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REVIEW

Deleterious point mutations in T-cell acute lymphoblastic leukemia: Mechanistic insights into leukemogenesis

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) is characterized by the leukemogenic transformation of immature T cells, which accumulate an array of genetic and epigenetic lesions, leading to a sustained proliferation of abnormal T cells. Genetic alterations in the DNA repair genes, protooncogenes, transcription factors, and epigenetic modifiers have been studied in the past decade using next-generation sequencing and high-resolution copy number arrays. While other genomic lesions like chromosomal rearrangements, inversions, insertions, and gene fusions have been well studied at functional level, the mechanism of generation of driver mutations in T-ALL is the subject of current investigation. Novel oncogenic mutations in the *TP53*, *BRCA2*, *PTEN*, *IL7R*, *RAS*, *NOTCH1*, *ETV6*, *BCL11B*, *WT1*, *DNMT3A*, *PRC2*, *PHF6*, *USP7*, *KDM6A* and an array of other genes disrupt the genetic and epigenetic homeostasis in T-ALL. In this review, we have summarized the mechanistic role of deleterious driver mutations in T-ALL initiation and progression. We speculate that the formation of non-B DNA structures could be one of the primary reasons for the occurrence of different genomic lesions seen in T-ALL, which warrants further investigation. Understanding the mechanism behind the genesis of oncogenic mutations will pave the way to develop targeted therapies that can improve the overall survival and treatment outcome.

KEYWORDS
chromosomal rearrangements, driver mutations, genomic instability, lymphoid cancer, non-B DNA structures

1 | INTRODUCTION

Among different cancers, treatment of leukemia remains a challenge due to its invasiveness, poor response to therapy, and frequent relapses. Globally, it is estimated that around 300 000 new cases of leukemia are detected annually, which accounts for 2.8% of all new cancer cases.[1,2] The 5-year event-free survival rate for ALL is 80% or more in children and younger adults.[3] According to WHO, the common subtypes of leukemia are acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL). ALL and CLL can be further subdivided into B-ALL/B-CLL and T-ALL/T-CLL depending on whether it

Abbreviations: AID, activation-induced cytidine deaminase; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; cALL, childhood acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; D2HG, D-2-hydroxyglutarate; DN, double-negative; DP, double-positive; ETP-ALL, early T-cell precursor ALL; EZH2, enhancer of zeste homolog 2; GSIs, γ-secretase inhibitors; HATs, histone acetyltransferase; HD, heterodimerization domain; HDACs, histone deacetylases; HSC, hematopoietic stem cell; IDH, isocitrate dehydrogenases; NLS, nuclear localization signals; PATRR, palindromic AT rich repeat; PHD, plant homeodomain; PHF6, plant homeodomain finger 6; PIP2, phosphatidylinositol (4, 5); SP, single-positive; SUZ12, suppressor of zeste 12 homolog; T-ALL, T-cell acute lymphoblastic leukemia; TSP, thymic seeding progenitors; USP7, ubiquitin-specific-processing protease 7; UTX, ubiquitously transcribed X.

has originated from B-lymphocytes or T-lymphocytes[4] (Figure S1). Pathogenesis of T-ALL involves abnormal proliferation and differentiation of a clonal population of immature thymocytes. Clinical symptoms of T-ALL include an increase in the number of white blood cells, bulky adenopathy, and disorders of the central nervous system.

The abnormal division of T cells is a direct consequence of the accumulation of a plethora of genetic abnormalities like chromosomal rearrangements t(5;14)(q35;q11), t(11;14)(p13;q11), activating mutations, loss-of-function mutations, gene fusions, inversions, deletions, gene duplications, and epigenetic mutations.[5,6] While genetic aberrations like translocations, large-scale deletions, and gene fusions in leukemia have been explored widely, the mechanism behind the generation of point mutations in protooncogenes, tumor suppressor genes, DNA repair genes, epigenetic regulatory enzymes, and so forth is being investigated in leukemia. For example, the tumor suppressor gene *TP53* is mutated in 15.7% of ALL cases with a higher mutation load in B-ALL compared to T-ALL,[7] while the signaling protein *NOTCH1* is mutated in 53% of T-ALL patients.[8] Similarly, several genes belonging to the category of epigenetic regulators, DNA repair, transcription factors are mutated at varying frequencies in T-ALL (Table 1). The aberrant expression of these mutated genes is associated with the relapse of T-ALL, poor prognosis, and increased antineoplastic resistance.[7,9-11] This review highlights the mechanistic role and implications of mutated genes in T-cell acute lymphoblastic leukemia. We also summarize how the dysregulation of such critical genes of the cell enhance the proliferative capacity of immature T lymphocytes, leading to the pathogenesis of T-ALL.

