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NOTCH1: a therapeutic target for T cell acute lymphoblastic leukemia/lymphoma

A B S T R A C T

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) is an aggressive tumor that generally affects children but also arises in adults, affecting 1,500 people per year in the United States. T-ALL is often associated with hyperleucocytosis, large mediastinal masses and leptomeningeal infiltration at diagnosis. Activating mutations in *NOTCH1* are present in about 50% of T-ALL cases and small molecule inhibitors of the γ-secretase complex (GSIs), which effectively abrogate NOTCH1 signaling, have been proposed for the treatment of T-ALL. Despite this interest, the clinical development of GSIs has been hampered by our incomplete understanding of the effector pathways controlled by NOTCH1, the lack of clinical responses to GSI therapy and the development of gastrointestinal toxicity secondary to inhibition of NOTCH signaling in the gut. However, recent studies have elucidated important downstream mechanisms of NOTCH1-induced transformation and uncovered the molecular basis of sensitivity and resistance to GSI therapy. Moreover, combination therapies of GSIs with glucocorticoids have been shown to induce potent antileukemic effects in glucocorticoid resistant T-ALL and to ameliorate the gastrointestinal toxicity associated with systemic inhibition of NOTCH signaling. These recent developments may lead to novel rationally-designed and highly effective therapies targeting NOTCH1 signaling in T-ALL.

NOTCH1, a central player in the pathogenesis of T-cell acute lymphoblastic leukemia/lymphoma

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) is an aggressive tumor that accounts for 10-15% and 25% of ALL cases in children and adults, respectively. T-ALL patients frequently present with hyperleukocytosis, mediastinal masses and central nervous system disease (CNS) at diagnosis. Over the last decade, highly active chemotherapy protocols have improved the clinical outcome of T-ALL patients and five-year survival rates range from 75-85% for children and adolescents and 40-50% for adults with this disease.[1-3] Despite this progress the treatment of T-ALL requires intensive therapies associated with higher toxicity than those used in B-precursor ALLs. Thus, the understanding of the molecular pathways involved in the pathogenesis of T-ALL and the identification of new highly effective and less toxic therapeutic strategies has become imperative.

The most prominent genetic abnormality in the pathogenesis of T-ALL is the presence of activating mutations in *NOTCH1*, which

are detected in over 50% of cases.[4] The NOTCH signaling pathway is an evolutionary conserved mechanism responsible for the direct transduction of extracellular signals into changes in gene expression in the nucleus. The NOTCH family of receptors is composed of four different proteins, NOTCH1-4, that share a similar structure. NOTCH1 is a transmembrane receptor composed of an extracellular subunit and a transmembrane and intracellular subunit, which interacts via a specialized heterodimerization domain (HD) (Figure 1). The NOTCH1 extracellular subunit contains EGF-like repeats involved in ligand-receptor interaction and 3 LIN-12/NOTCH repeats (LNRs), which stabilize the dimerization domain holding the two NOTCH subunits together in the resting state. The transmembrane-intracellular subunit of NOTCH1 is composed of a short extracellular juxtamembrane peptide followed by a transmembrane sequence and a series of cytoplasmatic domain including a RAM domain, nuclear localization signals (NLS), six ankirin repeat domains, a region rich in glutamine (OPA) and a C-terminal PEST domain, which together function as a ligand-activated transcription factor. Upon interaction with its ligands (Delta-like 1, 3 and 4; and Jagged 1 and 2), NOTCH1 undergoes a conformational change in the LNR repeats-HD domain complex, that leads to the proteolytic cleavage of the transmembrane-intracellular domain of the receptor, first by an ADAM metalloprotease, and subsequently by the γ-secretase complex (Figure 1). This final cleavage releases the intracellular domains of NOTCH1 (ICN1) from the membrane, allowing its translocation to the nucleus where it activates gene expression via association with the CSL DNA binding protein members of mastermind family of coactivators (Figure 1).

