Nephrol Dial Transplant (2004) 19 [Suppl 4]: iv6–iv10
doi:10.1093/ndt/gfh1034

Nephrology
Dialysis
Transplantation

Regulation of liver regeneration

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Abstract

Background. Hepatocyte regeneration proceeds along a sequence of distinctive phases and results in a precise reconstitution of the lost tissue mass. The molecular mechanisms regulating liver regeneration have recently been elucidated.

Methods. Important aspects of the regulatory steps of hepatocyte proliferation during regeneration are summarized.

Results. Hepatocytes normally remain in proliferative quiescence. Regeneration requires priming of hepatocytes to achieve competence for proliferation. This is the initiation phase, which is regulated by cytokines, including interleukin-6 and tumour necrosis factor-alpha. During the subsequent proliferation, the hepatocyte population is expanded (the expansion phase), it being chiefly regulated by hepatocyte growth factor and transforming growth factor-alpha. Upon sensing of the required cell mass, the proliferation response is terminated (termination phase) and is mainly mediated by transforming growth factor-beta and activins. These structured processes go in line with a remodelling response, resulting in the reconstruction of a vascularized liver lobule.

Conclusions. Liver regeneration proceeds in a highly ordered fashion, critical steps being regulated by several molecular mechanisms acting in a characteristic timely sequence.

Keywords: hepatocyte proliferation; hepatocyte regeneration; initiation; liver regeneration; regeneration regulation; termination

Introduction

The liver maintains a functional cell mass consisting of several specialized cell types, of which the hepatocytes form the major part. Hepatocytes are not terminally differentiated, rather, they are in proliferative quiescence (the G_0 phase), but can rapidly enter a cell division cycle upon stimulation. Hepatocyte regeneration proceeds along a sequence of distinctive phases: an initiation or priming phase, rendering hepatocytes in a state of replicative competence; a proliferation phase, where expansion of the entire hepatocyte population takes place; and a termination phase, where cell proliferation is suppressed to terminate regeneration at a defined set point. This classification does not mean that we are dealing with three more or less independent events; rather, the three phases are linked, and they share several mechanisms. In addition, proliferation in the expansion phase subsequently requires a complex re-design of the lobule, a remodelling process representing a 'fourth phase' of regeneration.

Liver regeneration: the timely sequence of morphological events

Hepatocyte proliferation starts after an interval of ~24 h, reflecting the priming of G_0 cells and the shift from G_0 to the G_1/S checkpoint. Proliferation starts in the periportal zone 1 and extends to zone 3 by 36–48 h. Proliferation of non-parenchymal cells lags behind, the delay amounting to 24–48 h or even later. After ~3 days, most hepatocytes proliferate (expansion), and this phase is associated with the formation of hepatocyte accumulations of eight to 10 cells (clusters) arranged around immature or disintegrating vascular channels. In fact, cluster formation is associated with the effacement of the normal sinusoidal network, accompanied by at least a partial loss of a typical space of Disse and a degradation of extracellular matrix (ECM) through metalloproteinases [1]. Clusters are depleted of mature hepatic stellate cells (HSC), possibly also related to an incomplete Disse space (own observations), and suggesting that hepatocytes might transiently engage in some sort of autonomous proliferation, related to the lack of locally produced stop signals. By 48 h post-partial hepatectomy (PH),

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insinuation of small vessels ensues (resinusoidalization), associated with resynthesis of ECM proteins, the reconstitution of a space of Disse, and the repopulation of this space with regenerating HSC. The resulting parenchymal unit is oversized, and the final achievement of a normal lobule requires termination and remodelling mechanisms.

The molecular regulation of the initiation phase

During initiation, hepatocytes are primed (rendered competent) for subsequent replication.

