

# Notch-driven carcinogenesis: The merging of hepatocellular cancer and cholangiocarcinoma into a common molecular liver cancer subtypes

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## COMMENTARY ON:

**Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice.** Villanueva A, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, Rodriguez-Carunchio L, Solé M, Thung S, Stanger BZ, Llovet JM. *Gastroenterology*. 2012 Dec;143(6):1660–1669.e7. Copyright© 2012. Abstract reprinted by permission from the AGA Institute.

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**Background & Aims:** The Notch signaling pathway is activated in leukemia and solid tumors (such as lung cancer), but little is known about its role in liver cancer.

**Methods:** The intracellular domain of Notch was conditionally expressed in hepatoblasts and their progeny (hepatocytes and cholangiocytes) in mice. This was achieved through Cre expression under the control of an albumin and  $\alpha$ -fetoprotein (AFP) enhancer and promoter (AFP-Notch intracellular domain [NICD]). We used comparative functional genomics to integrate transcriptome data from AFP-NICD mice and human hepatocellular carcinoma (HCC) samples ( $n = 683$ ). A Notch gene signature was generated using the nearest template prediction method.

**Results:** AFP-NICD mice developed HCC with 100% penetrance when they were 12 months old. Activation of Notch signaling correlated with activation of three promoters of insulin-like growth factor 2; these processes appeared to contribute to hepatocarcinogenesis. Comparative functional genomic analysis identified a signature of Notch activation in 30% of HCC samples from patients. These samples had altered expression in Notch pathway genes and activation of insulin-like growth factor signaling, despite a low frequency of mutations in regions of NOTCH1 associated with cancer. Blocking Notch signaling in liver cancer cells with the Notch activation signature using  $\gamma$ -secretase inhibitors or by expressing a dominant negative form of mastermind-like 1 reduced their proliferation *in vitro*.

**Conclusions:** Notch signaling is activated in human HCC samples and promotes formation of liver tumors in mice. The Notch signature is a biomarker of response to Notch inhibition *in vitro*.

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The Notch signaling cascade is a highly evolutionary conserved pathway controlling cell differentiation, proliferation, apoptosis, and ultimately cell fate. It is one of the few main hubs in a developmental network, along with Ras/MAP, Hedgehog, Wnt, TGF $\beta$ , and JAK/STAT pathways, and determines a binary cell fate in cells with equal developmental potential [1]. The core framework of the pathway consists of interaction between Notch receptors (i.e., Notch1 through Notch4) with five ligands (i.e., Jagged1, Jagged2, and Delta-like ligands 1, 3, and 4) with subsequent activation of downstream target genes. The key event in Notch receptor activation is its intramembrane proteolysis, which requires direct contact between the ligand bearing cell and the receptor expressing cell, and involves proteolytic processing of Notch, causing release and translocation to the nucleus of its intracellular domain (NICD). Within the nucleus, NICD interacts with transcriptional regulators, displaces transcriptional co-repressors, recruits co-activators, and, finally, executes transcriptional activation of target genes. Many target genes of the pathway, such as the basic helix-loop-helix proteins from Hairy enhancer of split (*Hes*) or Hairy related (*Hey* or *Hrt*) family, are universal for different cell types; while others are tissue specific, with the best example being the SRY-related transcriptional factor SOX9, which is essential for normal biliary system formation [2]. Notch-dependent cell fate events occur over relatively short developmental life spans and, despite “at a glance” simplicity of the signaling, have to occur with staking accuracy in an efficient manner. Mistakes in this high-fidelity process lead to pathology. The classic example of pathway dysregulation in the liver is Alagille syndrome characterized by a defective biliary tube formation due to mutation in Notch ligand Jagged1.

Notch dysregulation has also been implicated in oncogenesis. Thus, Notch dysregulation occurs in acute T-cell lymphoblastic leukemia where mutations in C-terminal PEST domain of Notch1 lead to constitutive activation of the pathway [3]. However, the role of Notch signaling in solid tumors is still emerging, and in liver malignancies, tumor suppressive versus tumor promoting action of Notch signaling has been a subject of debate [4–6]. In

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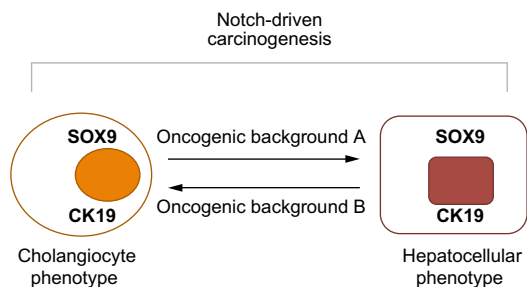
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Abbreviations: NICD, Notch receptor intracellular domain; Hes, Hairy enhancer of split; HCC, hepatocellular carcinoma; HPC, hepatic progenitor cells.



