The American Journal of Pathology, Vol. 176, No. 1, January 2010

DOI: 10.2353/ajpath.2010.090675

Rous-Whipple Award Lecture

Liver Regeneration after Partial Hepatectomy

Critical Analysis of Mechanistic Dilemmas

George K. Michalopoulos

From the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Liver regeneration after partial hepatectomy is one of the most studied models of cell, organ, and tissue regeneration. The complexity of the signaling pathways initiating and terminating this process have provided paradigms for regenerative medicine. Many aspects of the signaling mechanisms involved in hepatic regeneration are under active investigation. The purpose of this review is to focus on the areas still not well understood. The review also aims to provide insights into the ways by which current concepts of liver regeneration can provide understanding regarding malfunction of the regenerative process in liver diseases, such as acute liver failure. ***(Am J Pathol 2010, 176:2–13; DOI: 10.2353/ajpath.2010.090675)***

Liver regeneration after two-thirds partial hepatectomy (PHx) in rodents has become a useful paradigm of studying regenerative organ growth. The popularity of the model is based on two important aspects. *First*, the removal of the resected tissue is not associated with massive necrosis. The resected hepatic tissues are amenable to "clean" removal due to the multilobular structure of rat and mouse liver. Thus, regeneration of the residual lobes from its very beginning is mediated by processes relevant only to liver tissue and not to necrosis or acute inflammation. In contrast, in models involving necrosis of lobular zones induced by toxins (eg, CCl4), the events of first day after toxic injury are dominated by acute inflammation of the necrotic zones. Polymorphonuclear leukocytes and macrophages infiltrate the necrotic area to remove dead hepatocytes. *Second*, because PHx stimulates immediate initiation of regeneration without complications from inflammatory situations, and because PHx can be performed in a few minutes, the regenerative phenomena can be precisely timed, with a reference (time 0) point from the time of the performance of PHx. These two attributes of the model are the major reason for its usefulness, enhanced popularity, and acceptance through the years by many investigators.[1,2]

Although the model of PHx is a relatively "clean" one, it should be emphasized that in human disease settings, many processes involving innate immunity, tissue healing through removal of necrotic material, etc are of paramount importance and may have a significant role to play. The PHx model allows distinct understanding of the regenerative process per se, but for a better understanding of the regenerative process in the context of human liver disease, some of these nonregenerative aspects of the response to injury need to be eventually integrated with the PHx model.

Biochemical studies based on whole tissue homogenates and cellular localization techniques have defined the kinetics of cell proliferation and key intracellular events, such as activation of cell cycle–associated genes and key transcription factors (Cyclin D1,[3] Signal transducer and activator of transcription 3 (STAT3),[4] nuclear factor κB [NF-κB],[5] etc). Performance of PHx on different "knock-out" strains of mice deficient for specific genes has revealed controlling roles and influences of several signaling molecules, which, though not mitogenic for hepatocytes per se, nonetheless appear to contribute to optimization of the regenerative process. The absence of these important signals is associated with a delay but not elimination of regeneration.

Despite all of the advantages of live animal studies, determination of signaling pathways in live animals and whole tissues has inherent limitations. Tissues comprise several cell types, and data obtained from a liver tissue homogenate do not necessarily represent hepatocytes,

Accepted for publication August 25, 2009.

The Rous-Whipple Award is given by the American Society for Investigative Pathology to a senior pathologist with a distinguished career in experimental pathology research and continued productivity at the time of the award. George K. Michalopoulos, recipient of the 2009 ASIP Rous-Whipple Award, delivered a lecture entitled "Liver Regeneration," on April 19, 2009 at the annual meeting of the American Society for Investigative Pathology in New Orleans, Louisiana.

Address reprint requests to George K. Michalopoulos, M.D., Ph.D., University of Pittsburgh, Department of Pathology, School of Medicine, S-410 Biomedical Science Tower, Pittsburgh, PA 15261. E-mail: michalopoulosgk@msx.upmc.edu.

as they could also be affected by changes in stellate cells, macrophages (Kupffer cells), biliary ductules, and sinusoidal endothelial cells. The technique of isolation and culture of hepatocytes based on perfusion of liver by collagenase was introduced in the early 70s and allowed, for the first time, the isolation and culture of most of the hepatocytes from a rat, mouse, or human liver.[6,7] Hepatocytes were derived directly from the liver, without any intervening mitotic events. As such, these cultures were called "primary" cultures of hepatocytes.

Even though there are inherent limitations of primary cultures (loss of differentiation of hepatocytes and its restoration by extracellular matrix[8]), they have been very useful in delineating several aspects of hepatocyte biology. Different signals can be assessed for their capacity to stimulate hepatocyte DNA synthesis in chemically defined (serum-free) media. Typically a five- to tenfold increase in DNA synthesis above control levels is considered a significant effect and is most commonly seen by hepatocyte growth factor (HGF) and ligands of the epidermal growth factor receptor (EGFR), of which epidermal growth factor (EGF) and transforming growth factor α (TGFα) are the most commonly used.[8] Other substances, including norepinephrine,[9,10] prostaglandins,[11] tumor necrosis factor (TNF) α,[12] estrogens,[13] and insulin[14] are not significantly mitogenic by themselves but enhance the effects of HGF, EGF, and TGFα.[8] Hepatocyte proliferation is strongly induced by combining HGF and EGF but there is gradual loss of expression of hepatocyte-associated genes.[8] Addition of complex extracellular matrix products prevents loss of hepatocyte differentiation[15] but blocks the mitogenic effects of HGF and EGF. These findings have set paradigmatic backgrounds for understanding many of the events elicited by PHx. Based on a combination of effects seen both with whole animals and with primary cultures of hepatocytes, the signals associated with initiation and control of the proliferative events after PHx can be separated into two groups. Complete mitogens and auxilary mitogens.

Complete mitogens are mitogenic in hepatocyte cultures in chemically defined (serum-free) media. In addition, they cause liver enlargement and hepatocyte DNA synthesis when injected in sufficient doses into whole animals.[16–18] Currently, there are two groups of signals that fall in this category: 1) Hepatocyte growth factor (HGF) and receptor c-Met and 2) Ligands of the EGF R (EGF, TGFα, Heparin Binding-EGF, Amphiregulin).

Ablation of the signaling pathways associated with Auxiliary mitogens causes a delay but does not abolish liver regeneration. Typically, these substances are not mitogenic in hepatocyte cultures and when injected in animals do not cause hepatocyte DNA synthesis and liver enlargement. The list of these substances is relatively long and includes norepinephrine and the α1 adrenergic receptor,[9] TNF and TNFR1,[19] interleukin (IL) 6,[20,21] *Notch and Jagged*[22] (recombinant Jagged causes DNA synthesis in hepatocyte cultures), vascular endothelial growth factor (VEGF) and receptors I and II,[23] bile acids,[24] serotonin,[25] complement proteins,[26] leptin,[27] insulin,[28] fibroblast growth factor (FGF) 1 and FGF2.[29,30]

In some cases there is overlap in function between the two groups. For example, FGF1 and FGF2 and recombinant *Jagged 1* induce a moderate degree of hepatocyte proliferation in culture, but they have not been shown to cause hepatocyte proliferation when injected into normal rats or mice.

A recent review by this author[2] provides a detailed description of the multiplicity of pathways and cellular proliferation kinetics involved in initiation and termination of liver regeneration, the role of growth factors and cytokines in the process, and the capacity of hepatocytes and biliary epithelial cells to function as facultative stem cells for each other. Such information has also been provided in other reviews on the subject.[1,31,32] The purpose of this review is to concentrate on aspects of liver regeneration least understood and to provide recent and classic information that helps formulate the mechanistic dilemmas of the field. Such dilemmas are numerous and constitute either areas of active investigation or topics that are not easily amenable to experimental analysis, and thus difficult to assess in a mechanistic manner.

Is There a Single Signal Driving Liver Regeneration?

