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# Molecular Morphogenesis of T-Cell Acute Leukemia

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<http://dx.doi.org/10.5772/55144>

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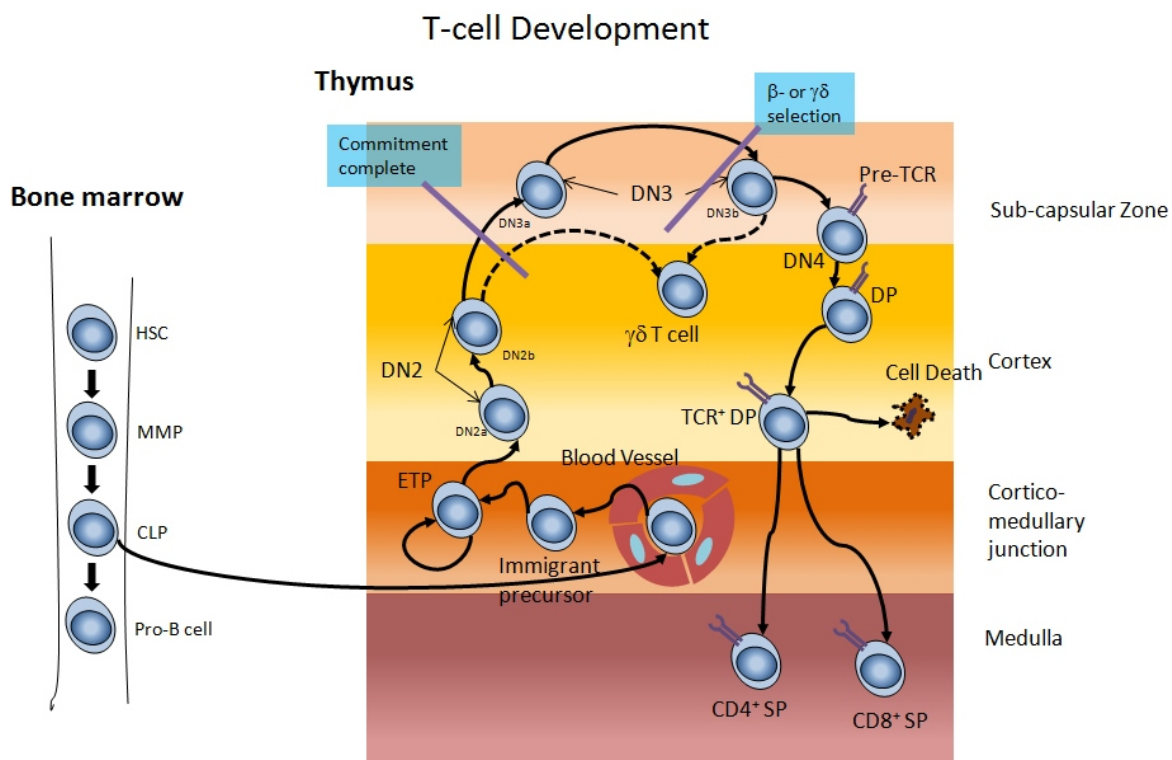
## 1. Introduction

Many molecular alterations are involved in the morphogenesis of T-cell acute leukemia (T-ALL), classified as lymphoblastic leukemia/lymphoma by the World Health Organization. T-ALL is a malignant disease of the thymocytes which accounts for approximately 15% of pediatric acute lymphoblastic leukemia (ALL) and 20-25% of adult ALL. Frequently, it presents with a high tumor load accompanied by rapid disease progression. About 30% of T-ALL cases relapse within the first two years following diagnosis with long term remission in 70-80% of children and 40% of adults [1]-[4]. This poor prognosis is a consequent of our insufficient knowledge of the molecular mechanisms underlying abnormal T-cell pathogenesis. Understanding the abnormal molecular changes associated with T-ALL biology will provide us with the tools for better diagnosis and treatment of lymphoblastic leukemia. Recent improvements in genome-wide profiling methods have identified several genetic aberrations which are associated with T-ALL pathogenesis. For simplification these molecular changes can be separated into 4 different groups: chromosome aberrations, gene mutations, gene expression profiles, and epigenetic alterations. This chapter will discuss these molecular changes in depth.

## 2. T-cell development

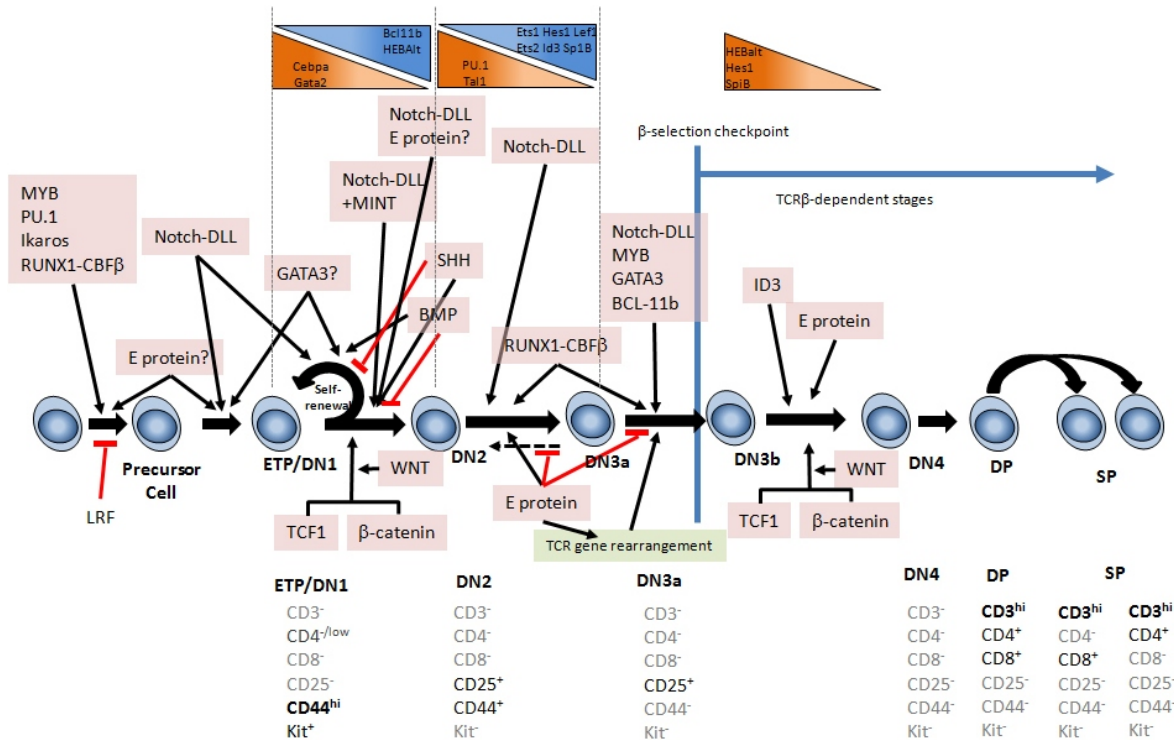
The progenitors for T lymphocytes arise in the bone marrow as long-term repopulating hematopoietic stem cells (LT-HSCs) (Figure 1). These cells then differentiate, generating short-term repopulating hematopoietic stem cells (ST-HSCs) and lymphoid-primed multipotent progenitor (LMPP)[5]-[7]. LMPPs, which migrate via the blood and a chemotaxis process to the thymus [8], phenotypically resemble early T-cell progenitors (ETP)[9],[10]. ETP cells, also called double negative 1 (DN1), are capable of differentiating into either T-cells or myeloid

cells and phenotypically belong to a  $CD3^+CD4^{low}CD8^+CD25^+CD44^+KIT^+$  (Figures 1 and 2). If ETP cells commit to the T-cell lineage they progress to double negative 2 (DN2), followed by double negative 3 (DN3) and finally to double negative 4 (DN4) T-cell development stages. This process starts with the downregulation of c-KIT receptor resulting in the cell surface phenotype  $CD4^+CD8^+CD25^+CD44^+$  for DN2 cells, next CD44 is lost for a cell surface phenotype of  $CD4^+CD8^+CD25^+CD44^-$  for DN3 cells, and finally CD25 is lost for a cell surface phenotype of  $CD4^+CD8^+CD25^-CD44^-$  for DN4 cells (Figures 1 and 2) [9],[11]-[13]. This differentiation from ETP to DN4 cells occurs within the thymus in intimate contact with the epithelial stromal cells, which express Notch ligands, essential growth factors (interleukin-7), and morphogens (sonic hedgehog proteins) important for T-cell development. Before differentiation into double positive cells (DP) which have the cell surface phenotype  $CD4^+CD8^+$ , DN4 cells lose their dependence on Notch ligand, interleukin-7 and sonic hedgehog (Shh) [14],[15]. Once they are DP cells, they undergo positive and negative selection. Following selection,  $\alpha\beta$  T-cell receptor (TCR)<sup>+</sup> T cells migrate from the thymus to secondary lymphoid organs to manifest their immune function. These mature cells are single positive (SP) with the cell surface phenotype of either  $CD4^+$  or  $CD8^+$  [9],[11].



