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REVIEW

Molecular pathogenesis and targeted therapies for NOTCH1-induced T-cell acute lymphoblastic leukemia

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ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic tumor resulting from the malignant transformation of immature T-cell progenitors. Originally associated with a dismal prognosis, the outcome of T-ALL patients has improved remarkably over the last two decades as a result of the introduction of intensified chemotherapy protocols. However, these treatments are associated with significant acute and long-term toxicities, and the treatment of patients presenting with primary resistant disease or those relapsing after a transient response remains challenging.

T-ALL is a genetically heterogeneous disease in which numerous chromosomal and genetic alterations cooperate to promote the aberrant proliferation and survival of leukemic lymphoblasts. However, the identification of activating mutations in the *NOTCH1* gene in over 50% of T-ALL cases has come to define aberrant NOTCH signaling as a central player in this disease. Therefore, the NOTCH pathway represents an important potential therapeutic target. In this review, we will update our current understanding of the molecular basis of T-ALL, with a particular focus on the role of the NOTCH1 oncogene and the development of anti-NOTCH1 targeted therapies for the treatment of this disease.

1. Introduction

Acute lymphoblastic leukemias (ALL) are characterized by the uncontrolled clonal proliferation of immature lymphoid cells which infiltrate the bone marrow. In T-cell acute lymphoblastic leukemias (T-ALL) the malignant clone is derived from T-cell progenitor cells and expresses immature immunophenotypic markers characteristic of the T-cell lineage. T-ALL represents about 15% of pediatric and 25% of adult ALLs and is typically associated with very high white cell counts, mediastinal masses with pleural effusions, and increased risk of leptomeningeal infiltration at diagnosis.[1] Although initially associated with a particularly bad prognosis, the introduction of intensified treatment protocols has improved the outcome of this disease and current therapies achieve five-year relapse-free survival rates of about 75% in children and 50% in adults.[2–8]

T-cell transformation is a multistep oncogenic process in which multiple lesions involving different oncogenes and tumor suppressor genes cooperate to disrupt the normal circuitry that controls cell proliferation, differentiation and survival during T-cell development.[9–12] Most of what we know about the molecular basis of T-ALL has been learned from the study of recurrent cytogenetic alterations.[9] The most common genetic alteration in T-ALL is the presence of deletions in the *CDKN2A* tumor suppressor locus containing the *P16/INK4A* and the *P14/ARF* tumor suppressor genes, which control cell cycle progression and p53 mediated apoptosis, respectively.[13] In addition, over 50% of T-ALLs harbor activating mutations in the NOTCH signaling pathway making of NOTCH1 the most prominent T-ALL specific oncogene[14] and defining T-ALL as a disease primarily characterized by aberrant NOTCH1 activation.[15,16] However, T-ALL is a heterogeneous disease in which different molecular groups, primarily defined by the expression of T-ALL transcription factor oncogenes, are associated with specific patterns of gene expression, a specific block in cell differentiation and distinct clinical characteristics.[10–12] Thus, T-ALL-associated chromosomal translocations typically result in the juxtaposition of a selective group of oncogenic transcription factors next to strong regulatory elements located in the vicinity of the T-cell receptor β (TCRB) gene in chromosome 7q34 or the T-cell receptor α-δ (*TCRAD*) locus in chromosome 14q11.[9,17] These T-ALL-specific transcription factor oncogenes include basic helix–loop–helix (bHLH) family members such as *TAL1*,[18–21] *TAL2*,[22] *LYL1*[23] and *BHLHB1*[24]; LIM-only domain (LMO) factors such as *LMO1* and *LMO2*[25–29]; *TLX1/HOX11*,[30–33] *TLX3/HOX11L2*,[34] *NKX2.5*[35,36] and *HOXA* homeobox genes[11,37]; *MYC*[38–42] and *MYB*[43] oncogenes; and *TAN1*, a truncated and constitutively activated form of the NOTCH1 receptor.[44,45] In some cases, these factors can also be

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activated in the context of other non-TCR-associated chromosomal abnormalities. This is the case for small deletions activating *TAL1*[46] and *LMO2*[47]; duplications of the *MYB* oncogene[48,49]; and the t(5;14)(q32;q11) translocation which activates the *TLX3* oncogene in chromosome 5 by relocating it to the vicinity of the *BCL11B* locus in chromosome 14.