2 | T-CELL DEVELOPMENTAL PROGRAM IN MICE AND HUMAN

T-cell development in thymus provides the favorable microenvironment for its maturation, differentiation, and lineage specification.[12] Natural competition between bone marrow-derived progenitors and intrathymic progenitors maintain the turnover of T lymphocytes in the thymus.[13] The persistence of intrathymic bone marrow precursors capable of self-renewing activates aberrant genetic programs, culminating into T-ALL.[10]

TABLE 1 Mutation frequency of a panel of driver genes in pediatric vs adult T-ALL

Gene	Types of mutation	Mutation freq. (%)		Major consequence of the mutation	Reference
		pT-ALL	Adult T-ALL		
TP53	Missense, frameshift, in-frame, splice site and nonsense mutations	23	15.7	Gain of oncogenic activity	7
BRCA2	Missense, nonsense, splice site	10	–	Loss of tumor-suppressor function, Fanconi anemia	35
BCL11B	Missense, frameshift deletion and insertion	8	14	T cells acquire an NK-cell like property and stop differentiating	11,56
ETV6	Frameshift insertion, missense, nonsense, splice site	5	14	Loss of transcription repression activity	63,80
GATA3	Missense, in-frame deletion, splice site insertion	8	3	Loss of its transcriptional activity	80
RUNX1	Frameshift, splice site, missense	–	10	Loss of transcription repression activity; juxtaposition with *ETV6*	80
DNMT3A	Frameshift, missense, nonsense, splice site	6-14	4-18	Mutation in catalytic domain decreases its methyltransferase activity	52,67,69
TET1	Missense	–	6-14	Aberrant distribution of histone modification marks	74
IDH1	Missense	–	2-6	Expression of myeloid genes in T-cells	10,74,77
IDH2	Missense	11-18	5-9	Expression of myeloid genes in T-cells	10,74,77
EZH2	Missense, frameshift insertion	6-11	11-18	Loss of PRC2 tumor suppressor activity	74,80
SUZ12	Frameshift deletion	5-10	6-11	Loss of PRC2 tumor suppressor activity	74,80
EED	Missense, frameshift insertion	2	5-10	Loss of PRC2 tumor suppressor activity	74,80
EP300	In-frame insertion, missense, splice site	19	2	Loss of histone acetyltransferase activity	74
PHF6	Frameshift, in-frame mutation, missense, nonsense, splice site	8	30-38	Aberrant expression of T-cell transcription factor, *TLX1* and *TLX3*	93
USP7	Frameshift, missense	1-4	Rare	Loss of deubiquitinase activity	64
HDAC5/7	–	–	1-4	Loss of histone deacetylase activity	74
KDM6A	Frameshift, missense	–	4.5	Loss of histone demethylase activity	65

Note: A cohort of genes harboring mutations in pediatric vs adult T-ALL and its role in the progression of T-ALL. The different mutation frequencies reported here are based on several previously published studies that analyzed pediatric and adult T-ALL patient cohorts. "pT-ALL" indicates pediatric T-ALL.

In the thymus, the progenitors fully commit to T-cell lineage by activating T-cell specific transcriptional program.[14] In both humans and mice, differentiation of progenitors into double-negative (DN) thymocytes commits them to the T-cell lineage. The productively rearranged TCRαβ complexes at this stage provide the necessary signal to progress into the $CD3^+CD4^+CD8^+$ (DP), double-positive stage.[15] The second phase of selection involves three key components consisting of negative selection to eliminate strong self-reactive clones, positive selection of T cells that can recognize self-antigens bound to self-MHC molecules, and death by neglect.[16] The positive selection gives rise to $CD4^+CD8$-$CD3^+$ or $CD8^+CD4$-$CD3^+$ (SP) single-positive cells that ultimately emerge as peripheral naïve T cells (Figure 52).[17] A fraction of peripheral T cells enter into a memory phase, in which long-term memory T cells protect against subsequent infection.[18] An array of transcription factors regulates the maturation, differentiation, and lineage specification of T cells. Notch signaling sets up the platform for induction of T-cell specific genes, *TCF7*, *GATA3*, and *BCL11B*, which orchestrates the T-cell program and seals off the T-cell commitment fate.[19,20]

3 | GENETIC PROFILE OF T-ALL

During the onset or progression of the T-cell developmental program, preleukemic clones accumulate a number of genetic alterations, which cooperate to disrupt the genetic equilibrium of the cell. T-ALL is one such cancer where a deleterious mutation remarkably changes the cancer genetics, requiring the development of targeted therapies against the specific mutations. The driver genes which majorly contribute to the mutational spectrum in T-ALL are listed below.