**Figure 1.** Stages in T-cell development. The different regions of the adult thymic lobule are indicated to the right. The progression of hematopoietic stem cells (HSC), multipotent progenitors (MPP), and the common lymphoid progenitors (CLPs) are shown to the left in the bone marrow. Lymphoid progenitors migrated through the blood to the thymus. The migration and differentiation from immigrant precursor to early T-cell precursors (ETP), to double negative (DN), to double positive (DP), and to single positive (SP) stages is illustrated within the distinct microenvironments of the thymus. Complete commitment to the T-cell lineage is indicated with a line between the DN2b and DN3a stages.  $\beta$  or  $\gamma\delta$  selection is indicated between the DN3a and DN3b stages. This figure is modified from Aifnatis 2008 and Rothberg 2008 [9],[11]

## Temporal gene expression and cell surface phenotype in T-cell development



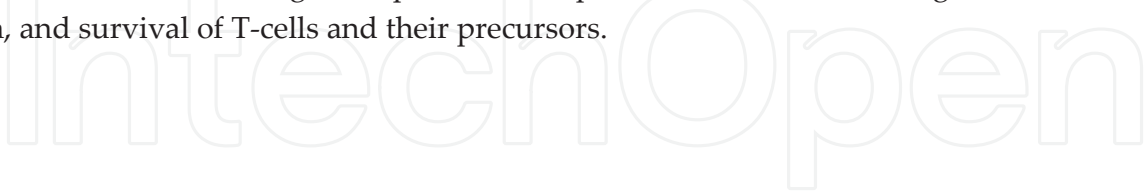
**Figure 2.** Regulatory factors in early T-cell development. The different stages of the cell differentiation are shown in the center starting with hematopoietic stem cells (HSC) and progressing to single positive cells. Above and below the line regulatory factors involved in the progression from one stage to another are indicated. Red lines indicated negatively active factors. The triangles at the top of the illustration indicate regulatory factors which are either upregulated or downregulated at indicated stages. For example, Tal1 expression decreases from the DN2 stage to the DN3a stage whereas Lef1 expression increased during that same transition. The solid blue line indicates the  $\beta$ -selection checkpoint with the long blue arrow indicating the TCR $\beta$ -dependent stages. At the bottom of the illustration the different cell surface phenotypes are shown below the corresponding stage in T-cell development. This figure is modified from Rothenberg 2008 [9].

## 3. Classifications

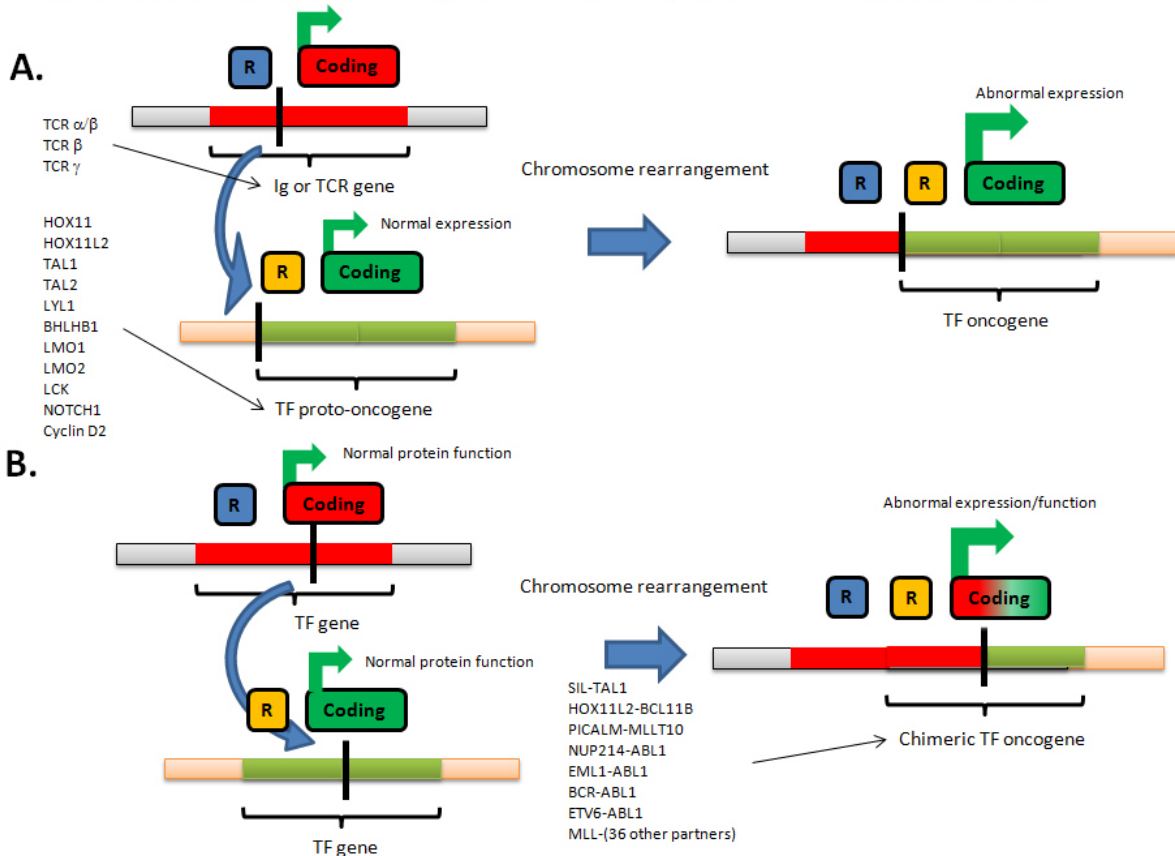
### 3.1. Recurring chromosomal aberrations

Chromosomal translocations which alter gene function were among the first clues to the genes and molecular mechanisms involved in abnormal T-cell biology. In T-ALL, approximately 50% of cases have cytogenetically detectable chromosomal abnormalities. There are at least two distinct molecular mechanisms of chromosomal translocations that can lead to abnormal T-cell biology (Figure 3). In one mechanism a strong regulatory element such as a promoter or enhancer is rearranged next to a gene resulting in abnormal expression of this gene. The affected gene typically encodes a transcription factor or a protein involved in cell cycle regulation. In the second mechanism the translocation results in a fusion protein. Frequently this fusion creates a novel protein that affects normal cell cycle regulation [16]. One of the

hallmark features of T-ALL is translocations involving T-cell receptor genes, which are observed in majority of T-ALL patients. The bulk of these recurring aberrations involve strong transcriptional regulator elements from the T-cell receptor (TCR) genes being juxtaposed with genes encoding transcription factors. These alterations are frequently caused by erroneous V(D)J recombination events during T-cell development. Overall these chromosomal abnormalities lead to aberrant gene expression and proteins that alter normal growth, differentiation, and survival of T-cells and their precursors.



Two Mechanisms of aberrant gene activities by chromosomal translocations



**Figure 3.** Two mechanisms of aberrant activities caused by chromosomal translocations. A. A strong promoter or enhancer is rearranged next to a proto-oncogene resulting in abnormal expression of the proto-oncogene. The TCR loci elements and recurring gene targets involved in T-cell leukemogenesis are indicated to the left. B. Chromosomal rearrangement between two transcription factors result in a chimeric transcription factor with oncogenic activity. Recurring gene fusions in T-cell leukemogenesis are indicated in the center below the arrow.