There has been a tendency in this field (and other areas of regenerative biology) to assign single causal relationships to initiation of liver regeneration by signals/agents whose blockade or deficiency leads to delay in the regenerative process. Delays in liver regeneration have been demonstrated by blocking of signals mediated by norepinephrine, Notch/Jagged, TNF, bile acids, serotonin, components of complement, and IL6 (see above). Regeneration completes when the remnant lobes enlarge to the size of the original liver, a process that typically requires about five to seven days in rat and mouse. Elimination of the signals of either direct or auxiliary mitogens causes delays in regeneration as manifested by delayed activation of transcription factors (STAT3, NF-κB) and delay or diminished magnitude of hepatocyte DNA synthesis in the first one to two days. The eventual completion of the regenerative process, despite the initial delays, demonstrates that there is no single signal that alone drives the regenerative process. There is a remarkable redundancy between signals so that many of the signaling agents overlap in function, and eventually provide the missing contributions of the blocked pathway, so that regeneration completes itself. However, it is often assumed that because regeneration eventually completes, any signal whose deletion merely delays regeneration is of no importance. This is not the case. Delay of regeneration is likely to have serious adverse effects on the life of the animal, when regeneration is critically needed to prevent loss of liver function and liver failure. Thus, all processes identified so far (and likely more to come) that are important for the optimization of the intracellular events after partial hepatectomy should be considered as important signaling contributors and likely to operate in tandem and provide the essential redundancy that confers a safety margin to liver regeneration and allows it to operate with maximal efficiency. The conse-

quences of elimination of the signals from both MET and EGFR have not been reported so far.

Studies on interference with specific signals tend to rely on mice with specific genetic deficiencies. The mice often demonstrate alterations in liver histology, which themselves have secondary effects whose contribution cannot be easily determined.[30,33] Many times there is no apparent alteration in histology and livers are considered "normal" with the exception of the missing signal. Secondary gene expression changes deriving from the original signaling block often are not considered in these studies. In view of the above, acute elimination of specific signals so that there are no long-term adaptive changes in gene expression or histological changes should be a useful complementary approach. As an example, targeted elimination of the HGF receptor from mouse hepatocytes leads to progressive fibro-fatty change in the livers.[33,34] When these livers are subjected to partial hepatectomy, there is a dampening of the response in the first proliferative cycle, and hepatocyte proliferation is decreased but measurable, down to one third of the control mice. Acute elimination ("knock-down") of Met signaling in rats on the other hand, by a ShRNA approach, results in complete elimination of Bromodeoxyuridine labeling and mitoses in the first proliferation cycle. Putting aside differences in the species used (mouse versus rats), the acute elimination of MET signaling is more effective in restricting the effects of the first proliferative cycle than the longer-term genetic approach, which results in adaptations imparting redundancy and thus potentially masking the inhibitory effects. (As an example of this concern, the reader is referred to the differences in responses seen in mice after acute removal of integrin-linked kinase by Adeno-Cre (causing massive hepatocyte apoptosis[35]) and slower removal of integrin-linked kinase by genetic approaches, causing massive adaptation of the liver and reprogramming of gene expression, leading to hepatocyte hyperproliferation.[36]

Role of Portal and General Circulation in Initiation of Liver Regeneration

Portal Circulation

After PHx in rats and mice, each residual lobe of liver retains its supply of hepatic artery and portal vein branches. Whereas the amount of arterial blood going into every lobe remains essentially the same, the relative proportion and amount of portal vein blood going into each lobe increases threefold. The reason for this is simple mathematics. PHx removes two thirds of the liver mass.[37] The entire portal circulation continues to traverse through a liver reduced to one third of its original size. Thus, the flow of portal blood per hepatocyte or unit liver mass theoretically increases threefold after PHx. Several signaling changes appear in liver tissue and hepatocyte nuclei within 15 minutes after PHx. These include increase in urokinase activity (within five minutes), β-catenin migration to hepatocyte nuclei (five minutes), and migration of Notch1 intracellular domain (NICD) to hepatocyte nuclei (15 minutes). The very rapid induction of these signaling changes suggests two alternatives. 1) The volume of portal flow per unit mass, which increases instantly, is a sufficient stimulus to initiate further cascades of signals such as the above. 2) The portal blood contains signaling molecule(s) whose relative three times increase per hepatocyte results in further signaling initiating regenerative activities.

These alternatives are not mutually exclusive. Several studies have shown that portal vein flow by itself is important in triggering some early changes, including induction of urokinase plasminogen activator gene expression[38] and activation of HGF. There is no easy scalable experimental approach, however, to proportionately correlate portal flow to the initiation of regenerative events and there is no clear answer as to whether the increase in portal vein flow is sufficient to initiate regeneration. When portal flow is restricted, and despite decreased activation of HGF the percentage of hepatocyte nuclei expressing PCNA (a marker of entry into cell cycle) does not appreciably change. Portal circulation contains increased concentrations of insulin (derived from pancreas) and EGF (derived from Brunner's glands of the duodenum).[39] EGF is a direct hepatocyte mitogen. It is not clear, however, whether a mere relative increase in EGF concentration would be sufficient to trigger the magnitude of the changes seen. EGF concentration, however, may be increasing in the portal circulation because norepinephrine rises in the blood after PHx,[9] and it is known that it can cause increased production of EGF from Brunner's glands.[40] Infusion of EGF into unoperated animals does cause increased DNA synthesis, but the scale is much smaller than that seen after PHx.[41]

General Circulation

Earlier studies had shown that in rats joined in parabiotic circulation, hepatectomy of one member of the pair resulted in DNA synthesis of the liver of the other member of the pair.[42] In addition, DNA synthesis was observed in transplanted grafts of hepatic tissue[43] as well as in isolated hepatocytes transplanted into the adipose tissue[44] when the liver of the recipient animal was subjected to PHx. All of these studies suggested that PHx results in generation of mitogenic signals that can trigger DNA synthesis in hepatocytes anywhere in the body. Attempts to identify such signal(s) by using DNA synthesis in hepatocyte cultures led to isolation of HGF.[45,46] It is now known that HGF does increase in peripheral blood within one hour after PHx,[47] that it is present in the active (heterodimeric) form,[48] and that the source of the early circulating HGF is hepatic pericellular and extracellular glycosaminoglycans, which are present in the pericellular or extracellular matrix associated with hepatocytes in periportal sites of hepatic lobules.[49,50] (There is synthesis of new HGF occurring in liver and lungs, but this does not occur until three hours after PHx [see below]). Because the methods used to identify the active mitogenic princi-

ple(s) in the blood after PHx used bioassays relying on hepatocyte proliferation in culture, they unavoidably would not detect changes in other signaling molecules that do not have this property. From subsequent studies we now know that in addition to the direct mitogen HGF, other "auxiliary mitogens" also increase in the peripheral blood, including norepinephrine, TNF,[51] IL6,[20] bile acids,[24] and insulin.[52] Of interest, hyaluronic acid, presumably derived from the extensive ECM remodeling after PHx, also increases in the peripheral blood[1] and so does TGFβ1,[1] also present in hepatic ECM bound to decorin. The kinetics of this response of transplanted hepatocytes in comparison with the proliferation kinetics of the orthotopic liver have not been studied.

Another recently studied component of the blood that should be a logically expected regulator of the regenerative response is glucose. Recent studies by Rudnick et al showed that there is persistent hypoglycemia after PHx and that administration of 10% Dextrose in the drinking water reversing the hypoglycemia had substantial inhibitory effects on the regenerative response.[53]

Ratio of Portal to Arterial Blood in the Extensive Hepatectomy Model and the "Small for Size" Transplanted Liver Graft

Partial hepatectomy is a well-tolerated procedure in which two thirds of the liver tissue is excised. On the other hand, several reports have indicated that 90% hepatectomy is mostly fatal.[54] The reason(s) for this is not clear. There is a similar issue related to orthotopic liver transplantation, under the name of (graft being) "small for size." In this situation, a small portion of the liver transplanted to an adult most often results in failure and the transplanted liver does not grow in size, whereas a liver portion at 60% of a typical adult liver mass typically responds much better and eventually grows to the size of an adult liver. The "small for size" situation has been investigated from the perspective of new hemodynamics and altered ratios of portal to arterial blood flow after transplantation. Several studies have shown that when the full portal vein flow has to traverse through a much reduced liver size, then the pressure building up in the portal vein effectively shuts down the flow through the portal arterioles and the liver becomes "dearterialized."[55–58] Adenosine may play a role in this phenomenon.[59] The failure to regenerate under these circumstances is not different from the situation in which PHx is accompanied by ligation of the hepatic artery, which also results in failure to regenerate. It is highly likely that the failure to regenerate after 90% hepatectomy in rodents is attributable to portal vein pressure impacting on hepatic artery flow, though other factors related to gene expression, growth factors, and cytokines have not been entirely ruled out as contributors to this phenomenon.

