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REVIEW

Elucidating the Metabolic Regulation of Liver Regeneration

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The regenerative capability of liver is well known, and the mechanisms that regulate liver regeneration are extensively studied. Such analyses have defined general principles that govern the hepatic regenerative response and implicated specific extracellular and intracellular signals as regulated during and essential for normal liver regeneration. Nevertheless, the most proximal events that stimulate liver regeneration and the distal signals that terminate this process remain incompletely understood. Recent data suggest that the metabolic response to hepatic insufficiency might be the proximal signal that initiates regenerative hepatocellular proliferation. This review provides an overview of the data in support of a metabolic model of liver regeneration and reflects on the clinical implications and areas for further study suggested by these findings. *(Am J Pathol 2014, 184: 309–321; http://dx.doi.org/10.1016/j.ajpath.2013.04.034)*

Liver diseases have a significant impact on human mor-bidity and mortality. Although disease-specific therapies exist for some insults, in all cases of liver injury host sur-vival and recovery depends upon the liver's remarkable capacity to regenerate. Therefore, liver regeneration has been subjected to rigorous experimental investigation for decades[1–3] with hope that mechanistic insights provided by such research will lead to novel, proregenerative strategies with which to improve the management of human liver diseases. Such analyses show that hepatic regenerative capability is conserved in all vertebrates where it has been studied, from fish to human, presumably because of the essential metabolic, synthetic, and detoxification functions subserved by liver. Although other body structures also regenerate in lower vertebrates (eg, the amputated fin of zebra fish), the liver is unique among mammalian visceral organs in the ability to recover from injury by regeneration instead of scar formation. Thus, elucidating the mechanisms that regulate hepatic regeneration might also inform efforts to promote regeneration in other human organs.

The best-characterized and most commonly used experi-mental paradigm for investigating the molecular, cellular, and physiological mechanisms that control liver regenera-tion has been surgical resection of a portion of the rodent liver.[4] In the most typically used version of this model (ie, two-thirds partial hepatectomy), the anesthetized rodent undergoes midventral laparotomy with sequential ligation and resection of the left and median hepatic lobes, followed by closure of the surgical wounds and recovery.[5] Afterward, a liver-specific regenerative response ensues, which includes activation of specific extracellular and intracellular signals, followed by alterations in gene and protein expression. These events, in turn, direct previously quiescent hepato-cytes and other cells in the remnant liver to reenter the cell cycle and proliferate, ultimately leading to restoration of the preresection liver/body mass ratio and normalization of hepatic function. Subsequently, hepatic lobular architecture, temporarily distorted by the regenerative response, is re-modeled, and the liver returns to its preregenerative state of proliferative inactivity.[1–3] Nonsurgical animal models,

based on controlled exposure to hepatotoxins (eg, carbon tetrachloride, thioacetamide, acetaminophen, and D-galactosamine[6]) or genetically induced hepatocellular injury (eg, the PiZ transgenic mouse model of α1-antitrypsin deficiency liver disease[7]), have also been studied to further elucidate the regulation of injury-induced hepatocellular proliferation and liver regeneration, with some of the regenerative signals identified in the partial hepatectomy model conserved in those paradigms.[8,9]

Experimental analyses using the models described above have defined several common characteristics of the typical hepatic regenerative response. For example, such studies show that the liver/body mass ratio, which is precisely regulated in health, is specifically restored by regeneration after hepatic injury.[1−4] This observation infers the existence of a master regulator of the liver/body mass ratio (ie, a hepatostat).[1−3] Interestingly, a recent report demonstrated that myostatin-null mice, which have skeletal muscle hypertrophy, exhibit a reduced liver/body mass ratio compared with wild-type littermates. That finding indicates that hepatic mass is not regulated in proportion to skeletal muscle mass, thereby illustrating a previously unrecognized degree of extrahepatic tissue specificity to liver mass regulation.[10] Analyses of liver regeneration have also revealed the seemingly unlimited proliferative potential of quiescent hepatocytes,[3] and established that these cells are the source from which recovered liver mass typically derives during regeneration.[11] Thus, liver regeneration does not necessarily depend on a stem cell; however, bipotential liver stem cells can be induced to expand within the liver under specific experimental circumstances.[12] These oval cells, named after their histological appearance, also have been identified in human liver diseases.[13]

The specific molecular mechanisms that control liver regeneration also have been experimentally examined. The importance of circulating factors in such regulation was first established by parabiotic analyses of regeneration,[14,15] and further suggested by the observation that periportal hepatocytes, which are closest to the afferent hepatic portal and systemic blood supplies, proliferate before centrilobular hepatocytes (furthest from those blood supplies) during this response.[16] Those observations motivated (still ongoing) efforts to discover these humoral factors and their intracellular targets. Such analyses have identified cytokines (eg, tumor necrosis factor α and IL-6), growth- and matrix-derived factors (eg, hepatocyte growth factor and epidermal growth factor receptor ligands), secondary messenger cascades and other intracellular events (eg, Wnt-dependent β-catenin signaling), transcription factors [eg, NF-κB, STAT3, cAMP regulatory element-binding protein, CCAAT-enhancer binding protein (C/EBP) β, activator protein 1, farnesoid X receptor (FXR), and liver X receptor (LXR)], and other signals as highly regulated in response to resection- or toxin-induced hepatic insufficiency.[1−3] Moreover, analyses of animal models in which these signals have been pharmacologically or genetically manipulated have demonstrated their

functional importance during hepatic regeneration.[1−3] After recovery of hepatic mass, architecture, and function, liver regeneration ceases. Although less well studied, specific factors (eg, transforming growth factor β,[1−3] integrin-linked kinase,[17] and glypican 3[18]) have been implicated in the precision with which regeneration is terminated after restoration of normal liver/body mass ratio. Nevertheless, despite the broad knowledge gained from these studies, the nature and identities of the most proximal events that initiate liver regeneration and those distal signals that terminate this process remain incompletely defined, and the essence of the hepatostat is still essentially unknown.