Approximately 35% of the observed cytogenetic abnormalities in T-ALL involve translocations that include the TCR alpha/delta chain at 14q11.2, the TCR beta chain at 7q34, and the TCR gamma chain at 7p14 (Table1). Among this group, rearrangements with the HOX11, HOX11L2, TAL1, TAL2, LYL1, BHLHB1, LMO1, LMO2, LCK, NOTCH1, and cyclin D2 genes are most frequently observed in patients [11]. Overexpression of LMO1, LMO2, or TAL1 is caused by rearrangements to the TCR delta chain in 3-9% of patients. About 3% of pediatric T-ALL is caused by ectopic TAL1(1p32) expression due to the t(1;14)(p32;q11) rearrangement [17]-[21]. Overexpression of HOX11(TLX1) is observed in greater than 30% of adult T-ALL when rearranged to the promoters of the TCR delta or TCR beta chains[22]. About 3-5% of patients have HOXA-TCR beta rearrangements. For example, the inv(7)(p15q34) and t(7;7)(p15;q34) rearrangement which results in up-regulation of the HOXA9, HOXA10 and HOXA11 genes [23],[24]. Rare translocations involving juxtaposition of the TCR gamma or the TCR alpha/delta chains to the LYL1 (19p13), TAL2 (9p32), or BHLH1(21q22) resulting in overexpression of these genes are also observed [25]-[28].

Several chromosomal translocations do not involve the TCR locus (Table1). In 10-25% of TAL1 positive T-ALL patients, TAL1 is expressed as result of an intrachromosomal deletion between the upstream ubiquitously expressed SIL gene as a result and TAL1 (SIL-TAL1)[29]-[31]. 20% of pediatric and 4% of adult cases of T-ALL have HOX11L2 (TLX3)-BCL11B fusion. This fusion causes ectopic expression of the HOX11L2/TLX3 gene [32],[33]. 8% of patients have the (10;11(p13;q14)/PICALM-MLLT10 rearrangement. In this case leukemogenesis is mediated through HOX gene upregulation via mistargeting of hDOT11 and H3K79 methylation [34],[35]. ABL1, a cytoplasmic tyrosine kinase, fusion genes have been identified in approximately 8% of T-ALL case. The NUP214-ABL1 fusion, which results in a constitutively active tyrosine kinase with oncogenic potential, occurs in 6% of both adult and children patients and is the most frequent ABL1 fusion gene observed. EMI1-ABL1, BCR-ABL1, and ETV6-ABL1 gene fusions are rarely observed in T-ALL but are frequent in other hematologic malignancies [36], [37]. ETV6, which is an important hematopoietic regulatory factor, fusion genes have been observed in both B-ALL (9.6%) and T-ALL patients (5%)[38],[39]. A significant cytogenetically visible deletion on chromosome 9p involves CDKN2A and CDKN2B genes, incidence of which varies from being rare to 70% in T-ALL cases [40]-[42]. In 5-10% of T-ALL patients, gene rearrangements involving MLL gene are observed. The MLL gene can fuse to at least 36 different translocation partner genes [43],[44]. Although there are a wide variety of chromosomal aberrations, the number of genes affected is relatively small. All of these genes are important for normal T-cell development.

### 3.2. Recurring genetic mutations

Several genes associated with T-ALL pathogenesis have mutations which are not cytogenetically visible. Some of the most frequently mutated genes are NOTCH1, FBXW7, PTEN, CDKN2A/B, CDKN1B, 6q15-16.1, PHF6, WT1, LEF1, JAK1, IL7R, FLT3, NRAS, BCL11B, and PTPN2 (Table2). Many of these genes were identified by gene expression profiling using microarrays or by whole genome sequencing analysis. Below some of these genes and their role in T-ALL is described briefly.

Recurring Translocations in T-ALL					
TCR Rearrangements			Non-TCR Rearrangements		
Gene	Rearrangemen t	Frequency	Gene	Rearrangement	Frequency
TAL1	t(1;14) (p32;q11) t(1;7)(p32;q34)	~3 of T-ALL	TAL1	STIL-TAL1 (1p32 deletion)	12-25% T-ALL
TAL2	t(7;9)(q34;q32)	rare	HOXA	PICALM-MLLT10 (t(10;11) (p13;q14)) MLL-MLLT1 (t(11;19) (q23;p13)) SET-NUP214 9q34 deletions	
LMO1	t(11;14) (p15;q11) t(7;11) (q34;p15)	6-8% of T-ALL	ABL1	EML1-ABL1 (t(9;14) (q34;q32)) BCR-ABL1 (t(9;22)(q34;q11)) ETV6-ABI1 (t(9;12)(q34;p13)) NUP214-ABL1	8% T-ALL for ABL1 6% T-ALL for NUP214
LMO2	t(11;14) (p13;q11) t(7;11) (q34;p13) 11p13 deletions		ETV6	ETV6-JAK2 (t(9;12)(p24;p13) ETV6-ARNT (t(1;12)(q21;p13))	Rare
HOX11	t(10;14) (q24;q11) t(7;10) (q34;q24)	30% of T-ALL			
HOX11L2	t(5;14) (q35;q32)	20% Childhood T-ALL 4% Adult T-ALL			
HOXA	Inv(7)(p15q34) t(7;7)(p15;q34)				
LYL1	t(7;19) (q34;p13)	rare			

**Table 1.** Table of recurring translocation involved in T-ALL. The rearrangements are divided into those involving TCR and non-TCR loci.

Recurring genetic alterations in T-ALL		
Gene	Alteration	Frequency
Notch1	Sequence mutations	~50% of T-ALL
FBW7	Sequence mutations	~20% of T-ALL
PTEN	Deletions/Sequence mutations	6-8% of T-ALL
CDKN2A/B	Deletions	30-70% of T-ALL
CDKN1B	Deletions/Sequence mutations	12% of T-ALL
6q15-16.1	Deletions	12% of T-ALL
PHF6	Deletions/Sequence mutations	16% of childhood T-ALL 38% of adult T-ALL
WT1	Frameshift mutations	13% childhood T-ALL 12% of adult T-ALL
LEF1	Focal deletions/sequence mutations	15% of childhood T-ALL
JAK1	Sequence mutations	18% of adult T-ALL
IL7R	Gain of function mutation	9% of T-ALL
FLT3	Internal tandem duplication	4% of adult T-ALL 3% of childhood T-ALL
NRAS	Sequence mutations	10% childhood T-ALL
BCL11	Deletions/Sequence mutations	9% of all T-ALL case 16% of T-ALL cases with HOX11 overexpression
PTPN2	Deletion	6% of T-ALL

**Table 2.** Table indicating recurring genetic alterations in T-ALL. The type of alteration and frequency of occurrence in T-ALL cases is indicated.

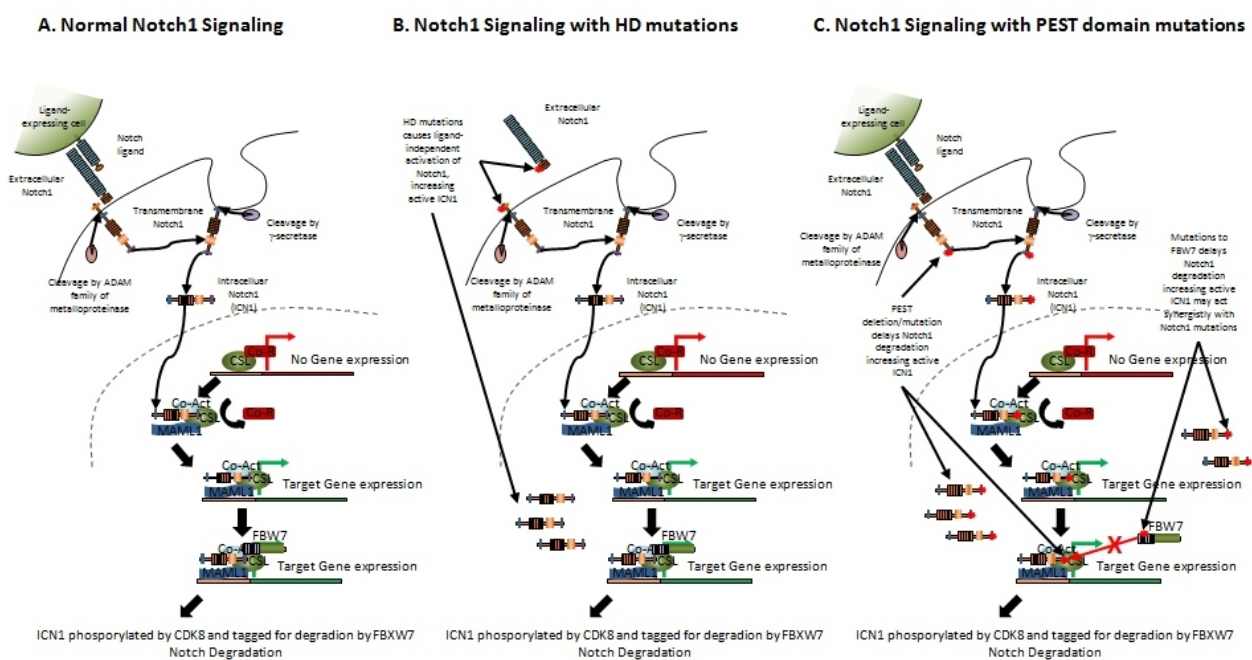
### 3.2.1. Notch1 signaling pathway in T-ALL

