Previews

Taking HSCs Down a Notch in Leukemia

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DOI 10.1016/j.stem.2011.04.010

The Notch signaling pathway is activated in the majority of T cell acute lymphoblastic leukemias (T-ALL). Adding to the complexity of Notch signaling in hematopoiesis, recently in *Nature*, Klinakis et al. (2011) demonstrate a tumor-suppressor function for the Notch pathway in myeloid malignancy.

In hematopoiesis, Notch1 has an essential role in T lymphocyte development. Activating mutations in Notch1 result in T-ALL (Weng et al., 2004). However, the importance of the Notch family in regulating other hematopoietic cell lineages, including hematopoietic stem cells (HSCs), remains controversial. There are four distinct Notch receptors in mammals, making it difficult to study the roles of Notch in hematopoiesis, due to potential redundancy between the receptors, even in compound conditional knockout mutants.

Notch signaling regulates the function of transcription factors of the *CBF1/RBP-Jκ, Suppressor of Hairless, LAG1* (CSL) family (Lai, 2004). When a Notch receptor is uniganded, CSL family members act as corepressors of gene transcription (Figure 1A). Upon binding of a Notch receptor to a Notch ligand, a number of different proteases sequentially cleave the Notch receptor: ADAM metalloproteases cleave the ligand-bound Notch receptor extracellularly, creating a membrane-bound intermediate, which is a substrate for gamma-secretase (γSE, Figure 1B) (Kopan and Ilagan, 2009). γSE cleaves the intramembrane form of the Notch intracellular domain (NICD), allowing it to translocate to the nucleus to bind CSL, Mastermind (MAM), and other coactivators, resulting in activation of gene transcription of target genes such as Hes1 (Figure 1C) (Kopan and Ilagan, 2009).

γSE consists of four conserved components: presenilin enhancer 2 (Pen-2), presenilin (PS), nicastrin (Ncstn), and anterior pharynx-defective 1 (Aph-1) (Figure 1B) (Laudon et al., 2007). Pen-2, PS, and Aph-1 are all transmembrane proteins, whereas Ncstn has both an extracellular and a transmembrane domain. Ncstn has been demonstrated to have two functions: (1) the extracellular domain binds

to membrane-bound intermediate γSE substrates such as those generated by ADAM cleavage of ligand-bound Notch and (2) the transmembrane domain is required to interact with other members of the γSE complex to allow it to move from the endoplasmic reticulum to the cell surface (De Strooper, 2005).

In their current study, Klinakis et al. (2011) generated a conditional *Nicastrin* allele (*Ncstn*^fl/fl) that they crossed with *Mx1*-Cre or *Vav*-Cre transgenic mice to generate hematopoietic *Ncstn* knockout mice (designated hereafter as *Ncstn*^−/−) (Klinakis et al., 2011). Both *Ncstn*^−/− strains produced identical phenotypes: they rapidly developed increased numbers of white cells (leukocytes) in the peripheral blood, spleen, and liver and died by 20 weeks of age. The hematopoietic cell phenotype present in both of the *Ncstn*^−/− strains was reminiscent of human chronic myelomonocytic leukemia (CMML), and bone marrow transplant studies showed that this hematopoietic disease was cell-autonomous.

Increased numbers of myeloid-biased Lineage^neg, Sca-1^+c-Kit^+ (LSK) CD150^+ CD48^+ cells were observed in *Ncstn*^−/− BM. This was accompanied by a significant reduction in the frequency of lymphoid-biased multipotent progenitor cells (MPPs). There were also significantly reduced numbers of megakaryocyte/ erythroid progenitors (MEPs) in the *Ncstn*^−/− BM, accompanied by increases in the numbers of granulocyte/monocyte progenitors (GMPs) in both BM and spleen. The impact of loss of Ncstn on HSCs was less clear, and would be difficult to ascertain in this model, as the cancer phenotype could complicate interpretation of the data. However, the numbers of cells with a phenotype most consistent with HSCs (LSK CD150^+CD48^−) were the same in

Ncstn^−/− and WT BM. Collectively, these data suggest that Ncstn (and, in turn, any pathway it regulates) may not play an important role in regulating HSC numbers, but does have an active role in lineage-fate commitment.