Signaling Contributions of Other Organs to Regeneration of Liver

As mentioned above, there is a rise of multiple signaling molecules in the peripheral blood after PHx. These include HGF, norepinephrine, TNF, IL6, bile acids, serotonin, TGFβ1, hyaluronic acid, etc. The origin(s) of each of these substances rising in the blood during liver regeneration is not clear (except for bile acids, which are generated only in the liver). Norepinephrine is normally cleared by the liver, thus a reduction in liver mass associated with diminished clearance may cause a rise in catecholamines. TNF and IL6 production increases in the liver after PHx, but the potential of production of these cytokines by other organs has not been investigated. The story related to HGF is more complex. There is a rise of active HGF protein in the plasma with a peak at one hour after PHx.[47] This is probably derived from remodeling of hepatic extracellular and pericellular matrix, in which HGF concentration decreases after PHx.[48,60] These events are mediated by matrix remodeling initiated by urokinase plasminogen activator and matrix metalloproteinase 9.[61,62] At three hours after PHx, there is a beginning of rise in HGF mRNA in the liver, primarily from stellate[63] and endothelial[23] cells. This is associated with increase in the concentration of active HGF protein in whole liver homogenates.[48] Of interest, however, is the fact that at the same time point and with the same time kinetics there is also an increase in HGF production in the lungs,[64] kidney, and spleen.[65] The increase in lungs is comparable with that seen in liver. The fate of PHx-stimulated HGF produced in lungs is not clear. There are no studies to determine whether it stays in the lungs, whether it is exported to the blood to function in an endocrine manner, or whether it has any effect on hepatic regeneration. The robustness and reproducibility of the phenomenon suggests that it should play a meaningful role. The nature of the signals that would lead to increased production of HGF in the lungs and other tissues after liver resection is not clear. Norepinephrine is known to stimulate production of HGF in mesenchymal cells[66] and it does rise after PHx,[9] thus being a reasonable candidate for causing the increase of HGF in several tissues. There have been no systematic studies, however, to address this relationship. The promoter region of the HGF gene includes an IL6 response element, but there is no direct evidence for IL6 inducing production of HGF.[67–69] LIF on the other hand, a cytokine that (similarly to IL6) is also acting through gp130, has been shown to stimulate production of HGF.[70] Whether these or other molecules are to account for the production of HGF by lungs, kidneys, and spleen, the cells involved in this phenomenon and its overall importance in liver regeneration are not clear at this point.

The peripheral nervous system (both sympathetic and parasympathetic) has also been implicated in liver regeneration. The mechanisms by which the parasympathetic nervous system may exert such controls (through the vagus nerve) are not clear.[71] The effects may not be directly on hepatocytes, as the vagus nerve has been

shown to affect vascular flow, etc. Resection of the sympathetic nervous system also leads to delay in liver regeneration.[9] The role of the sympathetic nervous system may be mediated via norepinephrine, because blockade of the α adrenergic receptor leads to similar results.[9] Recent studies have demonstrated that hepatic stellate cells may also be producing catecholamines including norepinephrine.[72] These cells express a variety of genes seen in neurons and astrocytes, including glial fibrillary acidic protein and many neurotrophin receptors,[73] and they may be the venue from which sympathetic functions affect the regenerative process.

Paracrine versus Autocrine Effects of Growth Factors during Liver Regeneration

Tyrosine phosphorylation of kinase sites of MET and EGFR can be demonstrated within 30 to 60 minutes after PHx. This is probably related to increased release and activation of HGF from intrahepatic stores as well as to EGF effects on its receptor. EGF is synthesized in Brunner's glands of the duodenum.[39] The synchrony of Tyr-phosphorylation of MET and EGFR is not entirely explained because EGF is always available to the liver. On the other hand, enhanced concentration of EGF in the portal blood has not been ruled out and norepinephrine (which has been shown to induce expression of HGF[66]) is also known to enhance production of EGF in Brunner's glands of the duodenum.[40] Other studies have shown "cross talk" of MET and EGFR in several cell lines,[74,75] and the interaction of the two receptors at the early steps after PHx has not been directly addressed.

There are many growth factors produced by hepatocytes during regeneration, and some of them may have not only paracrine but also autocrine effects. TGFα, FGF1, and FGF2 have been shown to be mitogenic to hepatocytes in culture, with TGFα being by far more active than FGF1 and FG2. The same growth factors, however, are also mitogenic for many nonparenchymal cell types, especially endothelial cells. Is TGFα acting as an autocrine mitogen for the hepatocytes that produce it? There are no critical and feasible experiments to answer this question *in vivo*. Mice deficient in TGFα have a normal response to PHx,[76] but this is probably attributable to redundant expression of other complementary EGFR ligands during regeneration, such as HB-EGF and Amphiregulin, both of which have been shown to have regulatory effects on liver regeneration.[77,78]

The paracrine effects of the same growth factors have not been critically demonstrated either, though there is an assumption that they must have an effect on endothelial and stellate cells. During regeneration, hepatocytes also produce purely angiogenic factors such VEGF and Angiopoietins 1 and 2.[79,80] Previous studies have shown that VEGF stimulates production of HGF in endothelial cells via VEGF receptor 1. In view of this, it is highly likely that the rise in HGF seen during liver regeneration is mediated by not only stellate cells but also the endothelial cells.[23] The peak in HGF mRNA expression in liver occurs 12 hours after PHx, a time in which most of the hepatocytes are already in S-phase.[1,2,31] HGF receptor is already Tyr-phosphorylated at 1 hour after PHx. Therefore, if HGF is playing a paracrine role to stimulate hepatocyte proliferation, this must be aimed toward the second (smaller) wave of DNA synthesis occurring at day 2 in the rat and day 3 after PHx in the mouse.[31] HGF however is also a mitogen for biliary epithelial cells[81] and endothelial cells, and the role of HGF produced from new HGF mRNA synthesis is not clear. "Knock-down" of HGF mRNA by silencing RNA was effective in decreasing the HGF mRNA expression, but it minimally affected proliferation of hepatocytes, suggesting that the effect of preexisting HGF protein (not affected by the silencing of mRNA) is more important in driving hepatocyte proliferation than that of the newly synthesized HGF.[82]

PDGF is a mitogen for hepatic stellate cells, and it is produced by hepatocytes during regeneration.[83] PDGF is not a mitogen for hepatocytes in culture. On the other hand, in transgenic mice with overexpression of PDGF in hepatocytes there is hepatic enlargement and eventually hepatic neoplasia.[84] It is possible that excess PDGF may lead to increase in numbers of hepatic stellate cells and overproduction of HGF, causing these effects. TNF is produced by macrophages, and its effects on regulation and timing of activation of NF-κB should be viewed as a paracrine effect between macrophages (Kupffer cells) and hepatocytes. On the other hand the sources of IL6, a regulator of activation and timing of STAT3, have not been clearly delineated. There is evidence that Kupffer cells produce IL6, and this may be the source of IL6 leading to the acute phase response. Other sources of production of IL6, however, including hepatocytes, have not been ruled out.

Growth Factors, Fas and TNF, Liver Regeneration, and Liver Failure

Current evidence from the existing literature on liver regeneration has shown that HGF and ligands of EGFR are major regulators of hepatocyte proliferation and that signaling mediated by MET and EGFR is a very early event of the regenerative process.[2] Studies in hepatocytes and other cell types have shown that activation of signaling by either of these two receptors leads to activation of NF-κB, STAT3, phosphoinositide 3-kinase (PI3K), and eventually Akt.[85–87] Although the two mitogenic receptors are capable of activating the full cascade of signaling leading to hepatocyte proliferation in hepatocyte cultures or in other cell types, studies with mice deficient in TNF receptor 1 (TNF R1$^{-/-}$ mice) have also shown that in the absence of TNF signaling NF-κB activation is delayed and deficient.[12,19] This demonstrates that the timing and optimal activation of NF-κB during liver regeneration is a TNF-dependent event. Similar results were seen in mice in which TNF was blocked by administering specific antibodies.[88] IL6 is apparently exercising similar effects on activation of STAT3.[20,89] Even though regeneration (as evidenced by restoration of hepatic mass) eventually completes in TNF R1$^{-/-}$ and IL6$^{-/-}$ mice, the importance of these regulatory events should not be underestimated

especially in the setting of human disease, where optimization of liver regeneration after catastrophic loss of hepatic parenchyma may be a serious determinant of survival. TNF effects on liver appear to be dependent on its capacity to activate NF-κB. In the absence of activation of NF-κB, TNF can lead hepatocytes to apoptosis.[90] NF-κB activation is associated with enhanced expression of NO synthase, which contributes to protection of hepatocytes from TNF-induced apoptosis.[91] Under conditions in which activation of NF-κB activation is not possible, TNF may lead to hepatocyte apoptosis and liver failure.[92,93] Studies in mice have shown that activation of NF-κB by TNF is dependent on Akt phosphorylation.[94] Although TNF can stimulate phosphorylation of Akt in primary mouse hepatocyte cultures, Akt activation via Tyr phosphorylation is a well-recognized effect of both MET and EGFR.[85,87] In mice in which MET was specifically removed only in hepatocytes, Akt activation was very delayed after PHx and activation of ERK1/2 did not occur.[33] Clinical studies and experimental models of liver failure have shown elevated levels of TNF and Fas ligand and an interaction between TNF and Fas in inducing hepatocyte apoptosis.[93,95]

