Collaborating Pathways that Functionally Amplify NOTCH1 Signals in T-Cell Acute Lymphoblastic Leukemia

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INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 15% of pediatric and 25% of adult ALL. With cure rates of ~80% in children and ~40% in adults, there is an urgent need to identify and understand the signaling pathways in T-ALL in order to develop the first targeted therapies for this cancer. The most prevalent oncogene is NOTCH1, which is mutated in about 60% of patient samples [1]. Normally, Notch is restrained by the heterodimerization domain (HD, Figure 1). Notch becomes activated once it engages ligand. γ-secretase cleaves Notch, thus releasing the intracellular domain of Notch (ICN). ICN binds Rbpj and Maml to activate transcription. ICN has a short half-life as phosphodegron motifs in its PEST domain are recognized by the ubiquitin ligase Fbxw7, which targets it for destruction [2,3]. PEST domain deletions improve ICN stability [4]. Mutations in the HD domain trigger ligand-independent activation [5,6]. Target genes of NOTCH1 that drive leukemia include MYC [7-9], HES1 [10], IGF-1R [11], TRIB2 [12], and IL7R [13]. Notch also activates the PI3K/Akt /mTor pathway [11,14,15].

Collaborative pathways confer resistance to NOTCH inhibitors

γ-secretase inhibitors (GSIs) block Notch signaling (Figure 1). GSIs are being tested in clinical trials in T-ALL and other NOTCH-driven cancers [16-18]. Initial reports show promising activity. However, resistance is an emerging problem. Approximately two-thirds of human T-ALL cell lines are resistant to GSI [1]. GSI depletes ICN in resistant cells just as it does in sensitive cells [7,15]. Thus, cancers resist GSI by activating collaborating pathways to bypass the effects of NOTCH1 inhibition. We previously showed that NOTCH1 mutations are moderate oncogenes in mouse models [19]. Collaborating oncogenic networks are essential to functionally enhance NOTCH1 signaling to leukemogenic levels. These findings have shifted the field toward identifying pathways that collaborate with the NOTCH pathway.

Collaborators that act as NOTCH1 co activators

A recent mass spectrometry screen identified coactivators that physically interact with the NOTCH1 complex such as the SWI/SNF remodeling complex PBAF, AF4p12, and the histone demethylases LSD1 and PHF8 [20]. These coactivators supported transactivation of classical NOTCH1 target genes and leukemic growth. Also identified were master regulatory transcription factors IKAROS, HEB, BCL11B, and RUNX1. Ikaros proteins antagonize Notch-transcriptional activity. Dominant-negative
IKAROS isoforms have been reported in human T-ALL [21,22] and collaborate with NOTCH1 in mouse models [23,24]. Runx sites have been imputed adjacent to Rbpj sites [25]. Although Runx1 has been reported to act as a tumor suppressor [26-28], a recent abstract suggests that Runx factors and NOTCH1 coordinately regulate oncogenic targets to drive proliferation in the majority of cases [29]. In addition, more than 90% of NOTCH1/Rbpj sites are co-bound with MYC, such as IL7R [30,31]. Although it has not been definitively tested whether these factors functionally collaborate with NOTCH1 in leukemogenesis, retroviral insertional mutagenesis screens suggest that Myc and Runx1 can accelerate NOTCH1-induced leukemia in mice [32-34].

Collaborators that intersect with the NOTCH1 pathway

Inactivating FBXW7 mutations occur in approximately 20% of human T-ALL cases [2,3]. Besides Notch1, Fbxw7 degrades other cellular substrates such as Myc [35,36], Mcl1 [37,38], and mTOR [39]. In T-ALL, FBXW7 mutations are mutually exclusive with PEST mutations, suggesting that they amplify NOTCH1 signals by improving ICN stability. FBXW7 mutations contribute to GSI resistance in cell lines likely by maintaining MYC protein levels despite loss of ICN. However, although MYC can rescue most human T-ALL cell lines treated with GSI, it cannot rescue all of them [7]. TAL1/SCL is a class II basic helix-loop-helix transcription factor that is over expressed in ~60% of human T-ALL cases [40]. TAL1 and NOTCH1 collaborate in mouse models [41]. TAL1 may amplify the NOTCH1 pathway in part by down regulating FBXW7 through miR-223 [42] and by directly inducing the NOTCH1 target gene TRIB2 [43]. However, TAL1 does not appear to contribute to GSI resistance. Finally, PTEN mutations occur in about 10% of human T-ALL cases [44]. PTEN mutations are thought to amplify NOTCH1 signals through the PI3K/AKT/mTOR pathway. Activation of PI3K/AKT/mTOR was shown to rescue the proliferation of some human T-ALL cell lines treated with GSI [45].

Collaborators with unknown mechanisms of interaction with the NOTCH1 pathway

Several pathways collaborate with NOTCH1 through unclear mechanisms. These pathways have typically been identified through mouse models of human T-ALL [46]. An example is the HOX family transcription factor TLX1 [47,48]. To determine if the NOTCH1-TLX1 collaboration was targetable, we developed a TLX1-initiated T-ALL mouse model in which the expression of TLX1 was repressed by doxycycline [48]. We treated these tumors with doxycycline, GSI, or both doxycycline and GSI [Figure 2A]. Mice receiving combined TLX1 and NOTCH1 suppression had the best response [Figure 2B-C]. Our work suggests that targeting collaborator proteins can improve the efficacy of anti-NOTCH therapy. Retroviral or transposon-mediated insertional mutagenesis screens in mice have been particularly effective in identifying collaborative pathways. The NOTCH1 locus is a frequent common insertion site. These insertions frequently lead to insertions in putative collaborators such as Rasgrf1, Lfng, Akt2, Erg, and Zmiz1 [34,49]. Zmiz1 is a co activator that is similar to Protein Inhibitor of Activated STAT (PIAS) family members. Our laboratory recently validated Zmiz1 as a NOTCH1 collaborator. Ectopic ZMIZ1 and leukemia-associated NOTCH1 alleles collaborated to induce T-ALL in mice [50]. ZMIZ1 and activated NOTCH1 were co-expressed in ~20% of patients across diverse oncogenic subsets [50]. Inhibition of ZMIZ1 in T-ALL cell lines slowed proliferation and overcame resistance to NOTCH inhibitors [50]. We identified MYC and IL7R as critical downstream target genes. However, the mechanism by which Zmiz1 interacts with the Notch pathway remains unclear.

CONCLUSION

Recent studies have revealed several collaborative pathways that functionally interact with the NOTCH1 pathway. As a result, clinical trials are underway combining GSI with agents that target collaborative pathways such as mTOR inhibitors [51,52]. However, none of the known collaborative pathways have been found to be sufficient to confer resistance [45,53]. The mechanism of many of these pathways remains elusive. Thus,
there is a critical need to identify and understand the signaling networks that functionally amplify NOTCH1 signals. Therapeutic agents that target these networks will be required to increase the effectiveness of T-ALL therapy, including NOTCH inhibitors. In the absence of these agents, advancing the treatment of T-ALL will be difficult.

REFERENCES


