The balance between Notch/Wnt signaling regulates progenitor cells' commitment during liver repair: Mystery solved?

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COMMENTARY ON:

Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, Ridgway RA, Seo SS, Spee B, Van Rooijen N, Sansom OJ, Iredale JP, Lowell S, Roskams T, Forbes SJ. Nat Med 2012 March 4;18(4):572–579. Copyright (2012). Abstract reprinted by permission from Macmillan Publishers Ltd.

http://www.ncbi.nlm.nih.gov/pubmed/22388089

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 chronic liver injury is promoted.*

In spite of the impressive regenerative potential of the liver as a result of the mitogenic capabilities of hepatocytes and cholangiocytes, liver repair often involves progenitor cells (HPC). This bipotent cell population, barely identifiable in normal livers, expands following liver injury. Depending on the type of damage including its acute or chronic nature, HPC may differentiate into mature cholangiocytes, hepatocytes or into a population of small cytokeratin 19-positive epithelial cells exhibiting cholangiocyte phenotypic markers that arrange themselves into tubeless structures. These "activated" (or "reactive") cholangiocytes express an array of inflammatory mediators as well as cytokines and their cognate receptors, and thus are able to orchestrate the functions of several cell types centered around the repair of the epithelial wound. Expansion of activated cholangiocytes is associated with persistent inflammation, mesenchymal cell activation, portal fibrosis and progression of liver disease [1–3].

While the mechanisms leading to the expansion of the HPC compartment are still unclear, a seminal paper recently published in the April issue of *Nature Medicine* addresses the mechanisms of HPC specification in chronic liver diseases [4]. This elegant work demonstrates that HPC specification differs according to which liver epithelial compartment (hepatocellular or biliary) is predominantly damaged and the consequent changes within the progenitor cell niche. It proposes the intriguing hypothesis that the switch is represented by two alternatively acting developmental mechanisms, canonical Wnt/β-catenin signaling or Notch signaling (Fig. 1).

Boulter *et al.* show that hepatocellular specification of HPC is determined by the release of Wnt3a by macrophages after phagocytosis of cellular debris from damaged hepatocytes. Wnt3a then acts on HPC to stimulate the nuclear translocation of β-catenin and its signaling. Wnt/β-catenin/T-cell-specific transcription factor/lymphoid enhancer-binding factor-1 (TCF/LEF-1) signaling is known to participate in several steps of bile duct development. Earlier works by Hu *et al.* have shown that Wnt3a is able to stimulate the proliferative activity of HPC *in vitro*, and, different from Boulter *et al.*, activation of canonical Wnt/β-catenin signaling is evident in proliferating HPC in mice *in vivo* also following the administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), a model of ductular reaction induced by obstructive cholestasis derived from precipitation of protoporphyrins within the intrahepatic bile ducts [5]. It is important to note that HPC behavior appears to be strongly model- and time-dependent. The choline-deficient, ethionine-supplemented diet (CDE) is a
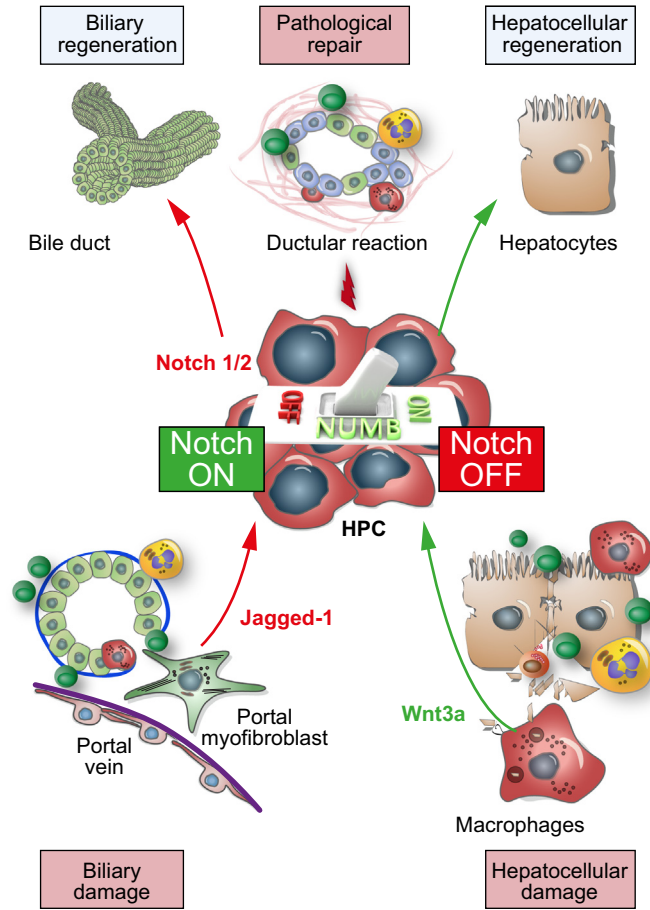
Fig. 1. Epithelial–stromal interactions behave as critical regulators of liver repair mechanisms in response to biliary or hepatocellular damage. By alternatively activating Notch and Wnt signaling, the stroma microenvironment orchestrates the divergent specification of HPC towards the biliary or hepatocellular lineage, respectively. In biliary regeneration, the interaction of HPC with myofibroblasts expressing Jagged1 results in the activation of Notch2 in HPC, leading to their biliary specification (red left-sided section). In contrast, during hepatocellular regeneration, HPC are activated by Wnt3a released by macrophages in response to phagocytosis of debris from apoptotic hepatocytes. Wnt3a then stimulates the nuclear translocation of β-catenin, which promotes HPC differentiation towards the hepatocellular lineage (green right-sided section). In this bimodal crosstalk between HPC and adjacent inflammatory stromal cells, Numb plays a key role as "switcher". HPC differentiation into mature cholangiocytes or hepatocytes leads to resolution. When these mechanisms fail, reactive cholangiocytes are activated in conjunction with persistent inflammation, mesenchymal cell activation, and portal fibrosis, ultimately leading to progression of liver disease (pathologic repair, center-sided section).

