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The role of the PTEN/AKT Pathway in NOTCH1-induced leukemia

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Abstract

Activating mutations in *NOTCH1* are the most prominent genetic abnormality in T-cell acute Lymphoblastic Leukemia (T-ALL) and inhibition of NOTCH1 signaling with γ-secretase inhibitors (GSIs) has been proposed as targeted therapy in this disease. However, most T-ALL cell lines with mutations in *NOTCH1* fail to respond to GSI therapy. Using gene expression profiling and mutation analysis we showed that mutational loss of *PTEN* is a common event in T-ALL and is associated with resistance to NOTCH inhibition. Furthermore, our studies revealed that NOTCH1 induces upregulation of the PI3K-AKT pathway via HES1, which negatively controls the expression of *PTEN*. This regulatory circuitry is evolutionary conserved from *Drosophila* to humans as demonstrated by the interaction of overexpression of *Delta* and *Akt* in a model of Notch-induced transformation in the fly eye. Loss of PTEN and constitutive activation of AKT in T-ALL induce increased glucose metabolism and bypass the requirement of NOTCH1 signaling to sustain cell growth. Importantly, PTEN-null/GSI resistant T-ALL cells switch their oncogene addiction from NOTCH1 to AKT and are highly sensitive to AKT inhibitors. These results should facilitate the development of molecular therapies targeting NOTCH1 and AKT for the treatment of T-ALL.

Keywords

T-cell lymphoblastic leukemia; NOTCH1; PTEN; AKT; γ-secretase inhibitor; oncogene addiction

Targeting NOTCH1 signaling in T-ALL

T-lineage acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic cancer that accounts for 10% to 15% of pediatric and 25% of adult ALL cases. [1, 2] Despite recent progress in the treatment of this disease the prognosis of T-ALL patients with primary resistant or relapsed disease is very poor, underscoring the need to develop more effective antileukemic drugs. [3-5]

The identification in the last years of aberrant activation of NOTCH1 signaling in the majority of T-ALL cases and the feasibility to inhibit the activation of NOTCH receptors with small molecule inhibitors of the gamma-secretase complex have created the opportunity to develop molecular therapies targeting the NOTCH1 signaling pathway in this disease. [6, 7]

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NOTCH signaling plays a critical role in lineage specification decisions by which equivalent multipotential precursor cells become committed to specific cell lineages during development, and has important roles in cellular differentiation, proliferation and apoptosis. [6-9] The fundamental components of the NOTCH pathway are the DSL ligands (Delta-like 1, 3 and 4; and Jagged 1 and 2), the NOTCH receptors (NOTCH1, 2, 3 and 4), and the CSL DNA binding protein, a transcription factor which mediates the conversion of NOTCH activating signals in the cell surface into changes in gene expression in the nucleus.

Both the DSL ligands and the NOTCH receptors are type I transmembrane proteins, and activation of the NOTCH signaling pathway is triggered by the interaction of a NOTCH receptor in one cell with a DSL ligand expressed in the surface of a neighboring cell. This ligand-receptor interaction induces two consecutive proteolytic cleavages, first by an ADAM metalloprotease at the cell surface and subsequently by the γ-secretase complex, which catalyzes the final cleavage step in the transmembrane domain of the receptor. After γ-secretase cleavage the resulting activated form of NOTCH (ICN1) rapidly translocates to the nucleus where it interacts with the CSL DNA binding protein. Binding of ICN1 to CSL recruits the MAML1 coactivator to the complex and induces the transcriptional activation of NOTCH-CSL target genes. [6, 7, 10]

The NOTCH signaling pathway plays a critical role in the hematopoietic system at multiple stages of T-cell development. During early hematopoiesis NOTCH signaling is required for the commitment of multipotent hematopoietic progenitors to the T-cell lineage. [2, 11-13] Immunodeficient mice reconstituted with bone marrow progenitors expressing a constitutively active form of Notch1 show ectopic T-cell development in the bone marrow and, in contrast with mice reconstituted with normal progenitor cells, fail to produce B lymphocytes. [2] Conversely, mice harboring a conditional deletion of *Notch1* in hematopoietic progenitors fail to develop T-cells, and show ectopic B-cell development in the thymus. [11]