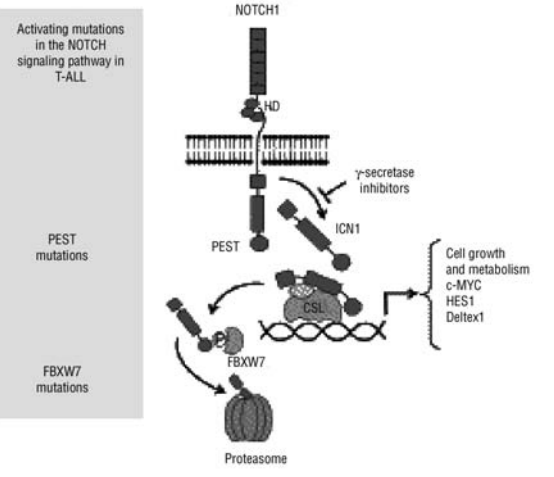

Figure 1. Schematic representation of the NOTCH1 signaling pathway. Upon interaction with its ligands, NOTCH1 undergoes a conformational change that leads to two subsequent proteolytic cleavages of the transmembrane-intracellular domain of the receptor. The second cleavage, mediated by the γ-secretase complex, releases the intracellular domain of NOTCH1 (ICN1) from the membrane. ICN1 then translocates to the nucleus where it associates with CSL and MAML1 to activate the expression of target genes which regulates cell growth and metabolism. The NOTCH1 signaling cascade is terminated by FBXW7/SCF mediated ubiquitination and subsequent proteasomal degradation of ICN1.

In the hematopoietic system, the NOTCH1 signaling pathway is strictly required for the commitment of multipotent hematopoietic progenitors to the T-cell lineage and to support cell growth, proliferation and survival at multiple stages of thymocyte development. Thus, NOTCH1 is involved in the progression through the DN1, DN2 and DN3 stages of thymocyte development,[5] in the regulation of TCRβ rearrangement[6] and in the lineage decision versus αβ or γδ T-cell lineages.[7] Oncogenic NOTCH1 signaling overrides these physiological roles of *NOTCH1* in T-cell development and promotes T-cell transformation. *NOTCH1* mutations are typically located in the HD and PEST domains of the receptor. *NOTCH1* mutations localized in the HD domain result in ligand-independent activation, while mutations located in the C-terminal PEST domain cause increased ICN1 stability

and aberrantly prolonged NOTCH1 activation. Recently, a new class of activating lesions in *NOTCH1* known as juxtamembrane expansion (JME) mutations have been described in 3.3% of primary T-ALL samples.[8] Notably, JME mutations result in very high levels of NOTCH1 signaling and may represent a unique mechanism of NOTCH1 activation. In addition, 20% of T-ALL cases show activation of NOTCH1 via mutations in the FBXW7 gene, which encodes a critical component of the ubiquitin ligase targeting ICN1 for degradation.[9-11] *FBXW7* induces ICN1 inactivation via binding to a phosphothreonin centered degron present in the PEST domain of NOTCH1 and targeting activated NOTCH1 to the proteasome (Figure 1). Thus *FBXW7* mutations are mechanistically related to NOTCH1 PEST mutations as they result in increased ICN1 protein stability. However, *FBXW7* mutations may also be associated with additional oncogenic functions as this F-box protein is also involved in the degradation of other important oncoproteins such as MYC, JUN and cyclin E. In about 25% of T-ALL cases HD mutations are associated with PEST or *FBXW7* mutations so that these leukemias have a dual mechanism of NOTCH1 activation that combines ligand independent activation and prolonged ICN1 stability.[4,11]

Recent progress in the identification of the transcriptional regulatory networks downstream of NOTCH1 has shown a close relationship between oncogenic NOTCH1 signaling and the transcriptional control of cell growth and metabolism.[12,13] Thus, NOTCH1 directly controls multiple genes involved in anabolic pathways and further promotes cell growth via direct transcriptional upregulation of *MYC*.[13-15] Importantly, small molecule inhibitors of the γ-secretase complex (GSIs) effectively abrogate NOTCH1 signaling, making NOTCH1 a promising therapeutic target for the treatment of T-ALL. Consistently, treatment of T-ALL cell lines harboring activating mutations in *NOTCH1* with CompE, a highly active GSI, induces cell cycle arrest in G1 and decrease rate of anabolic cell growth which results in a gradual reduction in cell size.[4,13,16] However, the antileukemic effects of GSIs in human T-ALL cell lines are primarily cytostatic, with minimal or no apoptosis which hampers their therapeutic efficacy.[13] Moreover, only a fraction of the T-ALL cell lines harboring mutations in *NOTCH1* respond with cell cycle arrest, suggesting that primary resistance to GSI therapy may be readily present in a significant fraction of T-ALLs.[16]