Rodents subjected to partial hepatectomy or exposed to hepatotoxic substances develop stereotypical alterations in hepatic and systemic metabolism.[19] These changes, which are among the earliest events to occur in response to experimentally induced hepatic insufficiency, begin with marked alterations in glycemia, followed by changes in circulating and hepatic metabolite levels. The functional importance of such changes for liver regeneration is implied by several experimental observations. For example, disruption of normal metabolism precedes the onset of regenerative hepatocellular proliferation and resolves with restoration of normal liver mass (Figure 1). Furthermore, various experimental strategies that suppress specific aspects of these metabolic alterations impair the ensuing hepatic regenerative response (Figure 2). This review summarizes and considers these data and other evidence in support of a metabolic model of liver regeneration in which the alterations in metabolism that occur in response to liver injury promote regenerative hepatocellular proliferation (Figure 3).

The Metabolic Response to Hepatic Insufficiency

After experimentally induced sublethal hepatic injury, the liver continues to perform essential metabolic and other functions necessary for survival. Still, many studies show that hepatic and systemic metabolism are rapidly and specifically altered in response to such regenerative stimuli (Figure 1).[19] For example, within hours of surgery, mice subjected to partial hepatectomy develop significant hypoglycemia compared with controls (Figure 1).[20] This finding, consistent with the essential role of liver in systemic glucose homeostasis, likely results (at least in part) from the acute removal of two-thirds of hepatic glycogen content and gluconeogenic capacity. Moreover, these data indicate that the liver does not entirely compensate for the acute hepatic functional compromise induced in mice and perhaps other animal models of regeneration; nonetheless, some adaptation after partial hepatectomy does occur, including induction of hepatic gluconeogenic machinery and suppression of liver glycolytic activity.[21] Those changes, which are determined, in part, via transcriptional regulation,[22] limit the post-hepatectomy decline in blood glucose but at the
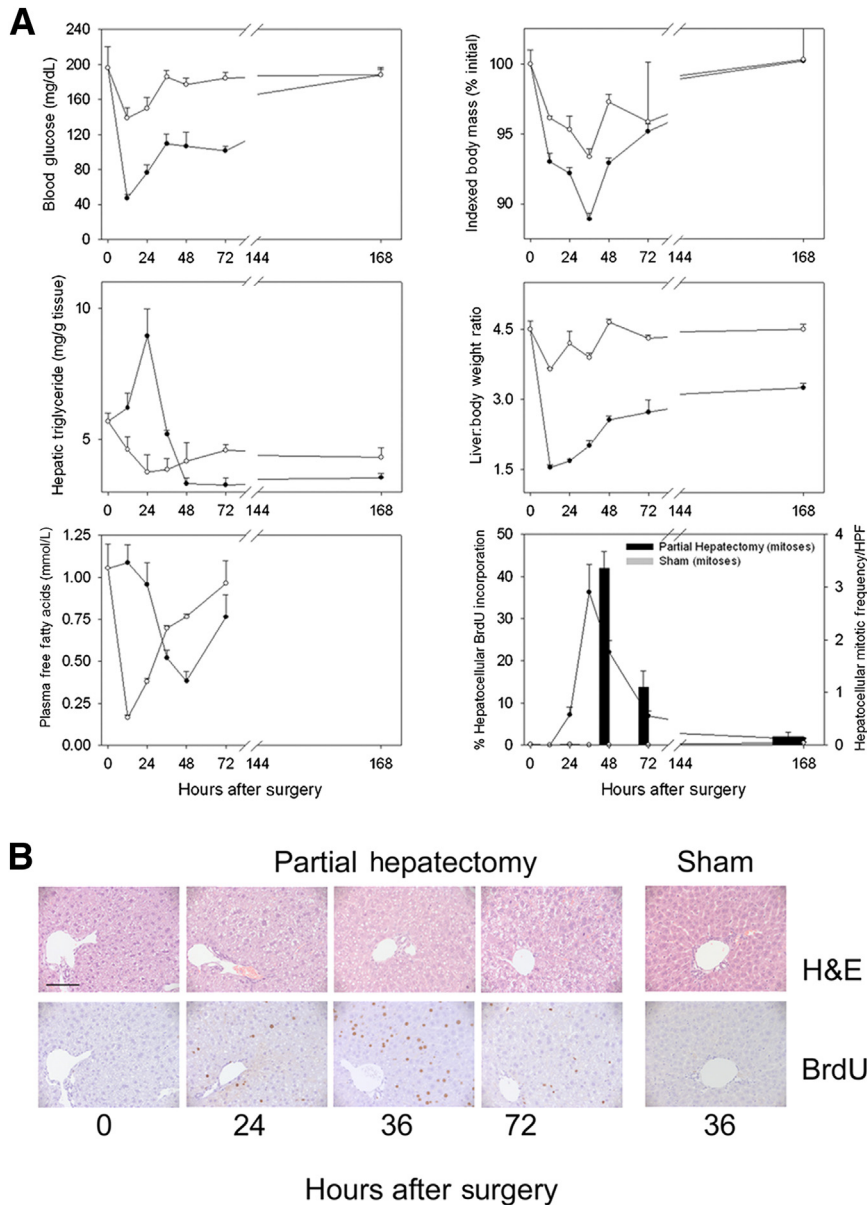
Figure 1 The metabolic and hepatocellular proliferative responses to partial hepatectomy. Blood glucose, indexed body mass, hepatic triglyceride content, liver/body weight ratio, plasma free fatty acids, percentage hepatocellular bromodeoxyuridine (BrdU) incorporation, and hepatocellular mitotic frequency per high-powered field (HPF; **A**) and liver histological features (H&E) and BrdU immunohistochemistry (**B**) at serial times after partial hepatectomy (**black circles**) or sham (**white circles**) surgery in mice. Scale bar = 100 μm.

