in the regulation of liver regeneration than those mentioned above. Further study will reveal more facts about the mechanisms of liver regeneration.

CONCLUSION

Liver regeneration is a very complex process, which is accompanied by a highly regulated intercellular and intracellular signal communication network. The extracellular signal molecules that regulate the progression of liver regeneration are produced by hepatic cells, endocrine glands and platelets in autocrine, paracrine, juxtacrine and endocrine modes. Most of these signal molecules are inactive precursors that need to be further processed into a mature form by activated proteases in ECM or on membrane of adjacent hepatic cells. The extracellular signaling interfaces with intracellular signals through their specific receptors. Liver regeneration is highly coordinated by the cross-talk between these signal

molecules and hepatic cells. Liver has a regenerative ability to restore functional hepatic mass after liver injury, but under some pathological conditions, the recovery of liver is not autonomous, so the study of the pathogeny of the diseased liver is more significant, to provide methods for treating patients with liver damage.

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