In addition to this early role in T-cell lineage commitment, NOTCH signaling participates, later on, in essential processes at multiple stages of thymocyte development. [14] Thus, NOTCH1 activity is required for lineage progression through the early DN1, DN2 and DN3 stages of thymocyte development; [15] participates in the regulation of T-cell receptor loci; [16] and regulates lineage decisions between αβ vs. γδ lineages. [17]

The first evidence linking aberrant NOTCH1 signaling to the pathogenesis of T-ALL came from the characterization of the t(7;9)(q34;q34.3) translocation, a rare recurrent chromosomal rearrangement present in less than 1% of human T-ALL cases. This translocation juxtaposes a truncated NOTCH1 gene next to the T-cell receptor B (*TCRB*) locus leading to the aberrant expression of an intracellular constitutively active form of NOTCH1.[18] Following this initial finding, the causative role of activated NOTCH1 in the pathogenesis of T-ALL was further demonstrated by animal models of NOTCH1 induced leukemia. Thus, mice reconstituted with hematopoietic progenitor cells transduced with viruses driving the expression of constitutively active forms of NOTCH1 develop T-cell tumors, [19] and transgenic mice expressing dominant active forms of NOTCH1 in hematopoietic progenitor cells or in immature thymocytes developed T-cell leukemias. [19, 20]

However, the role of *NOTCH1* in the pathogenesis of human T-ALL was not fully realized until the identification of activating mutations in *NOTCH1* in the majority of T-ALL patient samples.[21] Activating mutations in NOTCH1 result in ligand independent activation of the receptor or increased ICN1 protein stability in over 50% of T-ALLs.[21, 22][23][24, 25] Importantly, small molecule inhibitors of the γ-secretase complex (GSIs) effectively abrogate the function of the receptor encoded by these oncogenic *NOTCH1* alleles, making NOTCH1 a promising therapeutic target for the treatment of T-ALL. Initial studies treating human T-

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ALL cell lines harboring activating mutations in *NOTCH1* with GSIs demonstrated that inhibition of NOTCH signaling induces cell cycle arrest.[21,26,27] However, these same studies also demonstrated that despite high prevalence of *NOTCH1* mutations and the presence of high levels of ICN1 in these tumors, the majority of T-ALL cell lines failed to respond to NOTCH1 inhibition, suggesting that primary resistance to GSI treatment was readily present in the majority of these cell lines.[21]

Numerous mechanisms have been identified in the resistance of cancer cells to chemotherapy and molecularly targeted drugs. [28-30] General mechanisms of drug resistance typically decrease the effective intracellular concentration of the drug due to decreased drug uptake, increased drug export or drug metabolism. In the case of molecularly targeted drugs, resistance typically results from mutations that block the interaction of the drug with its specific molecular target. A common feature in all these mechanisms is that defective cellular responses are the result of persistent biochemical activity of the molecules and pathways that mediate drug response. Thus, the initial experiments aimed to identify the mechanisms of resistance to GSIs focused on the analysis of NOTCH1 activity. Surprisingly, these studies revealed that GSI treatment effectively blocked γ-secretase activity and as a result, induced decreased levels of ICN1 and transcriptional downregulation of NOTCH1 target genes in GSI-resistant T-ALL cell lines.[31] This observation indicated that the lack of response to GSIs occurs despite effective NOTCH1 inhibition, and pointed to the existence of alternative pathways promoting cell growth in the absence of NOTCH1 signaling in these tumors. Thus, we postulated that understanding of the mechanisms of sensitivity and resistance to GSIs in T-ALL required the identification of the key downstream target genes and pathways responsible for NOTCH1 induced transformation.

