EDITORIALS

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.[1,2] 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.[3] 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.[4] 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 Krupczak-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.[5]

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[6]) and interleukin 6 (IL-6) signaling pathways in the priming phase.[7,8] 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.[9]

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,[6] Taub,[8] and Michalopoulos and DeFrances[10]). 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.[11,12] 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.[13-16] Early development and subsequent differentiation of the liver involves other members of the forkhead transcription factor family
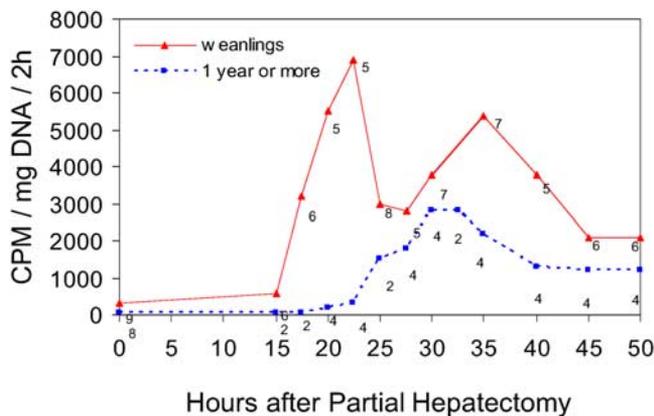
Fig. 1. 3H-thymidine uptake in young and old rats following partial hepatectomy. Modified and reprinted with permission from Bucher et al.[3]

(Foxa1, a2, and a3) and Foxm1b is present in virtually all embryonic tissues.[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[15] and tissue-specific knockout mice lacking Foxm1b in hepatocytes.[16] Wang et al. noted that mice lacking Foxm1b in the liver have a greatly reduced proliferative response to PH.[16] They further showed that over-expression of the human FoxM1B through adenoviral delivery to 12-month-old wild-type mouse liver reinstated the normal response to surgery.[19] FoxM1B over-expression 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,[20] skp2, and cyclin E,[21] and links Foxm1b expression closely to the progression of cell cycle. FoxM1B is able to increase the transcription of the cyclin B[22,23] and cyclin D[23] promoters in cotransfection assays, and thus impacts entry into both the S and G$_2$/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[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.[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[5] carries these important observations further and shows that growth hormone (GH) regulation of hepatocyte proliferation requires Foxm1b.[5] Growth hormone secretion diminishes with age. Moolten et al. showed that administration of GH to rats accelerated the response of hepatic DNA synthesis to PH,[24] but a mechanistic understanding was not forthcoming. Krupczak-Hollis et al.[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.
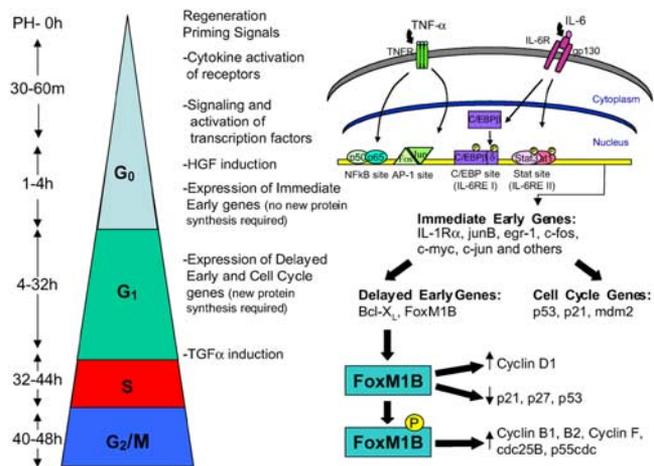

Fig. 2. Sequence of events in the regenerating liver after partial hepatectomy. Following two-thirds liver resection, the liver undergoes the "priming" phase, in which cytokines TNF-α and IL-6 activate their respective receptors in hepatocytes. This leads to the activation of several key transcription factors: NF-κB, AP-1, Stat 3, Stat 1, and C/EBP-β and -δ. These transcription factors induce the expression of immediate early genes. HGF is also expressed at this time and involved in the transition of quiescent hepatocytes into the G1 phase of the cell cycle. During the G1 phase, delayed early genes are expressed followed by induction of cell cycle–related genes, both of which require new protein synthesis for their production. Increased expression of FoxM1B and TGF-α occurs at the G1/S transition and is correlated with increased expression of cyclinD1 and decreased expression of cdk inhibitors. During the G2/M phase of the cell cycle, FoxM1B directly elevates cyclinB1, cyclinB2, and cdc25B expression. Additionally, FoxM1B is associated with increased cyclinF and p55cdc, which are involved in completion of the cell cycle following partial hepatectomy.

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.[25] 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[25]). 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 -δ nor 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 ($\sim$12 hours in mice).[25]

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.[15] 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.[26] Paradoxically, many studies in nonhuman organisms indicate that reduced levels of GH and IGF-1 correlate with increased longevity (see Bartke[27]). 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.[26,28] 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.[5] 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 un-
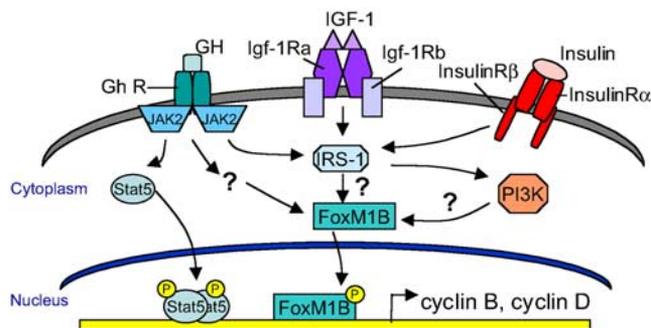

Fig. 3. Growth hormone, IGF-1, and insulin signal through their receptors and share a common downstream target in IRS-1. The regulation of Foxm1b protein might occur directly through IRS-1, through PI3 kinase, or through an entirely independent, as yet unknown, pathway.

wanted 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.[25]

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.

