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# Turning up our understanding of liver cancer by a notch

Amaia Lujambio<sup>1\*</sup> and Flavio Maina<sup>2\*</sup>

<sup>1</sup>Icahn School of Medicine at Mount Sinai, Oncological Sciences Department, Tisch Cancer Institute, 1470 Madison Avenue, New York (USA). [Amaia.lujambio@mssm.edu](mailto:Amaia.lujambio@mssm.edu)

<sup>2</sup>Aix Marseille Univ, CNRS, Developmental Biology Institute of Marseille (IBDM), UMR7288, Parc Scientifique de Luminy, Marseille (France). [flavio.maina@univ-amu.fr](mailto:flavio.maina@univ-amu.fr)

\* These authors contributed equally to this work.

## Corresponding authors:

Amaia Lujambio, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1470 Madison Avenue, Hess 6-111, 10029 New York (USA). Phone : [+1 212-824-9338](tel:+12128249338). email: [amaia.lujambio@mssm.edu](mailto:amaia.lujambio@mssm.edu)

Flavio Maina, IBDM, Parc Scientifique de Luminy, 13009 Marseille (France). Phone: +33.4.91.26.97.69. email: [flavio.maina@univ-amu.fr](mailto:flavio.maina@univ-amu.fr)

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The role of the Notch pathway in the regulation of developmental programs and tissue homeostasis has been extensively explored for decades.[1] The activity of Notch signalling in controlling several biological events is evolutionarily conserved. The canonical Notch pathway initiates at the cell membrane to regulate the transcription of a set of target genes and involves a signal-sending cell (expressing DSL ligands) and a neighbouring signal-receiving cell (expressing Notch receptors). Whereas the ligand blocks signalling in the same signal-sending cells (cis-inhibition), it induces two sequential cleavages of Notch in the signal-receiving cells, mediated by ADAM and the second by  $\gamma$ -secretase (Fig. 1). Consequently, the released Notch IntraCytoplasmic Domain (NICD) translocates into the nucleus, where it interacts with transcription factors inducing the recruitment of transcriptional co-activators and the release of transcriptional co-repressors.[2] The special feature of trans-activation in neighbouring cells and cis-inhibition in ligand expressing sending cells preserves the distinct fate of signal-receiving and signal-sending cells. Additionally, a non-canonical Notch pathway can operate in cells by post-translationally targeting Wnt/ $\beta$ -catenin signalling.[3] The interaction between Notch and Wnt/ $\beta$ -catenin signalling can be either synergistic or antagonistic, depending on the context.[3] Furthermore, the activity of Notch on cells and the biological consequences is broadened by the crosstalk with other signals.[4]

Multiple studies have linked Notch pathway deregulation to the initiation and the progression of several types of cancer. Interestingly, how Notch signalling acts in cancer is highly context dependent, acting as a tumour suppressor or an oncogene depending on the cellular and molecular milieu in which it operates.[5, 6] The oncogenic action of the Notch pathway is largely supported in several haematological malignancies, such as T cell lymphoblastic leukaemia (T-ALL), chronic lymphocytic leukaemia (CLL), and several forms of lymphoma.[4] Additionally, Notch signalling operates as a driver of tumour initiation and progression in solid malignancies, including breast, lung, or colorectal cancers. On the other hand, the activity of Notch as a tumour suppressor is exemplified in squamous cell carcinomas of various tissue types, where Notch loss-of-function mutations drive cancer progression.[4] Nevertheless, Notch can even act as a tumour suppressor or as an oncogene in patients affected by the same type of cancer, indicating that the precise role of Notch in cancer can be influenced by factors beyond the tumour type.[4] The duality of Notch function in cancer is likely linked to its ability to also regulate the interaction between tumour cells and cells in the tumour microenvironment, such as immune cells, endothelial cells, and fibroblasts.[7] Notwithstanding such context dependent dual action of Notch signalling in cancer, its implication in preserving self-renewal and expansion of cancer stem cells is corroborated by several reports.[8] Such properties in cancer stem cells attribute to the Notch pathway a role in the progression and recurrence of tumours. Finally, it has been reported that Notch signalling also triggers differentiation of neighbouring cancer cells,

thus contributing to tumour heterogeneity.[9] Collectively, these data suggest that Notch action in cancer initiation may differ from its role in cancer progression and resistance to therapy. In view of its implication in cancer, several efforts have been made to develop agents, with a set of them currently evaluated in clinical trials.[4, 10] These include blocking antibodies against the ligands of the Notch receptors and inhibitors of  $\gamma$ -secretase and of the Notch transcription complex. Nevertheless, their clinical development is challenged by the complexity of Notch pathway and by its involvement in tissue homeostasis maintenance.

