Published in final edited form as:

Gastroenterology. 2005 February ; 128(2): 503–506.

Liver Regeneration, Growth Factors, and Amphiregulin

GEORGE K. MICHALOPOULOS and **ZAHIDA KHAN**

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

The sequence of events triggering liver regeneration after acute loss of hepatic mass has been the subject of much investigation during the past 20 years.¹ Rapid changes in gene expression and activation of receptors and transcription factors occur immediately after partial hepatectomy (PHx).^{2,3} Several potential signaling stimuli are released in the liver or in circulation after the loss of hepatic parenchyma. These stimuli have effects on liver and on hepatocytes (or other hepatic cells) in culture. Such stimuli include cytokines (eg, tumor necrosis factor α , interleukin-6, norepinephrine, transforming growth factor [TGF]- β 1) and several growth factors. Cytokines are not direct mitogens for hepatocytes in culture and, after removal of their effects (eg, in mouse genetic models or by pharmacologic agents), liver regeneration is retarded or decreased, but hepatic mass is eventually restored nonetheless. Although this may suggest that the role of cytokines is not critical or essential, this should not imply that the events they mediate are not important or that they do not actually occur. The preponderance of evidence suggests that the cytokine effects facilitate optimal timing and orchestration of the early signaling events after PHx, summarized under the term priming of hepatocytes.⁴

The effects of growth factors are mediated through their receptors. Two receptor-ligand and growth factor signaling systems appear to be mainly involved in liver regeneration: hepatocyte growth factor and its receptor (Met) and the epidermal growth factor receptor (EGFR) and its relatively large family of ligands and coreceptors. The receptor for EGFR was the first one that was shown to play a role in liver regeneration. A seminal publication by Earp et al⁵ showed that EGFR was phosphorylated and downregulated after PHx, suggesting binding of ligands and endocytosis.

The signaling scenario related to EGFR family members and its ligands is quite complex. The EGFR itself (also known as ErbB or HER) is a member of a family of four. The other members are ErbB-2 (HER-2, NEU), ErbB-3 (HER-3), and ErbB-4 (HER-4).⁶ Of these, HER-4 is expressed in only a limited number of tissues and it does not appear to be expressed in adult or embryonic liver. There are many ligands for EGFR, including epidermal growth factor (EGF), TGF- α , amphiregulin, heparin-binding EGF (HB-EGF), crypto, epiregulin, and betacellulin.

All of these considerations are relevant in putting in context the significant findings presented by Berasain et al⁷ in this issue of *Gastroenterology*. The authors found that amphiregulin, a ligand for EGFR, is expressed early (within 30 minutes) during liver regeneration after PHx. In addition, they also found that liver regeneration is substantially decreased and retarded in mice with homozygous deletion of amphiregulin. The findings suggest a unique role for amphiregulin that cannot be substituted by the other ligands for EGFR. The time course of expression of amphiregulin corresponds well with the pattern of tyrosine phosphorylation of

the EGFR, which previous work has shown is dramatically enhanced at 60 minutes after PHx.²

Previous studies have shown that deletion of other EGFR ligands does not have much impact on liver regeneration or liver development. The first one to be investigated in that regard was TGF- α . Similar to amphiregulin, TGF- α expression also rises soon after PHx.⁸ TGF- α is a strong mitogen for hepatocytes in culture, and its production by hepatocytes (which also express EGFR) suggests that TGF- α drives liver regeneration via an autocrine loop. Careful measurement of the TGF- α protein levels, however, showed that the increase in TGF- α protein levels was rather modest.⁹ It is not clear whether TGF- α produced by hepatocytes during liver regeneration stimulates proliferation (or other functions) on hepatocytes or whether it provides paracrine mitogenic stimuli for adjacent cells expressing EGFR (stellate cells, endothelial cells, or bile duct epithelial cells). Surprisingly, mice with homozygous deletion of TGF- α have essentially normal liver regeneration and liver embryonic development.¹⁰ These findings were interpreted to be caused by the complementary action of the other EGFR ligands. In that scheme, however, it would be expected that removal of any other EGFR ligands would have no effect because stimulation of EGFR could be equally accomplished by the other members of the EGFR ligand family. The findings presented in the article by Berasain et al⁷ suggest that this is not the case. EGFR ligands are not equally interchangeable because removal of amphiregulin seriously affects liver regeneration whereas removal of TGF- α has no effect.

