Review On: Liver Regeneration

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Abstract— The liver is an important organ with in the body that has a central role in metabolic homeostasis. The liver is unique in its ability to regenerate in response to injury. A number of evolutionary safeguards have allowed liver to continue its complex functions in significant injury. Improved understanding of the regenerative process hassignificant benefit in the treatment of liver failure.This review provides an over view of the models of study currently used in liver regeneration, the molecular basis of liver regeneration, and the role of liver progenitor cells in regeneration of the liver. Hepatocyte regeneration proceeds along a sequence of distinctive phases and result in a precise reconstitution of the lost tissue mass.

ABBRIVIATION

DNA:- Deoxyribonucleic acid; EGF:-epidermal growth factor; FAH:-fumarylacetoacetate hydrolase; FGF:- fibroblast growth factor; HBEGF:- heparin-binding EGF-like growth factor; HGF:- hepatocyte growth factor; IGFBP:- insulin like growth factor binding protein; IL:- interleukin; MAPK:- mitogen-activated protein kinase; miRNA:- microRNA; mTOR:- mammalian target of rapamycin; NFkB:- nuclear factor kappa B; SOCS:- suppressors of cytokine signaling; STAT3:- signal transducer and activator of transcription 3; TGF:- transforming growth factor; TNF:- tumor necrosis factor; PHx:- partial hepatectomy; EGF:- epidermal growth factor; Ppar-α:-peroxisome proliferators activated receptor-α; TNFR-1:- tumor necrosis factor receptor-1; LIP:- liver enriched inhibitory protein; LAP:- liver enriched activated protein; ICAM:- intercellular adhesion molecule; FADD:- fas-associated death domain; FXR:- farnesoid x receptor; metR:- mesenchymal-epithelial transition factor receptor.

I. INTRODUCTION

Regeneration of the liver can be defined as hyperplasia, during which the remaining liver tissue expands to meet the metabolic needs of the organism. Unlike anatomic true regeneration, the expanding liver doesn't regain its original gross anatomic structure. It is also important to note the origin of cells used to replace the missing hepatocytes.[1] The liver can maintain its growth and mass. Surgical resection of hepatocyte loss caused by virus or chemical injury triggers hepatocyte replication while enlarged mass is corrected by apoptosis. [3]

Importantly, it is the main detoxifying organ, which removes wastes and xenobiotics from body by metabolic conversion and biliary excretion. The main cell type of the liver that carries out most of these functions is the parenchymal cells or hepatocyte, which makes up approx. 80% of hepatic cells. The other 20% comprises the non-parenchymal cells, which include endothelial, KUPFFER CELLS, lymphocytes and STELLATE CELLS.

The word ‘regeneration’ may be a misnomer because the lobes of the liver that are removed don't grow back, unlike the regeneration of limbs in amphibian models. Instead, there is a hyperplastic response that involves

the replication of virtually all of the mature functioning cells in the liver. [4]

Liver has all exiting circulation from the small and most of the large intestine, as well as spleen and pancreas, through the portal vein. Its location in relation to the food supply via the portal vein, and the unique gene and protein expression patterns of hepatocytes allow it to function as biochemical defense against toxic chemicals entering through the food and from absorbed food ingredients. Nutrients entering the liver are converted into proteins (albumin, most coagulation factors, several plasma carrier proteins etc. in the peripheral blood), lipids sent as lipoproteins into the other tissues, carbohydrates stored in liver as glycogen (the main glucose reserve used for stabilization of glucose levels in the blood). Synthesis of bile is essential for absorption of fat and lipophilic nutrients.

