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Notch signaling and new therapeutic options in liver disease

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Summary

Notch signaling is a crucial determinant of cell fate decision during development and disease in several organs. Notch effects are strictly dependent on the cellular context in which it is activated. In the liver, Notch signaling is involved in biliary tree development and tubulogenesis. Recent advances have shed light on Notch as a critical player in liver regeneration and repair, as well as in liver metabolism and inflammation and cancer. Notch signaling is finely regulated at several levels. The complexity of the pathway provides several possible targets for development of therapeutic agents able to inhibit Notch. Recent reports have shown that persistent activation of Notch signaling is associated with liver malignancies, particularly hepatocellular with stem cell features and cholangiocarcinoma. These novel findings suggest that interfering with the aberrant activation of the Notch pathway may have therapeutic relevance. However, further studies are needed to clarify the mechanisms regulating physiologic and pathologic Notch activation in the adult liver, to better understand the mechanistic role(s) of Notch in liver diseases and to develop safe and specific therapeutic agents.

Notch signaling is a developmental pathway that regulates several fundamental cellular processes including cell fate and differentiation. Four transmembrane Notch receptors (Notch-1, -2, -3, -4) and two types of ligands, Serrate/Jagged (Jag-1, -2) or Delta-like (Dll-1, -3, -4) constitute the Notch system, along with several other components that transduce and regulate the signal. Activation of Notch signaling requires a direct contact between cells expressing Notch ligands and cells expressing Notch receptors; often both the "transmitting" and the "receiving" cells are modified by their interaction. Initially cells express both Notch receptor and ligands, but as the interaction continues, one cell upregulates the ligands and down regulates the receptor, becoming a "transmitting cell", whereas the opposite holds true for the receiving cell [1]. Ligand-activated Notch receptors are cleaved by the γ-secretase complex, leading to the release of the Notch intracellular domain (NICD). NICD translocates into the nucleus where it, while binding the RBP-Jκ transcription factor, displaces the associated co-repressors and recruits associated co-activators (i.e., MAML1) [2–5]. The signal culminates with the expression of Notch target genes, such as the family of *Hes* and *Hey* related transcription factors. Regarding the liver, Notch partly controls also the expression of *Sox9* and *HNF1β*, key players in hepatic lineage commitment [6–8].

As expected from a signaling mechanism involved in organ morphogenesis, Notch is finely tuned in a tissue- and time-dependent fashion, and it is also controlled through post-translational modifications such as ubiquitination, glycosylation or endocytosis. Continuous Notch activation requires constant exposure to additional ligands, as NICD undergoes rapid proteasomal degradation [2–5]. Furthermore, the effects of Notch signaling depend upon the cell types involved and the presence of signals from other pathways, including Wnt and Hedgehog.

Studies based on rodent models of Notch loss or gain of function have demonstrated that Notch is involved in several stages of intrahepatic bile duct (IHBD) morphogenesis [9]. Jag-1-positive mesenchymal cells at the parenchymal/portal interface of the nascent portal space induce the expression of cholangiocytes-specific markers in adjacent hepatoblasts, committing them to the biliary lineage. Furthermore, by regulating Sox9 and HNF1β, Notch plays an essential role in the formation of the inner leaflet of the duplicating ductal plate and also in biliary tubule formation [6–8,10–16]. These data are consistent with the association of Alagille syndrome (AGS) (an autosomal dominant disorder characterized by ductopenia and cholestasis) with Jag-1 [17,18] (in some cases Notch-2 [19]) mutations. Beyond development, other important roles of Notch are emerging that significantly impact on liver physiology and diseases. As will be discussed below, several studies indicate that the Notch pathway plays a key role in maintaining liver tissue homeostasis in the post-natal life and is involved in the reparative reaction to biliary damage, as well as in liver carcinogenesis, metabolism and inflammatory responses. This review will focus on the involvement of Notch in liver repair and carcinogenesis and the possible therapeutic implications.