Activating or loss of function NOTCH1 mutations are observed in ~34-71% of T-ALL and is one of the most significant T-ALL oncogene [45]-[49]. NOTCH is involved in the regulation of several cellular processes including differentiation, proliferation, apoptosis, adhesion, and spatial development [50],[51]. The importance of NOTCH1 in leukemogenesis was first discovered in a rare translocation t(7;9) that fuses the intracellular form of NOTCH1 to the TCR beta promoter and enhancer sequences. This rare fusion leads to a truncated and constitutively activated form of NOTCH1 termed TAN1 [52]. Other Notch isoforms also show oncogenic activity. Notch2 sequences were able to induce leukemogenesis in cats and overexpression of Notch3 in mice resulted in multi-organ infiltration by T lymphoblasts [53],[54]. The majority of T-ALL cases with active Notch1 arise due to mutations in the Notch1's heterodimerization (HD) domain and/or the PEST domain (proline-, glutamic-acid-, serine-, and threonine-rich domain)[46]. Mutations in the HD domain appear to make the NOTCH1 receptor susceptible to ligand-independent proteolysis and activation (Figure 4b), whereas, mutations in the PEST domain interfere with recognition of the intracellular form of NOTCH1 by the FBW7 ubiquitin ligase (Figure 4c) [45],[46],[55]-[62]. Notch1 is a single-transmembrane receptor with an extracellular, transmembrane, and intracellular subunits. Initially the cell-membrane-bound Notch protein is a single protein. After maturation when the protein is cleaved into two



subunits the extracellular and intracellular subunits are linked non-covalently via the HD domains. On the extracellular domain multiple epidermal growth factor (EGF)-like repeats bind ligands namely, Delta-like ligand (DLL1), DLL2, DLL4, Jagged1 and Jagged 2. Ligand binding initiates two cleavage events by the ADAM family of metalloproteinases and the  $\gamma$ -secretase complex to release the intracellular form of NOTCH from the membrane. Two nuclear localization domains in NOTCH lead to its translocation to the nucleus [62]. Once in the nucleus, NOTCH associates with CSL (CBF1/suppressor of hairless/Lag1). Transcriptional activation of NOTCH-target genes begins once the NOTCH/CSL complex recruits the co-activator proteins like mastermind-like 1 and the histone acetyl transferase p300 (Figure 4a) [63]. The C-terminal domain of NOTCH contains the PEST domain. This domain is targeted for ubiquitination by FBW7 and subsequent proteasome-mediated degradation. Mutations in the PEST domain can increase the half-life of NOTCH protein resulting in aberrant activation of NOTCH-target genes [58],[59],[61]. Together, aberrant stabilization or activation of the intracellular form of NOTCH1 directly links to T-cell leukemogenesis.

### Notch Signaling and mutations



**Figure 4.** The Notch1 signaling pathway and mutations involved in aberrant Notch1 activation. A. Depiction of normal Notch1 signaling. Binding of Notch ligand to the extracellular Notch1 triggers a conformation change in the heterodimerization domain (HD). This allows cleavage first by a metalloproteinase of the ADAM family and then by  $\gamma$ -secretase. These cleavages release Notch1 from the membrane allowing it to translocate into the nucleus. Once in the nucleus, Notch1 associates with a transcriptional complex composed of CSL (CBF1/suppressor of hairless/lag1) and mastermind-like 1 (MAML1) to activate Notch1 target genes. Notch1 then becomes associated with FBW7 and is tagged for degradation following ubiquitination. B. Mutations in the HD domains (indicated by a red star) result in ligand independent cleavage allowing aberrant release of Notch1 from the membrane. C. Mutations in the PEST domain of Notch1 or mutations in FBW7 interfere with ubiquitination of Notch1. This allows accumulation of intracellular Notch1 by reducing its degradation. The figure is modified from Aifantis 2008 [11].

Because NOTCH1 plays a significant role in T-cell leukemogenesis, its regulation has been studied extensively. Nearly 40% of Notch-responsive genes are regulators of cell metabolism and protein biosynthesis [64]. c-MYC, a master regulator of multiple biosynthesis and metabolic pathways, is a direct transcriptional target of Notch1. Notch1 binding sites in the MYC promoter have been shown to be important for MYC expression in T-ALL [64]-[67]. Constitutively active Notch1 was shown to activate the NF- $\kappa$ B pathway [68], an important regulator of cell survival, cell cycle, cell adhesion and cell migration. This activation can occur by the direct transcriptional activation of Relb and Nfkb2 as well as via a Notch1 and IKK complex interaction. Another Notch1 target is PTEN (phosphatase and tension homologue). PTEN is negatively regulated by Notch1 through the activity of HES1 and MYC, resulting in the deregulation of the PI3K-AKT metabolic pathway [69]. Finally, Notch1 is also involved in the regulation of the NFAT signaling pathway, where it regulates the pathway by altering expression of calcineurin, a calcium-activated phosphatase [70]. Overall, these findings emphasize the role of Notch1 in inducing T-cell leukemogenesis through multiple cell signaling pathways capable of regulating cell survival, proliferation and metabolism.

As mentioned above, FBW7 (F-box and WD repeat domain containing 7), an E3 ubiquitin ligase located on chromosome 4q31.3, is observed to be mutated in T-ALL with a frequency ranging from 8.6% to 16% [59],[61],[71]. FBW7 is part of the SCF complex (SKP1-Cullin-1-F box protein complex), which can target MYC, JUN, cyclin E, and Notch1 for ubiquitination coupled proteosomal degradation [60]. The WD40 domain of FBW7 contains a degron-binding pocket domain. This domain recognizes phosphothreonine in the consensus sequence I/L/P-T-P-X-X-S/E of protein substrates. Roughly 20% of T-ALL patients have mutations in FBW7 that destroys the degron-binding pocket. Moreover, the degron sequence of Notch1 (LTPSPES) located in the distal portion of its PEST domain is found to be mutated in T-ALL, thus extending Notch1 half-life and altering downstream signaling cascades. Interestingly, T-ALL patients frequently have mutations in both the FBW7 degron binding pocket as well as in the Notch1 degron sequence (Figure 4c) [58],[59],[61]. These combined mutations elevate intracellular Notch1 activity and therefore, enhances leukemia manifestation. Current studies suggest FBW7 mutations induce T-cell leukemogenesis by disrupting Notch1 regulation.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is deleted or mutated in 6-8% of T-ALL cases. The major substrate of PTEN is PIP<sub>3</sub> (phosphatidylinositol-3,4,5-triphosphate). PTEN activity prevents the accumulation of PIP<sub>3</sub>, thus limiting or terminating activation of a cascade of PI3K-dependent signaling molecules. The expression of PTEN has been shown to be negatively regulated by Notch1. PTEN appears to be required for optimal negative selection in the thymus. Loss of PTEN is characterized by overexpression of the c-myc oncogene and induction of lymphomagenesis within the thymus [69],[72]. Therefore PTEN appears to be an important tumor-suppressor involved in T-cell leukemogenesis.

### *3.2.2. Cell cycle, apoptosis, and transcription regulators in T-ALL*

Deletions in CDKN2A and CDKN2B are significant secondary abnormalities in pediatric T-ALL. Loss of the tumor suppressor CDKN2A/B expression is observed in 30-70% of T-ALL cases and can occur due to chromosomal translocation, promoter hypermethylation, somatic

mutation, or gene deletions [40],[42]. CDKN2A and CDKN2B are located adjacent on chromosome 9p21. CDKN2A encodes p16<sup>INK4a</sup>(cyclin-dependent kinase inhibitor)/p14<sup>ARF</sup> while CDKN2B encodes p15<sup>INKb</sup>. These genes block cell division during the G<sub>1</sub>/S phase of the cell cycle by inhibiting cyclin/CDK-4/6 complexes [73],[74]. The principle mode of CDKN2A inactivation occurs via genomic deletions which can usually be detected by FISH [41]. Loss of function of CDKN1B (cyclin-dependent kinase inhibitor 1B) gene, located on 12p13.2, have been observed in 12% of T-ALL cases [75]. Similar to CDKN2A and CDKN2B, CDKN1B acts as a tumor suppressor. Inactivation of CDKN1B leads to overexpression of D-cyclins, thereby inhibiting the cells ability to maintain quiescence in G<sub>0</sub>. Therefore, CDKN2/B and CDKN1B play an important role in abnormal T-cell biology by regulating cell cycle progression.