In this issue of *Journal of Hepatology*, Zhu *et al.* have established the role of Notch in promoting the development of hepatocellular carcinoma (HCC) associated with non-alcoholic steatohepatitis (NASH). HCC is the most common type of liver cancer, which represents the 4<sup>th</sup> leading cause of cancer-related mortality.[11] 90% of all HCC cases develop in the context of liver damage, which can mainly be caused by hepatitis infection, alcohol abuse, or non-alcoholic fatty liver disease (NAFLD). Several genes and pathways have been involved with HCC pathogenesis; however, the role of Notch has been more controversial, with some supporting a pro-tumorigenic role and one, a tumour suppressive.[12-14] To better understand the role of Notch in HCC, Zhu *et al.* created a Notch transcriptional signature score based on the expression levels of 14 Notch pathway related genes and then used this score to stratify HCC patients into three subgroups: Notch-high (29.5%), -mid (41%), and -low (29.5%).[15] No association was observed between Notch score and mutations in the Notch pathway, which were much more infrequent than the activation of the Notch pathway. Instead, the authors found that the Notch-high HCCs were predominantly deprived of *CTNNB1* activating mutations, which are in general frequent in HCC and associated with the transcription of a set of genes  $\beta$ -catenin targets.[16] Notch-high HCC transcriptionally resembled cholangiocarcinoma-like HCC, with an enrichment of genes related to extracellular matrix (ECM), intratumoral fibrosis, and with an overall poor prognosis. These findings were elegantly corroborated in mice. In particular, using a mouse model of NASH, the authors showed that Notch-active hepatocytes are characterised by a transcriptional enrichment in ECM genes whereas metabolic pathways and  $\beta$ -catenin signalling are repressed, similar to human Notch-high HCCs. This was also the case in a mouse model of HCC induced by a combination of NASH-inducing diet and the carcinogen diethyl-nitrosamine, with a remarkable mutual exclusion of Notch and  $\beta$ -catenin activation in tumour and non-tumour areas. Additional experiments in a genetic mouse model harbouring a Notch activating mutation in combination with NASH-inducing diet further supported these interesting findings. These results indicate that Notch-triggered transcriptional changes in hepatocytes may persist in the tumorigenic program during the progression from NASH chronic liver disease to HCC in both mice and patients. Strikingly, Notch blockade in the HCC mouse model caused a switch towards  $\beta$ -catenin-active tumours, without changes in tumour frequency.

One of the most interesting findings from this study is the inverse correlation between Notch activity and *CTNNB1* mutations, which is reminiscent of the opposite function of Notch and Wnt/ $\beta$ -catenin in developmental processes and in chronic liver disease.[17] The possibility of a functional redundancy between Notch and  $\beta$ -catenin pathways can be ruled out as Notch-high tumours lack activation of  $\beta$ -catenin targets. A potential explanation could be that simultaneous activation of both pathways is lethal. However, the existence of a few human HCC tumours with a concomitant activation excludes this option or implies the necessity of a permissive molecular context to overcome lethal effects. Future studies will be needed to better dissect this unusual relationship. Another fascinating discovery is the shift in phenotype of HCC tumours when Notch pathway is inhibited, as this could have important therapeutic consequences. Will Notch-high tumours shift into  $\beta$ -catenin-activated tumours or will they undergo tumour regression as a result of oncogene addiction? The unchanged tumour frequency following Notch blockade reported by Zhu *et al.* would rather imply a shift, facilitated by the frequent intra-tumour heterogeneity of HCC. Preclinical studies using the appropriate mouse models and specific inhibitors will be required to address this relevant question. Furthermore, Notch activity as a driver of HCC was established in a NASH-related mouse model of HCC. Addressing whether the pathway activation is more prevalent in NASH-HCC patients or can also occur in other aetiologies will be important. Finally, Notch activation in hepatocytes can lead to either cholangiocarcinoma or HCC:[18] identifying the key signalling pathways that affect the tumour fate may help identify tumour-specific therapies. Collectively, these data indicate that in a context of obesity-related NASH, sustained Notch activation can trigger a tumorigenic program even in the absence of driving mutations.

#### **Figure Legend.**

**Canonical Notch signalling.** Notch ligands (DLL1, 3, 4 and Jag1-2), while blocking Notch receptors (Notch 1-4) in signal-sending cells (cis-inhibition), activate Notch in signal-receiving cells (trans-activation). Following activation, Notch is first cleaved by ADAM and then by  $\gamma$ -secretase complexes, leading to the release of the NICD (Notch IntraCytoplasmic Domain), which translocates into the nucleus. NICD interaction with transcription factors of the CSL family (CBF-1/Su(H)/LAG1) recruits CoAs (co-activators) by displacing CoRs (co-repressors), thus shifting target genes from repressed to activated states.

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