Microarray gene expression studies and ChIP-on-chip analysis have recently identified a major role of NOTCH1 in the regulation of cell growth and metabolism.[32] Thus, NOTCH1 inhibition with a GSI drives a gene expression signature dominated by the downregulation of genes involved in anabolic pathways such as ribosome biosynthesis, protein translation, RNA synthesis and nucleotide metabolism in T-ALL.[32-35] Notably, NOTCH1 also controls the expression of *MYC*, which in turn is also a broad transcriptional activator of anabolic genes and pathways involved in growth and metabolism.[32] This *NOTCH1-MYC* transcriptional network constitutes a feed-forward-loop circuit that confers increased robustness to the transcriptional regulation of cell growth downstream of NOTCH1 signaling. In addition, this model couples cell fate and developmental signals upstream of NOTCH1 with the activation of pathways promoting cell proliferation upstream of *MYC*, and establishes a fundamental relationship between *NOTCH1* and *MYC* in T-cell transformation. However, this *NOTCH1-MYC* feed forward loop regulatory network constitutes only a fraction of the transcriptional network that mediates the oncogenic and developmental effects of NOTCH1 signaling in T-cells. In this context, we hypothesized that aberrant activation of effector signaling pathways controlling cell growth and metabolism downstream of NOTCH1 could uncouple NOTCH1 signaling and cell proliferation rendering leukemic T-cells resistant to NOTCH inhibition with GSIs.

PTEN mutations confer resistance to GSI in T-ALL

To identify possible mechanisms of resistance to NOTCH1 inhibition in T-ALL, we analyzed global gene expression signatures associated with GSI sensitivity or resistance in a panel of leukemia cell lines using oligonucleotide microarrays. This analysis identified decreased levels of the *PTEN* tumor suppressor gene as the most consistent transcriptional feature associated with GSI-resistant cell lines.[31] Notably, Western blot and mutation analysis showed total absence or marked decrease in PTEN protein levels and the presence of biallelic loss of function mutations in *PTEN* in GSI-resistant T-ALL cells, respectively. In contrast, all GSI-sensitive

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cell lines analyzed expressed wild type *PTEN* transcripts and had readily detectable levels of PTEN protein.

The PTEN tumor suppressor gene encodes a lipid phosphatase responsible for the degradation of phosphatidylinositol triphosphate (PIP3) and the inhibition of the PI3K-AKT signaling pathway, which promotes cell growth, increased glucose uptake and oxidation, cell cycle progression and cell survival through multiple direct and indirect mechanisms. [36-41] The PI3K-AKT pathway is activated upon generation of PIP3 following the engagement of growth factor receptors at the plasma membrane and the subsequent activation of class I phosphatidylinositol 3-kinases (PI3Ks). The accumulation of PIP3 at the membrane recruits the AKT kinase inducing its phosphorylation and activation by the PDK1 and the mTOR-Rictor kinases. [42, 43]

Consistent with the presence of loss of function mutations in *PTEN*, all GSI-resistant T-ALL cell lines analyzed showed increased levels of AKT phosphorylation, indicative of constitutive PI3K-AKT activation. These results pointed to a close relationship between the PI3K-AKT signaling pathway, and GSI resistance.

Homozygous and heterozygous somatic mutations in *PTEN* have been described in a very broad range of human cancers. [44-46] However, *PTEN* mutations had only been reported sporadically in leukemias and lymphomas. [47-50] Thus, to determine whether our discovery of PTEN loss in GSI-resistant cell lines might also be relevant to primary human cancers, we examined the status of PTEN by immunohistochemistry and flow cytometry in T-cell lymphoblastic leukemia and lymphoma (T-ALL/LL) clinical samples. These analyses demonstrated complete loss of the PTEN protein in 17% of T-ALL/LL cases at diagnosis. In addition, mutation analysis demonstrated the presence of *PTEN* mutations in 8% of T-ALL samples. Consistent with the role of PTEN in the regulation of the PI3K-AKT signaling pathway, immunohistochemistry analysis showed increased levels of phosphoAKT in T-cell lymphoblastic tumors with loss of PTEN. Notably, the analysis of paired diagnostic and relapsed tumor samples showed patients with loss of PTEN at relapse indicating that PTEN loss can be associated with tumor progression in T-ALL. Overall these results demonstrate that PTEN loss and constitutive activation of PI3K-AKT signaling is a frequent event in T-cell lymphoblastic leukemias and lymphomas and is associated with resistance to NOTCH1 inhibition with GSIs.

These results led us to experimentally test the hypothesis that loss of *PTEN* and activation of AKT plays a causative role in resistance to GSIs. To address this question we first expressed a constitutively active form of AKT (MYR-AKT) in GSI-sensitive/PTEN-positive cells and showed that AKT activation is sufficient to rescue the reduction in cell growth induced by NOTCH1 inhibition with a GSI. Similarly, shRNA knock-down of *PTEN* in GSI-sensitive/PTEN-positive cells induced resistance to GSI treatment. These results mechanistically linked the loss of PTEN and constitutive activation of AKT with resistance to NOTCH1 inhibition in human T-ALL cell lines.