Removal of EGF has not been attempted by genetic mechanisms. Low levels of EGF are seen in male mice after removal of the salivary glands. Sialadenectomized mice have lower levels of EGF in the plasma and they also have deficient liver regeneration,¹¹ although the decrease in regeneration did not appear to be as intense as described in the article by Berasain et al.⁷ Deficient liver regeneration was also recently described in mice with homozygous deletions of another EGFR ligand, HB-EGF.¹² Liver regeneration was moderately decreased. Decreased DNA synthesis in the HB-EGF- deficient mice was observed at the early time points (30 and 36 hours) after PHx but it increased at subsequent times, becoming more than that seen in the wild-type animals.

The above findings clearly show that the EGFR ligands, despite the fact that they share the same receptor, have differing effects, not only during liver regeneration but also in most other biologic processes. To understand the basis for this phenomenon, one needs to dissect the complexity of the signaling of the ErbB receptor family members. ErbB signaling has evolved into a highly complex network of 4 closely related receptors and at least 10 different ligands. This diversification allows for a multilayered network of regulation for ErbB signal input, processing, and output. Although all EGFR ligands can induce ErbB signaling, their expression patterns differ and their effector functions are nonoverlapping, ranging from cell motility and proliferation to growth inhibition. Most EGF family members are synthesized as transmembrane precursors, and these may have juxtacrine interactions aside from their soluble counterparts.¹³ In addition, HB-EGF, amphiregulin, and the neuregulins all contain heparin-binding domains that allow retention by extra-cellular matrix components.

In trying to understand the selective effects removing some of the EGFR ligands, we need to focus on the intricate pathways of interaction among EGFR, the other members of the Erb family, and the 10 ligands involved in the process. The dimerization of ErbB receptors is an intricate process that defines their signaling profile. ErbB proteins can form homodimers or heterodimers, with the latter being more stable. There is a distinct hierarchy that prefers ErbB-2 in heterodimer formation, and this is influenced by the structure of the ligand itself. Crucial to understanding the findings of the work by Berasain et al⁷ is the fact that different ligands exhibit distinct abilities to recruit and direct specific ErbB partners, and this ligand affinity ultimately influences signal strength and duration. Of the 10 possible dimer combinations, activated

ErbB-2 heterodimers show the highest affinity ligand binding and a greater potency. ErbB-2 also exhibits a constitutively high basal kinase activity, and it is most closely linked to human cancer. ErbB2 is the major binding partner for EGFR.¹⁴ EGFR can be activated by EGF, TGF- α , HB-EGF, amphiregulin, betacellulin (BTC), and epiregulin. Unlike the other ErbB proteins, EGFR is coupled to the phospholipase C γ pathway. All ErbB receptors are coupled either directly or indirectly to the Ras-and Shc-activated mitogen-activated protein kinase pathways. ErbB-2 is a ligandless receptor because no direct ligand can induce ErbB-2 homodimers.¹⁵ Homodimers of ErbB-2 or ErbB-3 cannot activate ErbB signaling. The signal processing and output layers for ErbB receptors are a finely tuned network that involves receptor trafficking, as well as hidden multiple downstream cascades of enzymes, adaptor proteins, second messengers, and transcription factors. It should be noted that ErbB proteins, like other receptors, are synthesized, recycled, and shuttled to specific cellular locations even in the absence of any activating ligands. Once activated, the ErbB receptor-ligand complex is endocytosed in clathrin-coated pits. As a specific example, activated EGFR-ErbB-2 heterodimers are very potent for a number of reasons. ErbB-2-containing complexes are more stable at the cell surface.¹⁶ By decreasing the rate of ligand dissociation from the cognate receptor EGFR, ErbB-2 heterodimers remain at the cell surface longer and undergo a slower rate of endocytosis when compared with EGFR homodimers. Furthermore, once in early endosomes, internalized EGFR-ErbB-2 heterodimers are selected for recycling, whereas EGFR homodimers are continuously degraded.¹⁷ The preferential recycling of EGFR-ErbB-2 heterodimers allows a rapid return of this potent heterodimer to the cell of surface for another round of activation. ErbB-2 is also unique in that it is the only ErbB that is a substrate for the Hsp90 chaperone, which acts to further promote ErbB-2 folding and trafficking.¹⁸ Thus, even though ErbB-2 may be thought of as a ligandless receptor, its emerging role as a master regulator of ErbB signaling is quite extensive. The complexity of the pathways involved in ErbB receptor dimerization, ligand binding, endocytosis, and so on, create ample room for several alternative outcomes after ligand-receptor interactions. The protein structures formed from allosteric interactions between the ErbB receptors and their ligands determine the outcome of all further processing of the ligand-receptor complex and the signaling associated with it.