expense of glucose-derived hepatic ATP production. Glycogen in the remnant liver is also depleted in the hours after partial hepatectomy.[23] By 12 hours after surgery, animals exhibit a systemic catabolic response, characterized by declining lean- and adipose-tissue mass.[24] Systemic fat depletion also occurs in hepatotoxin-induced liver regeneration.[25] From 12 to 24 hours after surgery, marked steatosis develops in the regenerating mouse liver (Figure 1).[26] As with altered glycemia, liver triglyceride accumulates coincidentally with hepatic induction of an adipogenic transcriptional program.[26] Several observations suggest that this transient steatosis results from uptake by the regenerating liver of adipose-derived fat stores: i) serum free fatty acids, derived from hypoglycemia-induced adipose lipolysis, are significantly elevated in animals subjected to partial hepatectomy, compared with sham−operated controls, prior to accumulation of hepatic fat in regenerating liver (Figure 1),[19] ii) fatty liver dystrophy (*fld*) mice, which have a

genetic mutation (in *Lipin1*), resulting in a paucity of systemic adipose tissue, accumulate significantly less liver fat after partial hepatectomy than do their littermate controls,[24] and iii) suppression of *de novo* hepatic lipogenesis (by liver-specific genetic disruption of fatty acid synthase expression) does not prevent resection-induced hepatic steatosis.[27] Over this same time span, specific gluconeogenic-, ketogenic-, branched chain-, and urea cycle−related amino acids, likely derived from systemic proteolysis, also appear in the serum and accumulate in regenerating liver.[28] Hepatic ATP content coincidentally declines and AMP increases in the post-resection liver remnant,[29] with β-oxidation of fatty acids serving as the predominant source of new ATP production in regenerating liver.[19,30] These metabolic alterations precede the onset of resection- or toxin-induced hepatocellular proliferation, which is subsequently promoted by induction of cyclin expression and activation of cyclin-CDK complexes.[31]
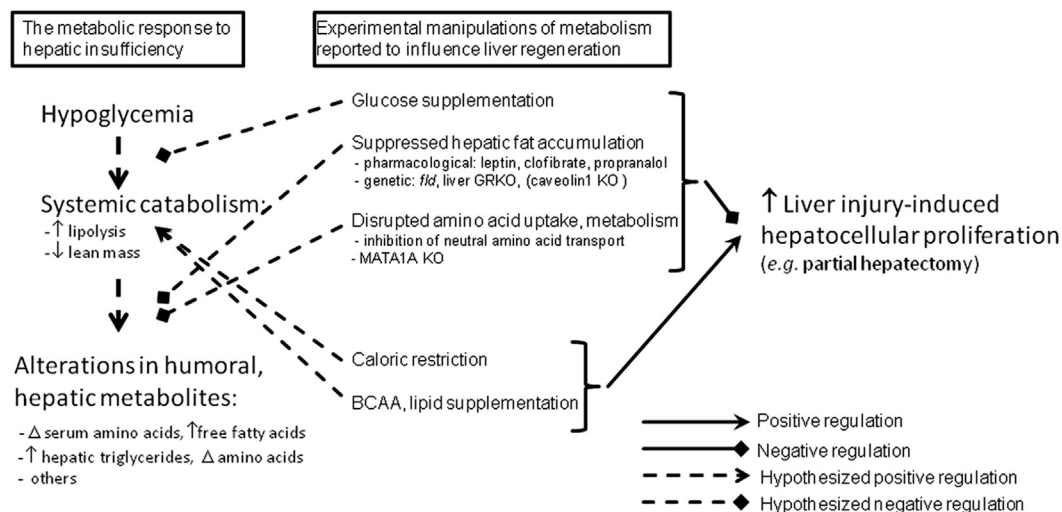
Figure 2 Evidence for the functional importance of the metabolic response to hepatic insufficiency during liver regeneration. Summary of the experimental manipulations of metabolism reported to influence liver regeneration that are discussed in the text.

In mice, peak hepatocellular DNA replication, one marker of such proliferation, occurs 36 hours after partial hepatectomy, with maximum hepatocellular mitotic progression taking place 48 to 72 hours after liver resection (Figure 1). As regeneration proceeds, these perturbations in hepatic and systemic metabolism resolve. For example, hepatic triglyceride levels decline with onset of hepatocellular proliferation, and blood glucose and body mass return toward normal as regenerative recovery of the liver/body mass ratio progresses (Figure 1).[20,24,26]

The Functional Importance of Altered Metabolism during Liver Regeneration

In addition to defining the metabolic response to hepatic insufficiency, experimental observations have implicated these perturbations as important physiological determinants of normal liver regeneration (Figure 2). Several examples are considered here:

The Metabolic and Hepatocellular Proliferative Responses to Partial Hepatectomy

Two-thirds partial hepatectomy results in significantly increased hypoglycemia, a greater decline in systemic body mass and adipose stores, and higher accumulation of hepatic triglyceride, as well as more robust hepatocellular proliferation, than does one-third hepatectomy.[24] Thus, the metabolic and hepatocellular proliferative responses to liver injury occur in proportion to each other, at least over a certain range. However, published experimental analyses of subtotal hepatectomy, in which 85% to 90% of the native liver is resected, show delayed and impaired liver regeneration and increased mortality.[32,33] Those data suggest that liver regeneration cannot rescue an animal below a threshold amount of remnant liver mass (and function), at least without additional

support. This consideration has potential clinical relevance in acute liver failure (ALF) and small (transplanted liver graft) for (host) size syndrome (SFSS), as discussed further in *Subtotal Hepatic Resection* under *Clinical Implications*.