Additional molecular alterations present in T-ALL include transcription factor fusion oncogenes such as *PICALM/MLLT10/CALM-AF10*,[50–52] *MLL-MLLT1/MLL-ENL*,[53,54] *SET/NUP214*,[55] *NUP98-RAP1GDS1*[56,57]; activation of signaling factors driving proliferation such as *LCK*,[58] *CCND2*,[59,60] *JAK1*,[61] *NUP214-ABL1*,[62] *EML1-ABL1*,[17] and *NRAS*[63,64]; and the loss of tumor suppressor genes in the RAS (*NF1*[65]) and PI3K (*PTEN*[66]) signaling pathways. However, the catalog of genetic alterations involved in the pathogenesis of T-ALL is not yet complete as shown by the recent identification of loss-of-function mutations in *WT1*,[67] the *PTPN2* phosphatase[68] and the *PHF6* tumor suppressor gene.[69]

2. NOTCH1 signaling pathway

The NOTCH1 receptor is a class I transmembrane protein that functions as a ligand-activated transcription factor (Fig. 1).[15] Thus, NOTCH1 directly transduces information from extracellular signals into changes in gene expression in the nucleus. The main components of NOTCH1 signaling include: the Delta/Serrate/LAG-2 (DSL) family of ligands (Delta-like 1, 3, and 4 as well as Jagged 1 and 2); the NOTCH1 receptor (NOTCH1); the RBPJ/CSL (CBF1/Su(H)/LAG-1) DNA-binding protein; and the mastermind-like family of coactivators. In resting conditions, NOTCH1 sits in the membrane as a heterodimeric complex composed of an N-terminal extracellular subunit (N_{EC}) and a C-terminal transmembrane and intracellular subunit (N_{TM}). The N_{EC} subunit interacts with Delta-like and Jagged ligands through 36 epidermal growth factor (EGF)-like repeat domains. In addition, it contains a negative regulatory region (NRR) composed of three Lin12/NOTCH repeats (LNRs). These LNR domains fold over and stabilize the heterodimerization domain (HD), which consists of the C-terminus of N_{EC} and the N-terminus of N_{TM} in close interaction, to prevent the spontaneous activation of the receptor in the absence of ligand. The N_{TM} subunit contains a transmembrane sequence followed by a series of cytoplasmic domains, including a RAM domain, a series of ankyrin repeats, a transactivator domain, and several nuclear localization signals, which collectively function as a ligand-activated transcription factor. The N_{TM} also contains a C-terminal PEST (proline (P), glutamic acid (E), serine (S), and threonine (T) rich) domain, which is responsible for the proteosomal degradation of activated NOTCH1 in the nucleus.[15]

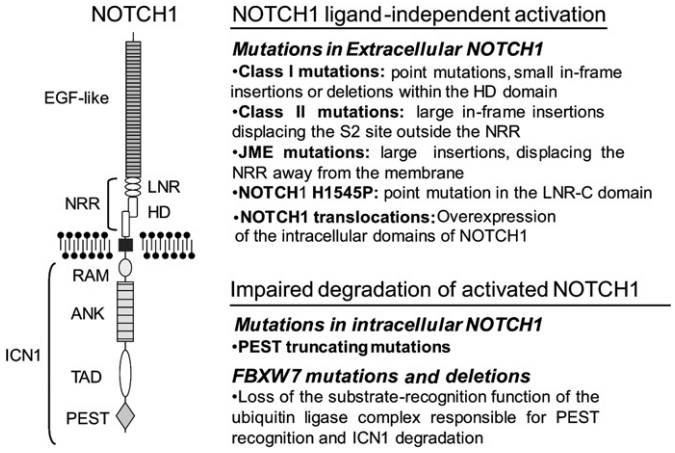

Fig. 1. NOTCH1 mutations in T-ALL. Schematic representation of the NOTCH1 receptor structure and types of NOTCH1 mutations found in T-ALL. EGF-like, EGF-like repeats involved in ligand recognition. NRR, negative regulatory region. LNR, Lin12/NOTCH repeats. HD, heterodimerization domain. RAM, RAM domain. ANK, ankyrin repeats. TAD, transactivation domain. PEST, proline, glutamic acid, serine and threonine rich domain. ICN1, intracellular NOTCH1.