In view of all the complex effects of EGFR and its ligands in liver regeneration, it is surprising that there are no apparent effects on liver embryonic development in mice with genetic deletion of EGFR. Several studies using different strains of mice have reported an array of findings that appear to be specific to the mouse strains. These effects range from embryonic lethality caused by placental abnormalities or neonatal death caused by pulmonary defects or lack of lethality during either embryonic or neonatal stage of life.¹⁹ All strains of mice, however, eventually present with abnormalities in the central nervous system, characterized by neurodegeneration seen in frontal cortex, olfactory bulb, and thalamus, with massive neuronal apoptosis and enhanced expression of c-fos.²⁰ Hepatic abnormalities have not been described in any of the strains. It is possible (but not very likely) that the lack of EGFR is compensated by actions of the other members of the receptor family, specifically ErbB-2 and ErbB-3, because ErbB-4 is not expressed in liver.²⁰ The alternative interpretation is that, although undoubtedly EGFR contributes signaling of some importance to liver during embryonic development, the impact is not critically vital. Equally unimpressive in terms of liver embryonic development are the effects of deletion of any one of the ligands of EGFR examined so far (TGF- α , HB-EGF, amphiregulin). Mice are born with apparently normal overall anatomy and histology and no abnormalities are seen in liver (or most other tissues). Although one could argue that removal of any one of the EGFR ligands is compensated by the presence of the others, the findings by Berasain et al⁷ strongly suggests that EGFR ligand-induced signaling is not interchangeable from one ligand to the other. The combination of the findings by Berasain et al⁷ and those from the genetic deletion studies of EGFR ligands suggest that the functions of the EGFR ligand

system are just not critically important for liver embryonic development as they appear to be for liver regeneration.

Overall, the findings of the accumulated literature suggest that the pathways of stimulation of hepatocyte proliferation are quite complex, but EGFR and Met are playing very important roles. Other signaling pathways such as Notch/Jagged²¹ and c-kit,²² norepinephrine and the α -1 receptor²³ are also involved. The integration of all these pathways into an easy scheme remains a challenge. In this complex signaling environment, the findings of Berasain et al⁷ clearly show that amphiregulin appears to play a specific and unique role.