Acute inhibition of MET by ShRNA leads to complete blockade of hepatocyte proliferation in the first 24 hours after PHx.[82] Smaller but similar events are seen by ShRNA knock-down of EGFR (Paranjpe and Michalopoulos, unpublished observations, 2009). Although the effects on hepatocyte proliferation are not surprising, MET knock-down resulted in unexpected elevation of expression of proapoptotic genes, decrease in expression of several antiapoptotic genes, and enhanced activation of caspase 3.[82] The reason for enhanced expression of proapoptotic genes after suppression of MET and EGFR is not clear. Several previous studies have shown that MET and EGFR protect cells from apoptosis induced by Fas and TNF.[87] Suppression of levels of MET may lead to increased levels of monomeric Fas because MET normally dimerizes with Fas and prevents it from forming Fas trimers required for Fas activation by Fas Ligand.[96] MET is also a potent activator of PI3K and Akt and MAPK, and decreased activation of MET may affect the balance of activation of NF-κB to the extent that the latter depends on Akt activation.[87,94] Similar effects on Akt activation and effects on NF-κB activation may be anticipated from decreased activation of EGFR, also a known potent activator of PI3K and Akt. The dependence of activation of NF-κB by TNF and the requirement for Akt phosphorylation is one of the settings in which interdependence between HGF and EGFR ligands and TNF may be a crucial determinant for a regenerative or apoptotic outcome. Further complicating the scenario, TNF directly induces MET expression via NF-κB activation in several hepatic-derived cell lines.[97]

There is considerable circumstantial evidence suggesting that acute liver failure in some circumstances may be attributable to a lack of normal interplay between EGFR, MET, and TNF and Fas. 1) Both TNF and Fas are reported increased in human ALF and in experimental models.[95] 2) The progenitor cell pathway through which biliary epithelial cells undergo transformation to become hepatocytes (oval cell pathway in rodents[98]) is activated in human cases of ALF.[99] There is experimental evidence from multiple models that this pathway does not become active unless hepatocyte proliferation is inhibited and there is demand for liver regeneration.[98] These findings suggest that in ALF, hepatocytes do not receive (or do not respond to) mitogenic signals, thus triggering the activation of the progenitor cell pathway. There is certainly no lack of HGF during human ALF, because the HGF levels in that condition are the highest recorded in the literature.[100] In view of this, any failure of the HGF/MET signaling system must be attributable to a lack of proper function of the MET receptor. A sizable number of hepatocyte nuclei (17%) are Ki-67 positive during liver failure. This, however, is not inconsistent with EGFR and MET dysfunction. Hepatocytes do become proliferating cell nuclear antigen–positive in large numbers when MET is silenced, but they do not complete the cell cycle.[82] There have been no direct assessments of the functionality and levels of MET and EGFR in ALF. 3) The scenario that ALF in humans may be attributable to dysfunctional interaction between MET/EGFR and TNF/FASL leading hepatocytes to apoptosis should be further investigated. Some of the concepts are summarized in Figure 1.

Mechanisms Leading to Proper Termination of Liver Regeneration

Most of the studies on liver regeneration have focused on pathways leading to its initiation after PHx. However, the mechanisms leading to its proper termination are equally interesting and much less understood. At the end of regeneration, liver mass is adjusted with relatively high accuracy to the prehepatectomy numbers.[101] There is a small wave of hepatocyte apoptosis at the end of regeneration in the rat and mouse, suggesting a pathway to correct for inappropriate increases in cell numbers.[21] TGFβ1 is a known suppressor of hepatocyte proliferation.[102] Its expression increases early (within 5 hours) after PHx, and it stays elevated until the end of regeneration.[103] Overexpression of TGFβ in transgenic mice (specific to hepatocytes, under control of Albumin promoter) causes massive elevation of TGFβ1 in the plasma (leading to glomerulonephritis!) but it does not appear to affect liver regeneration.[104] Hepatocyte-specific inactivation of TGFβ receptor I by itself does not prolong regeneration, unless combined with inactivation of the Activin receptor.[105] Activin itself is also one of the few known cytokines that selectively inhibit hepatocyte proliferation. On the other hand, TGFβ1 is also gradually eliminated from hepatic pericellular matrix as regeneration progresses[106] and its levels rise in the plasma with the same kinetics as that of HGF, suggesting "dumping" of TGFβ1 in the plasma as a result of the remodeling of the extracellular matrix, because TGFβ is bound to decorin, a protein present in the hepatocyte pericellular matrix.[107]

Perhaps the strongest evidence for a role for TGFβ in regulation of regeneration comes from a different angle. Injection of dominant negative constructs against TGFβ

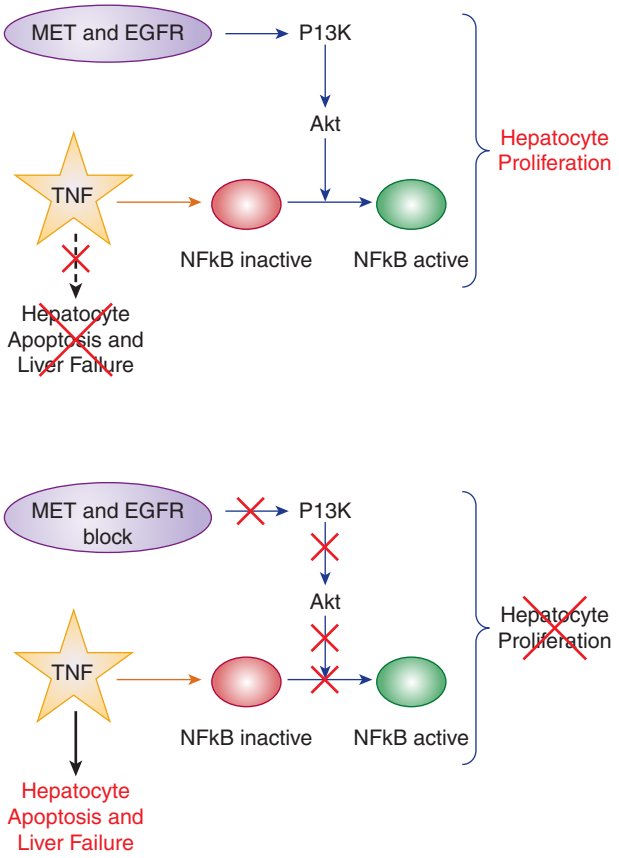

Figure 1. Hepatocyte proliferation after partial hepatectomy (PHx) depends on a concerted action between MET (the HGF receptor), EGFR (EGF receptor), and several cytokines, including tumor necrosis factor (TNF). TNF can have a promitogenic effect if it can activate NF-κB. If this activation does not occur, TNF can lead hepatocytes to apoptosis. NF-κB activation is dependent on activation of Akt. Both MET and EGFR are strong inducers of Akt activation. In the event that MET and EGFR fail to act, absence of Akt activation may tip the balance and lead TNF to an apoptotic role, potentially causing liver failure.

