Making the Liver Young Again

As the myths of the Greek and Roman gods reveal, the phenomenon of liver regeneration has been known to occur for centuries. Both the morphologic and functional changes that take place during this process have been studied in depth. Over the last two decades, considerable information has been acquired regarding the alterations in gene expression that accompany the recovery of the liver after injury or loss of tissue mass.

In 1964, Nancy Bucher and her colleagues published a kinetic profile of hepatocyte proliferation in the remnant liver, following two-thirds partial hepatectomy (PH) in young (3-4 weeks) and old (12-15 months) rats. A striking difference was observed in both the magnitude of the peak response in DNA synthesis and the time at which maximal DNA synthesis occurred (Fig. 1). In old rats, the response to PH was both delayed and reduced. Stocker and Heine performed a careful study to estimate the fraction of cells in the remnant liver that entered into the proliferative phase following two-thirds PH in young and old rats. Their observations showed that in young animals (2-3 months of age) proliferation in the remnant liver involved over 99% of the hepatocytes; whereas, in older animals (12 months) only 30% of the liver cells entered DNA synthesis. Some of the delay and loss of synchrony in response to PH in old animals may be accounted for by a reduction in the proliferative pool, suggesting that for old animals, the 30% of cells that do undergo division must replicate multiple times in order to reinstate total liver mass. An explanation for this difference in the regenerative response of older animals to liver injury or PH has been sought since these early observations. In this issue of HEPATOLOGY, the article by Kruppacz-Hollis et al. builds on several observations regarding the molecular basis for these age-related differences and, importantly, offers a potential therapeutic approach to relieve the proliferative block to liver injury observed in the elderly.

Priming Events

Investigators have divided the process of regeneration into a series of stages that include initial inductive or priming events through cellular mitosis (Fig. 2). Understanding the initiation signals that send the remnant liver down the proliferative path has been a goal of many investigations. A molecular delineation of these events would potentially enable early stimulation of hepatocyte proliferation, which could be a useful therapy in cases of liver injury. Various studies using targeted recombination to create “knockout” mice have strongly implicated tumor necrosis factor α (TNF-α) (see Fausto) and interleukin 6 (IL-6) signaling pathways in the priming phase. In addition, Greenbaum et al. have shown that the transcription factor, C/EBP-β, a downstream target of cytokine signaling, plays an important role in the outcome of the proliferative response.

Immediate Early Gene Activation

Additional pathways important to regeneration have recently been elucidated. Following the priming events just discussed, immediate early genes are induced, due in large part to post-translational modifications of the pre-existing transcription factors AP1, C/EBP-β, and -δ, Stat3, and nuclear factor κB (NF-κB) by cytokine priming. In addition, increased expression of immediate early genes occurs within the first few hours postsurgery and does not require new protein synthesis. Well-characterized immediate early genes that are induced include c-myc, c-fos, c-jun, EGR1, IGFBP-1, and hepatocyte growth factor (HGF), to name a few (see Fausto, Taub, and Michalopoulos and DeFrances). The array of immediate early genes occurs within the first few hours postsurgery and does not require new protein synthesis. Well-characterized immediate early genes that are induced include c-myc, c-fos, c-jun, EGR1, IGFBP-1, and hepatocyte growth factor (HGF), to name a few (see Fausto, Taub, and Michalopoulos and DeFrances). The array of immediate early genes has been partially established by gene expression profiling, giving a more complete picture of the molecular events that take place in priming and the immediate early phase. Following the immediate early phase, numerous changes in gene expression take place that require new protein synthesis. These events ultimately push the cell out of G0 and into G1 followed by the S phase of the cell cycle.

Foxm1b

Recently, the Foxm1b member of the Forkhead Box transcription factor family was shown to be an essential protein for hepatocyte cell growth. Early development and subsequent differentiation of the liver involves other members of the forkhead transcription factor family.

