HHS Public Access

Author manuscript

Hepatology. Author manuscript; available in PMC 2015 December 07.

Published in final edited form as:

Hepatology. 2012 October ; 56(4): 1577–1579. doi:10.1002/hep.25911.

The Role of Paracrine Signals During Liver Regeneration

Ben Z. Stanger, M.D., Ph.D.[1] and **Linda Greenbaum, M.D.**[2]

[1]Departments of Medicine and Cell and Developmental Biology Perelman School of Medicine University of Pennsylvania, Philadelphia, PA

[2]Departments of Cancer Biology and Medicine Thomas Jefferson University School of Medicine Philadelphia, PA

Abstract

During chronic injury a population of bipotent hepatic progenitor cells (HPCs) become activated to regenerate both cholangiocytes and hepatocytes. Here we show in human diseased liver and mouse models of the ductular reaction that Notch and Wnt signaling direct specification of HPCs via their interactions with activated myofibroblasts or macrophages. In particular, we found that during biliary regeneration, expression of Jagged 1 (a Notch ligand) by myofibroblasts promoted Notch signaling in HPCs and thus their biliary specification to cholangiocytes. Alternatively, during hepatocyte regeneration, macrophage engulfment of hepatocyte debris induced Wnt3a expression. This resulted in canonical Wnt signaling in nearby HPCs, thus maintaining expression of Numb (a cell fate determinant) within these cells and the promotion of their specification to hepatocytes. By these two pathways adult parenchymal regeneration during acute liver injury is promoted.

Comment

Adult organs have robust mechanisms for maintaining themselves during the decades in which they must function following embryonic development. In tissues with high rates of turnover—particularly the skin, intestine, and blood—stem cells provide the raw materials for organ homeostasis, whereas tissues with low rates of turnover such as the pancreas use replication as the prevailing mechanism for maintenance. The situation is somewhat more complex during regeneration, in which both replication and stem cell differentiation can contribute to repair. In the regenerating liver, the picture is particularly murky, as the primary mode of recovery is thought to be determined by the mechanism of injury. When a portion of the liver is removed surgically, for example, the liver regrows to its initial size through a process that is dominated by cell growth and division. Following the more physiologically relevant injury caused by toxin exposure, by contrast, a population of small cells emerges in the portal regions. Classically referred to as “oval cells” or “atypical ductal cells” (ADCs), these cholangiocyte-like cells have been proposed to act as “facultative” progenitors, mediating liver regeneration through a process that recapitulates differentiation of embryonic progenitors.[1–4]

Potential conflict of interest: Nothing to report.

During fetal development, hepatocytes and cholangiocytes (henceforth referred to as biliary epithelial cells, or BECs) are derived from a *bona fide* progenitor cell, the hepatoblast. Several signals influence the binary cell fate decision made by these progenitors. Specifically, signals from the Notch, Wnt, TGFβ, FGF, and Hippo signaling pathways all act to promote biliary differentiation at the expense of hepatocyte differentiation (reviewed[5]). Notch provides one of the most important signals for biliary differentiation, as both humans and mice with defects in hepatic Notch signaling exhibit bile duct paucity.[6–12] During development, Notch receptors (predominantly Notch2) are activated by the Jagged1 ligand, which is produced by cells in the portal vein mesenchyme.[13] Although some lineage-tracing and transplantation studies support the notion that ADCs act as true hepatic progenitor cells (HPCs),[14–18] other work suggests that replication of existing cells is the dominant mechanism for tissue regeneration even in the setting of toxin-induced injury.[19]

Why the liver might utilize two different methods for regeneration has been a longstanding question in the field. Even if ADCs do not function formally as liver-repopulating progenitor cells, their habitual appearance following a wide range of hepatic injuries suggests that they play an important role in liver regeneration, and thus the mechanism by which they emerge during liver damage is of great importance. Against this backdrop, Boulter et al. have undertaken a series of experiments aimed at understanding the nature of the cell populations that arise following toxin-mediated injury and the paracrine signals that influence their behavior. Using a combination of expression studies, macrophage depletion, and *ex vivo* coculture, the authors propose a model whereby the balance between Notch and Wnt signaling in ADCs determines the proper ratio of BECs and hepatocytes during liver regeneration. They report their findings in the March issue of *Nature Medicine*.[20]