Two alternative models emerged from these results. First, it is possible that NOTCH1 and PI3K-AKT signaling are parallel pathways controlling cell growth in leukemic lymphoblasts and that constitutive activation of AKT in PTEN-null cells provides alternative trophic signals that sustain cell growth upon inhibition of NOTCH1 signaling with GSIs. Alternatively, it is possible that NOTCH1 and PI3K-AKT signaling are closely interlinked in the control of cell growth and proliferation during normal T-cell development and that constitutive activation of NOTCH1 signaling promotes cell growth in part through PI3K-AKT. According to the latter model, mutational loss of *PTEN* would bypass the requirement of NOTCH1 to sustain cell growth.

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NOTCH1 regulates *PTEN* expression and the activity of the PI3K-AKT pathway via HES1 and MYC

Although the induction of resistance to NOTCH1 inhibition by constitutive activation of the AKT pathway does not strictly require a functional connection between NOTCH and the PI3K-AKT pathways, the effects of GSI treatment in T-ALL cells closely resembles the growth defect induced by nutrient deprivation, cytokine withdrawal and inhibition of the PI3K pathway. In addition, NOTCH1 activation has recently been shown to be essential to sustain AKT activation and glucose metabolism during thymocyte development.[51] These observations, together with the close association between the presence of *PTEN* mutations and GSI resistance led us to hypothesize that PTEN might be functionally linked to NOTCH1 signaling in T-ALL.

To test this possibility we analyzed the effects of NOTCH1 inhibition with a GSI in the PI3K-AKT pathway in PTEN-positive/GSI-sensitive T-ALL cells. These studies showed that blocking the activity of oncogenic NOTCH1 was followed by transcriptional upregulation of *PTEN*, increased PTEN protein levels and decreased activity of the PI3K-AKT pathway as shown by decreased phosphorylation of AKT and AKT targets. Furthermore, analysis of NOTCH1 inhibition in primary T-cell progenitors by removal of the NOTCH ligand Delta-like 1 results in downregulation of NOTCH1 target and induces transcriptional upregulation of *Pten*. These results demonstrate that NOTCH1 signaling regulates the PI3K-AKT pathway in human leukemic cells expressing mutant NOTCH1 receptors and also in primary mouse thymocytes expressing wild type NOTCH1. In addition, the effects of NOTCH signaling on *PTEN* expression are evidenced by pharmacologic inhibition of NOTCH signaling with a GSI and also upon withdrawal of NOTCH signals by removal of the stimulus provided by NOTCH ligands.

How does NOTCH signaling regulate the expression of *PTEN*? Given the well-established role of NOTCH1 as a transcriptional activator, we proposed that downregulation of *PTEN* transcripts by NOTCH1 signaling could be mediated by a transcriptional repressor downstream of NOTCH1.

Among the numerous signaling molecules and transcriptional factors controlled by NOTCH1 we selected *HES1*, as a possible mediator of the effects of NOTCH1 on *PTEN* expression. HES1 is a transcriptional repressor directly controlled by NOTCH1 [52] and has been shown to mediate important NOTCH1 functions in T-cell development [53]. In addition, analysis of *PTEN* regulatory sequences across different species demonstrated the presence of a conserved MYC-MAX canonical sequence in the *PTEN* proximal promoter, suggesting that NOTCH1 could regulate *PTEN* expression through MYC. ChIP-on-chip analysis of HES1 and MYC showed that both factors bind to the *PTEN* promoter in T-ALL cells. Notably, *HES1* induced a marked reduction in the activity of the *PTEN* promoter in reporter assays, while MYC expression induced a moderate increase in the *PTEN* promoter activity, which was effectively abrogated by HES1 expression. Thus transcriptional regulation of *PTEN* downstream of NOTCH1 seems to be controlled by a dual input consisting of negative signals from HES1 and positive signals by MYC. The balance between these two factors and the dominant effect of HES1 over MYC in the activity of the *PTEN* promoter is consistent with our observation of a moderate and slow upregulation of *PTEN* upon NOTCH1 inhibition and illustrates the essential role of combinatorial transcriptional regulatory systems to tune the kinetics and intensity of potentially oncogenic pathways controlling cell growth. Thus, the combined action of HES1 and MYC downstream of NOTCH1 in T-cell progenitors increases the activity of the PI3K-AKT signaling pathway in response to extracellular stimuli and promotes cell growth without inducing full oncogenic activation of AKT. In contrast, mutational loss of *PTEN* eliminates this transcriptional regulatory node, uncouples the PI3K-AKT pathway from extracellular