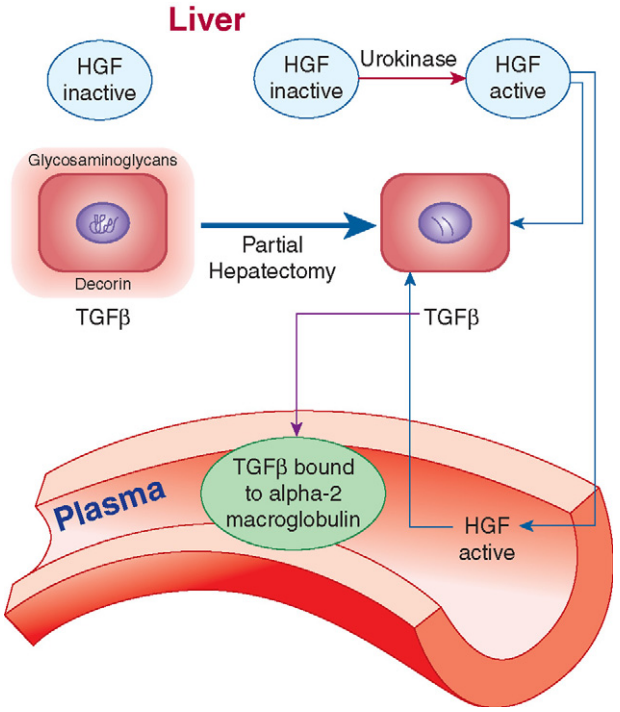

Figure 2. Interaction between HGF and TGFβ1 during initiation of liver regeneration. HGF is a mitogen for hepatocytes, whereas TGFβ1 is known as a mito-inhibitor. HGF binds to glycosaminoglycans and TGFβ1 to decorin, both of which are present in the pericellular matrix surrounding hepatocytes. After PHx, activation of urokinase leads to remodeling and degradation of the pericellular matrix. HGF is therefore released and activated by urokinase, where it can have a local mitogenic effect on hepatocytes. Concentrations of both HGF and TGFβ1 rapidly increase in plasma after PHx. Whereas HGF can exert effects on hepatocytes from plasma, TGFβ1 is neutralized by binding to α-2 macroglobulin. The tilt in the balance between HGF and TGFβ1 at the earliest stages of liver regeneration is a key component of the early stimuli leading hepatocytes to proliferation.

receptor ii or activin receptor II in normal, unoperated, rats stimulated DNA synthesis in hepatocytes.[108] The authors interpreted the data as suggesting that hepatocytes are under a constant "tonic" antagonism between growth factors and TGFβ in the immediate pericellular environment of the hepatocytes, and that both of growth factors and TGFβ have a small but constant agonistic effect in normal livers. Interference with the signaling of TGFβ unmasks the effect of the growth factors, driving hepatocytes to proliferation. To the extent that at the end of regeneration both growth factors (eg, HGF, HB-EGF) as well as TGFβ are restored in the hepatic extracellular matrix, it is fair to ask whether the termination of regeneration is a reverse process of the initiation. At the initiation of regeneration, remodeling of the extracellular matrix leads to HGF release and activation providing mitogenic signals for hepatocytes; TGFβ on the other hand is removed from the pericellular environment of hepatocytes and found elevated in the plasma, where it is known to be bound by α-2-macroglobulin.[109] Thus, at the initiation of regeneration the signaling power between HGF and TNGβ is shift toward HGF, whereas at the termination of regeneration, ECM (binding HGF) and decorin (binding TGFβ) bring in the pre-PHx balance to the pericellular environment of the hepatocytes (Figure 2).

If termination of regeneration is associated with reapposition of HGF and TGFβ in ECM, it is important to understand what are the mechanistic pathways associated with ECM during regeneration. Previous studies provided details on pathways and events associated with initial remodeling/degradation of ECM at the beginning of regeneration and restoration of ECM at the end of the process.[1,2,110–112] ECM however is not only a repository of growth regulators. ECM is a rich source of signaling to the cells it surrounds and such signaling is mediated via integrins. Recent studies showed that interference with signaling of integrins in the liver via liver-specific genetic elimination of integrin linked kinase led to enhanced proliferation of hepatocytes and biliary cells in the absence of PHx.[36] Liver was reorganized in terms of gene expression (initial overall down-regulation and subsequent up-regulation of hepatocyte associated genes) and became larger than normal. In hepatocyte cultures, ECM (in the form of exogenously added matrix such as collagen gels or extracts from EHS sarcoma, a.k.a "Matrigel") inhibits proliferation and enhances hepatocyte proliferation.[8] The findings of spontaneously enhanced hepatocyte proliferation and down-regulation of hepatocyte-associated

genes after interference with signaling by ECM/integrins in whole liver, is consistent with all of the previous literature of the effects of ECM in hepatocyte cultures. More to the point of termination of regeneration, however, mouse livers with hepatocytes deficient in integrin linked kinase did not properly terminate organ growth at the end of regeneration but kept on growing and reaching a size 59% greater than the original liver.[101] There was enhanced expression of HGF as well as enhanced hepatocyte nuclear expression of Yap (Yes-associated protein, target of hippo kinase and recently associated with size regulation of organs, including liver[113]) These findings suggest that ECM signaling via integrins has an important role to play in proper termination of liver regeneration.

The ECM is a complex mixture of proteins and glycosaminoglycans, including a myriad of components. One of these components is Glypican-*3* (GPC3). It is the most overexpressed protein in human hepatocellular carcinomas (HCC)[114] and other tumors, and it is a clinical marker for human liver cancer.[115] It is a protein present in abundance in the pericellular matrix of many epithelial cells including hepatocytes.[116] Loss-of-function deletion of GPC3 in humans result in the Simpson-Gholabi-Behmel (SGB) syndrome, which is associated with organomegaly including liver and most other internal organs, enlargement of bones and muscles, etc.[117] The findings associated with SGB syndrome suggest that though GPC3 is markedly produced by HCCs, it nonetheless may be a growth suppressor, perhaps produced by HCCs as a failed feedback of growth suppression to which HCCs cannot respond. Mice with loss-of-function deletion of GPC3 has similar symptoms.[118] We recently found that GPC3 expression increases during the end of liver regeneration and that inhibition of expression of GPC3 is associated with enhanced growth of hepatocytes in culture.[119] We also found that GPC3 is interacting with CD81, a tetraspanin family member associated with growth regulation and also with entry of hepatitis C virus into hepatocytes.[120] GPC3 and CD81 associate during liver regeneration at the time when hepatocyte proliferation ceases (day 2) and the time when proliferation of non-parenchymal cells is complete (days 5 to 6). There is more to be understood on GPC3 and its effects in termination of regeneration, but the findings underscore the complexity of ECM related pathways and their contribution to the process of termination of liver regeneration.

Is There a "Hepatostat"

The concept arises from older experiments involving liver transplantation in which livers from small animals (eg, baboons to humans, small dogs to large dogs) enlarge after transplantation to reach a liver size associated in proportion to the size of the recipient animal.[121] Additional support for the concept also arises from the numerous studies documenting that at the end of regeneration after PHx liver reaches the original prehepatectomy size with high precision, and then it stops.[101] There can be many contributing pathways related to this process, and they may be related to internal hormones (eg, estrogens causing increase liver size during pregnancy), ECM (see above), hepatic regulation of levels of free growth factors and growth inhibitors (eg, see above on ECM, HGF and TGFβ1), and perhaps many other mechanisms. The phenomenon is well documented, but it is not clear whether there are specific "sensor molecules" that trigger or suppress growth related signals so that the liver size is properly maintained. Small molecules such as glucose[53] and bile acids[24] or larger proteins such as Selenoprotein 1[101] may also play a role.

Other Regulatory Pathways: Thinking "Outside of the Box"

A recent publication[122] used inducible and rampant expression of a transposon in hepatocytes of $p53^{-/-}$ mice and noticed the appearance of many HCCs per mouse liver. It was assumed that the insertion of the transposon (*Sleeping Beauty*[123]) resulted in activation of growth promoting genes or inactivation of tumor suppressor genes. A metaanalysis of the supplemental data of this publication can be performed to demonstrate the frequency by which insertion of the transposon in proximity to specific genes was associated with HCC development. Of 67 tumors, there were 51 associated with transposon insertion related to EGFR, five tumors with insertion affecting both EGFR and MET and 10 tumors with no insertion related to either EGFR or MET. Of interest, no tumors had MET alone as an insertion hit without also an associated hit on EGFR. This may be related to the potential interactions of these two receptor tyrosine kinases in regulation of hepatocyte growth (see above). There were no other receptor tyrosine kinases involved in these hits, in accordance with the data on MET and EGFR and their unique role in hepatocyte growth regulation, discussed above. There were two "hits" associated with Tnfsf4, a member (No. 4) of the TNF superfamily, but no hits with TNF itself. In addition, however, to the above cast of the "usual" characters, there were multiple insertion "hits" associated with many other receptors and ligands, not known until now to have a role on hepatocyte growth regulation. These included olfactory receptors (77 hits), follistatin (a known binder and inactivator of Activin, a hepatocyte growth inhibitor[124]: 45 hits), glutamate receptors (metabotropic or inotropic: total of 37 hits), GABA receptor (14 hits: previous literature shows GABA inhibiting liver regeneration[125,126]), Ephrin receptors (16 hits), ADAM proteases (18 hits), Glypicans (13 hits), adrenergic receptors (7 hits: extensive older literature on adrenergic receptors and hepatocyte growth regulation, see above[9,10]). Multiple intracellular pathways associated with cell structure (catenins, cadherins), mitogenic signal transduction pathways (MAPK, PI3K, etc) and a variety of potassium and calcium channels had from 10 to 25 hits each. The results provide an independent validation of the role genes already known involved with hepatocyte growth regulation (eg, MET, EGFR, adrenergic receptors, etc) but also provide a glimpse on the totality and diversity of many other genes not yet considered as involved or studied extensively in relation to liver regeneration. The

findings are likely to stimulate research on these molecules of potential growth regulatory importance and further dissect the complexity of the regenerative response.