3.1 | DNA repair genes

3.1.1 | TP53

The tumor suppressor *TP53* undergoes a large number of genetic changes, among which gain-of-function mutations majorly contribute to its inactivation in hematopoietic and nonhematopoietic malignancies.[21] Interestingly, greater than 60% of human T-cell leukemia cell lines derived from relapse T-ALL cases showed activating mutation in both the p53 alleles.[7,22,23] The mutations and deletions of the *p53* gene in the initial diagnosis phase of both childhood and adult T-ALL were rarely observed, bringing into question the exact mechanism of p53-driven T-ALL.[23,24] An in-depth analysis of T-ALL patient samples from both diagnosis and relapse phase clearly shows that around 20% to 30% of relapse cases possess mutations in the *p53* gene.[23,25-27] This was associated with a poor treatment outcome, reduced duration of survival after the first remission, diminished response to reinduction therapy, and increased fatality.[7,23,26,28] On a functional level, mutant p53 helps cancer cells maintain high glycolytic output, protects against oxidative stress, blocks apoptosis, promotes metastasis, and increases antineoplastic resistance.[29]

p53 −/− mice spontaneously develop T and B cell lymphomas, owing to a loss of tumor-suppressive activity.[30] p53 loss is required for both tumor initiation and maintenance, making it a promising therapeutic strategy in cancer patients.[31] A study by Hsiao et al showed that transplantation of only the T-ALL cell line (CEM) harboring independent mutations of both p53 alleles was capable of causing tumor cell proliferation and hematological disorder in SCID mice[32] whereas the presence of either wild-type p53 or no p53 protein did not lead to such a phenotype. Further, infection of p53-negative Be-13T-ALL cells with viruses encoding dominant and activating mutant p53 genes resulted in the acquisition of metastatic potential and tissue invasiveness.[32]

Therefore, mutational activation of *p53* causes a genetic imbalance, especially in relapsed T-ALL cases, leading to the clonal selection of tumorigenic T cells.

3.1.2 | BRCA2

The Fanconi Anemia (FA) pathway maintains genomic integrity and stability by repairing DNA interstrand crosslinks and stalled replication forks.[33] Consequently, patients with Fanconi anemia develop congenital abnormalities along with an increased predisposition to leukemia.[34] BRCA2, identical to FANCD1, is a vital component of the Fanconi pathway, which suppresses the transformation of immature T cells in mice.[35] Mice harboring mutation in *Brca2* undergo extensive chromosomal breakage, rearrangements promoting the development of T-cell lymphomas.[36,37] Murine cells with truncated Brca2 accumulate DNA breaks, replicative failure, elevated p53 and p21 expression, and aberrant chromatid exchanges, reinforcing the role of BRCA2 in DNA repair.[38,39] *BRCA2*, a component of the Fanconi-BRCA DNA repair pathway harbors a monoallelic mutation in 23% of childhood T-ALL cases, conferring an incomplete Fanconi-BRCA pathway inactivation.[35] Thus, a partially active Fanconi pathway may be required for the T cells to fully transform into leukemic cells in cooperation with other oncogenic events.[35] Interestingly, the *BRCA2* haploinsufficient cells were prone to DNA damage and exhibited increased sensitivity to UV radiation.[35] An independent study by Hirsch et al led to the association of biallelic *BRCA2/FANCD1* mutations with an increased predisposition to solid tumors and early onset of T-ALL in the D1 subtype of FA.[40] However, further investigation is required to establish the exact role of *BRCA2* in T-ALL disease progression.

3.2 | Signaling pathways

Genomic alterations in the genes associated with the cell survival and proliferating pathways, namely, *RAS*, *PI3-AKT*, and *NOTCH*, have been recently discussed in numerous reviews.[6,41-43] Here, we summarize the impact of the somatic point mutations in altering the genomic landscape in T-ALL.

3.2.1 | IL-7-IL-7R

The IL-7-IL-7R signaling pathway required for the normal development and maintenance of the immune system, harbors mutations in 9% of pediatric T-ALL and 12% of adult T-ALL cases (Figure S3A).[2,44] Mice with IL-7 or IL-7R deficiency showed impaired thymocyte development and reduced numbers of peripheral T cells.[45] In association with mutated *PTEN*, these genetic aberrations impart a survival advantage to the tumor cells by upregulating PI3K/Akt/mTOR pathway. In addition, oncogenic mutations of the downstream signaling genes, for example, JAK/STAT and PI3K/AKT/mTOR, (~10%-30%)[2,46,47] make this pathway an ideal candidate for the development of targeted therapies in T-cell leukemia (Figure S3B).

