Review

Signals and Cells Involved in Regulating Liver Regeneration

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Abstract: Liver regeneration is a complex phenomenon aimed at maintaining a constant liver mass in the event of injury resulting in loss of hepatic parenchyma. Partial hepatectomy is followed by a series of events involving multiple signaling pathways controlled by mitogenic growth factors (HGF, EGF) and their receptors (MET and EGFR). In addition multiple cytokines and other signaling molecules contribute to the orchestration of a signal which drives hepatocytes into DNA synthesis. The other cell types of the liver receive and transmit to hepatocytes complex signals so that, in the end of the regenerative process, complete hepatic tissue is assembled and regeneration is terminated at the proper time and at the right liver size. If hepatocytes fail to participate in this process, the biliary compartment is mobilized to generate populations of progenitor cells which transdifferentiate into hepatocytes and restore liver size.

1. Introduction

1.1. Overview

The liver is a multi-functional organ that controls key physiological processes. These include nutrient processing following intestinal absorption, waste processing and excretion (urea cycle and bile synthesis), detoxification of xenobiotics, energy and nutrient storage and regulation, production of serum

proteins (coagulation factors, oncotic proteins, carrier proteins) and hormones (thrombopoietin [1], IGF1), as well as other functions.

The ability of the liver to carry out these normal duties is so essential that liver mass is maintained within a very narrow range in relation to the overall body mass. If there is loss or gain of liver mass, such as through liver injury or pregnancy, respectively, compensatory proliferation or apoptosis of cells allow restoration of original liver/body mass ratio once the stimulus is removed. The term "hepatostat" has been coined to describe this unique homeostatic relationship [2]. When the hepatostat is derailed and loss of liver function due to parenchymal injury falls below a critical point (build-up of toxic metabolites, inability to maintain glucose levels and blood pressure, along with coagulopathy), multi-organ failure and death follow.

The robust programmed proliferative response to loss of parenchymal function is widely known as "liver regeneration". A more accurate description may be "compensatory hyperplasia and hypertrophy", as resection of the liver does not induce spatial replacement of the part of the organ that was lost. Instead, the cells in the remaining portion proliferate and/or increase in size to restore the original liver mass [3]. Repopulation of the liver can be achieved via one of two mechanisms: (1) self-replication of individual cell types, or (2) transdifferentiation from facultative stem cells, or liver progenitor cells (LPC). In this review, we use the term "liver regeneration" to refer to restoration of the liver parenchyma by either of these processes.

Management of chronic liver injury and its sequelae are growing health care burdens worldwide [4]; knowledge of the principles and cellular compartments governing successful restoration of liver function after insult is the key to discovering therapeutic strategies applicable to human hepatic disease. This review highlights the molecular and cellular inputs necessary to complete liver regeneration after loss of parenchyma.
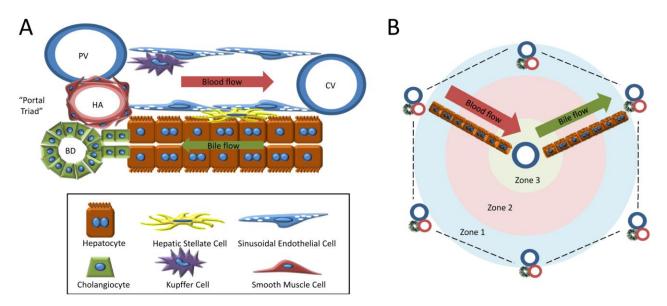
1.2. Cell Types of the Liver

The main unique cell types of the liver are hepatocytes, cholangiocytes (or biliary epithelial cells), Kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells. Hepatocytes are organized into cords that line an intricate, specialized capillary bed lined with fenestrated endothelial cells (Figure 1a). The vascular network is organized into a system that allows for unidirectional flow from branches of the inflow vascular supply, the portal vein and the hepatic artery, through the sinusoids to the central veins, which coalesce into the hepatic vein and connect to the inferior vena cava. The portal vein and hepatic artery are found in cluster along with a collecting bile duct, collectively referred to as the "portal triad" and spaced out at the corners of a roughly hexagonal unit ("the hepatic lobule", Figure 1b) that is repeated throughout the liver tissue. The lobule can be divided into three general zones: periportal (zone 1), pericentral (zone 3), and transitional (zone 2). The hepatocytes in Zone 1 to Zone 3 are exposed to increasing concentrations of processed xenobiotics/toxins and decreasing concentrations of oxygen (Figure 1b).

The hepatocyte is the parenchymal cell of the liver, performing all the essential functions of the organ described in the section above. In addition, the hepatocytes produce bile, secreted from the apical membrane into the bile canaliculi that run between hepatocytes and merge to form bile ducts, which are lined with cholangiocytes. Cholangiocytes and hepatocytes share a common precursor cell,

the hepatoblast, in development. This common lineage is attributed for the ability of cholangiocytes and hepatocytes to transdifferentiate in the setting of injury where one or the other cannot replicate sufficiently to replace its own cell compartment. The "non-parenchymal cell" population includes sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells. Sinusoidal endothelial cells are fenestrated to allow passage of macromolecules and lipoproteins in and out of the hepatocytes. Kupffer cells are resident liver macrophages, which along with other liver-associated immune cells play important roles in immune tolerance of the liver [5]. The hepatic stellate cell is a multifunctional cell of mesenchymal origin found to have roles in immune function, vitamin storage, matrix turnover, growth factor secretion, vascular tone, and perhaps progenitor cell niche. During injury, hepatic stellate cells become "activated" and transform into myofibroblast-like cells, depositing extracellular matrix and becoming contractile [6].

Figure 1. Architecture of the liver. **(a)** Schematic depicting the architecture and cell type composition of the hepatic sinusoid. Blood enters the lobule from branches of the portal vein (PV) and hepatic artery (HA) and progresses through the sinusoidal capillaries, collecting at the central veins (CV). Bile flows in the opposite direction towards the portal triad and exits through the bile ducts (BD). Hepatocytes are lined up along the sinusoids in "plates" 1-2 hepatocytes thick. Hepatic stellate cells reside in between the sinusoidal endothelial cells and hepatocytes in the "space of Disse". **(b)** Diagram illustrating the organization of the hepatic lobule, including vascular structures and relative zonality of the lobule. This unit is repeated throughout the liver. Representative plates of hepatocytes are shown for orientation; these rows of hepatocytes would fill the entire lobule and be lined with sinusoids.

During liver regeneration, whether following surgical resection or parenchymal injury and loss, all of the cell types of the liver can proliferate to replace their own cell population. When unable to do so, however, there are internal or external (e.g., hematopoietic) sources of progenitor cells that are able to differentiate into the various cells of the liver to restore cell compartments. These processes will be discussed in more detail below.

1.3. Models of Liver Regeneration and Growth

The two-thirds partial hepatectomy (PHx) model was first described in 1931 [7] and remains one of the most widely used models of liver regeneration. The rodent liver is multi-lobular, the two largest of which approximates 70% parenchymal mass. When these two lobes are excised via a straightforward surgical procedure [8], the cells of the remaining lobes restore liver mass over the course of 1-2 weeks. There are two key advantages to this approach: 1) the model is easily scalable, allowing investigators to study phenomena associated with minor (~30% PHx) to severe (~90% PHx) parenchymal loss simply by removing one less or one more lobe of the liver, and 2) because there is no injury to the remaining hepatocytes, PHx provides a "clean" model in which to study the timing and extent of contribution of different variables, which has been less optimally studied in injury models using hepatotoxins that are associated with necrosis/inflammation. However, the main drawback to this approach is the limited applicability of the PHx model for interpreting the dynamics of regeneration in human disease, which often involves components of hepatocyte death and inflammation. In addition, PHx alone does not require LPCs for successful regeneration, and thus is not a useful model to study LPC-mediated regeneration.

In comparison, several chemical injury models also exist. These cause hepatocyte injury and death, which activates an inflammatory response in addition to a regenerative response. One commonly used class of agents causes hepatocyte injury and death selectively in the pericentral zone (Zone 3); these include carbon tetrachloride [9,10] and acetaminophen [11–13]. They require metabolic activation by cytochrome P450 enzymes, a process which often generates hepatocyte-toxic free radicals. The CYP-expressing hepatocytes die first, creating a centrilobular distribution of injury and death. Allyl alcohol administration has been used to induce acute periportal injury [14]. There are two main advantages in using a hepatotoxin model to study regenerative response after parenchymal injury: (1) it more closely approximates the regenerative response that occurs in common human hepatic diseases, including the damage and inflammatory infiltrate, and (2) unlike PHx [15], there are few surgical complications with repeated administrations, so it is easy to extend the acute injury model to chronic injury and cirrhosis. An emerging concept from the hepatic toxicology field is the "progression of injury": primary injured/necrotic hepatocytes release phospholipases [16,17] and proteases [18] into the extracellular space, which then injure neighboring hepatocytes and delay/inhibit their ability to undergo normal regeneration. It is likely these processes occur in a variety of acute and chronic human liver diseases and intoxications, where there is also *in situ* hepatocyte injury and death. Therefore, despite the relative lack of clarity about cellular origins of signals occurring as a result of chemical injury models, the importance of synthesizing our understanding of ideal liver regeneration from PHx with data from chemical injury models cannot be understated. It may be the key to future understanding of the limitations of current interventions and to finding more suitable therapeutic targets for human disease and acute intoxication. These topics, however, are outside the scope of this review, and the reader is referred to other reviews discussing hepatic injury progression in chemical intoxication models for more detail [19,20].

A final model of liver growth is augmentative hepatomegaly (also known as "direct hyperplasia"), in which liver is stimulated to grow to a supraphysiological mass by growth factors, hormones [21–24], or xenobiotics. There are two classes of xenobiotics commonly used in this experimental model:

peroxisome proliferator-activated receptor (PPAR) family agonists [25] and *constitutive androstane receptor* (CAR) agonists [26]. Continuous administration of these chemicals induces liver growth until a new equilibrium is reached, which is different for each chemical. Upon removal of the hormone or xenobiotic treatment, however, the liver shrinks back to the original mass through hepatocyte apoptosis [27–29]. These phenomena suggest that the innate hepatostat is disrupted or readjusted in response to these xenobiotics, returning to normal when the chemical is removed, although it is not clear which pathways are relevant for this purpose. Due to the dependence on specific nuclear receptor pathways, there are some key differences between augmentative hepatomegaly and normal liver regeneration (compensatory hyperplasia) [30]. However, it has been shown that the same genetic alterations that enhance [31] or suppress [32] compensatory hyperplasia can also enhance [33,34] or suppress [35] augmentative hepatomegaly, so lessons learned from the augmentative hepatomegaly model may bear relevance to enhancing compensatory liver regeneration. There are scenarios where augmentative hepatomegaly is clinically relevant: (1) elevated estrogens during pregnancy are thought to increase liver weight to meet increased metabolic needs, (2) several prescribed drugs, such as phenobarbital [36], phenytoin, and diazepam, can bind to CAR and sensitize the patient to acetaminophen toxicity.

For consistency in interpretation, the signals and cellular dynamics to be covered in the main portion of this review focus on the extensive body of knowledge amassed from PHx studies in rodents.

2. Molecular Signals During Liver Regeneration after PHx

What initiates liver regeneration after PHx? Immediately upon removal of two-thirds of the liver in the standard PHx model, the entire hepatic vascular influx is forced to perfuse through only one-third of the original capillary bed. Consequently, there is an increase in portal and capillary pressure, as well as an increased availability of circulating growth factors and hormones. While studies are lacking in how the mechanical forces can directly influence gene expression during liver regeneration after PHx, there have been many observations that early changes in circulating factors, cell-cell and cell-matrix interaction, as well as intracellular signaling cascades dictate the kinetics of regeneration.

If portal circulation is maintained at normal pressures after PHx in rats using a portacaval shunt, there is an absence of hepatocyte hypertrophy and decreased protection against apoptosis compared to control PHx; these effects are attributed to lack of hepatocyte growth factor (HGF) activation [37]. Indeed, even the tonic maintenance of the hepatostat may be regulated in part by availability of circulating factors; in the absence of PHx, complete shunt of portal flow to the vena cava induces liver atrophy to one half of the original size [38]. In this portacaval shunt model, exogenously infused growth factors such as insulin, transforming growth factor-α (TGF-α), and HGF exhibited direct hepatotrophic effects, highlighting the importance of circulating growth factors in initiating hepatocyte growth and proliferation after a stimulus as well as maintenance of baseline liver size.

Extracellular matrix reorganization is another early feature after PHx that has profound effects on initiation of liver regeneration by releasing locally available latent growth factors. Urokinase-type plasminogen activator (uPA) activity increases within one minute of PHx, initiating a matrix remodeling cascade via plasminogen activation (within 15 minutes of PHx), which activates key

metalloproteinases (MMP) such as MMP-9 [39,40]. Importantly, uPA activates HGF in a manner utilizing its receptor uPAR, which starts increasing within one minute of PHx [41–43].

