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

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COMMENTARY ON:


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

Keywords: Hepatic progenitor cells; WNT; Notch; Numb.
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 Jagged1-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].
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 cholangio-
pathy 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. fur-
ther 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 activ-
ities 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 gener-
ate 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

This work was supported by NIH DK079005, NIH Yale Liver Cen-
ter, P30 DK34989, PSC Partner seeking a cure, Cariplo 2011-0470
and PRIN 2009 ARXY4T_005 to MS, and by Telethon GGP 09189
and Ateneo CPD 113799/11 to LF.

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