Consistent with the increased numbers of myeloid-biased immature progenitors observed in vivo, increased numbers of granulocyte/macrophage and, in particular, macrophage colonies, were formed from LSK cells from *Ncstn*^−/− mice compared to WT mice. The *Ncstn*^−/− colonies had enhanced serial replating potential compared to those obtained from WT BM and GMPs. Furthermore, whole transcriptome profiling of *Ncstn*^−/− GMPs revealed upregulation of several members of a core leukemic self-renewal signature defined by Krivtsov et al., 2006; in particular, Hoxa family members.

Although γSE participates in cleaving a number of different substrates, especially the amyloid precursor protein, the authors focused on the Notch pathway, since *Ncstn*^−/− mice exhibit known *Notch*^−/− phenotypes, including a block in T lymphocyte differentiation. To prove this connection, they generated Notch1, -2, and -3 compound null mutants (*Mx1-cre*^+N1^f/fN2^f/fN3). These mice phenocopied the hematopoietic defects observed in *Ncstn*^−/− mice, and deletion of both *Notch1* and *Notch2* were sufficient to recapitulate the phenotype.

To determine the underlying mechanisms for the CMML-like disease observed in *Ncstn*^−/− mice, the authors performed transcriptome analysis of LSK and GMP populations from WT and *Mx1*-Cre^+ *Ncstn*^f/f BM. This showed that a myeloid gene-expression program was initiated in *Ncstn*^−/− LSK cells as early as the CD150^+ HSC stage of differentiation and persisted at the CD150^− subset (which included

MPPs). Furthermore, gene expression in LSK cells that overexpressed Notch1 intracellular fragment (Notch1^{IC} LSK, generated using Mx1-Cre$^+$ Ef1α1-Notch1^{IC} mice) inversely correlated with that observed in Ncstn$^{-/-}$ LSK.

Of note, expression of the Notch target gene, Hes1, was downregulated in Ncstn$^{-/-}$ LSK and upregulated in Notch1^{IC} LSK. Hes1 is a transcriptional repressor; hence, the authors hypothesized that Hes1 could be responsible for the suppressive effects of Notch signaling on GMP-specific gene expression. Hes1 overexpression in c-Kit$^+$ progenitors from WT and Ncstn$^{-/-}$ BM shifted the differentiation of these cells from granulocytic (Gr-1$^+$) cells into megakaryocytic (CD41$^+$) cells after 7 days in methylcellulose, although increased numbers of Gr-1$^+$ cells were still observed in Ncstn$^{-/-}$ BM that overexpressed Hes1 compared to that of Hes1-overexpressing WT BM. Hes1 overexpression also suppressed the expression of key GM-lineage commitment genes such as Pu.1 and Cebpa in WT LSK cells due to direct binding of Hes1 on their promoters.

Given the striking phenotypic resemblance of Ncstn$^{-/-}$ hematopoiesis to that of human CMML (Figure 1D), the authors sequenced γSE/ Notch-pathway genes in human CMML samples and identified novel loss-of-function mutations in a subset of these samples. These mutations included Ncstn, another component of the γSE complex, Aph-1, and Notch-pathway-specific members MAM-like protein 1 (MAML1) and NOTCH2. Importantly, Notch-pathway mutations were restricted to CMML samples and were not found in other types of myeloproliferative disorders. These mutations were detected along with other mutations known to be involved in myeloid leukemia, such as

JAK2, KRAS, and TET2. Thus, mutations in the Notch pathway may act cooperatively with other oncogenic pathways in the initiation and maintenance of CMML.