Changes in cell junction proteins and related signaling are also early features of regeneration. One example is the transmembrane protein Notch. Its intracellular domain, cleaved when Notch is activated by transmembrane ligands existing on neighboring cells, migrates to the nucleus and initiates transcription within 15 minutes of PHx [44]. Another example is β-catenin, a protein that promotes cell-cycle progression by complexing with the Tcf transcription factor family to activate target genes. β-catenin translocates to the nucleus within 5 minutes of PHx and remains elevated in the nucleus over 24 hours [45,46].

The early events described above are illustrative of the broad changes that occur in the liver after PHx. These and innumerable other factors have been examined in various models of liver regeneration, raising the question of whether all factors found in reductionist animal studies actually play physiologically-relevant roles in liver regeneration. Loss of even the most potent growth factors (HGF, EGFR ligands) does not completely arrest regeneration; instead, delays of various lengths are seen to complete regeneration. High-throughput studies have been designed to address this question [47–49] and literature reviews have synthesized current knowledge into schemas to better understand the redundancy that occurs between metabolic, growth factor, and cytokine effects [50,51]. Below, we will explore in more depth a select cohort of intra- and extracellular signals shown to play roles in liver regeneration using the PHx model.

2.1. Hepatocyte Growth Factor (HGF)

HGF is one of only two described "complete mitogens" for hepatocytes (defined by its ability to induce DNA synthesis of primary hepatocytes in a chemically defined serum-free medium *in vitro* and liver enlargement when administered *in vivo*). It was one of the first isolated and studied circulating factors found to promote liver regeneration [52–55]. HGF binds to and activates the tyrosine receptor kinase MET, a multifunctional receptor involved in a number of cellular processes, such as proliferation, growth, survival, and metabolism [56–58]. Decrease of MET expression and activation using RNA interference (shRNA) is sufficient to inhibit mitoses and increase apoptosis related genes at 24 hours post-PHx, normally the peak of proliferation in the rat [59]. The mitogenic activity of HGF-initiated signaling requires the function of the transcription factor C/EBPβ [60].

HGF is utilized during liver regeneration after PHx in a biphasic manner. In the resting liver, HGF (inactive) is stored in the extracellular matrix of the liver, particularly in the connective tissue surrounding portal triads [61,62]. After PHx, the first wave of MET activation occurs within 30 minutes and peaks at 60 minutes [63]. This first round of HGF/MET signaling is thought to be derived from endogenously present HGF, as levels of both inactive and active HGF in the liver tissue decrease from baseline levels during the first three hours after PHx [64]. Early extracellular matrix remodeling events in the liver [39] allow the matrix-bound HGF to be activated and utilized, and some is released into the circulation as well [65]. The second phase of HGF utilization, this time from newly-synthesized HGF by stellate cells and endothelial cells [66–68], begins at three hours post-PHx and peaks at 24 hours after PHx [64,69].

Extrahepatic sources of HGF, such as platelets [53] and other organs (lung, kidney, spleen) [70,71], may also contribute to the pool of available HGF during liver regeneration, although the relative importance of these sources is not known. Systemic induction of HGF expression after PHx by different organs, including liver, may be a response to increases in circulating norepinephrine [72] or insulin-like growth factor [73], which have been shown to stimulate HGF mRNA transcription.

2.2. Epidermal Growth Factor Receptor Ligands

Ligands of the epidermal growth factor receptor (EGFR, or ErbB1) comprise the only other "complete mitogens" known besides HGF. Although there are other known ligands, the EGFR ligands studied in the context of liver regeneration to date are as follows: epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α), amphiregulin, and heparin binding-EGF-like growth factor (HB-EGF). TGF-α, amphiregulin, and HB-EGF are synthesized as transmembrane-anchored precursors that are then processed by extracellular proteases to their mature secreted forms.

EGF is mitogenic to hepatocytes, both in culture [74] and in the resting liver in rats when infused exogenously [75]. Although EGF is produced by multiple tissues, the two shown most relevant to liver regeneration include the Brunner's glands in the duodenum and salivary glands. The Brunner's glands provide a tonic supply of EGF via the portal circulation to the liver, where it is taken up and sequestered in the portal triad areas [76,77]. Increased circulating norephinephrine, as is seen after PHx, can augment Brunner's glands secretion of EGF [78]. Sialadenectomized rats exhibit severely blunted liver regeneration post-PHx, which can be rescued by exogenous EGF [79].

TGF-α is a more potent hepatocyte mitogen than EGF; this is thought to be mediated through differential ligand-receptor complex processing [80]. It is produced by hepatocytes during regeneration [81] and cleaved by specific proteases into the active growth factor [82,83]. In this way, it perhaps functions in an autocrine [84] or paracrine manner to contribute to liver regeneration. However, TGF-α null mice have no deficiency in liver regeneration [85].

Similar to TGF-α, amphiregulin is also produced by hepatocytes after PHx, and in culture its expression is regulated by interleukin-1β (IL-1β) and prostaglandin E2 [86,87]. Interestingly, the same study reports that amphiregulin knockout mice have impaired liver regeneration post-PHx, in contrast to TGF-α knockout mice.

HB-EGF is synthesized by Kupffer cells and endothelial cells during liver regeneration [88]. It has been found to be a potent mitogen for rat hepatocytes in culture [89]. In mice, HB-EGF is expressed in two-thirds PHx (where there is significant hepatocyte proliferation) but not one-third PHx (where there is minimal hepatocyte proliferation). However, exogenous HB-EGF given after one-third PHx is able to induce DNA replication in hepatocytes to comparable levels as with the two-thirds PHx at 24 hours [90]. HB-EGF transgenic mice have accelerated liver regeneration [91], and mice lacking HB-EGF have delayed regeneration [90].

Activation of the EGFR, as denoted by tyrosine phosphorylation, is present at baseline due to the steady flow of EGF from the Brunner's glands, as discussed above. After PHx, though, activation increases above baseline with similar kinetics as MET activation; peak EGFR activation is observed at 60 minutes post-PHx [63]. Although other ErbB family receptors can be upregulated in compensation,

loss of EGFR through shRNA interference in rats decreases DNA replication post-PHx [92], and hepatocyte targeted gene deletion in mice have higher rates of mortality post-PHx [93].

2.3. Tumor Necrosis Factor (TNF)

TNF is a pro-inflammatory cytokine produced by macrophages that has been shown to have pleiotropic roles during liver regeneration. On one hand, TNF mediates hepatocyte apoptosis and liver failure in mice in a number of toxicity models [94–99]; many of these studies also demonstrate a protective effect of NF-κB activation against the cytotoxic effects of TNF. On the other hand, a number of studies have demonstrated that loss of TNF function delays liver regeneration, whether by using TNF neutralizing antibodies [100], or TNF receptor 1 deficient mice [101]. It is likely that TNF signaling on cells already "primed" to survive and enter into proliferation can promote and enhance the same pathways, such as Akt and NF-κB activation [96,102] in response to growth factors [103,104]. In the absence of such signals, TNF then promotes death pathways.

2.4. Interleukin-6 (IL-6)

IL-6 is a multifunctional cytokine involved in initiating and mediating the acute phase response by hepatocytes during inflammation and other homeostatic disturbances [105–107]. It is secreted by immune cells, non-parenchymal cells, and hepatocytes under certain conditions [105] and signals through the IL6R-gp130 complex [108,109]. Plasma concentration of IL-6 increases after PHx, and mice lacking IL-6 display delayed liver regeneration and liver injury associated with loss of STAT3 activation and decreased cell cycle progression proteins such as Cyclin D1 [110]. A single dose of IL-6 prior to PHx could rescue the IL-6 deficient mice from liver damage and restore near normal regenerative capacity. Despite this marked phenotype, IL-6 alone is not mitogenic to hepatocytes in culture and administration of IL-6 to normal animals does not induce hepatocyte proliferation.

One potentially important role of IL-6 in liver regeneration, not fully explored, is its regulation of HGF. Recent studies suggest a feedback loop exists between IL-6 and HGF. The HGF gene promoter contains IL-6 response elements [111] and HGF has been shown to be synthesized in response to IL-6 by non-parenchymal cells of the liver [112,113]. HGF signaling through MET prevents NF-κB nuclear accumulation and decreases IL-6 synthesis in a GSK3β-dependent manner in bone marrow-derived macrophages [114], showing that HGF, in addition to its mitogenic and anti-apoptotic roles, may be a key anti-inflammatory mediator in liver repair [115].

2.5. Transforming Growth Factor-β (TGF-β)

These growth factor/cytokines, a subgroup of a larger TGF superfamily of ligands, include TGF-β1, 2, and 3, and their receptors are the TGF-β receptors type I, II, and III. Ligand binding to the type II receptor promotes complex formation with the type I receptor to initiate signaling as a heterodimer receptor complex [116]. The majority of studies in liver regeneration have focused on signaling mediated by TGF-β1.

Hepatocytes express all three TGF-β receptors [117,118]. It has been shown that locally, all the non-parenchymal cell types are capable of expressing TGF-β1, with Kupffer cells and endothelial cells

expressing the most at baseline, and hepatic stellar cells being the major cellular source during inflammatory and fibrogenic conditions [119,120]. There are conflicting reports about whether hepatocytes can express TGF-β1 during regeneration [119,121,122]. HGF and EGF-induced signaling can promote TGF-β expression in organoid cultures [123]. Hepatocytes are exquisitely sensitive to the mitoinhibitory effects of TGF-β1 in culture [124,125] and *in vivo* [126].

At baseline, extracellular but latent TGF-β1 can be detected throughout the lobule of the liver [127]. After PHx, matrix-bound TGF-β1 becomes activated and released into the circulation; increases in concentration are detected within one hour of PHx [128]. A strong "wave" of mature TGF-β1 immunolocalization can be observed in the initial stages of regeneration after PHx (12-48 hours), progressing in a periportal to pericentral direction. Likewise, a wave of hepatocyte proliferation is observed to follow immediately adjacent to this line, in TGF-β1-negative regions [127]. Despite the large increase in TGF-β1 activation, hepatocytes isolated between 24-72 hours after PHx are resistant to the mitoinhibitory effects of TGF-β1 in culture [129]. This may be due to a combination of downregulated TGF-β receptor expression [118] and inactivation of mature TGF-β1 by α2-macroglobulin in the circulation [130]. TGF-β1 mRNA levels increase by 4 hours after PHx and reach maximal expression by 72 hours [131]. Genetic deletion of the TGF-β type II receptor in hepatocytes enhances hepatocyte proliferation after PHx in mice but does not prolong regeneration [132] (see section 4 below for more discussion on TGF-β in termination of regeneration). Loss of the *β-2* spectrin protein, an adaptor protein involved in the TGF-β signaling pathway, also increases markers of proliferation at 24 hours; however, by 48 hours, those markers were decreased coincident to elevations in markers of cell cycle arrest (p53, p21) and DNA damage [133]. Consequently, the pleiotropic roles of TGF-β1 in liver regeneration are not fully understood.

2.6. Neurotransmitters

Norepinephrine is produced by cells of the sympathetic nervous system, where it functions as a neurotransmitter, and by the adrenal medulla, from which it is secreted into the circulation as a stress hormone. Furthermore, hepatic stellate cells have been observed to produce norepinephrine [134]. Norepinephrine concentrations increase in the liver after PHx with similar kinetics as HGF [135]. Although it lacks mitogenicity in itself, norepinephrine stimulates the production and potentiates the mitogenic effects of HGF [65,72] and EGF [78,136,137], and it weakens the mitoinhibitory effects of TGF-β [125,129]. These effects are attributed to SMAD7 [138], NF-κB [138], and STAT3 [139] activation by norepinephrine. Chemical inhibitors of α-1 adrenergic receptors delay liver regeneration [135].

Serotonin is another neurotransmitter with noted effects on liver regeneration. Although not produced by platelets, it is stored and secreted by them to regulate hemostasis and inflammatory processes. Mice lacking platelets or tryptophan hydroxylase 1 (the rate-limiting enzyme for peripheral serotonin synthesis) have deficient proliferative response at 48 hours after PHx, which can be rescued by exogenous serotonin administration [140]. The same group reports that a serotonin receptor agonist can promote sinusoidal endothelial cell fenestration and increased hepatocyte proliferative response after PHx in a VEGF-dependent manner [141]. Since VEGF stimulates HGF production, it is therefore possible that serotonin indirectly promotes hepatocyte proliferation through increasing HGF levels, although this has not been measured. Indeed, the exact mechanisms of serotonin action are not well

understood. Rats lacking the serotonin transporter (SERT) are unable to store serotonin in their platelets and blood serotonin concentrations are only 1–6% of normal levels; paradoxically, they exhibit no proliferative defect after PHx [142]. It may be possible that even trace amounts of serotonin in the SERT -/- rats are sufficient to exert its effects after PHx, or there may be inherent differences in serotonin sensitivity between the mouse and the rat.

2.7. Bile Acids

Hepatocyte proliferation has been long noted as a histologic feature of human cholestatic conditions. Now there is increasing evidence that bile acids promote hepatocyte proliferation during regeneration. The concentration of bile increases in the blood within several hours after PHx, and depletion of bile or deficiency in the bile-responsive transcription factor FXR delays regeneration [143]. Bile acids also activate the receptors LXR [144] and TGR5 [145] to increase resistance against bile acid toxicity in hepatocytes and cholangiocytes, respectively.