Glucose Supplementation Impairs Liver Regeneration

The functional importance of hypoglycemia during liver regeneration has been demonstrated by several studies showing suppression of liver resection— or hepatotoxin-induced hepatocellular proliferation by enteral or parenteral glucose supplementation.[20,30] Glucose supplementation also suppresses regeneration-associated hepatic fat accumulation.[30] Consistent with these data, dietary caloric restriction accelerates initiation of regenerative hepatocellular proliferation.[6,34] The mechanisms responsible for these effects have not been completely elucidated, although some progress has been made. For example, a recent study showed that supplemental glucose augments hepatic expression of C/EBPα, whose level and activity normally decline during early regeneration.[20] That study also reported increased hepatic expression of the CDK inhibitors, p21[Cip1] and p27[Kip1], in glucose-supplemented animals. Nonetheless, the precise mechanisms by which supplemental glucose increases the expression of these antiproliferative factors in regenerating liver remain undefined.

As expected, circulating insulin levels decline in response to partial hepatectomy-induced hypoglycemia and are augmented by exogenous glucose supplementation.[20] Conversely, systemic diversion of portal circulation (eg, portacaval shunting, which diverts pancreas-derived insulin away from the liver) causes atrophy of the liver lobe from which such flow is diverted, and insulin supplementation reverses such atrophy.[35] Thus, the functional role of alterations in glycemia and associated changes in hepatic insulin (and, perhaps, other hormonal) signaling during liver regeneration deserve further clarification. The glucose supplementation regimens used in previously described
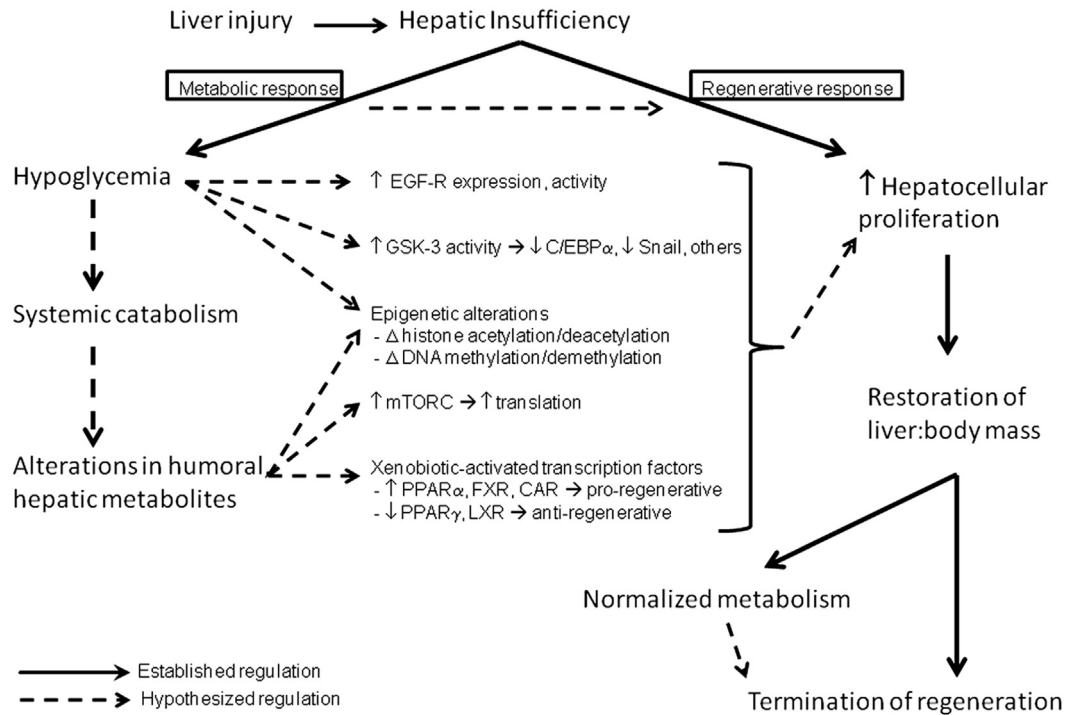
Figure 3 A metabolic model of liver regeneration. Summary of the candidate molecular mechanisms that link altered metabolism and regulation of liver regeneration that are discussed in the text.

studies of impaired liver regeneration do not entirely prevent hypoglycemia; moreover, they reduce, but do not completely abrogate, hepatocellular proliferation after partial hepatectomy.[20] Thus, the development of an experimental paradigm with which euglycemia is maintained in the setting of hepatic insufficiency might further inform mechanistic understanding of the links between glycemia and liver regeneration. Nevertheless, when considered collectively, these observations convincingly establish such metabolic alterations as involved in the initiation of liver regeneration.

Preventing Hepatic Fat Accumulation Suppresses Liver Regeneration

The impact of preventing hepatic steatosis, after partial hepatectomy and in other liver injury models, on the ensuing regenerative response has also been investigated. When hepatic fat accumulation is suppressed pharmacologically (eg, with clofibrate,[36] supraphysiological leptin supplementation,[26] or propranolol[37]) or genetically (eg, as in *fld*- or liver-specific glucocorticoid receptor-knockout mice), liver regeneration is inhibited.[24,26] However, other studies using different mouse models have reported seemingly contradictory results. For example, liver regeneration in caveolin 1—null mice, in which partial hepatectomy—induced hepatic steatosis is reduced, was reported to be impaired in one study but normal in another study.[38,39] Furthermore, hepatic triglyceride accumulation is diminished but regeneration proceeds normally after partial hepatectomy in liver fatty acid binding protein—null mice and in mice with intestine-specific deletion of the microsomal

triglyceride transfer protein.[27] More important, liver resection—induced hepatic fat accumulation was not entirely abrogated in either of those models, leading the investigators to speculate about the existence of a "threshold of adaptive lipogenesis" essential for regeneration but not crossed in those mice.[27] Nonetheless, when taken together with data showing that fat accumulates concomitantly with cell proliferation in primary hepatocyte culture,[40] these observations suggest that hepatocellular lipid accumulation promotes hepatocyte proliferation, at least under certain circumstances. Indeed, it has even been speculated that the anti-regenerative effect of glucose supplementation might be secondary to the suppressive action of such intervention on release of free fatty acids from systemic adipose.[30] Consistent with this consideration, both dietary and parenteral administration of various lipid-based formulations accelerates resection-induced[30] and toxin-induced[6] hepatocellular proliferation in experimental animals. Interestingly, as with inhibition of liver regeneration by glucose supplementation, the impaired regenerative response associated with reduced hepatic fat accumulation in *fld* mice is associated with augmented hepatic p21[Cip1] expression.[20,24]

