The Notch signaling pathway in hematopoiesis and hematologic malignancies

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The Notch signaling pathway plays a critical role in the development and maintenance of embryonic and adult tissues. Notch signaling is initiated when a cell expressing an appropriate ligand interacts with another cell expressing a Notch receptor. This interaction leads to two successive proteolytic cleavages of the receptor, mediated by the ADAM family and subsequently the γ-secretase enzyme complex. This liberates the intracellular domain of the Notch receptor (NICD), which translocates to the nucleus and binds to the DNA binding transcription factor RBP-J/CSL/CBF-1/Lag/Supperssor of hairless, forming a short-lived nuclear transcription complex. The functions of Notch are highly context-dependent and in addition to the well-known Hairy enhancer of split (HES) and Hairy related (Hey or Hrt) Notch target genes, a large number of genes have been identified that can be directly regulated by activated Notch.[1] Furthermore, several other signaling pathways interact with the Notch pathway,[2] further adding to the complexity of Notch signaling outcome. In the hematopoietic system, Notch signaling is essential for the generation of definitive embryonic hematopoietic stem cells[3] and controls several steps in T-cell development.[4] However, its role in regulating myeloid development remains controversial. In mammals, there are 4 highly homologous Notch receptors with partly overlapping functions, making it difficult to study the roles of Notch signaling in hematopoiesis. In addition, in the mouse, inactivation of Notch pathway genes in most cases causes embryonic lethality, thus restricting this approach to conditional or cell specific targeting of mutations. In their current study, after analyzing embryonic and adult hematopoiesis in Notch zebrafish mutants, Bugeon and colleagues report that Notch signaling affects cell fate decisions in myelopoiesis at the definitive but not primitive stage of hematopoiesis.[5] Zebrafish is a very useful model system to analyze developmental hematopoiesis. In addition to the possibility of following cell fate by imaging of transparent embryos *ex utero*[6] and the availability of methodologies for the analyses of the hematopoietic system,[7] further important tools are viable mutant zebrafish lines with defects in the Notch pathway. As in mammals, zebrafish hematopoiesis has 2 distinct waves: embryonic primitive hematopoiesis, which is analogous to the blood islands in the mammalian yolk sac, and definitive hematopoiesis emerging from hemogenic endothelial cells of the dorsal aorta in the aorta-gonad-mesonephros (AGM) region.[7,8] Bugeon and colleagues have now used the zebrafish mutant deadly seven (DES) and beamter (BEA) with disrupted function of the notch1a receptor and the deltaC Notch ligand, respectively, to analyze the development of myeloid cells in embryonic and mature zebrafish.[5] In mature fish, both strains had a decreased proportion of myelomonocytes and an increased percentage of lymphocytes while precursor numbers were unaltered in the kidney marrow, the functional equivalent of the bone marrow niche of mammals, and in the periphery, the coelomic cavity (Figure 1). Furthermore, knocking down Notch1a with translation blocking morpholinos in normal embryos resulted in a reduced number of definitive myeloid cells. No difference in the number of myeloid cells was observed during the primitive phase of hematopoiesis. Interestingly, the number of functional myeloid cells recruited to the wound site after tail fin wounding was reduced in the DES Notch1a mutant embryos, but not after inhibition of Notch signaling after treatment with DAPT, a γ-Secretase inhibitor, suggesting that a defect in Notch signaling results in a reduction in definitive myelopoiesis. The results of Bugeon and colleagues[5] are in line with several *in vitro* studies reporting promotion or requirement for Notch signaling for myeloid differentiation of murine stem and progenitor cells.[9-11] Importantly, by using zebrafish Notch mutants, the work by Bugeon *et al.*[5] demonstrates that Notch signaling has a role to play in physiological myelopoiesis. But previous studies also reported that activated Notch blocks myeloid differentiation[12] and represses a gene-expression program in blood stem and progenitor cells that is associated with differentiation along the myeloid lineage.[13] Interestingly, loss of function mutations of the Notch, but not of the RBP-J pathway, result in chronic myelomonocytic leukemia (CMML).[13,14] This raises the possibility that RBP-J-dependent and RBP-J-independent pathways initiated by Notch signaling have different and even opposing functions. Depending on the cellular context as determined by chromatin structure, RBP-J dependent/independent signal transduction and integration of other signaling pathways, the outcome of Notch activation may be highly variable. Further work using single cells and