12% of pediatric T-ALL cases have deletion in 6q15-16.1 [75]. The single most down regulated gene in this region is caspase 8 associated protein 2 (CASP8AP2). Deletion of CASP8AP2 probably interferes with Fas-mediated apoptosis. In gene expression profiling study, loss of CASP8AP2 was not observed in any pre-B-ALL samples [75], indicating deletions to 6q15-16.1 maybe a hallmark of T-ALL.

The X-linked plant homeodomain (PHD) finger 6 (PHF6) gene has inactivating mutations in 16% of pediatric and 38% of adult primary T-ALL cases [76]. Mutations in PHF6 are limited to male T-ALL cases. Consequently, this gene may be responsible for the increased incidence of T-ALL cases in males. Loss of expression of the PHF6 gene was associated with leukemia driven by abnormal expression of the homeobox transcription factor oncogenes. PHF6 gene encodes a protein with two plant homeodomain-like zinc finger domains. A recent study demonstrated that PHF6 copurifies with the nucleosome remodeling and deacetylation (NuRD) complex, implicating its role in chromatin regulation [77].

The WT1 (Wilms tumor) tumor suppressor gene is mutated in 13.2% of pediatric and 11.7% of adult T-ALL cases [78],[79]. The WT1 is known to be a transcriptional activator of the erythropoietin gene. Loss of WT1 expression results in diminished erythropoietin receptor (EpoR) expression in hematopoietic progenitors, suggesting that activation of the EpoR gene by Wt1 is an important mechanism in normal hematopoiesis [80]. WT1 mutations are frequently prevalent in T-ALL cases harboring chromosomal rearrangements associated with abnormal expression of the homeobox transcription oncogenes, HOX11, HOX11L2, and HOXA9 [79]. This suggests that the recurrent genetic mutations in WT1 are associated with abnormal HOX gene expression in T-ALL period

Lymphoid enhance factor 1 (LEF1) gene is mutated in 15% of pediatric T-ALL cases [81]. Inactivation of LEF1 was associated with increased expression of MYC and MYC targets, a gene expression signature consistent with developmental arrest at a cortical stage of T-cell differentiation. Interestingly, T-ALL cases with LEF1 mutation lacked overexpression of TAL1, HOX11, HOX11L2 and HOXA genes suggesting that LEF1 acts via different molecular pathways in T-cell leukemogenesis. In fact, The LEF family of DNA-binding transcription factors interacts with nuclear  $\beta$ -catenin in the WNT signaling pathway. The loss of LEF1 may result in the relief of transcriptional repression of MYC in T-ALL cases. It was reported that LEF1 probably contributes to T-ALL pathogenesis by acting in concert with NOTCH1 to

promote up-regulation of MYC expression. In this case LEF1 also relieves transcriptional repression of MYC to allow its maximum overexpression by Notch1 [81].

### 3.2.3. JAK/STAT signaling pathway in T-ALL

About 18% of adult and 2% of pediatric T-ALL cases have activating mutations in the Janus Kinase 1 (JAK1) [38]. The JAK family (JAK1, JAK2, JAK3, and TYK2) function as signal transducers to control cell proliferation, survival, and differentiation. They are nonreceptor tyrosine kinases that associate with cytokine receptors to phosphorylate tyrosine residues of the target proteins. This process regulates the recruitment and activation of STAT proteins. The JAK/STAT signaling cascade is an important regulator of normal T-cell development. Each JAK family member associates with a different subset of cytokine receptors. JAK1 regulates the class II cytokine receptors as well as receptors that use the gp130 or  $\gamma_c$  receptor subunit. These class of cytokine receptors are involved in controlling lymphoid development [82],[83]. The majority of the JAK1 kinase mutations observed in T-ALL cases result in unregulated tyrosine kinase activity. T-ALL cases with mutations in JAK1 appear to be associated with different T-ALL subgroups than patients harboring aberrant expressions of the homeobox transcription factors HOX11 and HOX11L2 [38]. JAK1 is involved in the regulation of both interleukin 7 receptor (IL7R) and protein tyrosine phosphatase non-receptor type 2 (PTPN2) [84],[85].

The interleukin 7 receptor (IL7R) has a gain-of-function mutation in exon 6 in 9% of T-ALL cases [85]. Several lines of evidence suggest IL7R plays an important role in T-cell leukemogenesis. IL-7 and IL7R signaling are essential for normal T-cell development. Deficiency of IL-7 and IL7R in mice caused reduction of non-functional T cells and showed an early block in thymocyte development [86]-[89]. Loss of IL7R function also results in severe combined immunodeficiency in humans [90]. Increased expression of IL7R was associated with spontaneous thymic lymphomas in mice. Furthermore, Notch1 has been shown to transcriptionally upregulate IL7R receptor gene [91]. Mutations in exon 6 of IL7R promotes de novo formation of intermolecular disulfide bonds between IL7R mutant subunits, which triggers constitutive activation of tyrosine kinase JAK1 regardless of regulation by IL-7,  $\gamma_c$  or JAK3. Gene expression profiles for IL7R mutations are generally associated with the T-ALL subgroup harboring HOX11L2 rearrangements and HOXA deregulation [85].

Inactivation of protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene is observed in ~6% of T-ALL cases [84],[92]. PTPN2 encodes a tyrosine phosphatase, located on chromosome 18p11.3-11.2, that negatively regulates JAK/STAT pathway and NUP214-ABL1 kinase activity. Loss of PTPN2 results in activation of the JAK/STAT pathway and increased T-cell proliferation by cytokines. Unlike JAK1 mutations, deletions in PTPN2 gene appear to be restricted to T-ALL cases which specifically overexpress HOX11 [84]. Therefore mutations in PTPN2 probably play a role in T-cell leukemogenesis by deregulating tyrosine kinase signaling.

Activating mutations in the FMS-like tyrosine kinase 3 (FLT3) gene are amongst the most common genetic aberrations in acute myeloid leukemia [93]-[95]. In T-ALL, FLT3 mutations



are relatively rare with a frequency of approximately 4% in adult and 3% in pediatric cases. [96]-[98]. FLT3 encodes a class III membrane tyrosine kinase that is expressed in early hematopoietic stem cells. Normally FLT3 is activated when bound by the FLT3 ligand (FL). This interaction causes receptor dimerization and kinase activity resulting in activation of downstream signaling pathways such as Ras/MAP kinase, PIK3/AKT, and STAT5. The most frequent FLT3 mutation involves a duplication of the juxtamembrane (JM) domain. This mutation leads to dimerization of FLT3 in the absence of FLT3 ligand (FL), autophosphorylation of the receptor and constitutive activation of the tyrosine kinase domain, which triggers uncontrolled proliferation and resistance to apoptotic signaling through activation of the PIK3/AKT, Ras/MAPK and JAK2/STAT pathways [98]-[100].