Amino Acid Metabolism and Liver Regeneration

Altered amino acid metabolism also occurs during and might influence normal liver regeneration. For example, a recent report showed that α-NH$_2$-butyric acid (Aab) accumulates in serum and liver after partial hepatectomy in mice.[28] Aab is generated by transamination of 2-oxobutyrate, which is produced by methionine and threonine catabolism.[41] Methionine

is primarily metabolized in the liver, and such metabolism is commonly deranged in chronic liver disease.[42] Furthermore, hepatic metabolism of S-adenosyl-methionine (SAMe), which is synthesized from methionine by methionine-adenosyl transferase 1A (MAT1A), is highly regulated during liver regeneration, and such regulation is disrupted in experimental and clinical liver disease and liver cancer.[42] Interestingly, perturbations in methionine metabolism induced by genetic disruption of MAT1A expression inhibit mouse liver regeneration.[43] Together, these findings suggest a correlation between increased metabolic flux from methionine to Aab and the initiation of liver regeneration. Other studies showed that the specific activities of (predominantly periportal) urea cycle and (primarily centrilobular) glutamine synthase enzymes are down-regulated during liver regeneration[44,45] and that pharmacological inhibition of hepatic neutral amino acid uptake impairs regeneration.[46] Finally, provision of supplemental branched-chain amino acids (BCAAs) was reported to promote liver regeneration in several studies.[47] Taken together, these observations implicate hepatic insufficiency-induced alterations in uptake and metabolism of amino acids as another aspect of the metabolic response to hepatic insufficiency, with functional importance during regenerative hepatocellular proliferation.

Candidate Molecular Mediators Linking Metabolism and Liver Regeneration

The previously summarized data (Figure 2) link alterations in metabolism to the regulation of liver regeneration but do not define the specific responsible molecular mechanisms. One possibility is that the metabolic response to hepatic insufficiency provides the liver with substrates necessary to meet the energy requirements of the hepatic regenerative response while simultaneously accommodating the glucose demands of the body mass. The liver might also meet systemic energy demand via ketone body synthesis. However, serum and hepatic levels of ketone bodies reportedly decline during experimental liver regeneration.[48,49] These changes in ketone metabolism, like those of glycemia and lipid metabolism, are accompanied by concordant changes in transcriptional regulation, with expression of the ketogenic enzyme, HMG-CoA synthase, down-regulated in regenerating liver.[50] Not exclusive of these considerations, the changes in metabolism that occur during liver regeneration are likely to generate anabolic precursors (nucleotides, amino acids, and lipids) necessary for the macromolecular biosynthetic requirements of regenerative cell proliferation. Consistent with this idea, the level of the less-active M2 isoform of the glycolytic enzyme pyruvate kinase (PK) increases, whereas that of the more active PK-L isoenzyme decreases, in regenerating liver.[51] This switch should divert glucose-derived carbon from glycolytic production of ATP to macromolecule precursor synthesis (or back to glucose). Increased PK-M2 is also associated with the Warburg effect,

ie, the aerobic glycolysis characteristic of cancer cell proliferation.[52] Together, these considerations suggest that alterations in metabolism contribute to both the energy and macromolecular precursor demands of the hepatic regenerative response. However, this conclusion does not explain the inhibitory effect of supplemental glucose on liver regeneration, nor can it account for the precision with which liver/body mass ratio is restored by regeneration after hepatic injury. Those observations, in particular, support the idea that the metabolic response to hepatic insufficiency is itself the source of a proregenerative signal. Although the specific molecular mediators connecting metabolism and regeneration await definitive identification, we discuss several attractive candidates specifically suggested by experimental analyses of hepatocellular proliferation (Figure 3).

Insights from Analysis of Xenobiotic-Induced Hepatocellular Proliferation

One candidate mechanistic link between metabolism and liver regeneration is suggested by analyses of chemical mitogen-induced hepatocellular proliferation. Administration of certain xenobiotic compounds to rodents has long been recognized to induce hepatocellular hypertrophy and hyperplasia, thereby increasing liver mass in the absence of liver injury.[53] Withdrawal of such agents is followed by a return to normal liver mass, providing additional evidence for the hepatostat. During the past two decades, several nuclear receptor transcription factors have been identified as specific and direct mediators of such xenobiotic-induced hepatocellular proliferation. For example, peroxisome proliferator-activated receptor (PPAR) α expression is required for development of the hepatomegaly that occurs in rodents exposed to clofibrate and Wy-14,636.[54] Similarly, the increase in liver mass that occurs with administration of 1,4-bis[2-(3,5-dichloropyridoxyloxy)] benzene (TCPOBOP) or phenobarbitol depends on the constitutive androstane receptor (CAR[55]). Interestingly, partial hepatectomy— and toxin-induced liver regeneration is altered in several models in which the expression or activity of certain xenobiotic-activated transcription factors has been manipulated. Moreover, analyses in mouse models in which hepatic integrin-linked kinase or glypican 3 expression is altered show that these signals, implicated in terminating regeneration after partial hepatectomy,[17,18] similarly affect at least some models of xenobiotic-induced hepatocellular proliferation.[56–58] Thus, the mechanisms that regulate chemically induced hepatomegaly overlap, at least to some degree, with those that direct hepatic insufficiency—induced liver regeneration. Conversely, disruption of other specific upstream signals implicated in partial hepatectomy—induced liver regeneration does not necessarily prevent xenobiotic-dependent changes in liver mass,[59–61] indicating that these chemically activated pathways can act downstream of or in parallel to at least some of the signals that promote such regeneration. Even so, characterization of hepatocellular proliferation and liver regeneration in models

with genetically or pharmacologically altered xenobiotic-responsive transcription factors has provided additional insight into the mechanisms that might link metabolism to liver regeneration. The following are some examples.