The authors begin their studies with a detailed immunohistochemical analysis and 3D reconstruction to characterize what they refer to as the hepatic progenitor cell “niche”—the population of nonparenchymal cells that arise alongside ADCs during liver injury. Using two different models: a murine choline deficient ethionine supplemented (CDE) model, which is thought to cause predominantly hepatocellular injury, and a DDC diet model, which is thought to cause predominantly biliary injury, the authors find two distinct patterns of infiltrating cells adjacent to the ADCs. Following hepatocyte injury, Kupffer cells were found in close proximity to the ADCs, whereas following biliary injury, ADCs were associated with portal fibroblasts and thick bands of collagen. Based on this difference in relative proximity, Boulter et al. hypothesized that these two cell populations (Kupffer cells and portal fibroblasts) might influence ADC behavior differently.

As portal fibroblasts express high levels of the Notch ligand Jagged1, Boulter et al. treated isolated ADCs with the γ-secretase inhibitor DAPT, which inhibits the Notch pathway. They observed a decrease in the expression of biliary markers, consistent with the known role of Notch signaling in biliary fate and identity. Furthermore, treatment of animals with DAPT *in vivo* led to a decrease in the number of ADCs. Interestingly, expression of the hepatocyte marker HNF4α was not increased by DAPT treatment, indicating that pharmacological inhibition of Notch was not sufficient to direct the ADCs to differentiate to the hepatocyte lineage.

The authors observed that a number of Wnt pathway target genes, including Numb, were activated in the ADCs in both patient and murine hepatocellular injury models. Hence, they investigated whether Numb, which inhibits Notch signaling by facilitating proteasome-mediated degradation of the Notch receptor, might induce ADCs to differentiate into hepatocytes. To test their hypothesis *in vivo*, they activated canonical Wnt signaling in ADCs by expressing a constitutively active form of β-catenin in these cells, an experiment that resulted in an increased number of hepatocytes exhibiting nuclear β-catenin in staining. Importantly, although the authors interpreted this finding as evidence that β-catenin activation directs ADCs to differentiate to the hepatocyte lineage, the absence of formal lineage tracing precludes such a conclusion.

Finally, Boulter et al. turned their attention to the cells that might be providing activating signals for these pathways. Having already concluded that Notch signals are derived from myofibroblasts, they sought to identify Wnt-producing cells in the injured livers and focused their attention on Kupffer cells. Several complementary lines of evidence indicated that these cells serve as a major source of Wnt ligand, including localization of Wnt-expressing macrophages adjacent to the ADCs and a demonstration that phagocytosis of hepatocellular debris by macrophages directly induces Wnt expression and paracrine activation of biliary markers in coculture experiments. Most convincingly, ablation of hepatic macrophages *in vivo* using liposomal clodronate (in the CDE model) caused an increase in ductular structures.

If one accepts the idea that ADCs function as progenitor cells, giving rise to both hepatocytes and BECs following toxin-mediated injury, then the study of Boulter et al. provides an interesting paradigm whereby the balance of Notch and Wnt signals (provided by myofibroblasts and macrophages, respectively) influences that cell fate decision. Given the controversial state of this proposition, however, their results need to be interpreted with great caution. The study does not employ lineage tracing, which might have more convincingly demonstrated their claims of shifts in lineage allocation, and much of the work relies on *in vitro* culture, where the lineage relationships and differentiation signals that exist *in vivo* can be overridden. Moreover, their model is at odds with observations from human liver disease, as patients often present with evidence of both hepatocellular injury and concomitant ductular cell expansion without evidence of significant portal fibroblast activation.