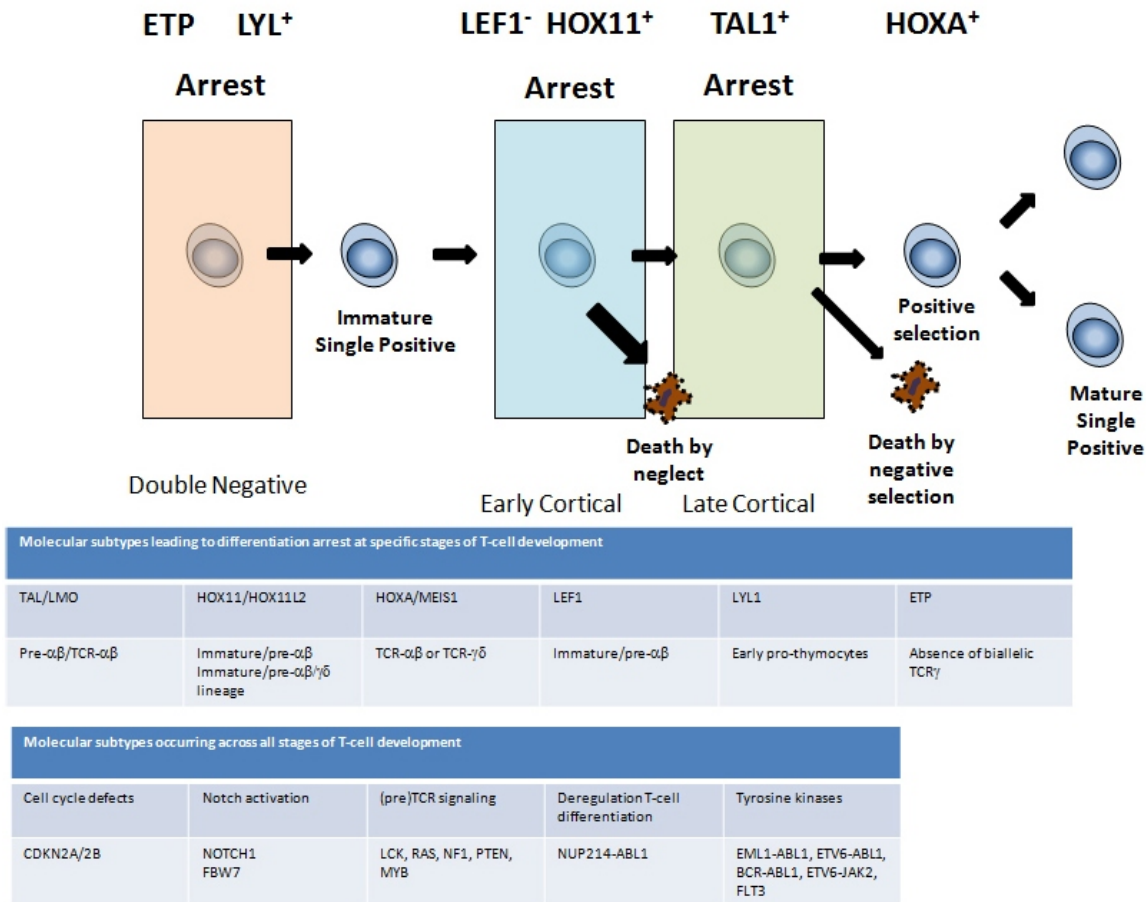
The B-cell chronic lymphocytic leukemia (CLL)/lymphoma 11B (BCL11B) gene has mutations in 16% of T-ALL patients with HOX11 overexpression. However, in unselected patients, deletions or missense mutations for BCL11B were observed in only 9% of cases. This suggests that BCL11 mutations probably occur across all subtypes of T-ALL [101]. BCL11B is located on human chromosome 14q32.2 and encodes a kruppel-like C<sub>2</sub>H<sub>2</sub> zinc finger protein which acts as a transcriptional repressor. Loss of function mutations in BCL11B gene in mice leads to developmental arrest of T-cell in DN2-DN3 stage, acquisition of NK-like features, and aberrant self-renewal activity. Transcriptional activation of IL-2 expression in activated T-cell is mediated by BCL11B via its interaction with p300 co-activator at the IL-2 promoter [102]-[106]. Because of BCL11B's role in normal T-cell development, it plays an important role in T-cell leukemogenesis.

Approximately 10% of childhood T-ALL cases have mutations in NRAS oncogene located on chromosome 1p13.2, which is involved in the malignant transformation of many cells [107]. The recurrence of NRAS mutations in T-ALL cases suggests that NRAS is involved in abnormal T-cell biology.

### 3.3. Gene expression profiles

Whole genome sequencing and gene expression profiles provide a more comprehensive view of the genetic alterations involved in T-cell leukemia. A recent microarray-based gene expression study classified T-ALL cases into major subgroups corresponding to leukemic arrest at different stages of thymocyte differentiation. Currently there are 3 subtypes of T-ALL cases which include the HOXA/MEISI, TLX1/3 and TAL1-overexpressing subtype [108], the LEF1-inactivated subtype [81], and the early T-cell precursor phenotype [109] (Figure 5). Leukemic arrest at early pro-T thymocytes (DN2 cells) were characterized by high levels of expression of the LYL1 gene. Arrest in early cortical thymocytes (DN3 cells) were characterized by changes in HOX11/TLX1 expression. Arrest in late cortical thymocytes (DP cells) were characterized by changes in the TAL1/LMO1 expression. Aberrant HOX11L2/TLX3 activation was also identified as being involved in T cell leukemogenesis (Figure 4) [108]. TAL1 and LYL1 are members of the basic helix-loop-helix (bHLH) family of transcription factors, LMO1 is member of the LIM-only domain genes (LMO), and HOX11 and HOX11L2 belongs to the homeobox gene family.

### Genetic abnormalities in T-ALL



**Figure 5.** Gene subtypes resulting in differentiation arrest at specific stages of T-cell development. The illustration shows the progression of T-cell development from the double negative stages to the mature single positive stage. The colored rectangles indicate stages of leukemic arrest. Overexpression of LYL, HOX11, TAL1, and HOXA lead to differentiation arrest at the double negative stage, early cortical stage, late cortical stage, and positive selection stage, respectively. Loss of Lef1 expression results in early cortical leukemic arrest. The table below indicates the molecular subtypes leading to differentiation arrest at specific stages of T-cell development and the molecular subtypes occurring across all the stages of T-cell development.

Recently whole genome sequencing of early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) identified several genes involved in abnormal T-cell biology [10]. 15% of T-ALL cases are ETP-ALL. Phenotypically ETP-ALL is negative for the cell surface markers CD1a and CD8, has little to no expression of CD5, and has aberrant expression of myeloid and hematopoietic stem cell markers. This study performed whole genome sequencing on 12 children with ETP-ALL. The frequency of the mutations identified from these 12 cases was then accessed in 94 cases of T-ALL. Of these 94 cases 52 cases had ETP and 42 had a non-ETP pediatric T-ALL. Even though an average of 1140 sequence mutations and 12 structural variations in the genome were identified per ETP case, they were able to narrow down the number of affected genes to 3 groups and 3 novel genes (DNM2, ECT2L, and RELN). 67% of the cases were characterized by activating mutations in genes involved in the regulation of cytokine receptor and RAS



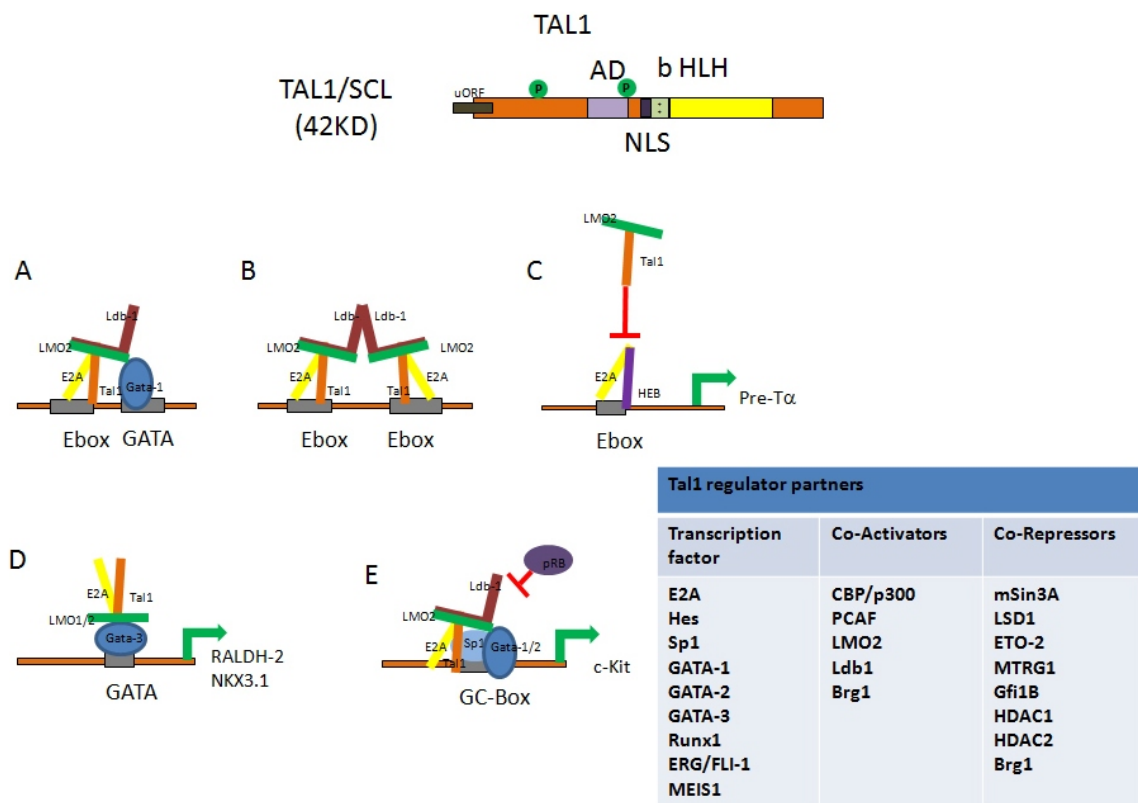
signaling. These genes included NRAS, KRAS, FLT3, IL7R, JAK3, JAK1, SH2B3 and BRAF. 58% of the cases were characterized by inactivating lesions that disrupted hematopoietic development. These genes included GATA3, ETV6, RUNX1, IKZF1, and EP300. 48% of the cases were characterized by changes in histone modifying genes (EZH2, EED, SUZ12, SETD2, and EP300) [10]. From gene expression profiling and whole genome sequencing we are beginning to obtain a more complete picture of the genes involved in abnormal T-cell biology.