Keywords: Notch signaling; Liver repair; Liver cancer; Notch inhibitors.
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Abbreviations: Jag-1, Jagged-1; Dll, Delta-like; NICD, Notch intracellular domain; RBP-Jκ, recombination signal binding protein immunoglobulin kappa J; MAML1, mastermind-like 1; Hes1, hairy enhancer of split-1; Hey1, hairy enhancer of split-related with YRPW motif1; Sox9, sex determining region Y-box 9; HNF1β, hepatocyte nuclear factor 1β; IHBD, intrahepatic bile duct; AGS, alagille syndrome; GSI, γ-secretase inhibitor; mAbs, monoclonal antibodies; HPC, hepatic progenitor cell; K7, cytokeratin-7; HSC, hepatic stellate cell; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; CSC, cancer stem cell; T-ALL, T cell acute lymphoblastic leukaemia; K19, cytokeratin-19; N1ICD, Notch-1 intracellular domain; N2ICD, Notch-2 intracellular domain; CCl_4, carbon tetrachloride.

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Better understanding of the Notch pathway and of its relevance in pathophysiological processes prompted the development of a broad spectrum of molecules able to interfere with its signaling by (1) blocking the activation of Notch receptors (γ-secretase inhibitors or GSIs), (2) blocking the binding of the ligand (monoclonal antibodies [mAbs], decoys) or (3) blocking the transcriptional activity of NICD (blocking peptides). Some of these molecules are in a preclinical phase or in an advanced phase I clinical trial for cancer treatment (reviewed in [20,21]) (see Fig. 1 and Table 1).

Notch signaling and liver repair

In chronic liver diseases, liver repair requires the concerted action of epithelial, mesenchymal and inflammatory cells. Central to the cross talk between these cell types are hepatic progenitor cells (HPCs or reactive cholangiocytes). This cell population, nearly absent in normal livers, expands significantly following liver injury and expresses an array of inflammatory mediators, cytokines and receptors that help establish the cellular crosstalk needed for epithelial healing. Unfortunately, continuous expansion of this reactive cell population is associated with persistent inflammation, mesenchymal cell activation, and portal fibrosis [22–24], leading to the deposition of the fibro-vascular stroma that is ultimately responsible for the architectural distortion of progressive liver diseases.

Several liver morphogenetic pathways are reactivated in HPCs during liver repair; for example, Notch acts in concert with Wnt [25] or Hedgehog [26], to restore liver architecture and function. In AGS, paucity of bile ducts is associated with impaired biliary differentiation of HPCs, consistent with the hypothesis that Notch is a default inducer of biliary specification. With comparison to other cholestatic diseases, in AGS, HPCs are decreased, while intermediate cytokeratin 7 (K7)-positive hepatocytes accumulate, suggesting that HPCs are forced towards the hepatocellular fate, or that transdifferentiation of hepatocytes into HPCs is blocked [22]. Of note, HNF1β, a transcription factor critical for biliary specification, is down-regulated in the accumulating K7-positive intermediate hepatocytes. Conversely, a reciprocal relationship between Hes1 and the transcription factor PDX-1 has been described [27].