PPARα Data

A specific role for PPARα expression during normal liver regeneration remains controversial based on conflicting reports about the magnitude of derangement of regeneration in PPARα-null mice.[27,62–64] Nevertheless, recent data implicating endogenous lipid metabolites as ligand activators of PPARα[65] raise the possibility that these and other naturally occurring PPARα ligands might link transient hepatic lipid accumulation after partial hepatectomy to subsequent initiation of regenerative hepatocellular proliferation. These data also suggest that other metabolites that accumulate in regenerating liver might serve as specific endogenous ligands for additional xenobiotic-binding transcription factors and, thereby, regulate liver regeneration.

FXR Data

FXR is a bile acid–activated transcriptional regulator, and global disruption of its expression results in marked impairment of liver regeneration in response to partial hepatectomy.[66] Furthermore, unoperated, bile acid–fed mice exhibit increased hepatocellular mitoses and hepatomegaly.[66] These observations suggest that the proportionately increased enterohepatic delivery of bile acids to the post-resection liver remnant might link altered metabolism to initiation of liver regeneration. However, hepatic bile acid content declines after partial hepatectomy in wild-type mice.[66] FXR is expressed in both liver and intestine, raising a related question as to whether disruption of hepatic or intestinal expression (or both) is responsible for impaired regeneration in global FXR-null animals. Recent studies using tissue-specific FXR-deleted mice showed that both liver- and intestine-specific FXR-null mice exhibit impaired regeneration in response to resection- and toxin-induced regenerative stimuli.[67,68] However, it is not clear if global FXR-null mice or either of the tissue-specific knockout models is resistant to the hepatomegaly-inducing effects of enteral bile acids. Intestinal FXR affects bile acid homeostasis in mice by inducing intestinal epithelial expression of fibroblast growth factor 15 (FGF-15), which is transported via portal circulation to the liver, where it suppresses bile acid synthesis.[69] Interestingly, FGF-15–null mice were recently reported to exhibit both impaired resection-induced hepatic regeneration and reduced enteral bile acid–stimulated hepatomegaly.[70] These data implicate FXR, FGF-15, and enterohepatic circulation of bile acids in the metabolic regulation of liver regeneration.

Other Xenobiotic-Activated Nuclear Receptors

Mice also develop hepatomegaly when treated with CAR- and pregnane X receptor (PXR)–activating ligands, such as phenobarbitol, TCPOBOP, and pregnenolone-16α-carbonitrile.[53,71] Although these chemically induced responses are dependent on expression of CAR or PXR, respectively,[55,72] the corresponding null mice show only modestly impaired liver regeneration after partial hepatectomy.[66,73] Whether naturally occurring metabolite ligands of CAR or PXR accumulate in regenerating liver remains unknown. Other xenobiotic-activated nuclear receptor transcription factors might negatively regulate liver regeneration. For example, PPARγ-null mice exhibit mildly accelerated regeneration,[74] and pharmacological activation of PPARγ[75] or LXR[76] suppresses regeneration. Unsaturated fatty acids, eicosanoids, and prostaglandins, which themselves affect liver regeneration, are naturally occurring PPARγ ligands,[77,78] whereas oxysterols are endogenous ligands for LXR.[76] A recent study also suggested that glucose itself might directly regulate LXR activity.[79] These data reenforce the intriguing, but unproved, hypothesis that hepatic insufficiency–induced alterations in hepatocellular metabolite levels affect liver regeneration by regulating xenobiotic-activated transcription factor activities (Figure 3).

Metabolically Regulated Extrahepatocellular and Intrahepatocellular Regenerative Signals

Many signaling molecules and pathways implicated in the regulation of liver regeneration are influenced by metabolism. However, the specific metabolic regulation of such signals during liver regeneration has generally not been established. Although it is beyond space constraints to consider all such examples herein, several provocative candidates are discussed.

EGF-R Data

Epidermal growth factor receptor (EGF-R) ligands are essential humoral regulators of hepatocellular proliferation in experimental models of liver regeneration.[1–3] In other models, hyperglycemia impairs EGF-R expression and activity,[80,81] suggesting that disruption of EGF-R–dependent signaling might contribute to the inhibitory effect of glucose supplementation on liver regeneration[20]; conversely, partial hepatectomy–induced hypoglycemia might promote such signaling (Figure 3).

GSK-3 Data

Glycogen synthase kinase (GSK)-3, encoded by two related genes (GSK-3α and GSK-3β), was originally identified based on and is named for its ability to phosphorylate glycogen synthase.[82] Such phosphorylation inactivates glycogen synthase activity, and inhibition of GSK-3 activity augments hepatic glycogen synthesis and improves glucose homeostasis in rodents.[82] Analyses of tissue- and isoform-specific functions of GSK-3 show that global disruption of GSK-3α expression, like pharmacological GSK-3 inhibition, augments murine insulin sensitivity and hepatic glucose metabolism in a mouse strain–specific manner.[83,84] Moreover,