3.2.2 | NOTCH1 signaling

The NOTCH1 protein, required during different stages of T-cell development, can undergo t(7;9) translocations leading to human T-ALLs (Figure 1A).[8,48] The *NOTCH1* heterodimerization domain *(HD)* mutants mainly result in in-frame deletions and insertions conferring ligand-hypersensitivity or ligand-independent NOTCH1 activation in T-ALL (Figure 1B).[49] The *NOTCH1ΔPEST* mutations lead to upregulation of activated NOTCH1 due to impaired degradation of the activated receptor by the proteasome complex (Figure 1C),[50] which, in turn, promotes cell growth, proliferation, premature entry into the S-phase, and a host of other mutagenic events.[51]

3.3 | Transcription factors

Deregulation of transcription factors by activating/loss-of-function mutations is a common mechanism by which T-cell progenitors gain oncogenic properties to progress into a malignant state. T-ALL is classified into three different subgroups; thymic-T-ALL, pediatric T-ALL, and adult T-ALL, based on the ectopic expression of one particular transcription factor (*TAL1*, *TLX1*, *TLX3*, *LMO2*) and other distinct genes,[52] some of which are described below.

3.3.1 | BCL11B

The transcription factor known to play a key role in maintaining the identity and differentiation of T cells is BCL11B, which is highly expressed at the DN stages of T-cell development.[53,54] It acts as a tumor suppressor gene in mice, while in humans, it displays a context-dependent protooncogenic or tumor-suppressive function.[54]

Several studies suggest that missense mutations or deletions in *BCL11B* (~15%), in human T-ALL cases, confer NK-cell like properties to T cells, resulting in sustained proliferation and survival of immature T cells.[55,56] The mutation load is significantly higher (~14%) in Exon 4 of *BCL11B* compared to the other three exons (Figure 2A).[11] These mutations disrupt the structure of the zinc finger domains and abrogate the transcription factor binding to DNA.[11] In addition, BCL11B has also been implicated in the development of T-cell lymphomas/leukemia by its frequent involvement in various translocations, for example, t(5;14) (q35;32).[57]