2.8. Insulin and Other Metabolic Regulators

The liver processes and stores the three macronutrients (carbohydrate, protein, and lipid). Altered regulation of macronutrients is tightly orchestrated during liver regeneration to promote successful DNA synthesis and cell replication, and disruption of these compensatory mechanisms leads to impaired regeneration [146,147].

Insulin is a key regulatory hormone for liver homeostasis and function. Liver is the first target organ for freshly secreted insulin from the beta islet cells in the pancreas. Without insulin, primary hepatocytes cannot survive in culture [148] and are unable to respond to mitogens [137,149]. In the absence of PHx, portacaval shunt induces liver atrophy to about one-third of the original size; injection of insulin in this model is able to restore liver mass through hepatocyte proliferation [38]. However, insulin alone does not induce hepatocyte proliferation or growth of liver [150]. Further, after PHx, rodents become hypoglycemic and hypoinsulinemic; external supplementation of dextrose suppresses hepatocellular proliferation [151,152]. It is therefore likely that while basal insulin is important for normal hepatocyte homeostasis, insulin action during hepatic insufficiency counteracts the metabolic stimuli necessary to initiate hepatic regeneration (such as lipolysis, described below).

The availability of certain amino acids is a rate-limiting step for hepatic regeneration. Protein deprivation significantly delays DNA synthesis after PHx [153], while supplementation with branched chain amino acids (BCAA) promotes liver regeneration [154]. Interestingly, other amino acids have no effect or inhibit regeneration. The specific effects of BCAA supplementation can be attributed to stimulation of protein and nucleotide synthesis [146].

Systemic lipolysis and hepatic lipid accumulation (steatosis) are early cardinal features of liver regeneration [155,156]. In addition, DNA synthesis after PHx is enhanced by exogenous administration of long chain fatty acids or L-carnitine (an essential co-factor for transport of acyl groups into the mitochondria for β-oxidation) [157]. It is thought that the suppressive effects of dextrose on regeneration are mediated indirectly through suppression of peripheral free fatty acid release by insulin [158]. However, the exact functions of hepatic steatosis (energy source? signaling?) after PHx are not well elucidated [147].

2.9. Wnt Family Proteins/β-catenin

As mentioned above, β-catenin nuclear translocation is one of the earliest events after PHx. β-catenin is normally sequestered in complex with E-cadherin at tight junctions of hepatocytes or tagged for ubiquitination and degradation; however when Wnt family proteins become available after PHx and activate the frizzled receptors, β-catenin is stabilized and can accumulate in the nucleus [45]. Wnt ligands and frizzled receptors are expressed by various liver cell types, both parenchymal and non-parenchymal cells [159]. Tyrosine receptor kinases such as MET have also been found to promote β-catenin stabilization and translocation to the nucleus [160,161]. β-catenin gene knockdown in rats [162] or liver-specific conditional knockout in mice [163] suppresses proliferation after PHx and delays regeneration.

2.10. Hedgehog

Hedgehog (HH) ligands bind to the receptor Patched, dissociating it from Smoothened and allowing Smoothened to initiate intracellular signaling. Both Indian and Sonic HH are expressed in the liver by various cell types [164,165], although it is not clear which cells express Patched. Inhibition of HH signaling using cyclopamine in mice caused dramatic inhibition of cell proliferation at 48 hours post-PHx and almost 100% mortality by 72 hours, despite no difference in serum liver injury markers at 24 hours and 48 hours post-PHx. In addition, activation of hepatic stellate cells and bile duct proliferation was also suppressed [166]. Further work is needed to elucidate the mechanisms behind these results, but they reveal HH signaling to be a major driving force behind liver regeneration. Modulation of this pathway, such as inhibition by glypican-3 [167], could therefore be a molecular switch to terminate liver regeneration (see below for more discussion on glypican 3).

2.11. Fibroblast Growth Factors (FGFs)

Fibroblast growth factors (FGF) are a large family of proteins (23 known members and counting), for which there are four known receptors (FGFR1-4). All four are expressed by different cell types in the liver, but hepatocytes express FGFR4 exclusively [168]. FGF1, FGF2 and FGF7/KGF can stimulate moderate DNA synthesis in cultured hepatocytes [169,170]. Disruption of all FGFR signaling through expression of a dominant negative receptor in hepatocytes resulted in reduced proliferation at 48 hours post-PHx [171], highlighting a role for parenchymal FGF signaling in regeneration.

3. Contribution of cell types to successful liver regeneration

3.1. Resident Liver Cells

After PHx, a well-orchestrated set of cell replications occurs amongst the resident cells of the liver in order to replace the lost liver mass and function. First and foremost, the hepatocytes undergo cell proliferation, with the peak of proliferation being at approximately 24 hours in the rat [172] and approximately 36–42 hours in the mouse [8]. Cholangiocytes respond to the same mitogenic signals and start proliferating almost as early as hepatocytes, while non-parenchymal cells initiate DNA

synthesis at a slower pace, with Kupffer cells and stellate cells peaking at 48–72 hours and sinusoidal endothelial cells at 96 hours [172–174]. There is no evidence to suggest that there are increased numbers of portal triads or lobules after completion of regeneration, and consequently, each lobule increases in size.

Most hepatocytes participate in cell proliferation during regeneration to restore liver mass. In the two-thirds PHx model in younger rats, over 95% of hepatocytes undergo DNA synthesis, and this only decreases to about 75% even in older rats [175–177]. Exogenous infusion of mitogens can improve the proliferative response in older animals, indicating that age-related decreases in hepatocyte proliferation are not due to inherent inability to proliferate (senescence) but perhaps changes in the ability to respond to extracellular environment and signals [22,178]. The remarkable capacity of hepatocytes to undergo innumerable proliferation cycles is demonstrated by successful regeneration after serial PHx (up to 12 documented [179]) and serial repopulation of small numbers of isolated hepatocytes into host animal livers with impaired native cells (calculated 69 doublings of each cell over six repopulations [180]). Indeed, during regeneration hepatocytes express markers of stem cells, or reprogramming factors, such as Oct4, Nanog, and KLF4 [181], lending even more credence to their self-renewal capabilities.

During the regenerative process, hepatocytes can secrete many growth factors to which non-parenchymal cells are responsive. These include TGF-α [84], FGFs [182], VEGF [67], and PDGF-A [183]. In turn, the non-parenchymal cells provide to hepatocytes many of the growth factors discussed in detail earlier in this review. New HGF is synthesized by stellate cells and endothelial cells [66–68]. EGF production increases from Brunner's glands in the duodenum, and HB-EGF is available from endothelial cells and macrophages [89]. Macrophages provide IL-6 and TNF-α. The secretion of growth factors by the different cell types to regulate hepatocyte proliferation during regeneration is summarized in Figure 2a.

Revascularization of the hepatic plates after proliferation occurs through a signaling "conversation" between hepatocytes and endothelial cells. Increases in expression of growth factor receptors on endothelial cells during regeneration, such as VEGF receptors Flk-1/KDR and Flt-1, allow them to be responsive to hepatocyte-derived VEGF [184]. The endothelial cells that surround avascular clumps of newly-replicated hepatocytes also selectively express the Angiopoietin receptor Tie-1 [184]. Activation of these VEGF and Angiopoietin receptors induces the endothelial cells to proliferate and invade in between the hepatocytes to form new sinusoids [173,185,186].
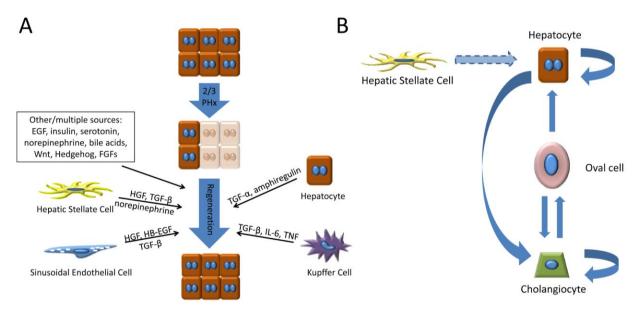
3.2. Progenitor Cells

3.2.1. Bone Marrow Derived Cells

Mobilization of bone marrow has been reported after PHx in rodents [187] and also in humans [188]. Circulating bone marrow-derived mesenchymal stem cells contribute to new sinusoidal endothelial cell repopulation and are a major source of newly synthesized HGF after PHx [189,190]. It has been a topic of discussion whether circulating bone marrow cells contribute to hepatocyte repopulation during regeneration. In experimental models in which hepatocyte proliferation was inhibited, hematopoietic lineage cells were able to repopulate a portion of the hepatocytes [191,192]. However, more recent

studies have suggested that the observations made from those experiments were in fact due to cell fusion events between the bone marrow hematopoietic stem cells and hepatocytes [193,194].

> **Figure 2.** Signals and cells contributing to cell repopulation during liver regeneration. **(a)** Upon loss of two-thirds of the liver mass after PHx, molecular signals from several cell types contribute to hepatocyte repopulation during regeneration. **(b)** Both hepatocytes and cholangiocytes undergo self-renewal in normal regeneration; however, transdifferentiation can occur via the relationships indicated. Dotted arrows indicate relationships based on newly emerging literature.

3.2.2. Oval/Progenitor Cells and Transdifferentiation

While there is still an ongoing search for conclusive proof of a resident liver stem cell population, a variety of studies have established that several liver cell types can act as facultative stem cells and transdifferentiate to replace the epithelial cell compartments during regeneration [195,196].

One of the best-described examples is the emergence of "oval cells" in experimental models (e.g., administration of the chemical AAF prior to PHx) and diseases where hepatocytes cannot proliferate [197–199]. Oval cells are named based on the shape of their nuclei, and they express cell markers of both hepatocytes and biliary cells as well as stem cell markers [200]; they can be induced to differentiate into either cell type (Figure 2b). They appear in the periportal areas, and pulse-chase labeling of these cells with tritiated thymidine in the AAF + PHx model indicate that over the course of regeneration, they acquire hepatocyte-associated markers and phenotypic characteristics [197]. Many of the growth factors discussed earlier in this review have been implicated in oval cell differentiation into hepatocytes, such as TGF-α, HGF, TGF-β, and Notch [201–204], although some oval-cell specific signaling pathways have been identified [205,206]. Some disagreement exists about the origins of oval cells; however, it is likely that they derive from the biliary cell compartment for the following reasons: (1) most "oval cell markers" are shared markers with biliary cells (cholangiocytes), (2) in situations where they appear, such as massive hepatocyte necrosis of the human liver, they emanate from and cluster around the portal tract [207], (3) early in oval cell-inducing experimental protocols, hepatocyte

markers (e.g., HNF4) appear in the nuclei of biliary cells of intact bile ducts prior to the appearance of oval cells, which also express these hepatocyte markers [208], and (4) toxin-mediated damage to the bile ducts prior to the AAF + PHx protocol can completely prevent the appearance of oval cells [209]. A proliferative biliary response, termed "ductular reaction", is seen in many human liver disease conditions in which there is attrition of hepatocytes, it is likely that oval cell-mediated regeneration is relevant to human liver regeneration [210].

Hepatocytes have also been noted to possess transdifferentiation capabilities under certain conditions, particularly hepatocytes immediately proximal to the portal tract [195]. In experiments where hepatocytes positive for the DPPIV marker were injected into DPPIV-negative rats that had been subjected to PHx and retrorsine intoxication, the regenerated chimeric liver possessed no DPPIV-positive bile ducts unless a biliary proliferative stimulus (e.g., bile duct ligation) also accompanied the procedure. In the combined hepatocyte injury and biliary proliferation scenario, approximately 1.5% of the bile ducts became DPPIV-positive. If the ability of the native cholangiocytes to proliferate was inhibited by the biliary-specific toxin methylene dianiline (DAPM), the number of DPPIV-positive bile ducts increased to nearly 50% after bile duct ligation + PHx/retrorsine [211], demonstrating the capacity of DPPIV-positive hepatocytes to participate in repopulation of the biliary cell compartment.

Emerging evidence points to a stem cell niche in the hepatic stellate cells of the liver [212]. One lineage tracing study used GFP to label cells that expressed GFAP (a stellate cell marker) before subjecting mice to a diet-based model of liver injury and oval cell activation. After the injury, GFP-positive cells lost stellate cell markers and acquired stem/oval cell markers. These transitional cells disappeared as GFP-positive hepatocytes emerged [213].

4. Termination of Liver Regeneration

The processes governing the termination of liver regeneration are not as well studied or understood. Below we examine a few of the signaling pathways found to play a role in organ size regulation at the termination of liver regeneration.