muscle-specific, but not liver-specific, disruption of GSK-3β also improves insulin sensitivity.[85] Nevertheless, these data establish the biological role of GSK-3 in glycemic control. Recent reports also demonstrate that pharmacological inhibition of GSK-3 activity or genetic suppression of GSK-3β expression inhibits liver regeneration.[86–88] Together, these observations raise the possibility that GSK-3 might link hepatic insufficiency−induced hypoglycemia to initiation of liver regeneration (Figure 3). Consistent with this consideration, GSK-3 also phosphorylates and, thereby, regulates proteins other than glycogen synthase, including transcription factors and cell cycle regulators involved in liver regeneration.[82] For example, C/EBPα is phosphorylated and inactivated by GSK-3, and an age-dependent decline in GSK-3β expression was reported to contribute to the reduced resection-induced regenerative capacity of older livers by disrupting such regulation.[88] As noted previously, hepatic C/EBPα expression is also augmented in glucose-supplemented animals subjected to partial hepatectomy.[20] Thus, suppression of GSK-3 activity might contribute to impaired regeneration in glucose-supplemented animals. GSK-3−dependent phosphorylation and degradation of the Snail transcription factor has been proposed as another mechanism by which GSK-3β might promote liver regeneration.[87] Despite these considerations, it is difficult to reconcile the expected positive effects of partial hepatectomy−induced hypoglycemia on GSK-3 activation (Figure 3) with GSK-3's recognized effects on other signaling pathways known to positively regulate liver regeneration. For example, β-catenin promotes liver regeneration[89,90] but is targeted for proteasomal degradation by GSK-3−dependent phosphorylation.[82] These observations suggest that distinct subcellular pools of GSK-3 exist, with the pool responsive to glycemic alterations and involved in promoting liver regeneration distinct from that which controls β-catenin degradation. Experimental models of liver regeneration might offer the opportunity to test this prediction.

mTORC Data

Mammalian target of rapamycin (mTORC), an intracellular protein complex composed of mTOR, regulatory-associated protein of TOR, and other proteins, integrates growth factor−dependent signals, together with nutrient and energy status, to control protein synthesis.[91] Activated mTORC promotes translation through phosphorylation of p70-S6 kinase 1 and eukaryotic initiation factor 4E binding protein. Amino acid availability, particularly that of leucine and other BCAAs, affects the ability of mTORC to interact with and phosphorylate its substrates.[91] Interestingly, pharmacological inhibition of mTOR suppresses cyclin D1 expression and hepatocellular proliferation in mice subjected to partial hepatectomy.[92,93] These data suggest a mechanism by which the metabolic response to hepatic insufficiency, and the accompanying hepatic accumulation of BCAA, might promote proregenerative hepatic protein expression. Indeed, this consideration is consistent with the previously mentioned beneficial effect of supplemental BCAA on liver regeneration

noted in various studies.[47] Growth factor−dependent activation of phosphoinositide-3 kinase/Akt signaling also stimulates mTORC activity[91] and promotes liver regeneration.[94–96] However, those findings seem contradictory to data indicating that mTORC activity is negatively regulated by the energy-sensitive AMP-activated protein kinase (AMPK[91]). This latter observation predicts that the declining ratio of ATP/AMP in regenerating liver, which should activate AMPK, would inactivate mTOR; however, decreased ATP/AMP has also been suggested as important for progression of regeneration.[29] Moreover, AMPK itself was recently implicated in the positive regulation of liver regeneration.[97] Perhaps the threshold level of ATP/AMP that triggers AMPK's inhibitory action on mTORC is below that which is crossed after partial hepatectomy to promote liver regeneration. Again, further study is needed to clarify these considerations.

Metabolic Influences on Epigenetic Regulation

A provocative, but unproved, idea is that alterations in metabolism influence liver regeneration by affecting epigenetic changes in histone protein acetylation. Indirect support for this hypothesis comes from data showing that glucose supplementation promotes histone protein acetylation in mammalian cell culture, with fatty acids unable to substitute for glucose in those models.[98,99] Thus, partial hepatectomy−induced alterations in glycemia might influence histone acetylation by reducing histone acetyltransferase acetyl-CoA substrate availability. Relevant to these points, total hepatic zinc-dependent histone deacetylase (Zn-HDAC) activity was recently reported to increase and global liver histone acetylation was reported to decrease in parallel with onset of hypoglycemia after partial hepatectomy.[100] The functional importance of Zn-HDAC activity was demonstrated by showing that suberoylanilide hydroxamic acid, a global inhibitor of Zn-HDAC activity, suppresses liver regeneration.[100] The possibility of metabolic regulation of hepatic Zn-HDAC activity during liver regeneration was also suggested by data showing that HDAC5, a class IIa Zn-HDAC whose subcellular localization is regulated by glycemia,[101] undergoes nuclear localization in response to partial hepatectomy.[100] A recent report identified the ketone body, β-hydroxybutyrate, as a potent inhibitor of specific Zn-HDACs *in vivo*,[102] raising the possibility that other endogenous metabolites that accumulate in regenerating liver as part of the metabolic response to hepatic insufficiency might have similar activity (Figure 3). Finally, SIRT1, a class III (ie, sirtuin) NAD-dependent HDAC, was recently shown to be essential for normal liver regeneration.[103] Together, these data suggest that altered metabolism might affect liver regeneration via epigenetic regulation of histone (and nonhistone protein) acetylation.

Metabolic alterations might also influence patterns of DNA methylation in regenerating liver. This consideration is particularly intriguing in the context of the previously mentioned impairment of liver regeneration observed in MAT1A knockout mice.[43] Biosynthesis of SAMe, which serves as a

methyl donor in DNA methylation reactions, is catalyzed by MAT1A, and hepatic SAMe levels are reduced in MAT1A-null mice.[104] Corroborating evidence for the functional importance of regulated changes in DNA methylation patterns during liver regeneration is suggested by older studies showing that azacytidine, which inhibits DNA methyltransferase activity, suppresses regeneration under certain circumstances.[105] Finally, one additional but highly speculative point regarding the potential relationships between metabolism, DNA methylation, and regenerative hepatocellular proliferation should be mentioned: mutations in isocitrate dehydrogenase, which catalyzes the production of α-ketoglutarate in the Kreb's cycle, have recently been identified in myeloid leukemia and other cancers.[106] α-Ketoglutarate also serves as a cofactor for DNA (cytosine) demethylation reactions,[107] and cancer patients with isocitrate dehydrogenase mutations exhibit global promoter hypermethylation.[106] α-Ketoglutarate is also the amino group acceptor for the reaction, catalyzed by alanine aminotransferase, which is highly expressed in liver and produces the gluconeogenic precursor, pyruvate, from alanine. Thus, alterations in the level of α-ketoglutarate, which likely occur together with the changes in glycolytic and gluconeogenic flux in regenerating liver, could affect patterns of DNA methylation via the mechanism discussed above. Nevertheless, the pattern, regulation, and functional importance of changes in hepatic DNA methylation during liver regeneration require further examination.