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signals, induces oncogenic levels of AKT, and bypasses the requirement of NOTCH1 signaling to maintain cell growth in T-ALL.

NOTCH1 and the PI3K-AKT pathways regulate glucose metabolism

The PI3K-AKT pathway controls numerous cellular functions associated with cell growth and regulates glucose metabolism at multiple levels [36, 54]. First, AKT activation promotes glucose uptake by the GLUT family of glucose transporters [55-58] and activates glycolysis by hexokinase and phosphofructokinase [59, 60]. Additionally, AKT inhibition of glycogen synthase kinase1 promotes glycogen synthesis [61] and blocks gluconeogenesis [58]. Most importantly, constitutive AKT signaling results in constitutive activation of aerobic glycolysis in tumor cells, a phenomenon known as the Warburg effect. [62, 63] The potential relevance of glucose metabolism in the sensitivity and resistance to NOTCH1 inhibition in T-ALL is highlighted by the prominent reduction in cell size of PTEN-positive/GSI-sensitive T-ALL cells treated with GSI and by evidence of a major role of NOTCH signaling in promoting glucose metabolism in developing thymocytes.[51]

Analysis of glucose metabolism in T-ALL cells showed that PTEN-null/GSI-resistant cells have higher levels of glucose uptake and glucose oxidation compared with PTEN-positive/GSI-sensitive cell lines. Furthermore, GSI treatment of PTEN-positive/GSI sensitive cells induced further reduction in glucose uptake and glucose oxidation, which were unaltered upon inhibition of NOTCH1 signaling in PTEN-null/GSI-resistant cells. Finally, treatment with methyl pyruvate, a membrane permeable metabolite that bypasses glycolysis and directly feeds the Krebs cycle, rescues the defects in cell size and cell cycle progression induced by GSI treatment in PTEN-positive/GSI-sensitive T-ALL cells (P. Real and A. Ferrando unpublished results). Overall, these results show a fundamental role for glucose metabolism in the maintenance of cell growth in T-ALL and mechanistically link the antitumor effects of GSIs with the inhibition of glucose uptake and oxidation in leukemic lymphoblasts.

Conservation of the NOTCH-AKT interplay in *Drosophila*

The growth and morphogenesis of the *Drosophila* eye depends on the activation of the Notch receptor by its ligands Delta and Serrate/Jagged in a conserved dorsal-ventral organizer, a specialized region of the eye imaginal disc that controls the growth and organization of the fly eye during development [64-66]. Ubiquitous overexpression of Delta in the developing fly eye induces a mild overgrowth which has been exploited to identify genes that interact the Delta-Notch pathway and induce tumors using forward genetic screens [64, 67]. In this screen, gain-of-expression mutations induced at random in the fly genome using the Gene Search (GS) system were combined with a gain of expression of *Delta* to isolate genetic alterations that synergize with Notch hyperactivation and induce an overt tumor phenotype [67].

A remarkable finding in this screen was the identification of a gain-of-expression mutation within the *Akt* locus that induced a tumor invasion phenotype when combined with *Delta* overexpression. Flies from this *Akt* mutant line show no spontaneous tumor phenotype. In contrast, when crossed with the *Delta* expressing line, activation of *Akt1* induces massive tumor overgrowths with a 100% penetrance and distal metastasis in 8% of the insects. Similarly, overexpression of the PI3K homolog (*Dp110*) with *Delta* also induced tumors showing that activation of Notch and PI3K-Akt have synergistic effects in tumor development in this model. A requirement of *Akt* for Notch-induced growth in non-tumoral conditions was also demonstrated by experiments in which Notch induced overgrowth was completely abrogated by inhibiting the PI3K/AKT pathway through overexpression of *Pten*. In addition, we showed using a null *Akt* allele (analyzed in mosaic patches of mutant cells), that eye overgrowth induced by expression of *Delta* is almost totally prevented in cells null for *Akt*. Finally, we showed also that, misexpression of the Notch modulator fringe induces a small eye phenotype, which can