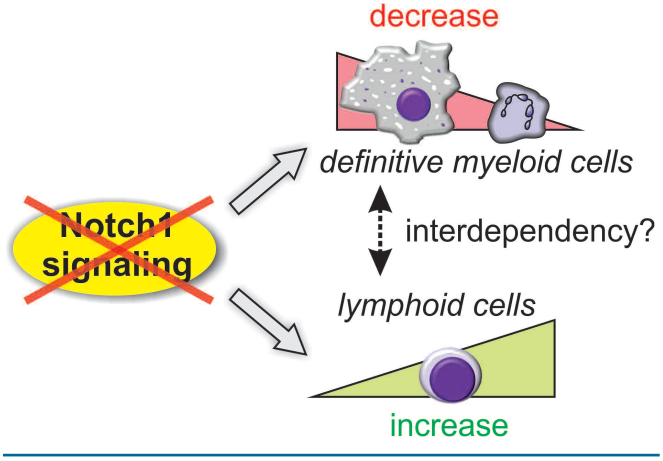

Figure 1. Notch1 signaling influences generation of both definitive myeloid cells and lymphoid cells.

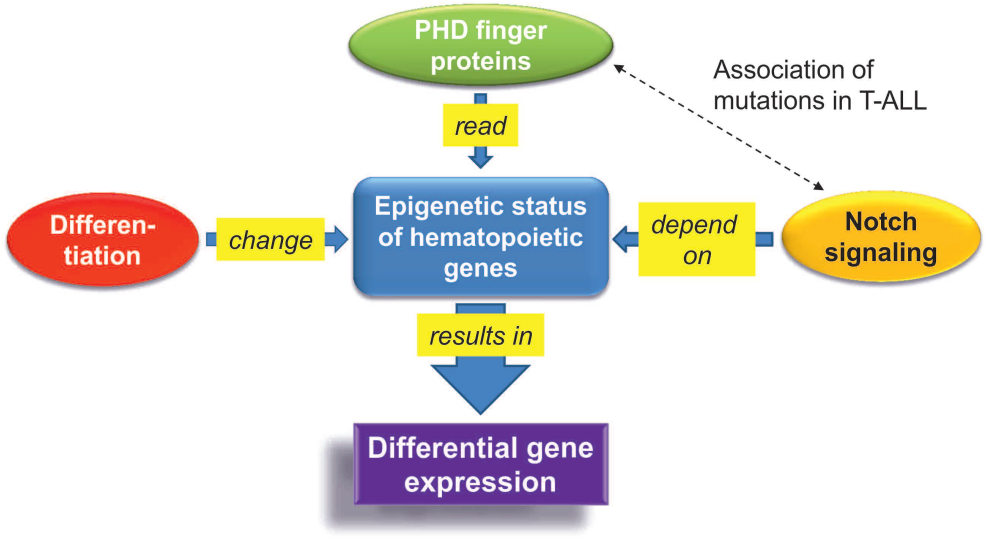

Figure 2. Model: the epigenetic status of hematopoietic genes serves as an integrative platform of gene regulation.

defined Notch effector molecules is needed to clarify the different roles of Notch signaling in myeloid hematopoiesis.

A constitutive activating mutation of human Notch1 was first described through analysis of T-cell acute lymphoblastic leukemias (T-ALLs) with balanced (7;9) translocations.[15] A number of further studies of murine and human leukemias later revealed the presence of acquired gain-of-function Notch1 mutations at frequencies from 30 to 80% in the mouse and around 60% in human T-ALL, clearly moving Notch1 to the center of T-ALL pathogenesis.[16] In their current study, Wang and colleagues report that in T-ALL, mutations in the plant homeodomain (PDH)-like finger 6 (PHF6) gene are frequently associated with mutations in the Notch1 receptor protein.[17] Importantly, Notch1 mutations were present in about 80% of T-ALL carrying a PHF6 mutation, clearly establishing a relationship between PHF6 and Notch1 in leukemogenicity (Figure 2). PHF6 is a tumor suppressor that is deleted or mutated in about 5-15% in pediatric and 20-40% in adult T-ALL.[17,18] PHD finger-containing proteins have been implicated in transcriptional regulation and as specialized reader modules that recognize the methylation status of histone lysine residues such as histone H3 lysine 4 (H3K4).[19] Recently, a correlation between the H3K4me3 status and cell-context dependent activation of Notch target genes has been shown.[20] It is thus tempting to speculate that loss of recognition of H3K4me3 sites at certain target genes lead to a change in target genes activated by Notch1 that contribute to leukemic transformation (Figure 2). The Notch pathway is certainly a primary drug target in T-ALL. In this regard, the results of the study of Wang *et al.*[17] suggest that the efficiency of therapy may depend on the simultaneous targeting of cooperating mutations such as the PHF6 mutation described here. Further functional studies on Notch1 signaling in normal hematopoiesis and in leukemic cells will help to understand the regulation of blood cell development as well as the mechanisms of malignant transformation, and will possibly contribute to the design of new treatments.

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