It is worth noting, however, that the phenotype of myeloproliferative disease in Ncstn$^{-/-}$ and Mx1-cre$^+$N1$^{f/f}$N2$^{f/f}$N3 mice was not detected in either CSL/ Rbp-Jk$^{-/-}$ mice or mice transplanted with BM overexpressing dominant-negative MAML (Han et al., 2002; Maillard et al., 2008). As the CSL signaling pathway

is downstream of both Ncstn and Notch, it raises the possibility that a CSL-independent pathway may be involved (Sanalkumar et al., 2010). The results of Klinakis et al. (2011) expand the repertoire of the Notch pathway in cancer and demonstrate that it can act as both an oncogenic and tumor-suppressor pathway, depending on the cell lineage affected and the nature of the mutation. The study further highlights the utility of murine models in expanding our understanding of human cancer genetics and in providing critical insights into gene function that can be of direct relevance to our understanding of human disease. Finally, it implicates that therapeutic modulation of the Notch pathway in hematopoietic diseases may be a double-edged sword.
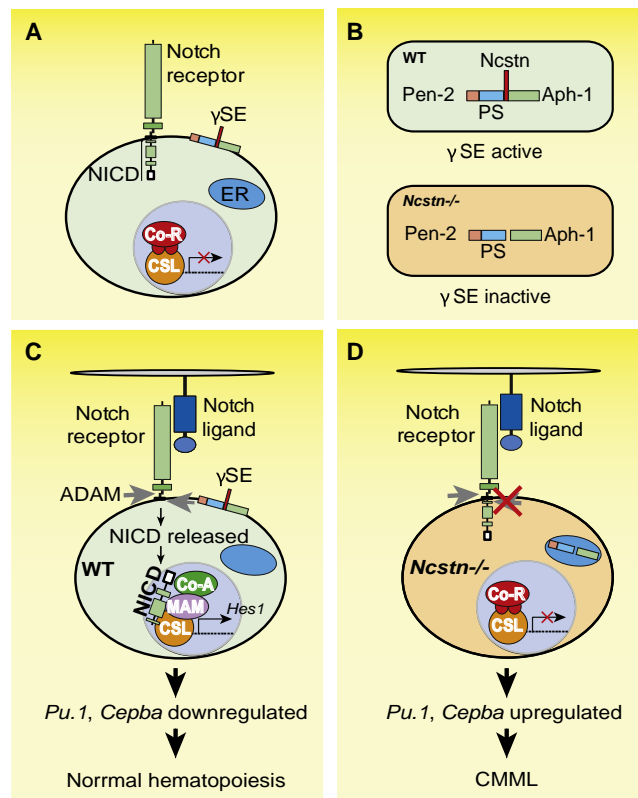

Figure 1. A Simplified Schematic Diagram of Notch Signaling and the γSE Complex in Immature Hematopoietic Progenitor Cells
(A) When the Notch receptor is unliganded, CSL is bound to a corepressor, and gene transcription is repressed.
(B) Essential components of the γSE protease are Ncstn, Pen-2, PS, and Aph-1. The complex is active in WT cells (green). In Ncstn knockout cells (orange), γSE complex is inactive.
(C) In WT cells, when the Notch receptor is liganded, an ADAM metalloprotease cleaves the extracellular portion of the Notch receptor, forming a membrane-bound intermediate, which is then cleaved by γSE. The resulting NICD then translocates to the nucleus, where it binds with CSL, MAM, and other coactivators to activate transcription of genes, including Hes1. Hes1 represses genes involved in myeloid commitment, including Pu.1 and Cebpa, resulting in normal differentiation of the immature progenitor cell.
(D) In Ncstn$^{-/-}$ cells, γSE cannot recognize the Notch receptor intermediate substrate and likely remains in the endoplasmic reticulum (ER). Hence, in liganded form, the Notch receptor cannot be cleaved accurately and NICD remains bound to the cell membrane, unable to translocate to the nucleus. Gene transcription of Notch pathway targets therefore remains repressed, target genes of Hes1 such as Pu.1 and Cebpa are elevated in immature progenitor cells, and CMML-like disease occurs.

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