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be rescued by overexpression of *Akt*. Thus Akt activation is necessary for NOTCH induced growth and also sufficient to restore cell growth upon inhibition of Notch signaling, an outcome that perfectly recapitulates the results obtained in the analysis of leukemic cells and shows that the functional interaction between Notch and AKT is evolutionary conserved.

Oncogene addiction

The model that emerges from these results places *NOTCH1* at the center of a transcriptional regulatory network controlling cell growth in T-ALL. Thus, NOTCH1 directly controls the expression of a broad number of genes involved in cell anabolic pathways, an effect that is further enhanced by direct transcriptional upregulation of the *MYC* oncogene and indirect transcriptional downregulation of the *PTEN* tumor suppressor gene via HES1. Given the redundancy of mechanisms promoting cell growth downstream of NOTCH1, it will not be surprising that the identification of additional edges and nodes in this network shows new effector genes and pathways involved in T-cell transformation. Nevertheless, the basic observation that PTEN loss is coupled with fundamental metabolic changes induced by constitutive activation of AKT suggests that GSI resistance may require significant rewiring of the metabolic machinery of these cells. Thus, we hypothesized that GSI resistance could occur at the expense of making PTEN-null T-ALL cells addicted to constitutive AKT signaling. In agreement with this model, retroviral expression of *PTEN*-induced decreased cell size and cell cycle arrest in PTEN-null/GSI-resistant cells. In addition, treatment of T-ALL cells with a phosphatidylinositol analog inhibitor of AKT demonstrated that GSI-resistant/PTEN-null T-ALL cells are significantly more sensitive to AKT inhibition than GSI-sensitive/PTEN-positive cells. These results suggest that, indeed, aberrant activation of the PI3K-AKT pathway makes PTEN-null T-ALL cells dependent on high levels of AKT signaling in a clear example of secondary oncogene addiction.

Concluding remarks and future directions

The introduction of highly intensive therapy has led to significant progress in the treatment of childhood leukemias over the last decades. However, 20 % of pediatric and 50% of adult T-ALL cases still relapse and ultimately die because of refractory disease. Furthermore, the intensified therapies used in the treatment of T-ALL are frequently associated with life-threatening and debilitating toxicities adding significant acute and chronic morbidity to this disease. In this context, the identification of activating mutations in *NOTCH1* present in over 50% of T-ALL patients at diagnosis [21] has brought enormous interest for the development of molecularly tailored therapies in T-ALL and prompted the initiation of a clinical trial to test the effectiveness of blocking NOTCH1 signaling with a small molecule GSI in this disease. Furthermore, a growing body of literature supports a role for aberrant NOTCH signaling in the pathogenesis of breast cancer, medulloblastoma, lung cancer, colon carcinomas, multiple myeloma and pancreatic cancer; [68-83] and GSIs targeting NOTCH signaling are in clinical trials for breast cancer and medulloblastoma. Thus, the successful development of anti NOTCH therapies and overcoming the resistance to NOTCH inhibitors in T-ALL may ultimately have a broad impact in the treatment of numerous human cancers.

Our studies have offered new insights on the role of NOTCH1 signaling in the regulation of the PTEN/AKT pathway and the mechanisms of resistance to GSIs in T-ALL. The identification of *PTEN* mutations as a frequent event in T-ALL highlights the importance of this tumor suppressor gene in the pathogenesis of T-ALL. Furthermore, the association of mutational loss of *PTEN* with resistance to GSIs should be tested prospectively in clinical trials testing the efficacy of GSIs in the treatment of T-ALL. Finally, the increased sensitivity of T-ALL lines resistant to GSI to inhibitors of the AKT pathway should be further studied using

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adequate animal models to analyze the underlying mechanisms and to test potentially new targeted therapies for the treatment of T-ALL.

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Abbreviations

T-ALL, T-cell acute lymphoblastic leukemia; GSI, Gamma Secretase Inhibitor; DSL, DELTA/Serrate/Lag1 ligands; ICN1, Intracellular NOTCH1.