Summary

Liver regeneration is both a clinically important process and a great model to study regenerative growth. Much is now understood about the signals driving and terminating the process, both in terms of ligand/receptor systems and in terms of whole body contributions, including circulatory events and signaling contributions from other organs. Studies with hepatocellular carcinomas suggest that there may be more pathways contributing to regulation of hepatocyte growth than those currently known.[122] Near future studies should shed some light on some of these. We can feel confident, however, that many of the basic signaling actors in this process have been already defined. The knowledge gained should be useful to understand liver regeneration not only as a successful homeostatic outcome, but also as a process that may sometimes fail, leading to catastrophic situations. Liver failure, essentially a failure of regeneration attributable to adverse circumstances, should be subjected to mechanistic analysis based on the knowledge already gained on regeneration, and perhaps therapeutic interventions may be designed with impact on human liver disease.

References

1. Michalopoulos GK, DeFrances MC: Liver regeneration. Science 1997, 276:60–66
2. Michalopoulos GK: Liver regeneration, J Cell Physiol 2007, 213: 286–300.
3. Nelsen CJ, Rickheim DG, Timchenko NA, Stanley MW, Albrecht JH: Transient expression of cyclin D1 is sufficient to promote hepatocyte replication and liver growth in vivo. Cancer Res 2001, 61:8564–8568
4. Cressman DE, Diamond RH, Taub R: Rapid activation of the Stat3 transcription complex in liver regeneration. Hepatology 1995, 21: 1443–1449
5. Kirillova I, Chaisson M, Fausto N: Tumor necrosis factor induces DNA replication in hepatic cells through nuclear factor kappaB activation. Cell Growth Differ 1999, 10:819–828
6. Seglen PO: Preparation of isolated rat liver cells. Methods Cell Biol 1976, 13:29–83
7. Strom SC, Jirtle RL, Jones RS, Novicki DL, Rosenberg MR, Novotny A, Irons G, McLain JR, Michalopoulos G: Isolation, culture, and transplantation of human hepatocytes. J Natl Cancer Inst 1982, 68:771–778
8. Block GD, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK: Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF. EGF and TGF alpha in a chemically defined (HGM) medium. J Cell Biol 1996, 132:1133–1149
9. Cruise JL, Knechtle SJ, Bollinger RR, Kuhn C, Michalopoulos G: Alpha 1-adrenergic effects and liver regeneration. Hepatology 1987, 7:1189–1194
10. Cruise JL, Houck KA, Michalopoulos GK: Induction of DNA synthesis in cultured rat hepatocytes through stimulation of alpha 1 adrenoreceptor by norepinephrine. Science 1985, 227:749–751
11. Kimura M, Osumi S, Ogihara M: Stimulation of DNA synthesis and proliferation by prostaglandins in primary cultures of adult rat hepatocytes. Eur J Pharmacol 2000, 404:259–271
12. Webber EM, Bruix J, Pierce RH, Fausto N: Tumor necrosis factor primes hepatocytes for DNA replication in the rat. Hepatology 1998, 28:1226–1234
13. Ni N, Yager JD: Comitogenic effects of estrogens on DNA synthesis induced by various growth factors in cultured female rat hepatocytes. Hepatology 1994, 19:183–192
14. McGowan JA, Strain AJ, Bucher NL: 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
15. Rana B, Mischoulon D, Xie Y, Bucher NL, Farmer SR: Cell-extracellular matrix interactions can regulate the switch between growth and differentiation in rat hepatocytes: Reciprocal expression of C/EBP alpha and immediate-early growth response transcription factors. Mol Cell Biol 1994, 14:5858–5869
16. Bucher NL, Patel U, Cohen S: Hormonal factors and liver growth. Adv Enzyme Regul 1977, 16:205–213
17. Patijn GA, Lieber A, Schowalter DB, Schwall R, Kay MA: Hepatocyte growth factor induces hepatocyte proliferation in vivo and allows for efficient retroviral-mediated gene transfer in mice. Hepatology 1998, 28:707–716
18. Liu ML, Mars WM, Zarnegar R, Michalopoulos GK: Collagenase pretreatment and the mitogenic effects of hepatocyte growth factor and transforming growth factor-alpha in adult rat liver. Hepatology 1994, 19:1521–1527
19. Yamada Y, Webber EM, Kirillova I, Peschon JJ, 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 [comment]. Hepatology 1998, 28:959–970
20. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R: Liver failure and defective hepatocyte regeneration in interleukin-6- deficient mice. Science 1996, 274:1379–1383
21. Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, Demetris AJ: Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology 1999, 29:403–411
22. Kohler C, Bell AW, Bowen WC, Monga SP, Fleig W, Michalopoulos GK: Expression of Notch-1 and its ligand Jagged-1 in rat liver during liver regeneration. Hepatology 2004, 39:1056–1065
23. LeCouter J, Moritz DR, Li B, Phillips GL, Liang XH, Gerber HP, Hillan KJ, Ferrara N: Angiogenesis-independent endothelial protection of liver: Role of VEGFR-1. Science 2003, 299:890–893
24. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, Dong B, Huang X, Moore DD: Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration, Science 2006, 312:233–236
25. Lesurtel M, Graf R, Aleil B, Walther B, Tian Y, Jochum W, Gaget C, Bader M, Clavien P: Platelet-derived serotonin mediates liver regeneration. Science 2006, 312:104–107
26. DeAngelis RA, Markiewski MM, Lambris JD: Liver regeneration: A link to inflammation through complement. Adv Exp Med Biol 2006, 586:17–34
27. Yamauchi H, Uetsuka K, Okada T, Nakayama H, Doi K: Impaired liver regeneration after partial hepatectomy in db/db mice. Exp Toxicol Pathol 2003, 54:281–286
28. Starzl TE, Porter KA, Putnam CW: Insulin, glucagon, and the control of hepatic structure, function, and capacity for regeneration. Metabolism 1976, 25:1429–1434
29. Kan M, Huang JS, Mansson PE, Yasumitsu H, Carr B, McKeehan WL: 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
30. Steiling H, Wustefeld T, Bugnon P, Brauchle M, Fassler R, Teupser D, Thiery J, Gordon JI, Trautwein C, Werner S: Fibroblast growth factor receptor signalling is crucial for liver homeostasis and regeneration. Oncogene 2003, 22:4380–4388
31. Fausto N, Campbell JS, Riehle KJ: Liver regeneration. Hepatology 2006, 43:S45–S53
32. Taub R: Liver regeneration: From myth to mechanism. Nat Rev Mol Cell Biol 2004, 5:836–847
33. Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C: Met provides essential signals for liver regeneration, Proc Natl Acad Sci USA 2004, 101:10608–10613
34. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS: Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair, Proc Natl Acad Sci USA 2004, 101:4477–4482
35. Gkretsi V, Mars WM, Bowen WC, Barua L, Yang Y, Guo L, St-Arnaud R, Dedhar S, Wu C, Michalopoulos GK: Loss of integrin linked kinase