MicroRNA expression profiling found 10 detectable miRNAs in human T-ALL cells, five of these miRNAs (miR-19b, miR-20a, miR-26a, miR-92, and miR223) were predicted to target tumor suppressors genes implicated in T-ALL [110]. These five miRNA's were able to accelerate leukemia development in a mouse model. Furthermore, it was shown that these five miRNAs produced overlapping and cooperative effects of the tumor suppressor genes IKAROS, PTEN, BIM, PHF6, NF1 and FBXW7 in T-ALL pathogenesis. miR223 appears to be the most overexpressed miRNA in leukemia. These results indicate the important role that miRNA's play in abnormal T-cell biology.

### 3.4. Basic helix-loop-helix proteins

As mentioned early, some of the most common recurrent chromosomal aberrations in abnormal T-cell biology involved chromosomal translocations of the TCR gene to the basic helix-loop-helix (bHLH) genes (MYC, TAL1, TAL2, LYL1, bHLHB1), the cysteine-rich (LIM-domain) genes (LMO1, LMO2), or the homeodomain genes (HOX11/TLX1), HOX11L2/TLX3, members of the HOXA cluster) (Table1). The most common bHLH gene with aberrant expression observed in T-ALL cases is the transcriptional regulator TAL1 (T-cell acute lymphocytic leukemia 1; also known as SCL). It was first identified in T-ALL patients with the t(1;14)(p32;q11) translocation [17],[18],[20],[21]. This chromosomal rearrangement, which is observed in 3% of cases, causes ectopic TAL1 expression by placing TAL1 under control of the TCR $\delta$  oncogene [19]-[21],[111]. 12%-25% of T-ALL cases have a submicroscopic 90-kb deletion that fuses the TAL1 coding sequence to the first exon of the SIL gene (SCL interrupting locus). This rearrangement leads to dysregulation of TAL1 expression [17],[29]-[31]. The majority of T-ALL cases, up to 60%, show ectopic TAL1 expression with no detectable TAL1 gene rearrangements [112]. Gene expression profiling has shown that ectopic expression of TAL1 results in leukemic arrest in late cortical thymocytes (Figure 5) [108]. These results show that activation of TAL1 gene is required for the leukemic phenotype of T-cells.

The TAL1 gene, located on chromosome 1p32, encodes a class II basic helix-loop-helix factor [113]. The protein binds DNA as a heterodimer with the ubiquitously expressed class I bHLH genes known as E-proteins such as E2A or HEB. These heterodimers recognize an E box sequence (CANNTG)[114]. TAL1 positively and negatively modulates transcription of targets gene as a large complex consisting of an E-protein, the LIM-only proteins LMO1/2, GATA1/2, Ldb1, and other associated coregulators. This complex usually binds a composite DNA elements containing an E box and a GATA-binding site separated by 9 or 10 bp (Figure 6) [115]-[117]. It was shown recently that in T-ALL cells TAL1, GATA-3, LMO1, and RUNX1 together form a core transcription regulatory circuit to reinforce and stabilize the TAL1-directed leukemogenic program [118].



**Figure 6.** Model of TAL1 complexes and target sites. A. TAL1 complex binding to an E-box and GATA box. B. TAL1 complex binding to a double E-box. C. TAL1 complex binding to a single E-box. D. TAL1 complex binding to a single GATA site showing activation of either the RALDH-2 or NKX3.1 genes. E. TAL1 complex binding to a GC-box with activation of c-kit. The table to the lower right shows the different TAL1 regulator partners. The partners are divided into three categories transcription factors, co-activators, or co-repressors.

TAL1 expression is essential for hematopoiesis. It is required for specification of hematopoietic stem cells during embryonic development and it is necessary for erythroid maturation. Normal expression of TAL1 is restricted to the DN1-DN2 subset of immature CD4<sup>+</sup>/CD8<sup>-</sup> thymocytes with ectopic expression resulting in leukemic arrest in late cortical thymocytes [108].

Two models have been proposed for TAL1-induced leukemogenesis. In the prevailing model TAL1 acts as a transcriptional repressor by blocking the transcriptional activities of E2A, HEB, and/or E2-2 through its heterodimerization with these E-proteins. TAL1 may mediate its inhibitory effect by interfering with E2A-mediated recruitment of chromatin-remodeling complex which activate transcription [114],[119]-[121]. It also been shown to associate with several corepressors including HDAC1, HDAC2, mSin3A, Brg1, LSD1, ETO-2, Mtgr1, and Gfi1-b (Figure 6) [122]. In human T-ALL TAL1 transcriptional repression may be mediated by TAL1-E2A DNA binding and recruitment of the corepressors LSD1 and/or HP1- $\alpha$  [123]. In the other model TAL1 induces leukemogenesis through inappropriate gene activation [124]. At least two genes RALDH2 and NKX3.1 are transcriptionally activated by TAL1 and GATA-3 dependent recruitment of the TAL1-LMO-Ldb1 complex [125],[126]. As a transcriptional activator TAL1 has been shown to associate with the coactivators p300 and P/CAF (Figure 6) [127],[128]. Both of these complexes contain HAT activities. The prevalence of histone-

modifying enzymes in TAL1 complexes suggests that one function of TAL1 is to regulate chromatin states of its target genes.

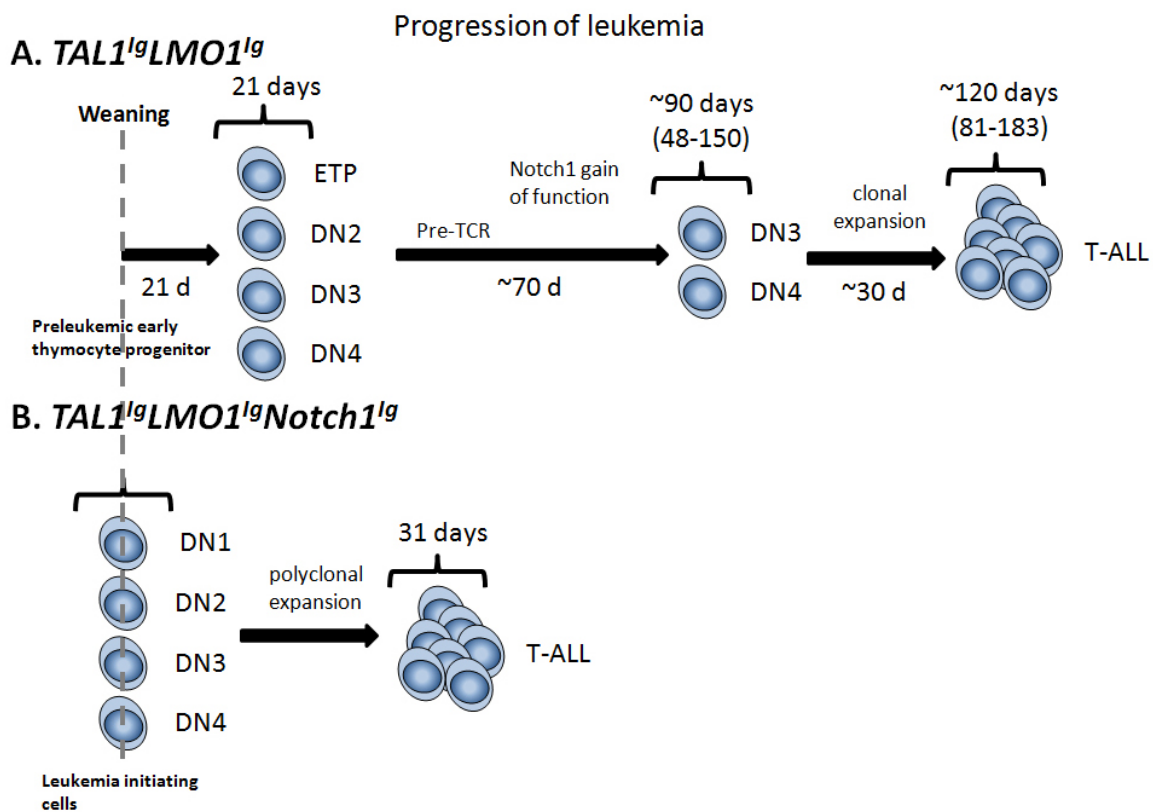
TAL1 and the lymphoblastic leukemia-derived sequence 1 (LYL1) share 90% sequence identity in their bHLH motif [26]. Like TAL1, LYL1 role in leukemogenesis was discovered by studying chromosomal rearrangements. It is expressed by adult hematopoietic cells and is overexpressed in T-ALL. Gene expression profiling showed that overexpression of LYL1 resulted in leukemic arrest at pro T-cell (Double negative) stage of T-cell differentiation (Figure 5) [108]. In mouse embryos LYL1 and TAL1 expression overlaps in hematopoietic development, developing vasculature and endocardium. At the molecular level LYL1 controls expression of several genes involved in the maturation and stabilization of the newly formed blood vessels [129]. Therefore, bHLH proteins play an important role in abnormal T-cell biology.