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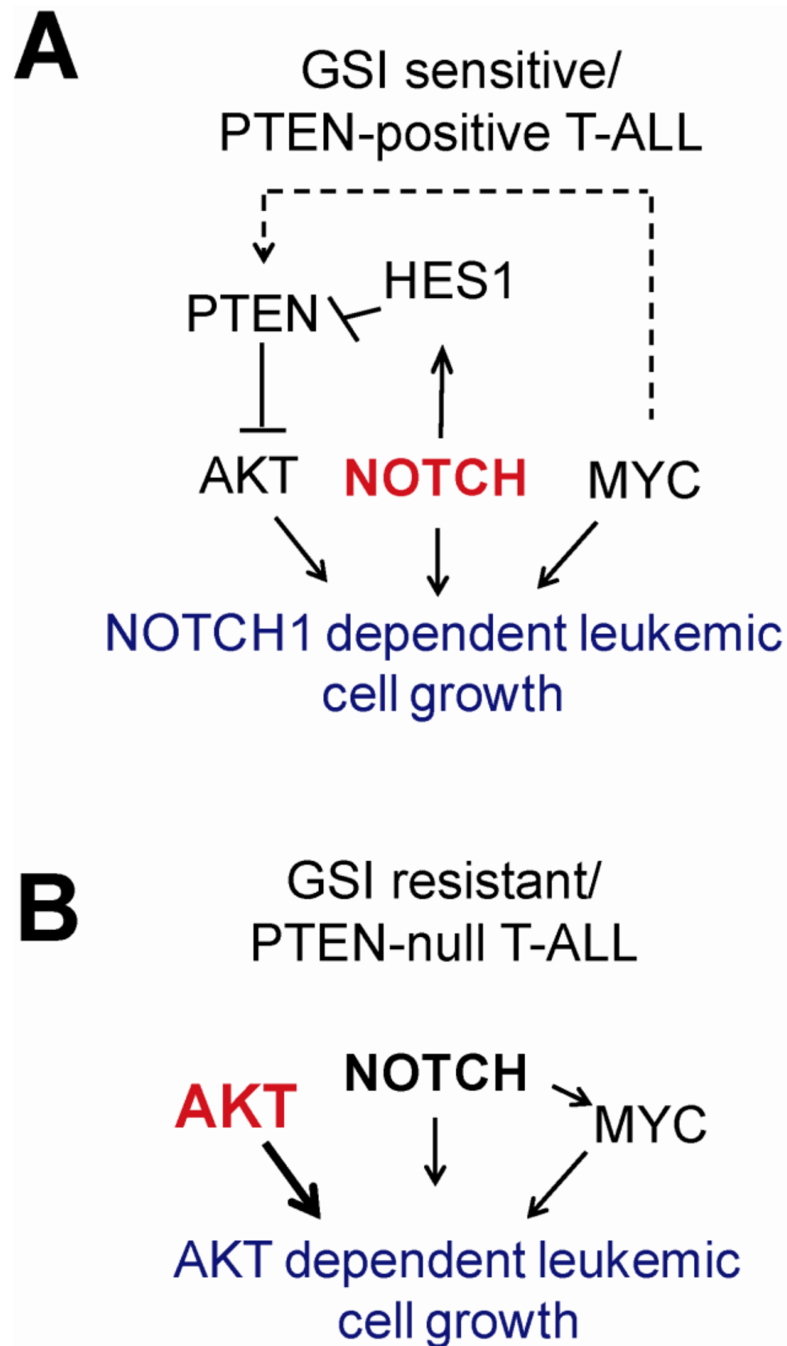

Figure 1. PTEN loss disrupts the circuitry controlling oncogenic cell growth in T-ALL downstream of NOTCH1 in T-ALL

A. NOTCH1 controls leukemic cell growth through multiple overlapping mechanisms including activation of target genes, upregulation of *MYC* and downregulation of *PTEN* via HES1. **B.** Mutational loss of *PTEN* induces constitutive activation of AKT and uncouples NOTCH1 signaling from the PI3K-AKT pathway. Thus, PTEN-null T-ALL cells become insensitive to inhibition of NOTCH1 signaling with GSIs.