The two most intriguing pieces of data provided by Boulter et al. are the *in vivo* findings following treatment with the γ-secretase inhibitor DAPT and macrophage ablation with clodronate. The observation that DAPT treatment abrogates the ADC response is consistent with the notion that Notch signaling is necessary for the differentiation of a presumptive progenitor cell, but it is also consistent with the possibility that Notch signaling (or another γ-secretase-dependent signal) is important for the expansion of preexisting BECs that give rise to ADCs. In either case, this finding has clear functional significance, and the identification of portal myofibroblasts as the likely source of Notch ligand during the process is a good starting point for future mechanistic studies. Likewise, the observation that macrophage ablation during liver injury changes the balance of ADCs during regeneration

supports a previously underappreciated role for these cells (and potentially Wnt signaling) in liver regeneration following toxin-mediated injury.

References

1. Desmet VJ. Intrahepatic bile ducts under the lens. J Hepatol. 1985; 1:545–559. [PubMed: 4056355]
2. Factor VM, Radaeva SA, Thorgeirsson SS. Origin and fate of oval cells in dipin-induced hepatocarcinogenesis in the mouse. Am J Pathol. 1994; 145:409–422. [PubMed: 8053498]
3. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. Science. 2008; 322:1490–1494. [PubMed: 19056973]
4. Yanger K, Stanger B. Facultative stem cells: fact and fancy. Dev Dyn. 2011; 240:521–529. [PubMed: 21312313]
5. Zong Y, Stanger BZ. Molecular mechanisms of liver and bile duct development. WIREs Dev Biol. 201210.1001/wdev.47
6. Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet. 1997; 16:243–251. [PubMed: 9207788]
7. Oda T, Elkahloun AG, Pike BL, Okajima K, Krantz ID, Genin A, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet. 1997; 16:235–242. [PubMed: 9207787]
8. McCright B, Lozier J, Gridley T. A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. Development. 2002; 129:1075–1082. [PubMed: 11861489]
9. Kodama Y, Hijikata M, Kageyama R, Shimotohno K, Chiba T. The role of notch signaling in the development of intrahepatic bile ducts. Gastroenterology. 2004; 127:1775–1786. [PubMed: 15578515]
10. Lozier J, McCright B, Gridley T. Notch signaling regulates bile duct morphogenesis in mice. PLoS One. 2008; 3:e1851. [PubMed: 18365007]
11. Geisler F, Nagl F, Mazur PK, Lee M, Zimber-Strobl U, Strobl LJ, et al. Liver-specific inactivation of Notch2, but not Notch1, compromises intrahepatic bile duct development in mice. Hepatology. 2008; 48:607–616. [PubMed: 18666240]
12. Zong Y, Panikkar A, Xu J, Antoniou A, Raynaud P, Lemaigre F, et al. Notch signaling controls liver development by regulating biliary differentiation. Development. 2009; 136:1727–1739. [PubMed: 19369401]
13. Hofmann JJ, Zovein AC, Koh H, Radtke F, Weinmaster G, Iruela-Arispe ML. Jagged1 in the portal vein mesenchyme regulates intrahepatic bile duct development: insights into Alagille syndrome. Development. 2010; 137:4061–4072. [PubMed: 21062863]
14. Wang X, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Grompe M. The origin and liver repopulating capacity of murine oval cells. Proc Natl Acad Sci U S A. 2003; 100:11881–11888. [PubMed: 12902545]
15. Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, et al. Locating the stem cell niche and tracing hepatocyte lineages in human liver. Hepatology. 2009; 49:1655–1663. [PubMed: 19309719]
16. Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet. 2010; 43:34–41. [PubMed: 21113154]
17. Dorrell C, Erker L, Schug J, Kopp JL, Canaday PS, Fox AJ, et al. Prospective isolation of a bipotential clonogenic liver progenitor cell in adult mice. Genes Dev. 2011; 25:1193–1203. [PubMed: 21632826]
18. Shin S, Walton G, Aoki R, Brondell K, Schug J, Fox A, et al. Foxl1-Cre-marked adult hepatic progenitors have clonogenic and bilineage differentiation potential. Genes Dev. 2011; 25:1185–1192. [PubMed: 21632825]

19. Malato Y, Naqvi S, Schurmann N, Ng R, Wang B, Zape J, et al. Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. J Clin Invest. 2011; 121:4850–4860. [PubMed: 22105172]

20. Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nat Med. 2012; 18:572–579. [PubMed: 22388089]