liver carcinogenetic diet that promotes the emergence of a large number of proliferating oval cells, expressing albumin and α-fetoprotein, with little parenchymal necrosis. As originally shown by Guest *et al.*, small ductules are formed by HPC after 10 weeks of CDE treatment [6]. Thus, it remains unclear whether the HPC (or oval cells) that accumulate during a CDE diet are en route to become hepatocytes or "activated" cholangiocytes or both.

A major novelty of the recent *Nature Medicine* paper is the role of Numb. Numb is a target of canonical Wnt signaling and its activation by Wnt3a has several consequences, including Notch receptor inhibition at the level of the intracellular domains [7], and inhibition of Hedgehog signaling [8], another major mechanism of biliary repair [2,3]. Boulter *et al.* present circumstantial evidence that specification of HPC is determined by a direct cell–cell interaction between Notch-expressing HPC and Jagged-1-expressing mesenchymal cells and hypothesize that this would be the default specification pathway for HPC in the absence of infiltrating macrophages and Wnt/β-catenin signaling [4]. Thus, macrophage-derived Wnt inhibits a default-activated Notch

signaling via Numb, allowing HPC to escape the biliary cell fate and acquire an hepatocellular specification. By alternatively activating Notch or Wnt/β-catenin signaling, myofibroblasts and macrophages orchestrate the divergent specification of HPC towards the biliary or hepatocellular lineage, respectively [4].

This seminal work certainly raises a number of open questions, worth being addressed in further studies. For example, the proposed role of macrophages in HPC specification towards the hepatocellular phenotype deserves further investigation. Paracrine factors secreted by macrophages have been previously shown to affect the HPC compartment. For example, the tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a member of the tumor necrosis factor (TNF)-α family and produced by natural killer cells and macrophages, induces a selective expansion of HPC in both DDC and CDE toxicity models [9,10]. In liver repair, the functions of macrophages strongly depend on their phenotype. Alternatively-activated rather than classically-activated macrophages are key drivers of abnormal liver reparative processes leading to excessive deposition of matrix [11]. There-

fore, phenotypic characterization of macrophages engaged in the crosstalk with the HPC compartment as well as the time frame of these interactions will provide additional insights into their role in HPC commitment.

Earlier work in Alagille syndrome (a ductopenic cholangiopathy caused by a genetic defect in Notch signaling) has shown that when Notch signaling is defective, hepatocyte nuclear factor (HNF)-1β, a transcription factor critical for biliary specification, is downregulated and HPC are forced towards the intermediate hepatocyte fate instead of the biliary fate [12]. Boulter *et al.* further suggest that Notch is a default inducer of biliary specification that can be activated by interaction with myofibroblasts. These conclusions rely on gene expression studies and on the use of Notch inhibitors that have several off-target effects. Thus, genetic models of Notch loss/gain of function will be needed to prove the relative role of Notch-1/Notch-2 in biliary specification during liver repair. As shown during development, the role of Notch may go well beyond HPC specification and involve branching tubular morphogenesis [13]. Effective biliary repair requires the formation of biliary tubules and regeneration of the branching architecture of the ductal system. If a proper branching structure is not regenerated, the final result will be parenchymal necrosis or vanishing bile duct syndrome and fibrosis, i.e., the final stage of several cholangiopathies. As Boulter's paper reminds us, liver repair is a complex mechanism that requires the concerted activities of several developmental mechanisms and interactions among multiple cell types. Understanding the role of Notch, Wnt and other morphogens in the regulation of biliary repair is a journey that has just begun, but solving this puzzle will generate significant benefits for liver patients.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgments

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