Molecular analysis of the response to GSI therapy showed no difference between GSI sensitive and GSI resistant T-ALL cell lines in terms of ICN1 clearance and transcriptional response to GSI therapy.[16] These results suggest that GSI resistance is not due to drug metabolism, increased drug export or decreased capability of the GSIs to interact with the γ-secretase complex. Instead, these findings support the hypothesis that activation of an alternative oncogenic pathway may bypass NOTCH1 signaling in GSI-resistant T-ALL cells rendering these tumors insensitive to NOTCH1 inhibition. Gene expression profiling and mutational analysis comparing GSI-sensitive and GSI-resistant T-ALL cell lines demonstrated mutational loss of PTEN in a broad panel of GSI-resistant T-ALL cell lines. Notably, PTEN functions as a critical negative regulator of the PI3K-AKT pathway and mutational loss of PTEN is associated with constitutively active AKT signaling which promotes increased cell growth.[16]

Further analysis demonstrated a mechanistic link between NOTCH1 and the PI3K-AKT pathway in normal T-cell development and T-ALL. NOTCH1 negatively regulates the expression of PTEN via upregulation of HES1, a transcriptional repressor, and facili-

tates the activation of the PI3K-AKT pathway in thymocyte progenitors and T-ALL cells.[16] In this context, *PTEN* mutations uncouple NOTCH1 and PI3K-AKT signaling by inducing constitutive activation of AKT which triggers NOTCH1 independent cell growth and confers resistance to GSI therapy. Importantly, PTEN loss is present in 17% of primary T-ALL samples suggesting that primary resistance to GSI therapy can be present in a significant fraction of T-ALL patients at diagnosis.[16] Drugs targeting the PI3K-AKT pathway such as mTOR and AKT inhibitors may be selectively active against tumors with PTEN loss and may represent an alternative therapeutic strategy for GSI-resistant T-ALLs.[16]

One of the most important obstacles in the clinical development of anti-NOTCH1 therapies has been the development of gastrointestinal toxicity secondary to inhibition of NOTCH signaling in the gut. Early studies in animal models showed that inhibition of NOTCH signaling in the intestinal epithelium results in secretory cell metaplasia characterized by increased numbers of goblet cells and a marked block in proliferation.[17] Consistent with these observations, T-ALL patients treated with the MK-0752 GSI showed a high incidence of gastrointestinal toxicity most probably associated with inhibition of NOTCH signaling in the gut.[18]

In a recent report, Real et al. have shown that inhibition of NOTCH signaling in the gut induces goblet cell metaplasia via upregulation of KLF4, a transcription factor responsible for goblet cell differentiation and an inhibitor of cell cycle progression in the intestinal epithelium.[19] Thus, KLF4 inhibitors may represent a strategy to ameliorate GSI induced gut toxicity. However, targeting KLF4 may prove difficult given the tumor suppressor role of this transcription factor.

A more feasible and direct strategy towards an effective and less toxic application of GSIs in the clinic is the use of these drugs in combination with other antileukemic agents. Following this rationale, Real *et al*.[19] tested the effects of combining a GSI with core drugs used in the treatment of T-ALL and showed that inhibition of NOTCH1 signaling can effectively reverse glucocorticoid resistance in T-ALL.[19] Moreover, using a xenograft leukemia model, these authors demonstrated that the treatment with DBZ, a highly active GSI, plus dexamethasone in combination resulted in a curative response in otherwise glucocorticoid resistant tumors and had a protective effect against GSI-induced gut toxicity. These results show that combination therapies with GSIs plus glucocorticoids may be effective against glucocorticoid-resistant T-ALL, uncover a critical role for glucocorticoids in the homeostasis of the intestinal epithelium and suggest that glucocorticoid cotreatment may protect T-ALL patients from the intestinal toxicity typically associated with anti-NOTCH1 therapies.

Final remarks

GSIs are the first family of drugs targeting NOTCH1 signaling in T-ALL. However, new developments may result in more specific and highly active NOTCH inhibitors for the treatment of this disease in the near future. A recent report by Li and coworkers shows an antibody based approach for the inhibition of NOTCH3; in particular, they used anti-NOTCH3 inhibitory antibodies that bind to the HD-LNR repeat complex and block the processing and activation of NOTCH3 receptor [20]. One can envision how a similar approach could be adopted for the inhibition of NOTCH1 signaling and how, should these antibodies recognize the mutant forms of NOTCH1, they may constitute new highly attractive drugs for the treatment of T-ALL.

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