Under physiologic conditions the NOTCH1 receptor is activated via interaction with a Jagged or Delta-like ligand molecule. This ligand–receptor interaction induces a conformational change in the NRR regulatory region and triggers the cleavage of the HD domain by the ADAM10 and ADAM17 metalloproteases at the cell surface.[70–74] This first activation-associated cleavage, also known as S2, is then followed by a second proteolytic cleavage (S3) catalyzed by the γ-secretase complex in the transmembrane region of the receptor.[70–72] Thus, the γ-secretase complex releases the intracellular domain of NOTCH1 (ICN1) into the cytosol and allows its translocation into the nucleus, where it associates with the RBPJ/CSL DNA-binding protein, recruits members of the mastermind (MAML) family of coactivators and p300, and through these interactions, activates gene expression.[15] Finally, recruitment of the RNA polymerase II holoenzyme to the ICN1-RBPJ/CSL-MAML transcriptional complex triggers the phosphorylation of the PEST domain of NOTCH1 by cyclin-dependent kinase 8 and recruits the FBXW7/SCF ubiquitin ligase complex, which ultimately mediates the polyubiquitination and proteasomal degradation of the activated receptor in the nucleus.[15]

3. NOTCH1 in T-cell development

The NOTCH signaling pathway is responsible for cell fate specification and tissue patterning in multiple cellular and tissue contexts during development. In the lymphoid system NOTCH signals provided by the thymic microenvironment are essential for the specification and development of T-cell progenitors.[75,76] Consistent with this model, conditional inactivation of NOTCH1 results in a complete ablation of T-cell lymphopoiesis and differentiation accompanied by ectopic B-cell development in the thymus.[77,78] Likewise, overexpression of an active, intracellular form of NOTCH1 in bone marrow progenitors results in ectopic pre-T-cell development in the bone marrow.[79]

Upon T-cell specification, thymocytes differentiate into αβ or γδ T-cell lineages, and while the development of γδ T-cells seems to be independent of NOTCH,[80,81] αβ T-cells require continuous NOTCH1 activation for their maturation to the DN3 stage of development.[81] During this process, several important factors required for T-cell development are transcriptionally controlled by NOTCH1, including the pre-T-cell receptor alpha (*PTCRA*),[82] the IL7 receptor alpha (*IL7RA*)[83] and *MYC*.[84] Both preTCR signaling and NOTCH activation are needed for growth and survival at the so called β-selection checkpoint,[80] at which point NOTCH1 signaling is critically required to sustain cell metabolism via activation of the PI3K–AKT cascade.[85]

4. Aberrant NOTCH1 activation in T-ALL

The first evidence of the role of *NOTCH1* in the pathogenesis of T-ALL resulted from the cloning of *TAN1*, a truncated and constitutively active form of NOTCH1, at the breakpoint of the t(7;9)(q34;q34.3) chromosomal translocation present in about 1% of T-ALL cases.[44] In this translocation, the *NOTCH1* locus in 9q34 is broken so that the derivative chromosome 9 retains the N-terminal domains of NOTCH1, including the NRR region, while the transmembrane and intracellular domains of the receptor are translocated to the derivative chromosome 7 where they are aberrantly expressed under the control of the *TCRB* regulatory sequences. Ultimately this rearrangement results in constitutive activation of NOTCH1 signaling due to the expression of high levels of N_{TM} and/or ICN1 in T-cell precursors (Fig. 1).[44,45] The pathogenic role of activated NOTCH1 in T-ALL was fully demonstrated when irradiated mice reconstituted with bone marrow progenitors expressing activated forms of NOTCH1 developed clonal hematopoietic tumors characterized as T-ALL.[86] In addition, T-cell tumors