References

1. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;276:60–66. [PubMed: 9082986]
2. Stolz DB, Mars WM, Petersen BE, Kim TH, Michalopoulos GK. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. *Cancer Res* 1999;59:3954–3960. [PubMed: 10463591]
3. Fausto N. Liver regeneration. *J Hepatol* 2000;32:19–31. [PubMed: 10728791]
4. Webber EM, Bruix J, Pierce RH, Fausto N. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology* 1998;28:1226–1234. [PubMed: 9794905]
5. Rubin RA, O'Keefe EJ, Earp HS. Alteration of epidermal growth factor-dependent phosphorylation during rat liver regeneration. *Proc Natl Acad Sci U S A* 1982;79:776–780. [PubMed: 6174980]
6. Earp HS, Dawson TL, Li X, Yu H. Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer research. *Breast Cancer Res Treat* 1995;35:115–132. [PubMed: 7612898]
7. Berasain C, García-Trevijano ER, Castillo J, Erroba E, Lee DC, Prieto J, Avila MA. Amphiregulin: an early trigger for liver regeneration in mice. *Gastroenterology* 2005;128:424–432. [PubMed: 15685553]
8. Webber EM, FitzGerald MJ, Brown PI, Bartlett MH, Fausto N. Transforming growth factor- α expression during liver regeneration after partial hepatectomy and toxic injury, and potential interactions between transforming growth factor- α and hepatocyte growth factor. *Hepatology* 1993;18:1422–1431. [PubMed: 8244268]
9. Russell WE, Dempsey PJ, Sitaric S, Peck AJ, Coffey RJ Jr. Transforming growth factor- α (TGF α) concentrations increase in regenerating rat liver: evidence for a delayed accumulation of mature TGF α . *Endocrinology* 1993;133:1731–1738. [PubMed: 8404616]
10. Russell WE, Kaufmann WK, Sitaric S, Luetke NC, Lee DC. Liver regeneration and hepatocarcinogenesis in transforming growth factor- α -targeted mice. *Mol Carcinog* 1996;15:183–189. [PubMed: 8597531]
11. Jones DE Jr, Tran-Patterson R, Cui DM, Davin D, Estell KP, Miller DM. Epidermal growth factor secreted from the salivary gland is necessary for liver regeneration. *Am J Physiol* 1995;268:G872–878. [PubMed: 7762671]
12. Mitchell C, Nivison M, Jackson LF, Fox R, Lee DC, Campbell JS, Fausto N. HB-EGF links hepatocyte priming with cell cycle progression during liver regeneration. *J Biol Chem*. 2004(e-pub ahead of print).
13. Wong ST, Winchell LF, McCune BK, Earp HS, Teixido J, Massague J, Herman B, Lee DC. The TGF- α precursor expressed on the cell surface binds to the EGF receptor on adjacent cells, leading to signal transduction. *Cell* 1989;56:495–506. [PubMed: 2464440]
14. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunakaran D, Lavi S, Ratzkin BJ, Yarden Y. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol* 1996;16:5276–5287. [PubMed: 8816440]
15. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, Yarden Y. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc Natl Acad Sci U S A* 1999;96:4995–5000. [PubMed: 10220407]

16. Lenferink AE, Pinkas-Kramarski R, van de Poll ML, van Vugt MJ, Klapper LN, Tzahar E, Waterman H, Sela M, van Zoelen EJ, Yarden Y. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. *Embo J* 1998;17:3385–3397. [PubMed: 9628875]
17. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 1998;12:3663–3674. [PubMed: 9851973]
18. Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol Med* 2002;8 (suppl):S55–S61. [PubMed: 11927289]
19. Sibilia M, Steinbach JP, Stingl L, Aguzzi A, Wagner EF. A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor. *EMBO J* 1998;17:719–731. [PubMed: 9450997]
20. Carver RS, Stevenson MC, Scheving LA, Russell WE. Diverse expression of ErbB receptor proteins during rat liver development and regeneration. *Gastroenterology* 2002;123:2017–2027. [PubMed: 12454858]
21. 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. [PubMed: 15057910]
22. Ren X, Hogaboam C, Carpenter A, Colletti L. Stem cell factor restores hepatocyte proliferation in IL-6 knockout mice following 70% hepatectomy. *J Clin Invest* 2003;112:1407–1418. [PubMed: 14597766]
23. Cruise JL, Knechtle SJ, Bollinger RR, Kuhn C, Michalopoulos G. Alpha 1-adrenergic effects and liver regeneration. *Hepatology* 1987;7:1189–1194. [PubMed: 2824312]