from mouse hepatocytes in vitro and in vivo results in apoptosis and hepatitis. Hepatology 2007, 45:1025–1034
36. Gkretsi V, Apte U, Mars WM, Bowen WC, Luo JH, Yang Y, Yu YP, Orr A, St-Arnaud R, Dedhar S, Kaestner KH, Wu C, Michalopoulos GK: Liver-specific ablation of integrin-linked kinase in mice results in abnormal histology, enhanced cell proliferation, and hepatomegaly. Hepatology 2008, 48:1932–1941
37. Higgins GM: A. RM: Experimental pathology of the liver, 1: Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931, 12:186–202
38. 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
39. Skov Olsen P, Boesby S, Kirkegaard P, Therkelsen K, Almdal T, Poulsen SS, Nexo E: Influence of epidermal growth factor on liver regeneration after partial hepatectomy in rats. Hepatology 1988, 8:992–996
40. Olsen PS, Poulsen SS, Kirkegaard P: Adrenergic effects on secretion of epidermal growth factor from Brunner's glands. Gut 1985, 26:920–927
41. Bucher NL, Patel U, Cohen S: Hormonal factors concerned with liver regeneration. Ciba Found Symp 1977, 55:95–107
42. Moolten FL, Bucher NL: Regeneration of rat liver: Transfer of humoral agent by cross circulation. Science 1967, 158:272–274
43. Leong GF, Grisham JW, Hole BV, Albright ML: Effect of partial hepatectomy on DNA synthesis and mitosis in heterotopic partial autografts of rat liver. Cancer Res 1964, 24:1496–1501
44. Jirtle RL, Michalopoulos G: Effects of partial hepatectomy on transplanted hepatocytes. Cancer Res 1982, 42:3000–3004
45. Zarnegar R, Michalopoulos G: Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. Cancer Res 1989, 49:3314–3320
46. 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
47. Lindroos PM, Zarnegar R, Michalopoulos GK: 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
48. Pediaditakis P, Lopez-Talavera JC, Petersen B, Monga SP, Michalopoulos GK: The processing and utilization of hepatocyte growth factor/scatter factor following partial hepatectomy in the rat. Hepatology 2001, 34:688–693
49. Masumoto A, Yamamoto N: Sequestration of a hepatocyte growth factor in extracellular matrix in normal adult rat liver. Biochem Biophys Res Commun 1991, 174:90–95
50. Liu ML, Mars WM, Zarnegar R, Michalopoulos GK: Uptake and distribution of hepatocyte growth factor in normal and regenerating adult rat liver. Am J Pathol 1994, 144:129–140
51. Yamada Y, Kirillova I, Peschon JJ, Fausto N: Initiation of liver growth by tumor necrosis factor: Deficient liver regeneration in mice lacking type I tumor necrosis factor receptor, Proc Natl Acad Sci USA 1997; 94:1441–1446
52. Leffert HL, Koch KS, Moran T, Rubalcava B: Hormonal control of rat liver regeneration. Gastroenterology 1979, 76:1470–1482
53. Weymann A, Hartman E, Gazit V, Wang C, Glauber M, Turmelle Y, Rudnick DA: p21 is required for dextrose-mediated inhibition of mouse liver regeneration. Hepatology 2009, 50:207–215
54. Makino H, Togo S, Kubota T, Morioka D, Morita T, Kobayashi T, Tanaka K, Shimizu T, Matsuo K, Nagashima Y, Shimada H: A good model of hepatic failure after excessive hepatectomy in mice. J Surg Res 2005, 127:171–176
55. Demetris AJ, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, Tan HP, Shaw-Stiffel T, Boig L, Novelli P, Planinsic R, Fung JJ, Marcos A: Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. Am J Surg Pathol 2006, 30:986–993
56. Smyrniotis V, Kostopanagiotou G, Kondi A, Gamaletsos E, Theodoraki K, Kehagias D, Mystakidou K, Contis J: Hemodynamic interaction between portal vein and hepatic artery flow in small-for-size split liver transplantation. Transpl Int 2002, 15:355–360
57. Lautt WW: Mechanism and role of intrinsic regulation of hepatic arterial blood flow: Hepatic arterial buffer response. Am J Physiol 1985, 249:G549–G556
58. Kelly DM, Demetris AJ, Fung JJ, Marcos A, Zhu Y, Subbotin V, Yin L, Totsuka E, Ishii T, Lee MC, Gutierrez J, Costa G, Venkataraman R, Madariaga JR: Porcine partial liver transplantation: A novel model of the "small-for-size" liver graft. Liver Transpl 2004, 10:253–263
59. Lautt WW, Legare DJ, d'Almeida MS: Adenosine as putative regulator of hepatic arterial flow (the buffer response). Am J Physiol 1985, 248:H331–H338
60. Mars WM, Liu ML, Kitson RP, Goldfarb RH, Gabauer MK, Michalopoulos GK: Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. Hepatology 1995, 21:1695–1701
61. Kim TH, Mars WM, Stolz DB, Michalopoulos GK: Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. Hepatology 2000, 31:75–82
62. Kim TH, Mars WM, Stolz DB, Petersen BE, Michalopoulos GK: Extracellular matrix remodeling at the early stages of liver regeneration in the rat. Hepatology 1997, 26:896–904
63. Schirmacher P, Geerts A, Pietrangelo A, Dienes HP, Rogler CE: 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
64. 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
65. 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
66. Broten J, Michalopoulos G, Petersen B, Cruise J: Adrenergic stimulation of hepatocyte growth factor expression. Biochem Biophys Res Commun 1999, 262:76–79
67. Liu Y, Bell AW, Michalopoulos GK, Zarnegar R: The mouse hepatocyte growth factor-encoding gene: Structural organization and evolutionary conservation. Gene 1994, 144:179–187
68. Liu Y, Michalopoulos GK, Zarnegar R: Structural and functional characterization of the mouse hepatocyte growth factor gene promoter. J Biol Chem 1994, 269:4152–4160
69. Liu Y, Michalopoulos GK, Zarnegar R: Molecular cloning and characterization of cDNA encoding mouse hepatocyte growth factor. Biochim Biophys Acta 1993, 1216:299–303
70. Tomida M, Saito T: The human hepatocyte growth factor (HGF) gene is transcriptionally activated by leukemia inhibitory factor through the Stat binding element. Oncogene 2004, 23:679–686
71. Tanaka K, Ohkawa S, Nishino T, Niijima A, Inoue S: Role of the hepatic branch of the vagus nerve in liver regeneration in rats. Am J Physiol 1987, 253:G439–G444
72. Oben JA, Diehl AM: Sympathetic nervous system regulation of liver repair. Anat Rec A Discov Mol Cell Evol Biol 2004, 280:874–883
73. Passino MA, Adams RA, Sikorski SL, Akassoglou K: Regulation of hepatic stellate cell differentiation by the neurotrophin receptor p75NTR. Science 2007, 315:1853–1856
74. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC: Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem 2000, 275:8806–8811
75. Presnell SC, Stolz DB, Mars WM, Jo M, Michalopoulos GK, Strom SC: Modifications of the hepatocyte growth factor/c-met pathway by constitutive expression of transforming growth factor-alpha in rat liver epithelial cells. Mol Carcinog 1997, 18:244–255
76. Russell WE, Kaufmann WK, Sitaric S, Luetteke NC, Lee DC: Liver regeneration and hepatocarcinogenesis in transforming growth factor-alpha-targeted mice. Mol Carcinog 1996, 15:183–189
77. Mitchell C, Nivison M, Jackson LF, Fox R, Lee DC, Campbell JS, 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
78. Berasain C, Garcia-Trevijano ER, Castillo J, Erroba E, Lee DC, Prieto J, Avila MA: Amphiregulin: An early trigger of liver regeneration in mice.[see comment]. Gastroenterology 2005, 128:424–432
79. Kraizer Y, Mawasi N, Seagal J, Paizi M, Assy N, Spira G: Vascular endothelial growth factor and angiopoietin in liver regeneration. Biochem Biophys Res Commun 2001, 287:209–215