- Models of study used in liver regeneration

Regeneration of the liver is studied by performing a surgical operation which removes 2/3 of the liver mass in rodents (rats and mice), a way referred to as 2/3 partial hepatectomy (PHx) (Higgins & Anderson 1931). Due to the multilobe structure of the rodent liver, three of the five liver lobes (representing 2/3 of the liver mass) are often removed by a simple surgical operation, without causing any tissue damage to the residual two lobes. The latter grow in size to restore an aggregate similar to the mass of the original five lobes. The process, in rats and mice, is complete within 5–7 days after surgery. [5]

Zebrafish have been recognized as an exceedingly important model of developmental biology because of their prolific production of offspring and transparent embryos, offering constant visualization and experimental manipulation. Furthermore, organogenesis occurs rapidly, with presence of nearly all major organ systems by 2 days post fertilization; a mature liver is visualized under standard light microscope by 5 days postfertilization. Forward genetic screening, the technique of targeting embryonic mutants defective in a particular process, has allowed researchers to identify essential genes for various processes of hepatogenesis within this vertebrate model. [1]

- Liver progenitor cells

Liver progenitor cells are thought of as the second line of defense against liver injury, becoming active when mature hepatocytes are prevented from proliferating. Ongoing work in animal and human models of disease has helped to delineate the role of liver progenitor cells in the physiological as well as the regenerating liver. The first liver progenitor cells to be identified, termed oval cells, were described in 1956 by Farber. Oval cells, named for the appearance of their nucleus, are small bipotent cells with a high nuclear-to-cytoplasmic ratio, and are capable of differentiation into both cholangiocytes and hepatocytes. These cells have been shown to activate in animal studies in which native hepatocytes have been blocked chemically from proliferation in the setting of liver injury, stimulating regeneration. Oval cells proliferate within the periportal region, dependent on growth factors produced by stellate cells, including HGF, FGF1, FGF2, and vascular endothelial growth factor. The oval cell, capable of production of albumin and alpha-fetoprotein become basophilic hepatocytes within 4–5 days of activation. Eventually, these cells can become mature hepatocytes. [1]

- Hepatocyte growth factor [HGF]

HGF is also protective in Fas-mediated liver injury and HGF levels correlate with protection against cirrhosis. In a rat model of liver cirrhosis that is mediated by dimethylnitrosamine, a high plasma level of human HGF (which was produced after human HGF gene transfection into skeletal muscles) was induced. This resulted in the activation of the Met receptor and a reduction in the increase in TGFβ1 that is normally associated with dimethylnitrosamine treatment. In addition, HGF infusion inhibits fibrogenesis and hepatocyte apoptosis, causes the resolution of fibrosis in an already cirrhotic liver, and improves the survival rate in liver-damaged rats. HGF blocks hepatocyte apoptosis, in part, through its ability to inactivate Rac1 by PI3K/AKT-mediated phosphorylation. It also seems to inhibit Fas signaling directly, through an interaction between Fas and the Met receptor. Again, as in the case of IL-6, it is not yet clear whether the effect of HGF on cirrhosis is limited to its effect on hepatocyte injury, or if it also directly reduces fibrogenic activation. [7]

- Tumor necrosis factor [TNF]

This is a protein known to have a variety of effects on many cells and tissues. Contrary to what its name implies, TNF can often have promitogenic effects on cells, depending on conditions which regulate activation of NFkB. If conditions favor activation of NFkB, then TNF may enhance other concurrently delivered growth signals. Alternatively, if activation of NFkB cannot be mediated by TNF, then TNF may elicit an apoptotic response. The status of free radicals, energy levels and other intracellular factors determine the emergence of complex pathway involving activation of NFkB by removal of the inhibitory IkB through phosphorylation mediated by the kinase IKK. One of the factors determining the activation of NFkB and the outcome of interaction of TNF with cells is altered integrin signaling. With all the matrix remodeling occurring during regeneration, such alterations in integrin signaling are bound to occur and they may be associated with directing the signaling of TNF towards a promitogenic effect. Antibodies against TNF administered at the time of hepatectomy decrease the regenerative response. Mice with genetic deletions of the TNF receptor 1 (TNFR1) have slow and deficient response following PHx. Activation of STAT3 and NFkB in these mice is diminished. Liver regeneration eventually becomes completed albeit much later. Even though deletion of NFkB components does not seem to affect liver regeneration, given the promitogenic effects of activated NFkB in many cells and tissues, it is likely that TNF exercises its effects on liver regeneration in major part by this pathway. TNF is involved in induction of TACE, a plasma membrane associated protease which controls activation of TGFα. Enhanced activation of TGFα cause's transactivation of EGFR. TNF is also a regulator of iNOS, and mice with deficiency in iNOS have defective liver regeneration. TNF is not a direct mitogen for hepatocytes. It does not induce DNA synthesis in primary cultures of hepatocytes in serum free media nor does it induce hepatocyte DNA synthesis when injected in whole animals. It does, however, enhance the mitogenic effects of direct mitogens such as HGF, both in vivo and in cell culture and is mitogenic for hepatocytes with transgenic expression of TGF α. TNF increases in plasma after PHx. Its cellular source is considered to be the hepatic macrophages (Kupffer cells) but production by other cell types has not been excluded. A stimulus that may induce TNF after PHx is endotoxin, produced by bacteria from the gut. Given the absence of direct mitogenic effects on hepatocytes, TNF should not be viewed as the initiator of liver regeneration, but rather as one of the many concurrent and contributory extracellular signals that all together orchestrate the early events of the response. [5]