A Metabolic Model of Liver Regeneration

Together, the data and considerations discussed here support a metabolic model of liver regeneration in which alterations in metabolism that occur in response to hepatic insufficiency provide energy and macromolecular precursors necessary for regeneration and generate specific molecular signals that initiate regenerative hepatocellular proliferation. Future studies should test the predictions suggested by this model (Figure 3).

Clinical Implications

Metabolic influences on hepatic regenerative capability have obvious potential relevance to human health. For example, the impairment of liver regeneration in experimental models of fatty liver disease, aging, fulminant liver failure, and other processes might be related to the perturbations in normal metabolism that accompany such conditions. These topics are briefly considered herein.

Fatty Liver

Unlike the transient steatosis that occurs during the normal regenerative response to hepatic insufficiency,[26] chronic hepatic steatosis is associated with impaired resection- and toxin-induced liver regeneration in many, but not all, experimental models.[19] Those studies suggest that the magnitude of hepatic steatosis is important in determining its

effect on liver regeneration. Chronic steatosis is also linked to adverse outcomes after liver resection in humans.[108] Taken together with the considerations raised herein, these data suggest that acute versus chronic hepatic steatosis, and accompanying differences in hepatic and systemic metabolism, likely exert distinct effects on growth factor, secondary messenger, genetic, and epigenetic signals and, thereby, have divergent influences on liver regeneration. Nevertheless, the specific mechanisms responsible remain enigmatic and require further investigation.

Aging

The aged liver has reduced regenerative capacity in response to resection. Although long recognized,[109] the mechanisms responsible remain incompletely defined. As already noted, specific anti-proliferative factors whose hepatic expression is induced in association with impaired regeneration in glucose-supplemented mice, including C/EBPα and p21[Cip1],[20] are also up-regulated in post-resection livers from aged animals.[110] Thus, age-related effects on glycemia might contribute to the impairment of partial hepatectomy—induced liver regeneration in old animals. Further support for this idea comes from data implicating effects on Zn-HDACs in the anti-regenerative influence of aging,[110] together with the previously mentioned study suggesting that metabolism influences Zn-HDAC expression, activity, and subcellular localization during liver regeneration.[100] Surprisingly, old age does not suppress the hepatocellular proliferative response to certain toxins[6] or xenobiotics (eg, TCPOBOP[111]). The relevance of these data to human liver regeneration requires further investigation. In particular, whether diminished resection-induced regeneration of the aged liver contributes to reduced survival of transplanted liver grafts from older donors reported in some studies[110] should be examined.

Subtotal Hepatic Resection

Published experimental analyses in which 85% to 90% of the native liver is resected (ie, subtotal hepatectomy) show delayed liver regeneration and increased mortality.[33] Those observations suggest the existence of a threshold amount of remnant liver mass below which regenerative recovery is inefficient. Unlike the effect on regeneration after two-thirds partial hepatectomy, glucose supplementation improves the outcomes from experimental subtotal hepatectomy.[32] The mechanisms responsible for these seemingly discordant effects of glycemia on the response to partial versus subtotal hepatectomy are entirely unknown. Elucidating those mechanisms might inform strategies to improve clinical management of patients with ALF and SFSS, for which subtotal hepatectomy in rodents has been used as an experimental model.

Metabolomic Biomarkers of Human Liver Regeneration

The considerations raised herein also suggest that metabolomic serum biomarkers of experimental liver

regeneration could permit more reliable, noninvasive assessment of human liver regeneration. Such tools would be especially useful in the clinical management of ALF and when there is concern for SFSS. ALF is a potentially devastating condition from which some patients die or undergo liver transplantation.[112] Others recover spontaneously, based in part on adequate regeneration of the liver. However, an early reliable distinction between those patients with ALF most likely to survive spontaneously and those at increased risk of death without liver transplantation remains extremely challenging.[112] Similarly, predicting spontaneous regenerative recovery in patients at risk of SFSS is difficult.[33] The novel metabolic model of liver regeneration proposed herein (Figure 3) predicts that specific patterns of change in circulating metabolites might distinguish progression of normal liver regeneration from an impaired response. Proof of principle for that idea was provided by the recent identification of Aab, mentioned earlier, as a sensitive and specific humoral biomarker of mouse liver regeneration, followed by subsequent demonstration of a significant correlation between serum Aab levels and spontaneous survival in a pilot analysis of pediatric patients with ALF.[28] Nonetheless, additional studies are needed to further characterize the humoral metabolomic signature of experimental liver regeneration and define its value in the evaluation and management of human liver disease.

Summary and Conclusions

Many studies implicate alterations in metabolism in response to experimentally induced hepatic insufficiency (Figure 1) as functionally important for normal liver regeneration (Figure 2). Such analyses also suggest candidate molecular mechanisms by which such linkage might occur (Figure 3). Together, these data support a metabolic model of liver regeneration in which the essence of the hepatostat is defined by hepatic metabolic function. Future research should interrogate the unique functional relationships between specific alterations in metabolism and individual signaling pathways during liver regeneration and investigate whether derangement of these interactions causes impaired liver regeneration in fatty liver disease, old age, ALF, SFSS, and perhaps other conditions. Ultimately, such work could lead to the development of pro-regenerative nutritional and metabolism-based strategies and more reliable, noninvasive, metabolomic biomarkers of liver regeneration to improve the management of human liver disease.

Acknowledgment

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