Initiation factors comprise interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-alpha). Following IL-6 binding to the gp130 receptor, activation of STAT3 and C/EBPbeta/nuclearfactor-IL-6 (NF-IL-6) takes place, TNF-alpha and IL-6 triggering the G_0/G_1 transition of the cell cycle. Following IL-6 binding to the gp130 receptor, activation of STAT3 and C/EBPbeta/nuclear factor-IL6 (NF-IL-6) take place. Signal transducer and activator of transcription (STAT) family proteins are transcription factors critical in mediating cytokine signalling, 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-alpha and IL-6 trigger the G_0/G_1 transition of the cell cycle. IL-6-deficient and TNFR-1-deficient animals fail to accomplish initiation and a hepatocyte regenerative response [2]. The IL-6-mediated pathway is further illustrated by C/EBPbeta/NF-IL-6 knockout mice, showing a blunted regeneration. C/EBPbeta controls the G_1/S checkpoint. IL-6 modulates the action of growth factors. Transgenic mice overexpressing IL-6 are growth-retarded and show increased suppressors of cytokine signalling in the liver, inhibiting the activation of STAT by growth hormone, IL-6, therefore, blunting hepatic growth hormone signalling [3]. 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 signalling operates rather via activation of protective pathways enabling hepatocyte proliferation [4].

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 (Pparalpha) induces the IL-6R; Pparalpha null mice lack IL-6R gene induction, and show delayed liver regeneration [5]. C/EBPbeta/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 [6].

Are there other priming factors? Rodents with dysfunctional leptin signalling exhibit a profound impairment of liver regeneration. Leptin-deficient ob/ob mice show an exaggerated activation of NF-kappaB and STAT3 during the initiation/priming phase, but an abrogation of TNF-alpha and IL-6 release at the time of the G_1/S transition, an effect correctable by leptin administration [7].

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 [8]. 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 G_0 to G_1 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 [9]. 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 subtilase, 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 G_1 and subsequent replicative cycling is dependent on hepatocyte growth factor (HGF) and transforming growth factor-alpha (TGF-alpha) signalling, 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 XIIa, 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 [10]. 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 sulphydryl 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 G_2 to M. CDK-mediated cell cycle progression is modulated by CDK inhibitors (CDKIs). Finally, the G_2/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 [11].

The remodelling of the growing lobule

What are the mechanisms involved in the correction of a growth overshoot? It appears that superfluous hepatocytes are eliminated through apoptosis. Interestingly, TNF-alpha not only induces regeneration, but also apoptosis. TNF-alpha-induced hepatocyte apoptosis is modulated by acidic sphingomyelinase (ASMase), as seen in ASMase knockout mice, by promoting the mitochondrial targeting of glycosphingolipids [12]. Furthermore, hepatocyte cytokeratins/-keratins (K) have an impact on cell survival. The K8/K18 system plays a significant role in providing resistance to stress and apoptosis in hepatocytes and in preserving hepatocyte integrity. K8/K18 provides resistance to Fas-mediated apoptosis through a modulation of Fas targeting to the cell surface. K18 is a caspase substrate, and transgenic mice overexpressing mutant human K18 develop hepatocyte fragility via keratin filament disruption, which predisposes hepatocytes to Fas- but not TNF-mediated apoptosis [13]. The mechanism apparently involved is that K18 may sequester TNFR1-associated death domain protein (TRADD) to attenuate interactions between TRADD and activated TNFR1.

The balance between apoptosis and hepatocyte survival is critical for appropriate remodelling of future fully regenerated lobules. Based on IGFBP-1-deficient mice it has been shown that IGFBP-1 functions as a hepatic survival factor counteracting the transforming growth factor-beta 1 (TGF-beta1) proapoptotic signal. Protection against caspase-8-mediated hepatocyte apoptosis is also accomplished by senescence marker protein-30 (SMP30), as based on studies with SMP30−/− mutant mice. A potent anti-apoptotic agent against TNF-alpha-mediated liver cell loss is the TNF superfamily member LIGHT binding to lymphotoxin-beta receptor, inhibiting caspase-3 processing on the apoptotic protease cascade.