- Norepinephrine

This is a neurotransmitter in the central and peripheral autonomic nervous system. Epinephrine and norepinephrine are released in peripheral circulation from nerve endings, as well as from the adrenal medulla. Interest in the role of norepinephrine in liver regeneration arose when it was shown that norepinephrine substantially enhances the mitogenic effects of EGF and HGF in hepatocyte cultures and it decreases the mito-inhibitory effects of TGFb1. In cultures of hepatocytes with balanced concentrations of EGF and TGFb1 such that the final effect is neutral (EGF mitogenic effect is balanced by the mito-inhibitory effect of TGFb1), addition of norepinephrine triggers high-level hepatocyte DNA synthesis. Norepinephrine induces synthesis of HGF in myofibroblasts. It is produced by and required for DNA

Synthesis of stellate cells in-vivo and in culture. Another effect of norepinephrine and epinephrine of potential importance to liver regeneration is enhancement of production of EGF by Brunner's glands of the duodenum. This has not been directly linked to liver regeneration but norepinephrine rises rapidly in plasma after PHx and it may have an effect on EGF production. Blockade of the alpha-1 adrenergic receptor by prazosin inhibits DNA synthesis after PHx for 72 h. It is not clear whether this effect reflects blockade of norepinephrine secreted peripherally or locally released by the stellate cells. [5]

- Bile acids and xenobiotics

It has been a long standing observation from liver pathology that hepatic cholestasis (chemically induced or due to mechanical biliary obstruction) is associated with proliferation of hepatocytes. A recent study provided evidence that bile acids increase in circulating blood after PHx and that depletion of bile acids leads to decreased regeneration. The elevation of bile acids in plasma occurs several hours after PHx,

thus it is unlikely that they contribute to the immediate early changes after PHx described above. None the less, the finding is very interesting. In the same study, mice with genetic deficiency of FXR, a transcription factor mediating nuclear events induced by bile acids, also have defective regeneration. There are several examples of xenobiotics ligating specific transcription factors or nuclear hormone receptors in hepatocytes and inducing liver enlargement. These include triiodothyronine (T3), agonists of PPARa, estrogens, barbiturates (acting on CAR and PXR), and others. Hepatic enlargement is mediated in part by hepatocyte proliferation and in part by hepatocyte enlargement. The signaling pathways, by which these chemicals exert these effects, are not clear. These pathways have not been shown so far to be associated with signaling patterns seen during liver regeneration. FXR is the first nuclear hormone receptor to be associated with proliferative events leading to regeneration of the liver and it sets a paradigm for discovery of other such nuclear hormone receptors as potentially having similar effects. [5]

- Insulin

Liver is the first recipient of all the insulin produced by the endocrine pancreas, since it is delivered through the portal vein. Diabetes (either through insulin deficiency or through insulin resistance) causes a mild to severe steatohepatitis that may even lead to cirrhosis. Diversion of the portal vein flow to vena-cava (portacaval shunt) forces insulin to bypass the liver. Liver atrophies to about 1/3 of its size. Administration of insulin directly to the liver in animals with experimental portacaval shunts reverses hepatic atrophy, and is associated with rapid hepatocyte proliferation. Hepatocytes in culture have diminished response to mitogens in the absence of insulin. Insulin is clearly a very important regulator of hepatocyte functions at all times and it is very likely involved in the metabolic adaptations that hepatocytes have to undergo to provide homeostatic functions during liver regeneration. Insulin, however, is not a direct mitogen for hepatocytes. [5]