4.1. Integrin-Linked Kinase (ILK)

As discussed earlier, one of the initiating events in regeneration after PHx is the remodeling of the extracellular matrix. Activation of metalloproteinases leads to the breakdown and release of proteins such as glysoaminoglycans, latent growth factors (HGF, TGF-β), and collagens. Later, when regeneration is nearing completion, new extracellular matrix is synthesized to re-establish the organ architecture. These processes are regulated by a fine balance of proteases (plasminogen activators, MMPs, plasmin) along with their inhibitors (TIMPs, PAI-1). Cells respond to the dynamic matrix turnover through integrins, which bind a variety of extracellular matrix components [214]. One intracellular mediator of integrin signaling is the integrin-linked kinase (ILK), which complexes with the proteins PINCH and parvin to mediate signaling [215].

The ILK signaling pathway serves to suppress hepatocyte growth. *In vitro*, hepatocytes cultured without extracellular matrix lose markers of hepatic differentiation and have enhanced proliferative capacity in response to HGF and EGF; when matrix preparations (such as Matrigel) are overlaid onto these cultures, the hepatocytes regain differentiation. ILK-deficient hepatocytes were not fully able to

re-differentiate [216]. Hepatocyte-specific deletion of ILK in mice induces increased proliferation of hepatocytes and cholangiocytes for the first three months of life. Expression of genes associated with hepatocyte differentiation decreases over this time period. By three months of age, the mice have livers twice the size of normal, increased extracellular matrix accumulation around hepatocytes, altered matrix profile, and enhanced hepatocyte-associated genes (in contrast to earlier time points) [217]. When subjected to PHx, regeneration is prolonged and the final weight of the liver exceeds the original weight prior to surgery [31]. These observations highlight the essential role of extracellular matrix-mediated signaling in controlling liver size and suppression of proliferation at the termination of liver regeneration.

4.2. Glypican 3

Glypican 3 is a GPI-linked protein on the plasma membrane of hepatocytes that does not possess a signaling domain. It is one of the most highly expressed proteins in hepatocellular carcinoma and is measured clinically as a diagnostic marker [218]. It was thought that glypican 3 might be a growth promoter; however, loss-of-function mutations in humans (Simpson-Golabi-Behmel syndrome) are associated with organ enlargement [219], suggesting that it is a growth suppressor that is overexpressed in cancer but unable to halt the accelerated growth of the neoplastic cells. Studies in liver regeneration after PHx also support a growth-suppressing role of glypican 3. Expression of glypican 3 only begins at day 2 after PHx, reaching maximal levels at day 5. Suppression of glypican 3 in cultured rat hepatocytes enhances proliferation [220]. Further, overexpression of glypican 3 on hepatocytes in transgenic mice suppresses proliferation after PHx [32]. The mechanism behind these effects of glypican 3 is not clear, but one possibility is through modulation of the Hedgehog signaling pathway, which has been shown to be negatively regulated by glypican 3 during development [167].

4.3. TGF-β and Activins

As discussed above in section 2, TGF-β signaling is strongly mitoinhibitory for hepatocytes. It is purged from the liver at the initiation of regeneration after PHx but begins to be expressed in later stages. Re-establishment of local stores of TGF-β may represent one arm of the balance in holding hepatocytes in quiescence at the end of regeneration and in the resting liver. When a plasmid encoding a dominant negative TGF-β type II receptor is expressed in normal rat livers by adenovirus infection, DNA synthesis was significantly elevated starting at day 3 and peaking at day 7 after infusion [221]. No PHx was performed in this study, suggesting the increased proliferation may be a response to locally available growth factors (e.g., HGF) with the loss of tonic TGF-β signaling. TGF-β is bound to the matrix by decorin, a GPI-linked protein on hepatocytes, which has its own inhibitory effects on MET and EGFR function [222,223].

Activins are members of the TGF-β superfamily that have also been implicated in growth suppression in liver regeneration. Adenovirus injection of a dominant negative activin type II receptor also elevates hepatocellular DNA synthesis [221]. In addition, inhibition of activin using follistatin prolongs hepatocyte proliferation after PHx in mice, and this effect of follistatin is especially pronounced in mice lacking TGF-β type II receptors, which otherwise do not exhibit increased proliferation after PHx due to compensatory activin signaling [132].

4.4. Yes-Associated Protein (YAP)

YAP is the mammalian homologue of the Hippo kinase pathway target Yorkie (Yki) in Drosophila, a growth regulatory pathway [224]. YAP promotes the transcription of genes relating to cell differentiation and proliferation. When YAP is phosphorylated through activation of the kinase pathway (Mst1/2 in mammals), it is expelled from the nucleus and targeted for degradation. Overexpression of YAP in hepatocytes results in massive hepatic enlargement [225], and likewise genetic loss of Mst1/2 has similar consequences [226,227]. Compellingly, recent studies show that increased YAP nuclear localization correlates with perturbations causing liver enlargement [31,33,34], and likewise decreased nuclear YAP correlates with growth suppression [32,35]. Could YAP be the molecular "switch" that governs the hepatostat? More studies are needed to understand the signaling pathways that interact with and influence the activity of the Mst1/2 kinases and YAP localization, especially how this regulation connects to extracellular signals mediated by receptor tyrosine kinases and integrins. It is very likely, however, that this pathway is indeed a major component of the hepatostat.

5. Conclusions

The partial hepatectomy model has taught us that the process of liver regeneration is complex and intricate yet buffered by many redundant pathways. There is much evidence to suggest that liver regeneration in humans progresses similarly to rodents and many of the same growth factors and signaling pathways are relevant. These lessons learned from the PHx model have helped to inform clinical knowledge and strategies to bolster regeneration after surgical resection or transplant in patients [228,229], but also they serve as launching pads from which we can start to tease out the dysregulation of regeneration that occurs in chronic liver disease and cancer.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

1. Wolber, E.M.; Jelkmann, W. Thrombopoietin: The novel hepatic hormone. *News Physiol. Sci.* **2002**, *17*, 6–10.
2. Michalopoulos, G.K. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am. J. Pathol.* **2010**, *176*, 2–13.

3. Miyaoka, Y.; Ebato, K.; Kato, H.; Arakawa, S.; Shimizu, S.; Miyajima, A. Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. *Curr. Biol.* **2012**, *22*, 1166–1175.

4. Lim, Y.S.; Kim, W.R. The global impact of hepatic fibrosis and end-stage liver disease. *Clin. Liver Dis.* **2008**, *12*, 733–746, vii.

5. Parker, G.A.; Picut, C.A. Immune functioning in non lymphoid organs: The liver. *Toxicol. Pathol.* **2012**, *40*, 237–247.

6. Friedman, S.L. Hepatic stellate cells: Protean, multifunctional, and enigmatic cells of the liver. *Physiol. Rev.* **2008**, *88*, 125–172.

7. Higgins, G.M.; Anderson, R.M. Experimental pathology of the liver – Restoration of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **1931**, *12*, 186–202.

8. Mitchell, C.; Willenbring, H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat. Protoc.* **2008**, *3*, 1167–1170.

9. de Toranzo, E.G.; Gomez, M.I.; Castro, J.A. Carbon tetrachloride activation, lipid peroxidation and liver necrosis in different strains of mice. *Res. Commun. Chem. Pathol. Pharmacol.* **1978**, *19*, 347–352.

10. DeCicco, L.A.; Rikans, L.E.; Tutor, C.G.; Hornbrook, K.R. Serum and liver concentrations of tumor necrosis factor alpha and interleukin-1beta following administration of carbon tetrachloride to male rats. *Toxicol. Lett.* **1998**, *98*, 115–121.

11. Jaeschke, H.; McGill, M.R.; Williams, C.D.; Ramachandran, A. Current issues with acetaminophen hepatotoxicity—a clinically relevant model to test the efficacy of natural products. *Life Sci.* **2011**, *88*, 737–745.

12. Hinson, J.A.; Roberts, D.W.; James, L.P. Mechanisms of acetaminophen-induced liver necrosis. *Handb. Exp. Pharmacol.* **2010**, 369–405.

13. David Josephy, P. The molecular toxicology of acetaminophen. *Drug Metab. Rev.* **2005**, *37*, 581–594.

14. Lee, J.H.; Ilic, Z.; Sell, S. Cell kinetics of repair after allyl alcohol-induced liver necrosis in mice. *Int. J. Exp. Pathol.* **1996**, *77*, 63–72.

15. Pound, A.W.; McGuire, L.J. Repeated partial hepatectomy as a promoting stimulus for carcinogenic response of liver to nitrosamines in rats. *Br. J. Cancer.* **1978**, *37*, 585–594.

16. Bhave, V.S.; Donthamsetty, S.; Latendresse, J.R.; Cunningham, M.L.; Mehendale, H.M. Secretory phospholipase A(2)-mediated progression of hepatotoxicity initiated by acetaminophen is exacerbated in the absence of hepatic COX-2. *Toxicol. Appl. Pharmacol.* **2011**, *251*, 173–180.

17. Bhave, V.S.; Donthamsetty, S.; Latendresse, J.R.; Muskhelishvili, L.; Mehendale, H.M. Secretory phospholipase A2 mediates progression of acute liver injury in the absence of sufficient cyclooxygenase-2. *Toxicol. Appl. Pharmacol.* **2008**, *228*, 225–238.

18. Limaye, P.B.; Bhave, V.S.; Palkar, P.S.; Apte, U.M.; Sawant, S.P.; Yu, S.; Latendresse, J.R.; Reddy, J.K.; Mehendale, H.M. Upregulation of calpastatin in regenerating and developing rat liver: role in resistance against hepatotoxicity. *Hepatology* **2006**, *44*, 379–388.

19. Mehendale, H.M. Once initiated, how does toxic tissue injury expand? *Trends Pharmacol. Sci.* **2012**, *33*, 200–206.

20. Mehendale, H.M. Tissue repair: an important determinant of final outcome of toxicant-induced injury. *Toxicol. Pathol.* **2005**, *33*, 41–51.

21. Columbano, A.; Ledda-Columbano, G.M. Mitogenesis by ligands of nuclear receptors: an attractive model for the study of the molecular mechanisms implicated in liver growth. *Cell Death Differ.* **2003**, *10* (Suppl. 1), S19–S21.

22. Columbano, A.; Simbula, M.; Pibiri, M.; Perra, A.; Deidda, M.; Locker, J.; Pisanu, A.; Uccheddu, A.; Ledda-Columbano, G.M. Triiodothyronine stimulates hepatocyte proliferation in two models of impaired liver regeneration. *Cell Prolif.* **2008**, *41*, 521–531.

23. Malik, R.; Habib, M.; Tootle, R.; Hodgson, H. Exogenous thyroid hormone induces liver enlargement, whilst maintaining regenerative potential—a study relevant to donor preconditioning. *Am. J. Transplant.* **2005**, *5*, 1801–1807.

24. Francavilla, A.; Carr, B.I.; Azzarone, A.; Polimeno, L.; Wang, Z.; Van Thiel, D.H.; Subbotin, V.; Prelich, J.G.; Starzl, T.E. Hepatocyte proliferation and gene expression induced by triiodothyronine *in vivo* and *in vitro*. *Hepatology* **1994**, *20*, 1237–1241.

25. Pyper, S.R.; Viswakarma, N.; Yu, S.; Reddy, J.K. PPARalpha: energy combustion, hypolipidemia, inflammation and cancer. *Nucl. Recept. Signal.* **2010**, *8*, e002.

26. Qatanani, M.; Moore, D.D. CAR, the continuously advancing receptor, in drug metabolism and disease. *Curr. Drug Metab.* **2005**, *6*, 329–339.

27. Ledda-Columbano, G.M.; Coni, P.; Simbula, G.; Zedda, I.; Columbano, A. Compensatory regeneration, mitogen-induced liver growth, and multistage chemical carcinogenesis. *Environ. Health Perspect.* **1993**, *101* (Suppl. 5), 163–168.

28. Bursch, W.; Taper, H.S.; Lauer, B.; Schulte-Hermann, R. Quantitative histological and histochemical studies on the occurrence and stages of controlled cell death (apoptosis) during regression of rat liver hyperplasia. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **1985**, *50*, 153–166.

29. Columbano, A.; Ledda-Columbano, G.M.; Coni, P.P.; Faa, G.; Liguori, C.; Santa Cruz, G.; Pani, P. Occurrence of cell death (apoptosis) during the involution of liver hyperplasia. *Lab. Invest.* **1985**, *52*, 670–675.

30. Columbano, A.; Shinozuka, H. Liver regeneration *versus* direct hyperplasia. *FASEB J.* **1996**, *10*, 1118–1128.

31. Apte, U.; Gkretsi, V.; Bowen, W.C.; Mars, W.M.; Luo, J.H.; Donthamsetty, S.; Orr, A.; Monga, S.P.; Wu, C.; Michalopoulos, G.K. Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrin-linked kinase. *Hepatology* **2009**, *50*, 844–851.

32. Liu, B.; Bell, A.W.; Paranjpe, S.; Bowen, W.C.; Khillan, J.S.; Luo, J.H.; Mars, W.M.; Michalopoulos, G.K. Suppression of liver regeneration and hepatocyte proliferation in hepatocyte-targeted glypican 3 transgenic mice. *Hepatology* **2010**, *52*, 1060–1067.