Abbreviations: PH, partial hepatectomy; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; NF-κB, nuclear factor κB; HGF, hepatocyte growth factor; GH, growth hormone; TGF-α, transforming growth factor α.

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(Foxa1, a2, and a3) and Foxm1b is present in virtually all embryonic tissues.\textsuperscript{13,17,18} The level of Foxm1b transcripts decreases to undetectable levels in nondividing, differentiated tissues. Thus, Foxm1b has been thought to be tightly regulating or regulated by entry of the cell into S phase.

The laboratory of Dr. Robert Costa has contributed significantly to our understanding of the role of Foxm1b through the use of transgenic mice over-expressing the human protein\textsuperscript{15} and tissue-specific knockout mice lacking Foxm1b in hepatocytes.\textsuperscript{16} Wang et al. noted that mice lacking Foxm1b in the liver have a greatly reduced proliferative response to PH.\textsuperscript{16} They further showed that overexpression of the human FOXM1B through adenoviral delivery to 12-month-old wild-type mouse liver reinstated the normal response to surgery.\textsuperscript{19} FOXM1B overexpression in transgenic animals accelerated the initiation of DNA synthesis following PH by approximately 8 hours. Early entry into S phase in response to FoxM1B is similar to the effects of over-expression of other critical components of the cell cycle machinery, including cyclin D,\textsuperscript{20} skp2, and cyclin E,\textsuperscript{21} and links Foxm1b expression closely to the progression of cell cycle. FoxM1B is able to increase the transcription of the cyclin B\textsuperscript{22,23} and cyclin D\textsuperscript{23} promoters in cotransfection assays, and thus impacts entry into both the S and G\textsubscript{2}/M phases of the cell cycle. Other important components in the regulation of the cell cycle during regeneration appear to be controlled by Foxm1b, including cdc25A and B\textsuperscript{19} phosphatases, which activate cyclin/CDK complexes. In addition, a reduction in the cyclin-dependent kinase inhibitors, P27 and P21, also occurs when FoxM1B is over-expressed in hepatocytes.\textsuperscript{16,19} The multiplicity of Foxm1b control points suggests that the activity of this Forkhead factor is a major determinant in the process of division in hepatocytes.

**Role of Growth Hormone**

The exciting report presented in this issue of *Hepatology*\textsuperscript{5} carries these important observations further and shows that growth hormone (GH) regulation of hepatocyte proliferation requires Foxm1b.\textsuperscript{5} Growth hormone secretion diminishes with age. Moolten et al.\textsuperscript{24} showed that administration of GH to rats accelerated the response of hepatic DNA synthesis to PH, but a mechanistic understanding was not forthcoming. Krupczak-Hollis et al.\textsuperscript{5} show that GH treatment of old animals increases Foxm1b expression and the levels of DNA synthesis both at the control (nonsurgery) levels as well as in response to PH. The increase in Foxm1b in GH-treated old mice reached levels equivalent to those induced in young animals in response to surgery. The requirement for FoxM1B in GH-stimulated proliferation was shown by treating the mice deficient for this transcription factor in hepatocytes with GH and observing that these animals failed to accelerate DNA synthesis in contrast to the mice with functional Foxm1b. Their data strongly support the conclusion that Foxm1b must be present for GH to stimulate hepatocyte proliferation.
Further Study Needed

The observations presented here, and in previous manuscripts from the Costa laboratory, stimulate several questions for further study.

**Do Hepatocytes Undergoing Proliferation Following Primary Mitogen Stimulation Require Foxm1b?**

Columbano and Ledda-Columbano have summarized the knowledge regarding the induction of hepatocyte proliferation in the absence of injury. Various ligands such as the halogenated hydrocarbon TCPOBOP, PPAR ligands, retinoic acids, or T3 can act as primary mitogens *in vivo* through steroid nuclear receptors (see Columbano and Ledda-Columbano). These agents do not require priming events resulting from liver injury to initiate cell cycling. Primary mitogen activation of proliferation does not involve cytokine activation of the transcription factors AP-1, Stat3, NF-κB, and C/EBP-β and α or elevation of transforming growth factor α (TGF-α) or HGF. Nor is there elevation of many of the immediate early genes described above. Following treatment with TCPOBOP, the kinetics of DNA synthesis are greatly altered with accelerated entry into S phase (~12 hours in mice).