- Interleukin 6 [IL-6]

The most detailed analyses of hepatoprotection have been carried out using IL-6-deficient animals. The role of its upstream regulator TNFα is less clear, as TNFα can both protect against, and exacerbate, liver injury. However, NF-κB signaling, as mediated by the TNFα response, is clearly anti-apoptotic. IL-6 ameliorates acute toxic liver injury and, after acute CCl4 treatment104, IL-6 mice develop increased hepatocellular injury and defective regeneration. This is accompanied by a significant reduction of STAT3 and NF-κB activation and reduced DNA-synthesis and mitotic responses in hepatocytes. Unlike after partial hepatecto my, after CCl4 treatment, increased hepatocyte apoptosis is noted in IL-6 livers. Pretreatment with IL-6 before CCl4 reduces acute CCl4 -mediated injury and apoptosis, and accelerates regeneration in both wild type and IL-6 livers. Fas ligation, which causes mainly apoptosis (but causes little necrosis, in contrast to CCl4 toxicity), provides further insight into the mechanisms by which IL-6 reduces hepatocyte apoptosis. IL-6 mice are more susceptible to Fas-mediated death than wild-type animals, and IL-6 hepatocytes have an increased sensitivity to Fas. After Fas activation, IL-6 livers show several alterations in the apoptotic pathways that involve caspase-8 and -3, as well as the release of cytochrome c (as a result of increased mitochondrial permeability). IL-6 livers have reduced levels of the anti-apoptotic factors BCL2 and BCL-XL, and increased degradation of the caspase-8 inhibitor FLIP (Fas-associated death domain (FADD)-like IL-1-converting enzyme (FLICE)-inhibitory protein) after Fas treatment. STAT3 is the main effector of IL-6-mediated hepatoprotection, which blocks apoptotic injury in two ways: first, by the induction of anti-caspase regulators; and second, by the reduction of oxidative injury owing to the upregulation of an antioxidant protein. Likewise, IGFBP1, another pro-regeneration factor, is highly protective against Fas-mediated injury that occurs by pathways other than those stimulated by IL-6 and STAT3. After Fas treatment, IGFBP1 deficiency is associated with massive hepatocyte apoptosis and caspase activation that is corrected by pretreatment with IGFBP1. Preliminary data indicate that IGFBP1 is important in the regulation of two APOPTOGENIC factors, matrix metalloproteinase [MMP] 9 and TGF-β. [4]

Hepatocyte are not terminally differentiated, rather, they are in proliferative quiescence (G0 phase), but can rapidly enter a cell division cycle upon stimulation hepatocyte regeneration proceeds along a sequence of distinctive phases.

A. An initiation or priming phase- rendering hepatocyten a state of replicative competence.
B. Proliferation Phase where expansion of the entire hepatocyte population takes place.
C. Termination Phase where cell proliferation is suppressed to terminate regeneration at a defined set point.[6]

Hepatocytes need to be primed before they can fully respond to the growth factors HGF (Hepatocyte Growth Factor), TGFα (Transforming Growth Factor Alpha), and EGF (Epidermal Growth Factor) in vitro. Priming requires the cytokines TNF and IL-6 in addition to other agents that prevent cytotoxicity. Reactive Oxygen Species and glutathione content can determine whether the TNF effect on hepatocytes is proliferative or apoptotic. At least four transcription factors, NFKB, STAT3 (which are strongly induced by TNF), AP-1 and CIEBPP play major roles in the initiation of liver regeneration. [3]