33. Donthamsetty, S.; Bowen, W.; Mars, W.; Bhave, V.; Luo, J.H.; Wu, C.; Hurd, J.; Orr, A.; Bell, A.; Michalopoulos, G. Liver-specific ablation of integrin-linked kinase in mice results in enhanced and prolonged cell proliferation and hepatomegaly after phenobarbital administration. *Toxicol. Sci.* **2010**, *113*, 358–366.

34. Donthamsetty, S.; Bhave, V.S.; Kliment, C.S.; Bowen, W.C.; Mars, W.M.; Bell, A.W.; Stewart, R.E.; Orr, A.; Wu, C.; Michalopoulos, G.K. Excessive hepatomegaly of mice with hepatocyte-targeted elimination of integrin linked kinase following treatment with 1,4-bis [2-(3,5-dichaloropyridyloxy)] benzene. *Hepatology* **2011**, *53*, 587–595.

35. Lin, C.W.; Mars, W.M.; Paranjpe, S.; Donthamsetty, S.; Bhave, V.S.; Kang, L.I.; Orr, A.; Bowen, W.C.; Bell, A.W.; Michalopoulos, G.K. Hepatocyte proliferation and hepatomegaly induced by phenobarbital and 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene is suppressed in hepatocyte-targeted glypican 3 transgenic mice. *Hepatology* **2011**, *54*, 620–630.

36. Kodama, S.; Negishi, M. Phenobarbital confers its diverse effects by activating the orphan nuclear receptor car. *Drug. Metab. Rev.* **2006**, *38*, 75–87.

37. Marubashi, S.; Sakon, M.; Nagano, H.; Gotoh, K.; Hashimoto, K.; Kubota, M.; Kobayashi, S.; Yamamoto, S.; Miyamoto, A.; Dono, K.; Nakamori, S.; Umeshita, K.; Monden, M. Effect of portal hemodynamics on liver regeneration studied in a novel portohepatic shunt rat model. *Surgery* **2004**, *136*, 1028–1037.

38. Francavilla, A.; Starzl, T.E.; Porter, K.; Foglieni, C.S.; Michalopoulos, G.K.; Carrieri, G.; Trejo, J.; Azzarone, A.; Barone, M.; Zeng, Q.H. Screening for candidate hepatic growth factors by selective portal infusion after canine Eck's fistula. *Hepatology* **1991**, *14*, 665–670.

39. Kim, T.H.; Mars, W.M.; Stolz, D.B.; Petersen, B.E.; Michalopoulos, G.K. Extracellular matrix remodeling at the early stages of liver regeneration in the rat. *Hepatology* **1997**, *26*, 896–904.

40. Kim, T.H.; Mars, W.M.; Stolz, D.B.; Michalopoulos, G.K. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* **2000**, *31*, 75–82.

41. Mars, W.M.; Zarnegar, R.; Michalopoulos, G.K. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am. J. Pathol.* **1993**, *143*, 949–958.

42. Mars, W.M.; Kim, T.H.; Stolz, D.B.; Liu, M.L.; Michalopoulos, G.K. Presence of urokinase in serum-free primary rat hepatocyte cultures and its role in activating hepatocyte growth factor. *Cancer Res.* **1996**, *56*, 2837–2843.

43. Mars, W.M.; Liu, M.L.; Kitson, R.P.; Goldfarb, R.H.; Gabauer, M.K.; Michalopoulos, G.K. Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. *Hepatology* **1995**, *21*, 1695–1701.

44. Kohler, C.; Bell, A.W.; Bowen, W.C.; Monga, S.P.; Fleig, W.; Michalopoulos, G.K. Expression of Notch-1 and its ligand Jagged-1 in rat liver during liver regeneration. *Hepatology* **2004**, *39*, 1056–1065.

45. Monga, S.P.; Pediaditakis, P.; Mule, K.; Stolz, D.B.; Michalopoulos, G.K. Changes in WNT/beta-catenin pathway during regulated growth in rat liver regeneration. *Hepatology.* **2001**, *33*, 1098–1109.

46. Monga, S.P. Role of Wnt/beta-catenin signaling in liver metabolism and cancer. *Int. J. Biochem. Cell Biol.* **2011**, *43*, 1021–1029.

47. Haber, B.A.; Mohn, K.L.; Diamond, R.H.; Taub, R. Induction patterns of 70 genes during nine days after hepatectomy define the temporal course of liver regeneration. *J. Clin. Invest.* **1993**, *91*, 1319–1326.

48. Mohn, K.L.; Laz, T.M.; Melby, A.E.; Taub, R. Immediate-early gene expression differs between regenerating liver, insulin-stimulated H-35 cells, and mitogen-stimulated Balb/c 3T3 cells. Liver-specific induction patterns of gene 33, phosphoenolpyruvate carboxykinase, and the jun, fos, and egr families. *J. Biol. Chem.* **1990**, *265*, 21914–21921.

49. Strey, C.W.; Winters, M.S.; Markiewski, M.M.; Lambris, J.D. Partial hepatectomy induced liver proteome changes in mice. *Proteomics* **2005**, *5*, 318–325.

50. Taub, R. Liver regeneration: from myth to mechanism. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 836–847.

51. Fausto, N.; Campbell, J.S.; Riehle, K.J. Liver regeneration. *Hepatology* **2006**, *43*, S45–S53.

52. Zarnegar, R.; Michalopoulos, G. Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. *Cancer Res.* **1989**, *49*, 3314–3320.

53. Nakamura, T.; Teramoto, H.; Ichihara, A. Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6489–6493.

54. Nakamura, T.; Nishizawa, T.; Hagiya, M.; Seki, T.; Shimonishi, M.; Sugimura, A.; Tashiro, K.; Shimizu, S. Molecular cloning and expression of human hepatocyte growth factor. *Nature.* **1989**, *342*, 440–443.

55. Zarnegar, R.; Muga, S.; Enghild, J.; Michalopoulos, G. NH2-terminal amino acid sequence of rabbit hepatopoietin A, a heparin-binding polypeptide growth factor for hepatocytes. *Biochem Biophys. Res. Commun.* **1989**, *163*, 1370–1376.

56. Wang, X.; DeFrances, M.C.; Dai, Y.; Pediaditakis, P.; Johnson, C.; Bell, A.; Michalopoulos, G.K.; Zarnegar, R. A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. *Mol. Cell.* **2002**, *9*, 411–421.

57. Fafalios, A.; Ma, J.; Tan, X.; Stoops, J.; Luo, J.; Defrances, M.C.; Zarnegar, R. A hepatocyte growth factor receptor (Met)-insulin receptor hybrid governs hepatic glucose metabolism. *Nat. Med.* **2011**, *17*, 1577–1584.

58. Nakamura, T. Hepatocyte growth factor as mitogen, motogen and morphogen, and its roles in organ regeneration. *Princess Takamatsu Symp.* **1994**, *24*, 195–213.

59. Paranjpe, S.; Bowen, W.C.; Bell, A.W.; Nejak-Bowen, K.; Luo, J.H.; Michalopoulos, G.K. Cell cycle effects resulting from inhibition of hepatocyte growth factor and its receptor c-Met in regenerating rat livers by RNA interference. *Hepatology.* **2007**, *45*, 1471–1477.

60. Wang, B.; Gao, C.; Ponder, K.P. C/EBPbeta contributes to hepatocyte growth factor-induced replication of rodent hepatocytes. *J. Hepatol.* **2005**, *43*, 294–302.

61. Schuppan, D.; Schmid, M.; Somasundaram, R.; Ackermann, R.; Ruehl, M.; Nakamura, T.; Riecken, E.O. Collagens in the liver extracellular matrix bind hepatocyte growth factor. *Gastroenterology* **1998**, *114*, 139–152.

62. Liu, M.L.; Mars, W.M.; Zarnegar, R.; Michalopoulos, G.K. Uptake and distribution of hepatocyte growth factor in normal and regenerating adult rat liver. *Am. J. Pathol.* **1994**, *144*, 129–140.

63. Stolz, D.B.; Mars, W.M.; Petersen, B.E.; Kim, T.H.; Michalopoulos, G.K. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. *Cancer Res.* **1999**, *59*, 3954–3960.

64. Pediaditakis, P.; Lopez-Talavera, J.C.; Petersen, B.; Monga, S.P.; Michalopoulos, G.K. The processing and utilization of hepatocyte growth factor/scatter factor following partial hepatectomy in the rat. *Hepatology* **2001**, *34*, 688–693.

65. Lindroos, P.M.; Zarnegar, R.; Michalopoulos, G.K. Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration. *Hepatology* **1991**, *13*, 743–750.

66. Schirmacher, P.; Geerts, A.; Pietrangelo, A.; Dienes, H.P.; Rogler, C.E. Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* **1992**, *15*, 5–11.

67. LeCouter, J.; Moritz, D.R.; Li, B.; Phillips, G.L.; Liang, X.H.; Gerber, H.P.; Hillan, K.J.; Ferrara, N. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science* **2003**, *299*, 890–893.

68. Maher, J.J. Cell-specific expression of hepatocyte growth factor in liver. Upregulation in sinusoidal endothelial cells after carbon tetrachloride. *J. Clin. Invest.* **1993**, *91*, 2244–2252.

69. Zarnegar, R.; DeFrances, M.C.; Kost, D.P.; Lindroos, P.; Michalopoulos, G.K. Expression of hepatocyte growth factor mRNA in regenerating rat liver after partial hepatectomy. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 559–565.

70. Kono, S.; Nagaike, M.; Matsumoto, K.; Nakamura, T. Marked induction of hepatocyte growth factor mRNA in intact kidney and spleen in response to injury of distant organs. *Biochem. Biophys. Res. Commun.* **1992**, *186*, 991–998.

71. Yanagita, K.; Nagaike, M.; Ishibashi, H.; Niho, Y.; Matsumoto, K.; Nakamura, T. Lung may have an endocrine function producing hepatocyte growth factor in response to injury of distal organs. *Biochem. Biophys. Res. Commun.* **1992**, *182*, 802–809.

72. Broten, J.; Michalopoulos, G.; Petersen, B.; Cruise, J. Adrenergic stimulation of hepatocyte growth factor expression. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 76–79.

73. Skrtic, S.; Wallenius, V.; Ekberg, S.; Brenzel, A.; Gressner, A.M.; Jansson, J.O. Insulin-like growth factors stimulate expression of hepatocyte growth factor but not transforming growth factor beta1 in cultured hepatic stellate cells. *Endocrinology* **1997**, *138*, 4683–4689.

74. McGowan, J.A.; Strain, A.J.; Bucher, N.L. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP. *J. Cell. Physiol.* **1981**, *108*, 353–363.

75. Bucher, N.L.; Patel, U.; Cohen, S. Hormonal factors concerned with liver regeneration. *Ciba Found. Symp.* **1977**, 95–107.

76. St Hilaire, R.J.; Hradek, G.T.; Jones, A.L. Hepatic sequestration and biliary secretion of epidermal growth factor: evidence for a high-capacity uptake system. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 3797–3801.

77. St Hilaire, R.J.; Jones, A.L. Epidermal growth factor: its biologic and metabolic effects with emphasis on the hepatocyte. *Hepatology* **1982**, *2*, 601–613.

78. Olsen, P.S.; Poulsen, S.S.; Kirkegaard, P. Adrenergic effects on secretion of epidermal growth factor from Brunner's glands. *Gut* **1985**, *26*, 920–927.

79. Jones, D.E., Jr.; Tran-Patterson, R.; Cui, D.M.; Davin, D.; Estell, K.P.; Miller, D.M. Epidermal growth factor secreted from the salivary gland is necessary for liver regeneration. *Am. J. Physiol.* **1995**, *268*, G872–G878.

80. Reddy, C.C.; Wells, A.; Lauffenburger, D.A. Receptor-mediated effects on ligand availability influence relative mitogenic potencies of epidermal growth factor and transforming growth factor alpha. *J. Cell. Physiol.* **1996**, *166*, 512–522.

81. Webber, E.M.; FitzGerald, M.J.; Brown, P.I.; Bartlett, M.H.; Fausto, N. Transforming growth factor-alpha expression during liver regeneration after partial hepatectomy and toxic injury, and potential interactions between transforming growth factor-alpha and hepatocyte growth factor. *Hepatology* **1993**, *18*, 1422–1431.

82. Luetteke, N.C.; Lee, D.C. Transforming growth factor alpha: expression, regulation and biological action of its integral membrane precursor. *Semin. Cancer Biol.* **1990**, *1*, 265–275.

83. Lee, D.C.; Sunnarborg, S.W.; Hinkle, C.L.; Myers, T.J.; Stevenson, M.Y.; Russell, W.E.; Castner, B.J.; Gerhart, M.J.; Paxton, R.J.; Black, R.A.; Chang, A.; Jackson, L.F. TACE/ADAM17 processing of EGFR ligands indicates a role as a physiological convertase. *Ann. N. Y. Acad. Sci.* **2003**, *995*, 22–38.

84. Mead, J.E.; Fausto, N. Transforming growth factor alpha may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1558–1562.

85. Russell, W.E.; Kaufmann, W.K.; Sitaric, S.; Luetteke, N.C.; Lee, D.C. Liver regeneration and hepatocarcinogenesis in transforming growth factor-alpha-targeted mice. *Mol. Carcinog.* **1996**, *15*, 183–189.