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 [14]. The cation-independent mannose 6-phosphate receptor (CIMPR) is overexpressed in hepatocytes during regeneration. Induction of its gene occurs in mid G_1 phase, and CIMPR mediates latent proTGF-beta activation, thus acting by targeting TGF-beta to hepatocytes during the termination response of regeneration [15]. 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 betaA chain) and follistatin (an activin-binding protein) inhibit and promote hepatocyte proliferation, respectively. Activin A is an autocrine inihibitor 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 [16], a phenomenon possibly involved in rendering hepatocytes responsive to mitogenic stimuli.

How is the termination factor system inactivated during the expansion phase of regeneration? Recent results demonstrate that two transcriptional repressors of the TGF-beta/Smad pathway, SnoN and Ski, are up-regulated during regeneration, and they antagonize

TGF-beta-mediated termination via binding to Smad proteins [17].

Termination of hepatocyte regeneration requires, and goes in parallel with, the restauration of the normal sinusoidal and littoral phenotypes and the repopulation by HSC

In parallel to the later stages of hepatocyte regeneration and to the termination response, insinuation of vascular channels (the later sinusoids; resinusoidalization), formation of the perisinusoidal space of Disse, re-equipment of Disse's space by HSC, and the re-synthesis of the perisinusoidal ECM take place. These are processes that are crucial not only for the reconstruction of the regenerated lobule by remodelling, but also for the termination of regeneration as such, because sinusoidal and littoral cells are the principal sources of termination signals.

Resinusoidalization requires endothelial cell proliferation, exerting an important influence on hepatic regeneration. The role of endothelial cells increasing in number during resinusoidalization is underlined by the recent finding that mice administered circulating VEGF-A had enlarged livers due to increased proliferation of hepatocytes and non-parenchymal cells, and the sinusoidal endothelial cell-derived paracrine mediators promoting growth were IL-6 and HGF [18]. Therefore, it can be assumed that the shaping of the future lobule also critically depends on a close interaction between vascular endothelial cells and hepatocytes.

The repopulation of the developing liver lobule by HSC and their differentiated offspring, in particular their homing to the newly formed Disse space in the phase of resinusoidalization, depends on proliferation and migration of these cells. HSC show a migration response to PDGF-BB, to EGF, and to TGF-beta1, a key molecule of termination, augmenting the chemoinvasive response of HSC to PDGF and bFGF. The appropriate number of stellate cells after a proliferative surge is controlled by apoptosis. TGF-beta1 and IFN-alpha downregulate apoptosis in stellate cells, whereas CD95/CD95L and p53 protein promote apoptosis. The mechanisms involved include the TRAIL receptor-2/death receptor-5 expressed on activated stellate cells [19].

A resynthesis of the previously degraded perisinusoidal ECM is required for full regeneration, and the ECM is synthesized by HSC transiently differentiated to myofibroblasts. This is illustrated by mice deficient in the Forkhead Box (Fox) f1 transcription factor, showing defective stellate cell activation and abnormal liver regeneration associated with aberrantly elevated TGF-beta1 expression [20]. The importance of only a transient and well controlled ECM increase is documented by observations obtained with mice bearing a mutated collagen-I gene conferring collagenase resistance (r/r mice); these animals exhibit a persistent activation and a reduced apoptosis of stellate cells, a failure of ECM degradation, and a diminished hepatocyte regeneration [21]. Epithelial cells require contact with ECM to inhibit detachment-induced apoptosis (anoikis), and the reconstitution of a perisinusoidal ECM will therefore stabilize the newly formed hepatocyte population. The ERK and PI-3K/Akt signalling pathways have been identified to inhibit anoikis [68]. In the context of the relationship between cell loss and ECM formation it is of interest to note that human HSC can engulf hepatocyte apoptotic bodies followed by up-regulation of collagen alpha (I) mRNA in a protein kinase-dependent pathway [22].

The final construction of the liver lobule also depends on the function of ductules and peribiliary stellate/myofibroblastic cells, ductular/myofibroblastic 'units' probably serving as pacemakers of remodelling, similar to processes occurring in pancreatic regeneration [23].

Conflict of interest statement. None declared.

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