- The molecular regulation of the initiation phase

During initiation, hepatocytes are primed for subsequent replication. Initiation factors comprise interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Following IL-6 binding to the gp130 receptor, activation of STAT3 and C/EBP beta/nuclear factor-IL-6 (NF-IL-6) takes place, TNF- α and IL-6 triggering the G0/G1 transition of the cell cycle. Following IL-6 binding to the gp130 receptor, activation of STAT3 and C/ EBP beta/nuclear factor-IL6 (NF-IL-6) take place. Signal transducer and activator of transcription (STAT) family proteins are transcription factors critical in mediating cytokine signaling, and STAT3 is frequently activated in human cancers. NF-IL-6 is a transcription factor for IL-6, binding to an IL-1- responsive element in the IL-6 gene; it is homologous with C/EBP, a hepatocyte and adipocyte specific transcription factor. TNF-α and IL-6 trigger the G0/G1 transition of the cell cycle. IL-6-deficient and TNFR-1-deficient animals fail to accomplish initiation and a hepatocyte regenerative response. The IL-6-mediated pathway is further illustrated by C/EBP beta/NF-IL-6 knockout mice, showing a blunted regeneration. C/EBP beta controls the G1/S checkpoint. IL-6 modulates the action of growth factors. Transgenic mice overexpressing IL-6 are growth-retarded and show increased suppressors of cytokine signaling in the liver, inhibiting the activation of STAT by growth hormone, IL-6, therefore, blunting hepatic growth hormone signaling.

- Is the IL-6/gp130 pathway directly involved in the regulation of DNA synthesis?

Gp130 knockout mice have shown that activation of transcription factors is gp130 dependent, but the signaling operates rather via activation of protective pathways enabling hepatocyte proliferation.

- What are the mechanisms stimulating the expression of IL-6 and TNF-alpha, and their receptors/targets during liver regeneration?

Peroxisome proliferators activated receptor-alpha (Ppar-alpha) induces the IL- 6R; Ppar-alpha null mice lack IL-6R gene induction, and show delayed liver regeneration. C/EBP beta/NF-IL-6 binds to the IL-6 promoter region and enhances expression of IL-6. Promoter interaction is modulated by C/EBP homologous protein (CHOP), which interferes with NF-IL-6 action insofar as CHOP dimerizes more preferentially with an inhibitory isoform of NF- IL-6 [liver-enriched inhibitory protein (LIP)] than with a positively acting isoform [liver-enriched activator protein (LAP)], CHOP up-regulating IL-6 production without binding to its promoter.

Are there other priming factors?

Rodents with dysfunctional leptin signaling exhibit a profound impairment of liver regeneration. Leptin-deficient ob/ob. mice show an exaggerated activation of NF-kappa B and STAT3 during the initiation/priming phase, but an abrogation of TNF-alpha and IL-6 release at the time of the G1/S transition, an effect correctable by leptin administration.

- What are the sources of priming/initiation factors in hepatocyte regeneration?

The main cellular sources for IL-6 and TNF-alpha are non-parenchymal cells, i.e. sinusoidal endothelia, Kupffer cells and HSC. ICAM- 1-deficient mice exhibit impaired hepatocyte regeneration because ICAM-1 triggers release of TNF-alpha and IL-6 from Kupffer cells. Subsequent to priming/initiation, several immediate early-phase genes related to hepatocyte proliferation are induced within 2 h. They comprise c-fos, c-jun and others. c-Jun serves as a

major c-Jun-N-terminal kinase (JNK) target in proliferation. JNK is strongly activated minutes after PH; the JNK/c-Jun pathway is a critical component of the early proliferative response and induces the G0 to G1 transition via cyclin D1. A further rapidly induced factor is insulin like growth factor (IGF) binding protein 1 (IGFBP-1). IGFBP-1-deficient mice display reduced and delayed hepatocyte DNA replication after PH. Hepatocyte growth is furthermore regulated by human/murine fibrinogen-related protein-1 (H/MFREP-1), a liver- specific protein of the fibrinogen superfamily, eventually acting as a 'molecular facilitator' in the regenerative response. Some of the proteins involved in priming, expansion and termination may be modified by enzymes converting (inactive) proproteins via limited proteolysis to the active species. Nine mammalian proprotein convertases have been identified so far, eight belonging to the yeast kexin subfamily of subtilases. A K-like subtilases, neural apoptosis regulated convertase 1 (NARC-1), peaks on days 2–3 post PH, whereas other convertases (PC5, PACE4, furin) peak on day 1, suggesting that these enzymes are active in the bioavailability of growth factors. Furthermore, the bioavailability of growth factors depends on the liberation of these factors from storage in the ECM by the action of plasmin and plasmin-like enzymes, as found in experiments using mice deficient in urokinase or in plasminogen activator (uPA), showing an impaired liver regeneration.