86. Berasain, C.; Garcia-Trevijano, E.R.; Castillo, J.; Erroba, E.; Lee, D.C.; Prieto, J.; Avila, M.A. Amphiregulin: an early trigger of liver regeneration in mice. *Gastroenterology* **2005**, *128*, 424–432.

87. Michalopoulos, G.K.; Khan, Z. Liver regeneration, growth factors, and amphiregulin. *Gastroenterology* **2005**, *128*, 503–506.

88. Kiso, S.; Kawata, S.; Tamura, S.; Higashiyama, S.; Ito, N.; Tsushima, H.; Taniguchi, N.; Matsuzawa, Y. Role of heparin-binding epidermal growth factor-like growth factor as a hepatotrophic factor in rat liver regeneration after partial hepatectomy. *Hepatology* **1995**, *22*, 1584–1590.

89. Ito, N.; Kawata, S.; Tamura, S.; Kiso, S.; Tsushima, H.; Damm, D.; Abraham, J.A.; Higashiyama, S.; Taniguchi, N.; Matsuzawa, Y. Heparin-binding EGF-like growth factor is a potent mitogen for rat hepatocytes. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 25–31.

90. Mitchell, C.; Nivison, M.; Jackson, L.F.; Fox, R.; Lee, D.C.; Campbell, J.S.; Fausto, N. Heparin-binding epidermal growth factor-like growth factor links hepatocyte priming with cell cycle progression during liver regeneration. *J. Biol. Chem.* **2005**, *280*, 2562–2568.

91. Kiso, S.; Kawata, S.; Tamura, S.; Inui, Y.; Yoshida, Y.; Sawai, Y.; Umeki, S.; Ito, N.; Yamada, A.; Miyagawa, J.; Higashiyama, S.; Iwawaki, T.; Saito, M.; Taniguchi, N.; Matsuzawa, Y.; Kohno, K. Liver regeneration in heparin-binding EGF-like growth factor transgenic mice after partial hepatectomy. *Gastroenterology* **2003**, *124*, 701–707.

92. Paranjpe, S.; Bowen, W.C.; Tseng, G.C.; Luo, J.H.; Orr, A.; Michalopoulos, G.K. RNA interference against hepatic epidermal growth factor receptor has suppressive effects on liver regeneration in rats. *Am. J. Pathol.* **2010**, *176*, 2669–2681.

93. Natarajan, A.; Wagner, B.; Sibilia, M. The EGF receptor is required for efficient liver regeneration. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 17081–17086.

94. Bradham, C.A.; Plumpe, J.; Manns, M.P.; Brenner, D.A.; Trautwein, C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. *Am. J. Physiol.* **1998**, *275*, G387–G392.

95. Hatano, E.; Bennett, B.L.; Manning, A.M.; Qian, T.; Lemasters, J.J.; Brenner, D.A. NF-kappaB stimulates inducible nitric oxide synthase to protect mouse hepatocytes from TNF-alpha- and Fas-mediated apoptosis. *Gastroenterology* **2001**, *120*, 1251–1262.

96. Hatano, E.; Brenner, D.A. Akt protects mouse hepatocytes from TNF-alpha- and Fas-mediated apoptosis through NK-kappa B activation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, G1357–G1368.

97. Leist, M.; Gantner, F.; Bohlinger, I.; Tiegs, G.; Germann, P.G.; Wendel, A. Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. *Am. J. Pathol.* **1995**, *146*, 1220–1234.

98. Leist, M.; Gantner, F.; Jilg, S.; Wendel, A. Activation of the 55 kDa TNF receptor is necessary and sufficient for TNF-induced liver failure, hepatocyte apoptosis, and nitrite release. *J. Immunol.* **1995**, *154*, 1307–1316.

99. Leist, M.; Gantner, F.; Kunstle, G.; Bohlinger, I.; Tiegs, G.; Bluethmann, H.; Wendel, A. The 55-kD tumor necrosis factor receptor and CD95 independently signal murine hepatocyte apoptosis and subsequent liver failure. *Mol. Med.* **1996**, *2*, 109–124.

100. Akerman, P.; Cote, P.; Yang, S.Q.; McClain, C.; Nelson, S.; Bagby, G.J.; Diehl, A.M. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. *Am J. Physiol.* **1992**, *263*, G579–G585.

101. Yamada, Y.; Webber, E.M.; Kirillova, I.; Peschon, J.J.; Fausto, N. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: Requirement for type 1 but not type 2 receptor. *Hepatology* **1998**, *28*, 959–970.

102. Iimuro, Y.; Nishiura, T.; Hellerbrand, C.; Behrns, K.E.; Schoonhoven, R.; Grisham, J.W.; Brenner, D.A. NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. *J. Clin. Invest.* **1998**, *101*, 802–811.

103. Webber, E.M.; Bruix, J.; Pierce, R.H.; Fausto, N. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology* **1998**, *28*, 1226–1234.

104. Webber, E.M.; Godowski, P.J.; Fausto, N. In vivo response of hepatocytes to growth factors requires an initial priming stimulus. *Hepatology* **1994**, *19*, 489–497.

105. Gauldie, J.; Northemann, W.; Fey, G.H. IL-6 functions as an exocrine hormone in inflammation. Hepatocytes undergoing acute phase responses require exogenous IL-6. *J. Immunol.* **1990**, *144*, 3804–3808.

106. Castell, J.V.; Gomez-Lechon, M.J.; David, M.; Fabra, R.; Trullenque, R.; Heinrich, P.C. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology* **1990**, *12*, 1179–1186.

107. Streetz, K.L.; Wustefeld, T.; Klein, C.; Manns, M.P.; Trautwein, C. Mediators of inflammation and acute phase response in the liver. *Cell. Mol. Biol. (Noisy-le-grand)* **2001**, *47*, 661–673.

108. Peters, M.; Muller, A.M.; Rose-John, S. Interleukin-6 and soluble interleukin-6 receptor: Direct stimulation of gp130 and hematopoiesis. *Blood* **1998**, *92*, 3495–3504.

109. Wang, Y.; Nesbitt, J.E.; Fuentes, N.L.; Fuller, G.M. Molecular cloning and characterization of the rat liver IL-6 signal transducing molecule, gp130. *Genomics* **1992**, *14*, 666–672.

110. Cressman, D.E.; Greenbaum, L.E.; DeAngelis, R.A.; Ciliberto, G.; Furth, E.E.; Poli, V.; Taub, R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* **1996**, *274*, 1379–1383.

111. Liu, Y.; Michalopoulos, G.K.; Zarnegar, R. Structural and functional characterization of the mouse hepatocyte growth factor gene promoter. *J. Biol. Chem.* **1994**, *269*, 4152–4160.

112. Sun, R.; Jaruga, B.; Kulkarni, S.; Sun, H.; Gao, B. IL-6 modulates hepatocyte proliferation via induction of HGF/p21cip1: regulation by SOCS3. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1943–1949.

113. Kariv, R.; Enden, A.; Zvibel, I.; Rosner, G.; Brill, S.; Shafritz, D.A.; Halpern, Z.; Oren, R. Triiodothyronine and interleukin-6 (IL-6) induce expression of HGF in an immortalized rat hepatic stellate cell line. *Liver Int.* **2003**, *23*, 187–193.

114. Coudriet, G.M.; He, J.; Trucco, M.; Mars, W.M.; Piganelli, J.D. Hepatocyte growth factor modulates interleukin-6 production in bone marrow derived macrophages: Implications for inflammatory mediated diseases. *PLoS One* **2010**, *5*, e15384.

115. Gong, R. Multi-target anti-inflammatory action of hepatocyte growth factor. *Curr. Opin. Investig. Drugs* **2008**, *9*, 1163–1170.

116. Wrana, J.L.; Attisano, L.; Carcamo, J.; Zentella, A.; Doody, J.; Laiho, M.; Wang, X.F.; Massague, J. TGF beta signals through a heteromeric protein kinase receptor complex. *Cell* **1992**, *71*, 1003–1014.

117. Gruppuso, P.A.; Mead, J.E.; Fausto, N. Transforming growth factor receptors in liver regeneration following partial hepatectomy in the rat. *Cancer Res.* **1990**, *50*, 1464–1469.

118. Chari, R.S.; Price, D.T.; Sue, S.R.; Meyers, W.C.; Jirtle, R.L. Down-regulation of transforming growth factor beta receptor type I, II, and III during liver regeneration. *Am. J. Surg.* **1995**, *169*, 126–131; discussion 131–122.

119. Bissell, D.M.; Wang, S.S.; Jarnagin, W.R.; Roll, F.J. Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. *J. Clin. Invest.* **1995**, *96*, 447–455.

120. Ikeda, H.; Nagoshi, S.; Ohno, A.; Yanase, M.; Maekawa, H.; Fujiwara, K. Activated rat stellate cells express c-met and respond to hepatocyte growth factor to enhance transforming growth factor beta1 expression and DNA synthesis. *Biochem. Biophys. Res. Commun.* **1998**, *250*, 769–775.

121. Nakatsukasa, H.; Evarts, R.P.; Hsia, C.C.; Thorgeirsson, S.S. Transforming growth factor-beta 1 and type I procollagen transcripts during regeneration and early fibrosis of rat liver. *Lab. Invest.* **1990**, *63*, 171–180.

122. Carr, B.I.; Huang, T.H.; Itakura, K.; Noel, M.; Marceau, N. TGF beta gene transcription in normal and neoplastic liver growth. *J. Cell. Biochem.* **1989**, *39*, 477–487.

123. Michalopoulos, G.K.; Bowen, W.C.; Mule, K.; Luo, J. HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. *Gene Expr.* **2003**, *11*, 55–75.

124. McMahon, J.B.; Richards, W.L.; del Campo, A.A.; Song, M.K.; Thorgeirsson, S.S. Differential effects of transforming growth factor-beta on proliferation of normal and malignant rat liver epithelial cells in culture. *Cancer Res.* **1986**, *46*, 4665–4671.

125. Houck, K.A.; Cruise, J.L.; Michalopoulos, G. Norepinephrine modulates the growth-inhibitory effect of transforming growth factor-beta in primary rat hepatocyte cultures. *J. Cell. Physiol.* **1988**, *135*, 551–555.

126. Russell, W.E.; Coffey, R.J., Jr.; Ouellette, A.J.; Moses, H.L. Type beta transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 5126–5130.

127. Jirtle, R.L.; Carr, B.I.; Scott, C.D. Modulation of insulin-like growth factor-II/mannose 6-phosphate receptors and transforming growth factor-beta 1 during liver regeneration. *J. Biol. Chem.* **1991**, *266*, 22444–22450.

128. Michalopoulos, G.K.; DeFrances, M.C. Liver regeneration. *Science* **1997**, *276*, 60–66.

129. Houck, K.A.; Michalopoulos, G.K. Altered responses of regenerating hepatocytes to norepinephrine and transforming growth factor type beta. *J. Cell. Physiol.* **1989**, *141*, 503–509.

130. O'Connor-McCourt, M.D.; Wakefield, L.M. Latent transforming growth factor-beta in serum. A specific complex with alpha 2-macroglobulin. *J. Biol. Chem.* **1987**, *262*, 14090–14099.

131. Braun, L.; Mead, J.E.; Panzica, M.; Mikumo, R.; Bell, G.I.; Fausto, N. Transforming growth factor beta mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 1539–1543.

132. Oe, S.; Lemmer, E.R.; Conner, E.A.; Factor, V.M.; Leveen, P.; Larsson, J.; Karlsson, S.; Thorgeirsson, S.S. Intact signaling by transforming growth factor beta is not required for termination of liver regeneration in mice. *Hepatology* **2004**, *40*, 1098–1105.

133. Thenappan, A.; Shukla, V.; Abdul Khalek, F.J.; Li, Y.; Shetty, K.; Liu, P.; Li, L.; Johnson, R.L.; Johnson, L.; Mishra, L. Loss of transforming growth factor beta adaptor protein beta-2 spectrin leads to delayed liver regeneration in mice. *Hepatology* **2011**, *53*, 1641–1650.

134. Oben, J.A.; Roskams, T.; Yang, S.; Lin, H.; Sinelli, N.; Torbenson, M.; Smedh, U.; Moran, T.H.; Li, Z.; Huang, J.; Thomas, S.A.; Diehl, A.M. Hepatic fibrogenesis requires sympathetic neurotransmitters. *Gut* **2004**, *53*, 438–445.

135. Cruise, J.L.; Knechtle, S.J.; Bollinger, R.R.; Kuhn, C.; Michalopoulos, G. Alpha 1-adrenergic effects and liver regeneration. *Hepatology* **1987**, *7*, 1189–1194.

136. Cruise, J.L.; Michalopoulos, G. Norepinephrine and epidermal growth factor: dynamics of their interaction in the stimulation of hepatocyte DNA synthesis. *J. Cell. Physiol.* **1985**, *125*, 45–50.