3. Bucher NLR, Swaffield MN, DiTroia J, F. The influence of age upon the incorporation of thymidine-2-C^{14} into the DNA of regenerating rat liver. Cancer Res 1964;24:509-512.

4. Stocker E, Heine W-D. Regeneration of liver parenchyma under normal and pathological conditions. Mol Cell Biol 1971;144:400-408.

5. Krupczak-Hollis K, Wang X, Dennewitz MB, Costa RH. Growth hormone stimulates proliferation of old-aged regenerating liver through Forkhead Box m1b. HEPATOLOGY 2003;38:1552-1562.

6. Fausto N. Liver regeneration. J Hepatol 2000;32:19-31.

7. 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.

8. Taub R. Liver regeneration 4: transcriptional control of liver regeneration. FASEB J 1996;10:413-427.

9. Greenbaum LE, Li W, Cressman DE, Peng Y, Ciliberto G, Poli V, Taub R. CCAAT enhancer-binding protein beta is required for normal hepatocyte proliferation in mice after partial hepatectomy. J Clin Invest 1998;102:996-1007.

10. Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997;276:60-66.

11. Su AI, Guidotti LG, Pezacki JP, Chisari FV, Schultz PG. Gene expression during the priming phase of liver regeneration after partial hepatectomy in mice. Proc Natl Acad Sci U S A 2002;99:11181-11186.

12. Kelley-Loughnane N, Sabla GE, Ley-Ebert C, Aronow BJ, Bezerra JA. Independent and overlapping transcriptional activation during liver development and regeneration in mice. HEPATOLOGY 2002;35:525-534.

13. Korver W, Roose J, Clevers H. The winged-helix transcription factor Trident is expressed in cycling cells. Nucleic Acids Res 1997;25:1715-1719.

14. Ye H, Kelly TF, Samadani U, Lim L, Rubio S, Overdier DG, Roebuck KA, et al. Hepatocyte nuclear factor 3/fork head homolog 11 is expressed in proliferating epithelial and mesenchymal cells of embryonic and adult tissues. Mol Cell Biol 1997;17:1626-1641.

15. Ye H, Holterman AX, Yoo KW, Franks RR, Costa RH. Premature expression of the winged helix transcription factor HFH-11B in regenerating mouse liver accelerates hepatocyte entry into S phase. Mol Cell Biol 1999;19:8570-8580.

16. Wang X, Kiyokawa H, Dennewitz M, Costa R. The Forkhead Box m1b transcription factor is essential for hepatocyte DNA replication and mitosis during mouse liver regeneration. Proc Natl Acad Sci U S A 2002;99:16881-16886.

17. Kaestner KH, Hiemisch H, Luckow B, Schutz G. The HNF-3 gene family of transcription factors in mice: gene structure, cDNA sequence, and mRNA distribution. Genomics 1994;20:377-385.

18. Kaestner KH, Katz J, Liu Y, Drucker DJ, Schutz G. Inactivation of the winged helix transcription factor HNF3alpha affects glucose homeostasis and islet glucagon gene expression in vivo. Genes Dev 1999;13:495-504.

19. Wang X, Krupczak-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 Cdc25B expression. J Biol Chem 2002;277:44310-44316.

20. 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.

21. Nelsen CJ, Hansen LK, Rickheim DG, Chen C, Stanley MW, Krek W, Albrecht JH. Induction of hepatocyte proliferation and liver hyperplasia by the targeted expression of cyclin E and skp2. Oncogene 2001;20:1825-1831.

22. Leung TW, Lin SS, Tsang AC, Tong CS, Ching JC, Leung WY, Gimlich R, et al. Over-expression of FoxM1 stimulates cyclin B1 expression. FEBS Lett 2001;507:59-66.

23. Wang X, Quail E, Hung NJ, Tan Y, Ye H, Costa RH. Increased levels of forkhead box M1B transcription factor in transgenic mouse hepatocytes prevent age-related proliferation defects in regenerating liver. Proc Natl Acad Sci U S A 2001;98:11468-11473.

24. Moolten FL, Oakman NJ, Bucher NL. Accelerated response of hepatic DNA synthesis to partial hepatectomy in rats pretreated with growth hormone or surgical stress. Cancer Res 1970;30:2353-2357.

25. Columbano A, Ledda-Columbano GM. Mitogenesis by ligands of nuclear receptors: an attractive model for the study of the molecular mechanisms implicated in liver growth. Cell Death Differ 2003;10 Suppl 1:S19-21.

26. Harman SM, Blackman MR. The effects of growth hormone and sex steroid on lean body mass, fat mass, muscle strength, cardiovascular endurance and adverse events in healthy elderly women and men. Horm Res 2003;60:121-124.

27. Bartke A. Is growth hormone deficiency a beneficial adaptation to aging? Evidence from experimental animals. Trends Endocrinol Metab 2003;14:340-344.

28. Blackman M, Sorkin J, Munzer T, Bellantoni M, Busby-Whitehead J, Stevens T, Jayme J, et al. Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. JAMA 2002;288:2282-2292.