**How Is Foxm1b Regulated?** Earlier reports suggest that Foxm1b accumulation after PH is only partially the result of transcriptional activation and that mRNA stabilization may be an important mechanism whereby Foxm1b becomes elevated. Is mRNA stabilization specific to Foxm1b or is it a more general phenomenon with multiple mRNAs showing a similar elevation via this process?

**When Would Treatment With Growth Hormone Be a Useful Therapy?** GH has been administered to older individuals with positive outcomes, such as an increase in lean body mass and bone density and a decrease in adipose tissue mass. Paradoxically, many studies in nonhuman organisms indicate that reduced levels of GH and IGF-1 correlate with increased longevity (see Bartke). The use of GH in older adults as an age-defying agent is hotly debated, in part because the frequency of adverse side effects such as an increase in the onset of diabetes is relatively high. However, these studies used relatively long-term exposure to GH (26 weeks). It is likely that GH treatment for regeneration that accompanies liver transplantation, gene or cell therapy, or for rapid recovery after hepatic failure would be short term and therefore, efficacious. Another issue of potential concern is the demonstration by Krupczak-Hollis et al. that liver cell proliferation is also elevated in normal, GH-treated, nonregenerative livers. This observation would also militate against long-term hormonal therapy on the premise that more cell division than necessary would enable unwanted fixation of mutations that might lead to cancer. However, as Columbano and Ledda-Columbano point out, some hormones, operating through nuclear hormone receptors, decrease neoplastic growth despite stimulation of normal hepatocytes.

**What Are the Pathway(s) for GH Signaling in the Primed, Regenerating Liver and Are the Same or Different Ones Being Stimulated in the Nonsurgery Controls?** GH stimulation of DNA synthesis in young and old livers not undergoing surgery reached levels similar to those observed following PH in the young mice. Thus, priming by injury seems unnecessary for GH stimulation of proliferation. A portion of the signaling pathway for GH is shown in Fig. 3. An immediate downstream target of the GH receptor is JAK 2, with potential involvement of IRS-1 and Stat5. IRS-1 is also a direct target of the IGF-1 and insulin receptors, and PI3 kinase is subsequently regulated by IRS-1. This commonality among the signaling pathways suggests additional experimentation to determine whether JAK2 and/or IRS-1 is required for GH signaling of proliferation and/or Foxm1b elevation and how the GH responses differ from IGF-1/insulin stimulation.

The elegant studies presented here significantly advance our understanding of the molecular regulation of liver regeneration and identify a hormone regulator of that process. As with many exciting discoveries, they also underscore the realization that, although hepatocyte proliferation has been the subject of much research for a very long time, we still have a great deal to learn.

**Stephanie MacKey**

**Pallavi Singh**

**Gretchen J. Darlington, Ph.D.**

**Huffington Center on Aging**

**Baylor College of Medicine**

**Houston, TX**
References

1. Aeschylus. Prometheus Bound. 430 B.C.E.
2. Ovid. Metamorphoses. 1 A.C.E.
16. Wang X, Krueczak-Hollis K, Tan Y, Dennewitz M, Adami GR, Costa RH. Increased Forkhead Box M1B (FoxM1B) levels in old-aged mice stimulated liver regeneration through diminished p27Kip1 protein levels and increased Cdk2B expression. J Biol Chem 2002;277:44310-44316.
19. Wang X, Krueczak-Hollis K, Tan Y, Dennewitz M, Adami GR, Costa RH. Increased Forkhead Box M1B (FoxM1B) levels in old-aged mice stimulated liver regeneration through diminished p27Kip1 protein levels and increased Cdk2B expression. J Biol Chem 2002;277:44310-44316.