### 3.5. LIM domain proteins

Aberrant expression of the LMO1 and LMO2 proteins is observed in 45% of T-ALL cases. The discovery of the LMO1 and LMO2 genes adjacent to the chromosomal translocations t(11;14)(p15q11) and t(11;14)(p13;q11) was the first indication that these proteins were involved in T-cell leukemogenesis [130]-[132]. The LMO family (LMO1, LMO2, LMO3, and LMO4) encodes genes that have two cysteine-rich zinc coordinating LIM domains. The LIM domain is found in a variety of proteins including the homeodomain-containing transcription factors, kinases, and adaptors. Despite the presence of 2 zinc finger motifs, LMO1 and LMO2 genes do not appear to bind DNA. Instead the LMO proteins probably act as scaffolding protein to form multiprotein complex through their interaction with the LIM domain binding protein 1 (LDB1) (Figure 6) [116].

Leukemogenesis by aberrant expression of LMO1 or LMO2 is thought to occur via two mechanisms. In the first mechanism aberrant expression or abnormal LMO proteins forms a dysfunctional multiprotein complexes that alters the expression of the target genes by directly binding to their promoters [133]-[136]. In the second mechanism abnormal LMO1 or LMO2 complexes displace the LMO4 complex. This results in arrest of T-cell development at the DP stage [137].

LMO2 function is necessary for normal T-cell development. In fact, LMO2 has been shown to interact with several factors involved in aberrant T-cell biology. As mentioned above TAL1 may regulate its target genes through the TAL1-LMO-Ldb1 complex (Figure 6). Ectopic expression of LMO1 and LMO2 leads to accumulation of immature DN T cells in mice with subsequent leukemia manifestation with a long latency, suggesting the role of LMO is important for the development of tumors but is not self-sufficient [26],[138],[139]. Ectopic expression of both TAL1 and LMO1 in mice accelerated the progression to leukemogenesis (Figure 7). In this case thymic expression of the TAL1 and LMO1 oncogenes induced expansion of the ETP/DN1 to DN4 population and lead to T-ALL in ~120 days. The acquisition of a Notch1 gain-of-function mutation was proposed to be the rationale behind this increase in leukemia penetrance. In fact, thymic expression of all three oncogenes Notch1, TAL1 and LMO1 induced T-ALL with high penetrance in 31 days, the time necessary for clonal expansion (Figure 7) [140]. These studies suggest that aberrant LMO proteins are key players in abnormal T-cell biology.



**Figure 7.** Model of progression to leukemia via TAL1, LMO1 and Notch1. The dashed line indicates the time of weaning. The number of days to differentiation arrest and finally T-ALL are shown above the cell stages. A. Shows the numbers of days to full T-ALL in mice with TAL1 and LMO1 oncogenes. Note the 70 day delay for a Notch1 gain of function mutation. B. Shows the number of days to full T-ALL in mice with TAL1, LMO1, and Notch1 oncogenes. Note the delay is ~30 days the time necessary for clonal expansion. This figure is modified from Tremblay et al 2010 [140].

### 3.6. Homeobox genes

Dysregulated expression of HOX-type transcription factors occurs in 30-40% of T-ALL cases [23],[24],[32]. The HOX genes play an important role in hematopoiesis [141]. The majority of the HOXA, HOXB and HOXC genes clusters are expressed in hematopoietic stem cells and immature progenitor compartments. Furthermore, these genes are down regulated during differentiation and maturation of hematopoiesis [142],[143]. In T-ALL dysregulation of the HOXA gene cluster is a frequent recurring aberration. Upregulation of HOXA9, HOXA10, and HOXA11 occurs in T-ALL cases when the TCR beta regulatory elements are juxtaposed with these genes [16].

Two orphan HOX proteins (HOX11 and HOX11L2) have been implicated in T-cell leukemogenesis [144]. Overexpression of HOX11 is observed in 30% of T-ALL cases because of two recurring translocation events. This gene is also frequently overexpressed in T-ALL cases in the absence of genetic rearrangements. Mice deficient in HOX11 fail to develop a spleen, implicating its role in spleen organogenesis [145]. Normally HOX11 is not expressed in thymocytes. Ectopic expression of HOX11 in T-cells caused a block at the DP stage of T-cell differentiation (Figure 5). This is consistent with genetic profiling studies which showed that overexpression



of HOX11 results in leukemic arrest at early cortical thymocytes stage (Figure 5) [108]. Overexpression of HOX11 in hematopoietic stem cells of mice developed T-cell leukemia. However, the long latency of tumorigenesis suggests other genetic abnormalities are required [146]-[148]. It should be noted that nearly all HOX11 T-ALL cases have activating NOTCH1 mutation. It has been proposed that HOX11 binding to the Groucho-related TLE corepressor was necessary for maximal transcriptional regulation of Notch1-responsive genes. This suggests that HOX11 and Notch1 may synergistically regulate transcription in T-ALL [149].

### 3.7. Epigenetic modifications

Aberrant changes in DNA methylation and histone modifications occur frequently in all cancers. Estimates vary but studies suggest that there are approximately 100 epigenetic changes for every DNA based genetic mutation. Consequently epigenetic modifications will almost certainly play an important role in T-cell leukemogenesis.

Comparative genomic hybridization data of T-ALL primary samples has shown recurrent deletions in 25% of T-ALL cases in EZH2 and SUZ12 genes. These genes are members of the polycomb repressor complex 2 (PRC2) and involved in establishing the repressive H3K27me3 mark. Activation of Notch1 was shown to cause the loss of the H3K27me3 mark by antagonizing the activity of PRC2. This data implicates histone modifications and PRC2 as important regulatory factors in T-cell leukemogenesis [150].

The CpG island methylator phenotype (CIMP) has been used to characterize T-ALL patients. The CIMP+ phenotype has a large number of hypermethylated genes with the CIMP- having a low number of hypermethylated genes. Analysis of the methylation status of 20 genes, the majority of which are implicated in abnormal T-cell biology, in 61 pediatric T-ALL patients and 11 healthy children showed a difference in the CIMP pattern. On average patients had 2.4 hypermethylated loci where none of the normal individual's loci were hypermethylated [151]. Therefore changes in the patterns of CpG island methylation at critical genes can be associated with specific tumorigenesis and consequently may be playing an important role in T-cell leukemogenesis.

## 4. Summary

Although there are a large number of genes involved in the molecular morphogenesis of T-cell leukemogenesis, many of the genes act through related pathways. This has helped us clarify the different genetic subtypes of T-ALL improving our risk stratification of T-ALL. Furthermore understanding the different genetic subtypes is allowing for personalized chemotherapy. Powerful new tools such as next-generation sequencing aid in identifying more relevant recurring lesions in leukemogenesis. This is resulting in the development of better therapeutic agents and methods. Because of improved supportive care, better risk stratification and personalized chemotherapy the 5-year survival of pediatric acute lymphoblastic leukemia has increase to 85% [152]. Even though we have made significant progress in the understanding

of the molecular morphogenesis of T-ALL there are still significant gaps in our knowledge of the genes involved in leukemogenesis.

## Acknowledgements

We are grateful to members of the Huang laboratory for their suggestions and comments. We also apologize to those whose work could not be cited due to space constraints. This work was supported by grants from the National Institute of Health (R01 HL090589 and R01 HL091929 to SH; 5T32CA9126-34 to BP; R01HL095674 to YQ).

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