80. Sato T, El-Assal ON, Ono T, Yamanoi A, Dhar DK, Nagasue N: Sinusoidal endothelial cell proliferation and expression of angiopoietin/Tie family in regenerating rat liver. J Hepatol 2001, 34:690–698
81. Joplin R, Hishida T, Tsubouchi H, Daikuhara Y, Ayres R, Neuberger JM, Strain AJ: Human intrahepatic biliary epithelial cells proliferate in vitro in response to human hepatocyte growth factor. J Clin Invest 1992, 90:1284–1289
82. Paranjpe S, Bowen WC, Bell AW, Nejak-Bowen K, Luo JH, Michalopoulos GK: 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
83. Pinzani M: PDGF and signal transduction in hepatic stellate cells. Front Biosci 2002, 7:d1720–d1726
84. Campbell JS, Hughes SD, Gilbertson DG, Palmer TE, Holdren MS, Haran AC, Odell MM, Bauer RL, Ren HP, Haugen HS, Yeh MM, Fausto N: Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma, Proc Natl Acad Sci USA 2005, 102:3389–3394
85. Delehedde M, Sergeant N, Lyon M, Rudland PS, Fernig DG: Hepatocyte growth factor/scatter factor stimulates migration of rat mammary fibroblasts through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways. Eur J Biochem 2001, 268:4423–4429
86. Puri N, Salgia R: Synergism of EGFR and c-Met pathways, cross-talk and inhibition, in non-small cell lung cancer. J Carcinog 2008, 7:9
87. Xiao GH, Jeffers M, Bellacosa A, Mitsuuchi Y, Vande Woude GF, Testa JR: Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways, Proc Natl Acad Sci USA 2001, 98:247–252
88. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, Diehl AM: Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992, 263:G579–G585
89. Li W, Liang X, Leu JI, Kovalovich K, Ciliberto G, Taub R: Global changes in interleukin-6-dependent gene expression patterns in mouse livers after partial hepatectomy. Hepatology 2001, 33: 1377–1386
90. Imose M, Nagaki M, Naiki T, Osawa Y, Brenner DA, Asano T, Hayashi H, Kato T, Moriwaki H: Inhibition of nuclear factor kappaB and phosphatidylinositol 3-kinase/Akt is essential for massive hepatocyte apoptosis induced by tumor necrosis factor alpha in mice. Liver Int 2003, 23:386–396
91. Hatano E, Bennett BL, Manning AM, Qian T, Lemasters JJ, Brenner DA: NF-kappaB stimulates inducible nitric oxide synthase to protect mouse hepatocytes from TNF-alpha- and Fas-mediated apoptosis. Gastroenterology 2001, 120:1251–1262
92. Hatano E: Tumor necrosis factor signaling in hepatocyte apoptosis, J Gastroenterol Hepatol 2007, 22 Suppl 1:S43–S44
93. Kuhla A, Eipel C, Siebert N, Abshagen K, Menger MD, Vollmar B: Hepatocellular apoptosis is mediated by TNFalpha-dependent Fas/FasLigand cytotoxicity in a murine model of acute liver failure. Apoptosis 2008, 13:1427–1438
94. Hatano E, Brenner DA: 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
95. Singhal S, Chakravarty A, Das BC, Kar P: Tumour necrosis factor-alpha and soluble Fas ligand as biomarkers in non-acetaminophen-induced acute liver failure, Biomarkers 2009, 14:347–353
96. Wang X, DeFrances MC, Dai Y, Pediaditakis P, Johnson C, Bell A, Michalopoulos GK, Zarnegar R: A mechanism of cell survival: Sequestration of Fas by the HGF receptor Met. Mol Cell 2002, 9:411–421
97. Dai JY, Defrances MC, Zou C, Johnson CJ, Zarnegar R: The Met protooncogene is a transcriptional target of NFkappaB: Implications for cell survival. J Cell Biochem 2009, 107:1222–1236
98. Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS: In vivo differentiation of rat liver oval cells into hepatocytes. Cancer Res 1989, 49:1541–1547
99. Limaye PB, Alarcon G, Walls AL, Nalesnik MA, Michalopoulos GK, Demetris AJ, Ochoa ER: Expression of specific hepatocyte and cholangiocyte transcription factors in human liver disease and embryonic development. Lab Invest 2008, 88:865–872
100. Tsubouchi H, Hirono S, Gohda E, Nakayama H, Takahashi K, Sakiyama O, Kimoto M, Kawakami S, Miyoshi H, Kubozono O, Arakai N, Daikohara Y, Hashimoto S: Human hepatocyte growth factor in blood of patients with fulminant hepatic failure. I Clinical aspects, Dig Dis Sci 1991, 36:780–784
101. Apte U, Gkretsi V, Bowen WC, Mars WM, Luo JH, Donthamsetty S, Orr A, Monga SP, Wu C, Michalopoulos GK: Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrin-linked kinase. Hepatology 2009, 50:844–851
102. Houck KA, Michalopoulos GK: Altered responses of regenerating hepatocytes to norepinephrine and transforming growth factor type beta. J Cell Physiol 1989, 141:503–509
103. Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, 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
104. Sanderson N, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, Roberts AB, Sporn MB, Thorgeirsson SS: Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. Proc Natl Acad Sci USA 1995, 92:2572–2576
105. Oe S, Lemmer ER, Conner EA, Factor VM, Leveen P, Larsson J, Karlsson S, Thorgeirsson SS: Intact signaling by transforming growth factor beta is not required for termination of liver regeneration in mice. Hepatology 2004, 40:1098–1105
106. Jirtle RL, Carr BI, Scott CD: Modulation of insulin-like growth factor-II/mannose 6-phosphate receptors and transforming growth factor-beta 1 during liver regeneration [published erratum appears in J Biol Chem 1991, 266:24860]. J Biol Chem 1991, 266:22444–22450
107. Dudas J, Kovalszky I, Gallai M, Nagy JO, Schaff Z, Knittel T, Mehde M, Neubauer K, Szalay F, Ramadori G: Expression of decorin, transforming growth factor-beta 1, tissue inhibitor metalloproteinase 1 and 2, and type IV collagenases in chronic hepatitis. Am J Clin Pathol 2001, 115:725–735
108. Ichikawa T, Zhang YQ, 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
109. LaMarre J, Hayes MA, Wollenberg GK, Hussaini I, Hall SW, Gonias SL: An alpha 2-macroglobulin receptor-dependent mechanism for the plasma clearance of transforming growth factor-beta 1 in mice. J Clin Invest 1991, 87:39–44
110. Mohammed FF, Pennington CJ, Kassiri Z, Rubin JS, Soloway PD, Ruther U, Edwards DR, Khokha R: Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. Hepatology 2005, 41:857–867
111. Rudolph KL, Trautwein C, Kubicka S, Rakemann T, Bahr MJ, Sedlaczek N, Schuppan D, Manns MP: Differential regulation of extracellular matrix synthesis during liver regeneration after partial hepatectomy in rats. Hepatology 1999, 30:1159–1166
112. Gallai M, Sebestyen A, Nagy P, Kovalszky I, Onody T, Thorgeirsson SS: Proteoglycan gene expression in rat liver after partial hepatectomy. Biochem Biophys Res Commun 1996, 228:690–694
113. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D: Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell 2007, 130:1120–1133
114. Luo JH, Ren B, Keryanov S, Tseng GC, Rao UN, Monga SP, Strom S, Demetris AJ, Nalesnik M, Yu YP, Ranganathan S, Michalopoulos GK: Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. Hepatology 2006, 44:1012–1024
115. Filmus J, Capurro M: Glypican-3 and alphafetoprotein as diagnostic tests for hepatocellular carcinoma. Mol Diagn 2004, 8:207–212
116. Song HH, Filmus J: The role of glypicans in mammalian development. Biochim Biophys Acta 2002, 1573:241–246
117. Pilia G, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, 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
118. Cano-Gauci DF, Song HH, Yang H, McKerlie C, Choo B, Shi W, Pullano R, Piscione TD, Grisaru S, Soon S, Sedlackova L, Tanswell AK, Mak TW, Yeger H, Lockwood GA, Rosenblum ND, Filmus J: Glypican-3-deficient mice exhibit developmental overgrowth and some of the abnormalities typical of Simpson-Golabi-Behmel syndrome. J Cell Biol 1999, 146:255–264
119. Liu B, Paranjpe S, Bowen WC, Bell AW, Luo JH, Yu YP, Mars WM, Michalopoulos GK: Investigation of the role of glypican 3 in liver regeneration and hepatocyte proliferation. Am J Pathol 2009, 175:717–724

120. Cocquerel L, Kuo CC, Dubuisson J, Levy S: CD81-dependent binding of hepatitis C virus E1E2 heterodimers. J Virol 2003, 77:10677–10683
121. Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M: Baboon-to-human liver transplantation [see comment]. Lancet 1993, 341:65–71
122. Keng VW, Villanueva A, Chiang DY, Dupuy AJ, Ryan BJ, Matise I, Silverstein KA, Sarver A, Starr TK, Akagi K, Tessarollo L, Collier LS, Powers S, Lowe SW, Jenkins NA, Copeland NG, Llovet JM, Largaespada DA: A conditional transposon-based insertional mutagenesis screen for genes associated with mouse hepatocellular carcinoma. Nature Biotechnol 2009, 27:264–274
123. Yant SR, Wu X, Huang Y, Garrison B, Burgess SM, Kay MA: High-resolution genome-wide mapping of transposon integration in mammals. Mol Cell Biol 2005, 25:2085–2094
124. Kogure K, Zhang YQ, Maeshima A, Suzuki K, Kuwano H, Kojima I: The role of activin and transforming growth factor-beta in the regulation of organ mass in the rat liver. Hepatology 2000, 31:916–921
125. Minuk GY, Gauthier T: The effect of gamma-aminobutyric acid on hepatic regenerative activity following partial hepatectomy in rats. Gastroenterology 1993, 104:217–221
126. Minuk GY: Gamma-aminobutyric acid and the liver. Dig Dis 1993, 11:45–54