- Regulation of the proliferation/expansion phase

Progression of primed/competent hepatocytes through G1 and subsequent replicative cycling is dependent on hepatocyte growth factor (HGF) and transforming growth factor-alpha (TGF-α) signaling, after which the proliferation process seems to proceed autonomously under the control of cyclins and cyclin-dependent kinases. HGF is secreted as an inactive single chain protein (scHGF), proteolytically cleaved at the Arg–Val–Val site to form the active two-chain HGF (tcHGF). The HGF receptor, c-met, can bind both forms, but is only activated by tcHGF. Cleavage is accomplished by uPA, tissue-type plasminogen activator, coagulation factor XII a, and hepatocyte growth factor activator (HGFA). The activity of HGFA is itself regulated by two Kunitz type serine protease inhibitors, HGFA inhibitor type 1(HAI-1) and type 2 (HAI-2), having different roles.

- What is the cellular source of HGF?

Expression of HGF has been detected in HSC, hepatic macrophages and in sinusoidal endothelial cells. This suggests that an important hepatocyte growth response derives from a distinctive interaction between parenchymal and non-parenchymal cells. In addition to HGF and TGF- alpha, other HGFs have been identified, including hepassocin, augmenter of liver regeneration (ALR), a mammalian FAD-dependent sulfhydryl oxidase, epithelial growth factor (EGF), heparin-binding epidermal growth factor-like growth factor (HB-EGF) and lal-1 (liver annexin like-1), all of which are not further discussed here.

Cell cycle progression itself is regulated by cyclin expression and activation of cyclin-dependent kinases (CDKs). For example, S phase entry and progression critically depends on the formation of cyclin E-CDK2 and cyclin A-CDK2 complexes. Cyclin B associates with CDK1 (Cdc2) to achieve progression from G2 to M. CDK-mediated cell cycle progression is modulated by CDK inhibitors (CDKIs). Finally, the G2/M transition in hepatocytes is regulated by a cell cycle-dependent nuclear protein, citron kinase as a down-stream target of Rho-GTPase, and citron kinase loss in knockouts induces apoptosis in a subset of cells.

- Termination

Subsequent to the expansion phase, the growth response must finally be terminated. Major factors involved in the termination response comprise TGF-beta and the activins.TGF-beta and activin regulate hepatic organ mass and tonically inhibit DNA synthesis in hepatocytes. The significance of TGF-beta1 in decreasing hepatocyte growth is shown in rats expressing a truncated type II TGF-beta receptor, enhancing hepatocyte regeneration after liver injury. The cation-independent mannose 6-phosphate receptor (CIMPR) is over expressed in hepatocytes during regeneration. Induction of its gene occurs in mid G1 phase, and CIMPR mediates latent pro TGF-beta activation, thus acting by targeting TGF-beta to hepatocytes during the termination response of regeneration. The action of TGF-beta itself is mediated by Smads serving as intracellular signals in this pathway. In addition to an inhibition of hepatocyte proliferation, TGF-beta1 also induces hepatocyte apoptosis by a c-Jun-independent mechanism, and this effect may contribute to the termination response.

Activin A (the homodimer of the inhibin beta A (chain) and follistatin (an activin-binding protein) inhibit and promote hepatocyte proliferation, respectively. Activin A is an autocrine inhibitor of initiation of hepatocyte DNA synthesis. It can also induce a reduction of liver mass, and promotes apoptosis in hepatocytes, blocked by follistatin. This response is dependent on activin receptors and on Smad2 protein relocated to the nucleus. Activins and their receptors and follistatin exhibit a distinctive timely expression pattern during regeneration. After rat liver injury or PH, hepatocyte activin A receptors are down-regulated by 24 h and normalize by 72 h, a phenomenon possibly involved in rendering hepatocytes responsive to mitogenic stimuli. [6]

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