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**Fig. 1. Liver cancer phenotype in Notch-driven liver carcinogenesis depends on an oncogenic background.** Notch contributes to the development of HCC and cholangiocarcinoma; we propose it is the oncogenic background (A or B), rather than Notch itself, that determines the malignant cellular phenotype.

cholangiocarcinoma oncogenesis, two recent studies have demonstrated that upon Notch dysregulation, adult hepatocytes can undergo an oncogenic phenotypic switch to acquire characteristics typically attributed to cholangiocarcinoma, including CK19 and SOX19 expression. Indeed, using a mouse model of hepatocyte fate tracing, Fan *et al.* have demonstrated that Notch and AKT signaling pathways cooperate to transform fully differentiated normal adult hepatocytes into precursors of highly aggressive intrahepatic cholangiocarcinoma [7], while Sekiya *et al.*, employing a mouse model of intrahepatic cholangiocarcinoma, where hepatocytes and cholangiocytes were labeled with heritable reporters, have shown that cholangiocarcinoma cells were also derived from a Notch-driven conversion of adult hepatocytes [8].

Recently, Villanueva *et al.* took a step forward and aimed at elucidating the role of the Notch signaling cascade in hepatocellular carcinoma (HCC), utilizing a genetically engineered mouse model and comparative functional genomics [9]. The group reported that aberrant Notch signaling throughout liver development and into adulthood promotes hepatocyte carcinogenesis in a mouse model. They identified a molecular Notch signature in one third of human HCCs of different etiologies. Pathologic examination of HCCs associated with Notch signaling upregulation demonstrated a subset of tumors enriched with cells positive for CK19, a marker of biliary epithelial cells. The prior and later data raise the question: how can one pathway, Notch signaling, contribute to two different liver cell cancers, HCC and cholangiocarcinoma? This could be explained by a concept of dysregulated Notch signaling in hepatic progenitor cells (HPC), which are able to differentiate into either hepatocytes or cholangiocytes, as nicely reviewed by Strazzabosco *et al.* [10]. This fate-determination of the bipotent HPC into hepatocytes or cholangiocytes in response to an injury can be directed by the inflammatory cells in the microenvironment (e.g., macrophages-derived signals) [11]. Presumably, the inflammatory cells in chronic liver disease could influence the oncogenic transformation of HPC into either hepatocellular or cholangiocellular carcinoma. However, we want to pose another rather provocative question: can the different phenotypes of cells observed in HCCs with Notch signature reflect the ability of mature hepatocytes to acquire diverse “phenotypic masks?” (Fig. 1). We further propose that tumors can be categorized into a molecular cancer genotype, irrespective of histological subtypes. In the Villanueva *et al.* study, this would be a Notch-driven genotype causing an HCC phenotype. In other studies, it would be a Notch-driven genotype, with a predominant cholangi-

ocarcinoma phenotype. This concept suggests that cross-talk between oncogenic signaling pathways determines the malignant cells histological phenotype. In fact, Villanueva *et al.* also demonstrated co-activation of IGF, AKT, and mTOR pathways in Notch signature positive HCCs. In contrast, dysregulation of Notch signaling in another oncogenic context may drive a cholangiocarcinoma phenotype.

In summary, based on the topical and comprehensive study of Villanueva *et al.*, we can conclude that Notch signaling cascade is activated in a subset of human HCCs and also, from the work of others, in cholangiocarcinoma. With current advances in biotechnologies, it is becoming possible to classify tumors based on their predominant oncogenic driver rather on their histological phenotype. Perhaps Notch targeted therapies should be applied to both HCC and cholangiocarcinoma in a distinct molecular cancer genotype, termed “Notch-associated liver cancer.”

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### 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.

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