137. Cruise, J.L.; Houck, K.A.; Michalopoulos, G.K. Induction of DNA synthesis in cultured rat hepatocytes through stimulation of alpha 1 adrenoreceptor by norepinephrine. *Science* **1985**, *227*, 749–751.

138. Kanamaru, C.; Yasuda, H.; Takeda, M.; Ueda, N.; Suzuki, J.; Tsuchida, T.; Mashima, H.; Ohnishi, H.; Fujita, T. Smad7 is induced by norepinephrine and protects rat hepatocytes from activin A-induced growth inhibition. *J. Biol. Chem.* **2001**, *276*, 45636–45641.

139. Han, C.; Bowen, W.C.; Michalopoulos, G.K.; Wu, T. Alpha-1 adrenergic receptor transactivates signal transducer and activator of transcription-3 (Stat3) through activation of Src and epidermal growth factor receptor (EGFR) in hepatocytes. *J. Cell. Physiol.* **2008**, *216*, 486–497.

140. Lesurtel, M.; Graf, R.; Aleil, B.; Walther, D.J.; Tian, Y.; Jochum, W.; Gachet, C.; Bader, M.; Clavien, P.A. Platelet-derived serotonin mediates liver regeneration. *Science* **2006**, *312*, 104–107.

141. Furrer, K.; Rickenbacher, A.; Tian, Y.; Jochum, W.; Bittermann, A.G.; Kach, A.; Humar, B.; Graf, R.; Moritz, W.; Clavien, P.A. Serotonin reverts age-related capillarization and failure of regeneration in the liver through a VEGF-dependent pathway. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 2945–2950.

142. Matondo, R.B.; Punt, C.; Homberg, J.; Toussaint, M.J.; Kisjes, R.; Korporaal, S.J.; Akkerman, J.W.; Cuppen, E.; de Bruin, A. Deletion of the serotonin transporter in rats disturbs serotonin homeostasis without impairing liver regeneration. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2009**, *296*, G963–G968.

143. Huang, W.; Ma, K.; Zhang, J.; Qatanani, M.; Cuvillier, J.; Liu, J.; Dong, B.; Huang, X.; Moore, D.D. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science.* **2006**, *312*, 233–236.

144. Uppal, H.; Saini, S.P.; Moschetta, A.; Mu, Y.; Zhou, J.; Gong, H.; Zhai, Y.; Ren, S.; Michalopoulos, G.K.; Mangelsdorf, D.J.; Xie, W. Activation of LXRs prevents bile acid toxicity and cholestasis in female mice. *Hepatology* **2007**, *45*, 422–432.

145. Keitel, V.; Haussinger, D. TGR5 in the biliary tree. *Dig. Dis.* **2011**, *29*, 45–47.

146. Holeček, M. Nutritional modulation of liver regeneration by carbohydrates, lipids, and amino acids: a review. *Nutrition* **1999**, *15*, 784–788.

147. Rudnick, D.A.; Davidson, N.O. Functional Relationships between Lipid Metabolism and Liver Regeneration. *Int. J. Hepatol.* **2012**, 549241.

148. Michalopoulos, G.; Pitot, H.C. Primary culture of parenchymal liver cells on collagen membranes. Morphological and biochemical observations. *Exp. Cell Res.* **1975**, *94*, 70–78.

149. Lai, H.S.; Chung, Y.C.; Chen, W.J.; Chen, K.M. Rat liver regeneration after partial hepatectomy: effects of insulin, glucagon and epidermal growth factor. *J. Formos. Med. Assoc.* **1992**, *91*, 685–690.

150. Starzl, T.E.; Francavilla, A.; Porter, K.A.; Benichou, J.; Jones, A.F. The effect of splanchnic viscera removal upon canine liver regeneration. *Surg. Gynecol. Obstet.* **1978**, *147*, 193–207.

151. Weymann, A.; Hartman, E.; Gazit, V.; Wang, C.; Glauber, M.; Turmelle, Y.; Rudnick, D.A. P21 is required for dextrose-mediated inhibition of mouse liver regeneration. *Hepatology* **2009**, *50*, 207–215.

152. Caruana, J.A.; Whalen, D.A.; Anthony, W.P.; Sunby, C.R.; Ciechoski, M.P. Paradoxical effects of glucose feeding on liver regeneration and survival after partial hepatectomy. *Endocrine Res.* **1986**, *12*, 147–156.

153. McGowan, J.; Atryzek, V.; Fausto, N. Effects of protein-deprivation on the regeneration of rat liver after partial hepatectomy. *Biochem. J.* **1979**, *180*, 25–35.

154. Holeček, M.; Šimek, J.; Palička, V.; Zadák, Z. Effect of glucose and branched chain amino acid (BCAA) infusion on onset of liver regeneration and plasma amino acid pattern in partially hepatectomized rats. *J. Hepatol.* **1991**, *13*, 14–20.

155. Glende, E.A.; Morgan, W.S. Alteration in liver lipid and lipid fatty acid composition after partial hepatectomy in the rat. *Exp. Mol. Path.* **1968**, *8*, 190–200.

156. Gazit, V.; Weymann, A.; Hartman, E.; Finck, B.N.; Hruz, P.W.; Tzekov, A.; Rudnick, D.A. Liver regeneration is impaired in lipodystrophic fatty liver dystrophy mice. *Hepatology* **2010**, *52*, 2109–2117.

157. Blaha, V.; Simek, J.; Zadak, Z. Liver regeneration in partially hepatectomized rats infused with carnitine and lipids. *Exp. Toxicol. Pathol.* **1992**, *44*, 158, 165–168.

158. Nakatani, T.; Ozawa, K.; Asano, M. Differences in predominant energy substrate in relation to the resected hepatic mass in the phase immediately after hepatectomy. *J. Lab. Clin. Med.* **1981**, *97*, 887–898.

159. Zeng, G.; Awan, F.; Otruba, W.; Muller, P.; Apte, U.; Tan, X.; Gandhi, C.; Demetris, A.J.; Monga, S.P. Wnt'er in liver: expression of Wnt and frizzled genes in mouse. *Hepatology.* **2007**, *45*, 195–204.

160. Apte, U.; Zeng, G.; Muller, P.; Tan, X.; Micsenyi, A.; Cieply, B.; Dai, C.; Liu, Y.; Kaestner, K.H.; Monga, S.P. Activation of Wnt/beta-catenin pathway during hepatocyte growth factor-induced hepatomegaly in mice. *Hepatology* **2006**, *44*, 992–1002.

161. Monga, S.P.; Mars, W.M.; Pediaditakis, P.; Bell, A.; Mule, K.; Bowen, W.C.; Wang, X.; Zarnegar, R.; Michalopoulos, G.K. Hepatocyte growth factor induces Wnt-independent nuclear translocation of beta-catenin after Met-beta-catenin dissociation in hepatocytes. *Cancer Res.* **2002**, *62*, 2064–2071.

162. Sodhi, D.; Micsenyi, A.; Bowen, W.C.; Monga, D.K.; Talavera, J.C.; Monga, S.P. Morpholino oligonucleotide-triggered beta-catenin knockdown compromises normal liver regeneration. *J. Hepatol.* **2005**, *43*, 132–141.

163. Tan, X.; Behari, J.; Cieply, B.; Michalopoulos, G.K.; Monga, S.P. Conditional deletion of beta-catenin reveals its role in liver growth and regeneration. *Gastroenterology* **2006**, *131*, 1561–1572.

164. Rangwala, F.; Guy, C.D.; Lu, J.; Suzuki, A.; Burchette, J.L.; Abdelmalek, M.F.; Chen, W.; Diehl, A.M. Increased production of sonic hedgehog by ballooned hepatocytes. *J. Pathol.* **2011**, *224*, 401–410.

165. Witek, R.P.; Yang, L.; Liu, R.; Jung, Y.; Omenetti, A.; Syn, W.K.; Choi, S.S.; Cheong, Y.; Fearing, C.M.; Agboola, K.M.; Chen, W.; Diehl, A.M. Liver cell-derived microparticles activate hedgehog signaling and alter gene expression in hepatic endothelial cells. *Gastroenterology* **2009**, *136*, 320–330 e322.

166. Ochoa, B.; Syn, W.K.; Delgado, I.; Karaca, G.F.; Jung, Y.; Wang, J.; Zubiaga, A.M.; Fresnedo, O.; Omenetti, A.; Zdanowicz, M.; Choi, S.S.; Diehl, A.M. Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. *Hepatology* **2010**, *51*, 1712–1723.

167. Capurro, M.I.; Xu, P.; Shi, W.; Li, F.; Jia, A.; Filmus, J. Glypican-3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. *Dev. Cell.* **2008**, *14*, 700–711.

168. Kan, M.; Wu, X.; Wang, F.; McKeehan, W.L. Specificity for fibroblast growth factors determined by heparan sulfate in a binary complex with the receptor kinase. *J. Biol. Chem.* **1999**, *274*, 15947–15952.

169. Houck, K.A.; Zarnegar, R.; Muga, S.J.; Michalopoulos, G.K. Acidic fibroblast growth factor (HBGF-1) stimulates DNA synthesis in primary rat hepatocyte cultures. *J. Cell. Physiol.* **1990**, *143*, 129–132.

170. Strain, A.J.; McGuinness, G.; Rubin, J.S.; Aaronson, S.A. Keratinocyte growth factor and fibroblast growth factor action on DNA synthesis in rat and human hepatocytes: Modulation by heparin. *Exp. Cell Res.* **1994**, *210*, 253–259.

171. Steiling, H.; Wustefeld, T.; Bugnon, P.; Brauchle, M.; Fassler, R.; Teupser, D.; Thiery, J.; Gordon, J.I.; Trautwein, C.; Werner, S. Fibroblast growth factor receptor signalling is crucial for liver homeostasis and regeneration. *Oncogene* **2003**, *22*, 4380–4388.

172. Grisham, J.W. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. *Cancer Res.* **1962**, *22*, 842–849.

173. Widmann, J.J.; Fahimi, H.D. Proliferation of mononuclear phagocytes (Kupffer cells) and endothelial cells in regenerating rat liver. A light and electron microscopic cytochemical study. *Am. J. Pathol.* **1975**, *80*, 349–366.

174. Tanaka, Y.; Mak, K.M.; Lieber, C.S. Immunohistochemical detection of proliferating lipocytes in regenerating rat liver. *J. Pathol.* **1990**, *160*, 129–134.

175. Stocker, E.; Heine, W.D. Regeneration of liver parenchyma under normal and pathological conditions. *Beitr. Pathol.* **1971**, *144*, 400–408.

176. Stocker, E.; Heine, W.D. Proliferation and regeneration in liver and kidney of juvenile rats. Autoradiographic studies after continuous infusion of 3H-thymidine (author's transl). *Verh. Dtsch. Ges. Pathol.* **1971**, *55*, 483–488.

177. Stocker, E.; Schultze, B.; Heine, W.D.; Liebscher, H. Growth and regeneration in parenchymatous organs of the rat. Autoradiographic investigations with 3 H-thymidin. *Z. Zellforsch. Mikrosk. Anat.* **1972**, *125*, 306–331.

178. Schmucker, D.L.; Sanchez, H. Liver regeneration and aging: a current perspective. *Curr. Gerontol. Geriatr. Res.* **2011**, *2011*, 526379.

179. Stocker, E.; Wullstein, H.K.; Brau, G. Capacity of regeneration in liver epithelia of juvenile, repeated partially hepatectomized rats. Autoradiographic studies after continous infusion of 3H-thymidine (author's transl). *Virchows Arch. B Cell. Pathol.* **1973**, *14*, 93–103.

180. Overturf, K.; al-Dhalimy, M.; Ou, C.N.; Finegold, M.; Grompe, M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. *Am. J. Pathol.* **1997**, *151*, 1273–1280.

181. Bhave, V.S.; Paranjpe, S.; Bowen, W.C.; Donthamsetty, S.; Bell, A.W.; Khillan, J.S.; Michalopoulos, G.K. Genes inducing iPS phenotype play a role in hepatocyte survival and proliferation *in vitro* and liver regeneration *in vivo*. *Hepatology* **2011**, *54*, 1360–1370.

182. Kan, M.; Huang, J.S.; Mansson, P.E.; Yasumitsu, H.; Carr, B.; McKeehan, W.L. Heparin-binding growth factor type 1 (acidic fibroblast growth factor): a potential biphasic autocrine and paracrine regulator of hepatocyte regeneration. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7432–7436.

183. Borkham-Kamphorst, E.; Kovalenko, E.; van Roeyen, C.R.; Gassler, N.; Bomble, M.; Ostendorf, T.; Floege, J.; Gressner, A.M.; Weiskirchen, R. Platelet-derived growth factor isoform expression in carbon tetrachloride-induced chronic liver injury. *Lab. Invest.* **2008**, *88*, 1090–1100.

184. Ross, M.A.; Sander, C.M.; Kleeb, T.B.; Watkins, S.C.; Stolz, D.B. Spatiotemporal expression of angiogenesis growth factor receptors during the revascularization of regenerating rat liver. *Hepatology* **2001**, *34*, 1135–1148.