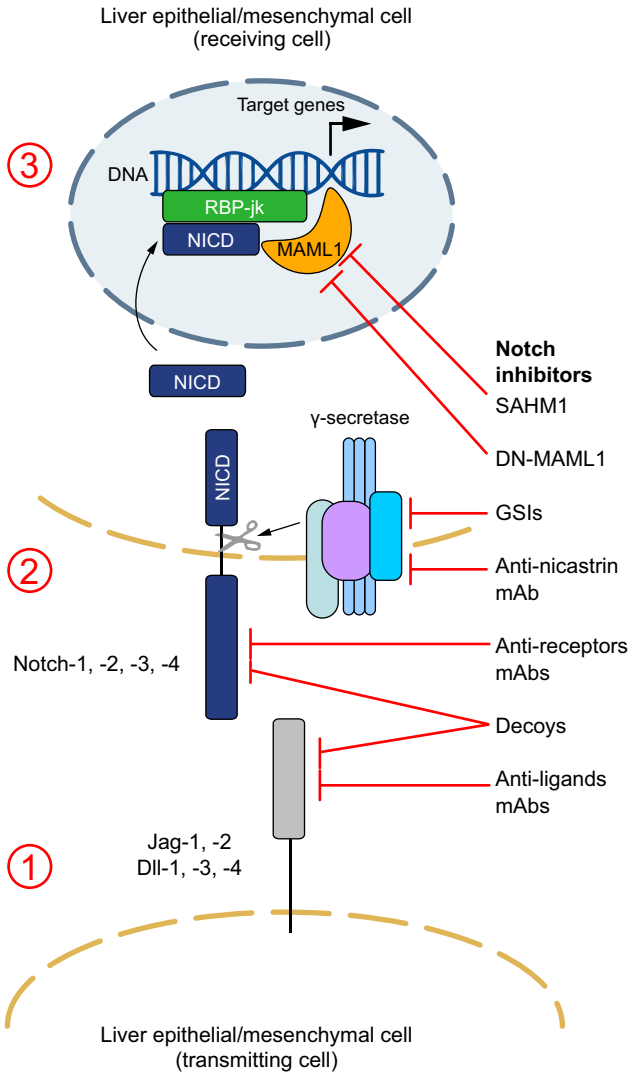

Fig. 1. Schematic representation of Notch signaling and potential inhibitory strategies. Notch pathway requires different steps to transmit the signal from the "transmitting cell" to the adjacent "receiving cell". (1) Ligand/receptor binding is the very first step that leads to signaling activation. Notch inhibition can be achieved by interfering with this step. Recent Notch neutralizing antibodies proved to be highly specific for the target isoforms of receptors/ligands. They target the Notch regulatory region (NRR) on the extracellular portion of the receptors and can selectively recognize Notch1 (NRR1 mAb [56,57]), Notch2 (NRR2 mAb [57]) or Notch3 (NRR3 mAb [58]) receptors. Other mAbs compete with endogenous ligand at the ligand-binding domain level [56]. Immunostrategies directed against ligands (i.e., mAbs recognizing Jag1, Dll4, Dll1) showed inhibited tumor growth and angiogenesis [59]. These antibodies are now in phase I trials investigation (OMP-59R5 [anti-Notch2-3] and OMP-21M18 [anti-Dll4]). The high specificity of mAbs decreases the toxicity that can derive from pan-Notch inhibition. mAbs can target the desired Notch molecule that is aberrantly upregulated, sparing the other isoforms. Soluble proteins mimicking Notch receptors or ligands but lacking the transmembrane portion necessary for signal activation can be used as Decoys to compete with endogenous Notch1 [60] Jag1 [61] and Dll1 [62]. (2) The next fundamental step relies upon γ-secretase dependent receptor-proteolysis. GSIs are the most investigated Notch-inhibiting compounds, since they have already been tested in clinical trials to treat Alzheimer's disease [63]. GSIs are potent non-selective Notch inhibitors that target the activating proteolysis of Notch intracellular domain operated by the γ-secretase enzyme and thus inhibit non-specifically all four Notch receptors isoforms. GSIs are being tested in phase I clinical trials for T-all leukaemia, breast cancer and other solid tumors, either alone or in combination with standard of care treatment. Although appealing, GSIs based therapy suffers from some drawbacks. GSI might have off target effects on other γ-secretase dependent pathways, and long term GSI treatment leads to intestinal toxicity as a result of combined Notch-1 and -2 inhibition. Therefore, alternative strategies have been designed, such as immunotherapy for the extracellular domain of nicastrin (i.e., one of the subunit of the γ-secretase complex). This antibody recognizes nicastrin in the active enzymatic complex, thus acting as pan-Notch inhibitor [64]. (3) Receptor cleavage allows the release of the NICD, which translocates to the nucleus where it binds the DNA-binding partner RBP-jk and recruits the co-activator MAML1, necessary for Notch target gene transcription. Also, cell permeable blocking peptides (dominant negative [DN]-MAML1, stapled peptide SAHM1) can be used to interfere with the formation of the nuclear complex NICD/CSL and inhibit the transcriptional activity of NICD. These newly designed molecules reviewed in [20] are promising but need further investigation. (mAbs, monoclonal antibodies; GSIs, γ-secretase inhibitors; NICD, notch intracellular domain).