185. Martinez-Hernandez, A.; Amenta, P.S. The extracellular matrix in hepatic regeneration. *FASEB J.* **1995**, *9*, 1401–1410.

186. Wack, K.E.; Ross, M.A.; Zegarra, V.; Sysko, L.R.; Watkins, S.C.; Stolz, D.B. Sinusoidal ultrastructure evaluated during the revascularization of regenerating rat liver. *Hepatology* **2001**, *33*, 363–378.

187. Fujii, H.; Hirose, T.; Oe, S.; Yasuchika, K.; Azuma, H.; Fujikawa, T.; Nagao, M.; Yamaoka, Y. Contribution of bone marrow cells to liver regeneration after partial hepatectomy in mice. *J. Hepatol.* **2002**, *36*, 653–659.

188. Zocco, M.A.; Piscaglia, A.C.; Giuliante, F.; Arena, V.; Novi, M.; Rinninella, E.; Tortora, A.; Rumi, C.; Nuzzo, G.; Vecchio, F.M.; Bombardieri, G.; Gasbarrini, A. CD133+ stem cell mobilization after partial hepatectomy depends on resection extent and underlying disease. *Dig. Liver Dis.* **2011**, *43*, 147–154.

189. Wang, L.; Wang, X.; Xie, G.; Hill, C.K.; DeLeve, L.D. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. *J. Clin. Invest.* **2012**, *122*, 1567–1573.

190. Wang, L.; Wang, X.; Chiu, J.D.; van de Ven, G.; Gaarde, W.A.; Deleve, L.D. Hepatic Vascular Endothelial Growth Factor Regulates Recruitment of Rat Liver Sinusoidal Endothelial Cell Progenitor Cells. *Gastroenterology* **2012**, *143*, 1555–1563.

191. Lagasse, E.; Connors, H.; Al-Dhalimy, M.; Reitsma, M.; Dohse, M.; Osborne, L.; Wang, X.; Finegold, M.; Weissman, I.L.; Grompe, M. Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat. Med.* **2000**, *6*, 1229–1234.

192. Petersen, B.E.; Bowen, W.C.; Patrene, K.D.; Mars, W.M.; Sullivan, A.K.; Murase, N.; Boggs, S.S.; Greenberger, J.S.; Goff, J.P. Bone marrow as a potential source of hepatic oval cells. *Science* **1999**, *284*, 1168–1170.

193. Terada, N.; Hamazaki, T.; Oka, M.; Hoki, M.; Mastalerz, D.M.; Nakano, Y.; Meyer, E.M.; Morel, L.; Petersen, B.E.; Scott, E.W. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* **2002**, *416*, 542–545.

194. Wang, X.; Willenbring, H.; Akkari, Y.; Torimaru, Y.; Foster, M.; Al-Dhalimy, M.; Lagasse, E.; Finegold, M.; Olson, S.; Grompe, M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature.* **2003**, *422*, 897–901.

195. Kuwahara, R.; Kofman, A.V.; Landis, C.S.; Swenson, E.S.; Barendswaard, E.; Theise, N.D. The hepatic stem cell niche: identification by label-retaining cell assay. *Hepatology* **2008**, *47*, 1994–2002.

196. Dolle, L.; Best, J.; Mei, J.; Al Battah, F.; Reynaert, H.; van Grunsven, L.A.; Geerts, A. The quest for liver progenitor cells: a practical point of view. *J. Hepatol.* **2010**, *52*, 117–129.

197. Evarts, R.P.; Nagy, P.; Nakatsukasa, H.; Marsden, E.; Thorgeirsson, S.S. In vivo differentiation of rat liver oval cells into hepatocytes. *Cancer Res.* **1989**, *49*, 1541–1547.

198. Trautwein, C.; Will, M.; Kubicka, S.; Rakemann, T.; Flemming, P.; Manns, M.P. 2-acetaminofluorene blocks cell cycle progression after hepatectomy by p21 induction and lack of cyclin E expression. *Oncogene* **1999**, *18*, 6443–6453.

199. Limaye, P.B.; Alarcon, G.; Walls, A.L.; Nalesnik, M.A.; Michalopoulos, G.K.; Demetris, A.J.; Ochoa, E.R. Expression of specific hepatocyte and cholangiocyte transcription factors in human liver disease and embryonic development. *Lab. Invest.* **2008**, *88*, 865–872.

200. Petersen, B.E.; Goff, J.P.; Greenberger, J.S.; Michalopoulos, G.K. Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat. *Hepatology* **1998**, *27*, 433–445.

201. Evarts, R.P.; Hu, Z.; Fujio, K.; Marsden, E.R.; Thorgeirsson, S.S. Activation of hepatic stem cell compartment in the rat: role of transforming growth factor alpha, hepatocyte growth factor, and acidic fibroblast growth factor in early proliferation. *Cell Growth Differ.* **1993**, *4*, 555–561.

202. Darwiche, H.; Oh, S.H.; Steiger-Luther, N.C.; Williams, J.M.; Pintilie, D.G.; Shupe, T.D.; Petersen, B.E. Inhibition of Notch signaling affects hepatic oval cell response in rat model of 2AAF-PH. *Hepat. Med.* **2011**, *3*, 89–98.

203. Thenappan, A.; Li, Y.; Kitisin, K.; Rashid, A.; Shetty, K.; Johnson, L.; Mishra, L. Role of transforming growth factor beta signaling and expansion of progenitor cells in regenerating liver. *Hepatology* **2010**, *51*, 1373–1382.

204. Erker, L.; Grompe, M. Signaling networks in hepatic oval cell activation. *Stem Cell Res.* **2007**, *1*, 90–102.

205. Jakubowski, A.; Ambrose, C.; Parr, M.; Lincecum, J.M.; Wang, M.Z.; Zheng, T.S.; Browning, B.; Michaelson, J.S.; Baetscher, M.; Wang, B.; Bissell, D.M.; Burkly, L.C. TWEAK induces liver progenitor cell proliferation. *J. Clin. Invest.* **2005**, *115*, 2330–2340.

206. Mavier, P.; Martin, N.; Couchie, D.; Preaux, A.M.; Laperche, Y.; Zafrani, E.S. Expression of stromal cell-derived factor-1 and of its receptor CXCR4 in liver regeneration from oval cells in rat. *Am. J. Pathol.* **2004**, *165*, 1969–1977.

207. Theise, N.D.; Saxena, R.; Portmann, B.C.; Thung, S.N.; Yee, H.; Chiriboga, L.; Kumar, A.; Crawford, J.M. The canals of Hering and hepatic stem cells in humans. *Hepatology* **1999**, *30*, 1425–1433.

208. Nagy, P.; Bisgaard, H.C.; Thorgeirsson, S.S. Expression of hepatic transcription factors during liver development and oval cell differentiation. *J .Cell. Biol.* **1994**, *126*, 223–233.

209. Petersen, B.E.; Zajac, V.F.; Michalopoulos, G.K. Bile ductular damage induced by methylene dianiline inhibits oval cell activation. *Am. J. Pathol.* **1997**, *151*, 905–909.

210. Demetris, A.J.; Seaberg, E.C.; Wennerberg, A.; Ionellie, J.; Michalopoulos, G. Ductular reaction after submassive necrosis in humans. Special emphasis on analysis of ductular hepatocytes. *Am. J. Pathol.* **1996**, *149*, 439–448.

211. Michalopoulos, G.K.; Barua, L.; Bowen, W.C. Transdifferentiation of rat hepatocytes into biliary cells after bile duct ligation and toxic biliary injury. *Hepatology* **2005**, *41*, 535–544.

212. Kordes, C.; Sawitza, I.; Muller-Marbach, A.; Ale-Agha, N.; Keitel, V.; Klonowski-Stumpe, H.; Haussinger, D. CD133+ hepatic stellate cells are progenitor cells. *Biochem. Biophys. Res. Commun.* **2007**, *352*, 410–417.

213. Yang, L.; Jung, Y.; Omenetti, A.; Witek, R.P.; Choi, S.; Vandongen, H.M.; Huang, J.; Alpini, G.D.; Diehl, A.M. Fate-mapping evidence that hepatic stellate cells are epithelial progenitors in adult mouse livers. *Stem Cells.* **2008**, *26*, 2104–2113.

214. Xu, C.; Yang, Y.; Yang, J.; Chen, X.; Wang, G. Analysis of the role of the integrin signaling pathway in hepatocytes during rat liver regeneration. *Cell Mol Biol Lett.* **2012**, *17*, 274–288.

215. Legate, K.R.; Montanez, E.; Kudlacek, O.; Fassler, R. ILK, PINCH and parvin: the tIPP of integrin signalling. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 20–31.

216. Gkretsi, V.; Bowen, W.C.; Yang, Y.; Wu, C.; Michalopoulos, G.K. Integrin-linked kinase is involved in matrix-induced hepatocyte differentiation. *Biochem. Biophys. Res. Commun.* **2007**, *353*, 638–643.

217. Gkretsi, V.; Apte, U.; Mars, W.M.; Bowen, W.C.; Luo, J.H.; Yang, Y.; Yu, Y.P.; Orr, A.; St-Arnaud, R.; Dedhar, S.; Kaestner, K.H.; Wu, C.; Michalopoulos, G.K. Liver-specific ablation of integrin-linked kinase in mice results in abnormal histology, enhanced cell proliferation, and hepatomegaly. *Hepatology* **2008**, *48*, 1932–1941.

218. Hippo, Y.; Watanabe, K.; Watanabe, A.; Midorikawa, Y.; Yamamoto, S.; Ihara, S.; Tokita, S.; Iwanari, H.; Ito, Y.; Nakano, K.; Nezu, J.; Tsunoda, H.; Yoshino, T.; Ohizumi, I.; Tsuchiya, M.; Ohnishi, S.; Makuuchi, M.; Hamakubo, T.; Kodama, T.; Aburatani, H. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res.* **2004**, *64*, 2418–2423.

219. Pilia, G.; Hughes-Benzie, R.M.; MacKenzie, A.; Baybayan, P.; Chen, E.Y.; Huber, R.; Neri, G.; Cao, A.; Forabosco, A.; Schlessinger, D. Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nat. Genet.* **1996**, *12*, 241–247.

220. Liu, B.; Paranjpe, S.; Bowen, W.C.; Bell, A.W.; Luo, J.H.; Yu, Y.P.; Mars, W.M.; Michalopoulos, G.K. Investigation of the role of glypican 3 in liver regeneration and hepatocyte proliferation. *Am. J. Pathol.* **2009**, *175*, 717–724.

221. Ichikawa, T.; Zhang, Y.Q.; Kogure, K.; Hasegawa, Y.; Takagi, H.; Mori, M.; Kojima, I. Transforming growth factor beta and activin tonically inhibit DNA synthesis in the rat liver. *Hepatology* **2001**, *34*, 918–925.

222. Buraschi, S.; Pal, N.; Tyler-Rubinstein, N.; Owens, R.T.; Neill, T.; Iozzo, R.V. Decorin antagonizes Met receptor activity and down-regulates {beta}-catenin and Myc levels. *J. Biol. Chem.* **2010**, *285*, 42075–42085.

223. Zhu, J.X.; Goldoni, S.; Bix, G.; Owens, R.T.; McQuillan, D.J.; Reed, C.C.; Iozzo, R.V. Decorin evokes protracted internalization and degradation of the epidermal growth factor receptor via caveolar endocytosis. *J. Biol. Chem.* **2005**, *280*, 32468–32479.

224. Avruch, J.; Zhou, D.; Fitamant, J.; Bardeesy, N. Mst1/2 signalling to Yap: gatekeeper for liver size and tumour development. *Br. J. Cancer.* **2011**, *104*, 24–32.

225. Dong, J.; Feldmann, G.; Huang, J.; Wu, S.; Zhang, N.; Comerford, S.A.; Gayyed, M.F.; Anders, R.A.; Maitra, A.; Pan, D. Elucidation of a universal size-control mechanism in Drosophila and mammals. *Cell* **2007**, *130*, 1120–1133.

226. Lu, L.; Li, Y.; Kim, S.M.; Bossuyt, W.; Liu, P.; Qiu, Q.; Wang, Y.; Halder, G.; Finegold, M.J.; Lee, J.S.; Johnson, R.L. Hippo signaling is a potent *in vivo* growth and tumor suppressor pathway in the mammalian liver. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1437–1442.

227. Song, H.; Mak, K.K.; Topol, L.; Yun, K.; Hu, J.; Garrett, L.; Chen, Y.; Park, O.; Chang, J.; Simpson, R.M.; Wang, C.Y.; Gao, B.; Jiang, J.; Yang, Y. Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1431–1436.

228. Pahlavan, P.S.; Feldmann, R.E., Jr.; Zavos, C.; Kountouras, J. Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. *J. Surg. Res.* **2006**, *134*, 238–251.

229. Humar, A.; Kosari, K.; Sielaff, T.D.; Glessing, B.; Gomes, M.; Dietz, C.; Rosen, G.; Lake, J.; Payne, W.D. Liver regeneration after adult living donor and deceased donor split-liver transplants. *Liver Transpl.* **2004**, *10*, 374–378.