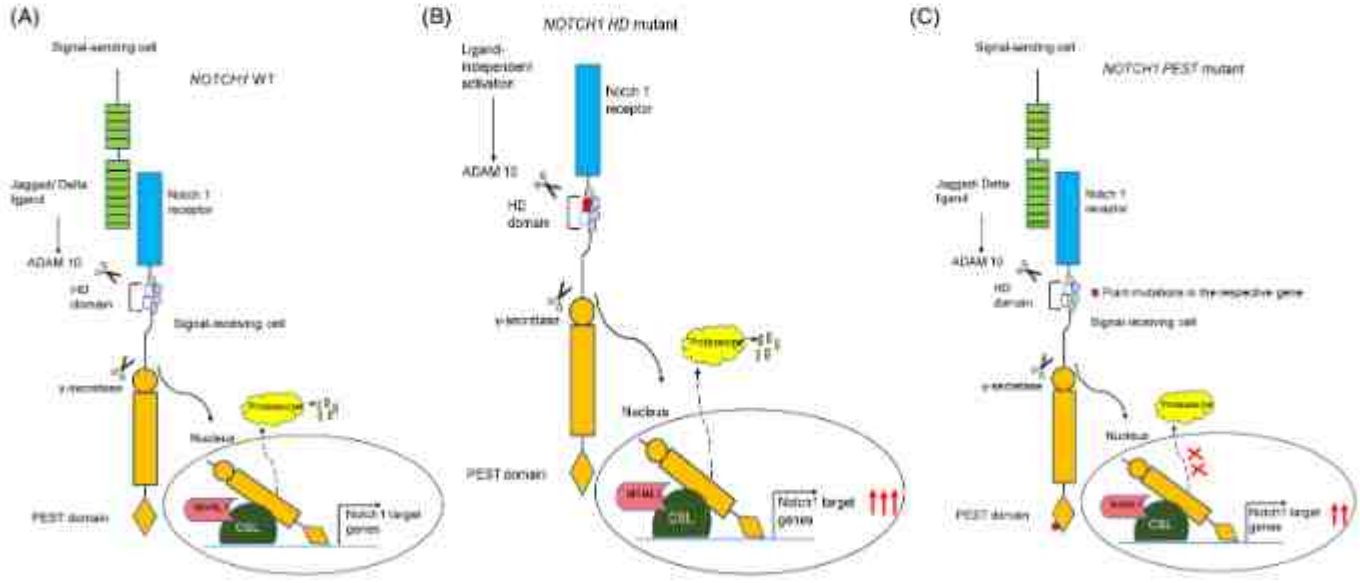

FIGURE 1 Aberrant NOTCH 1 signaling in T-ALL. A, Binding of the Notch 1 receptor to Jagged/Delta ligand triggers the proteolytic cleavage of the receptor, first by an ADAM metalloprotease followed by the γ-secretase complex, which releases the intracellular domains of NOTCH1 from the membrane. In the nucleus, this domain interacts with DNA via the CSL DNA binding protein and recruits the MAML1 coactivator to activate the expression of NOTCH1 target genes. Once the target genes are activated, the NOTCH1 receptor is degraded in the cytoplasm by the proteasome complex. B, Mutation in the HD domain leads to ligand-independent activation of NOTCH1 and overexpression of NOTCH1 target genes, enhancing cell survival and proliferation. C, Mutation in the NOTCH1 PEST domain leads to constitutive activation of NOTCH1 signaling by preventing the degradation of the receptor by the proteasome complex. Red squares indicate point mutations [Color figure can be viewed at wileyonlinelibrary.com]

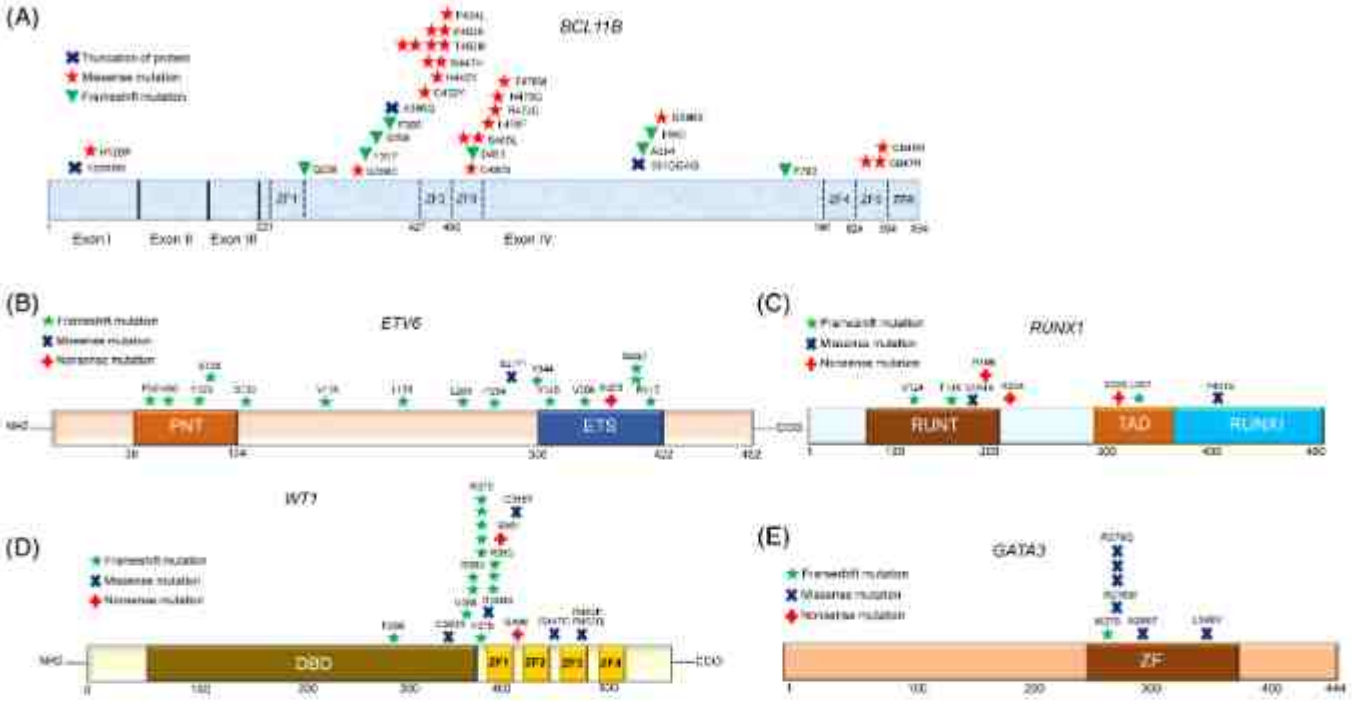

FIGURE 2 Schematic representation showing point mutations in the transcription factors in T-ALL. A, The zinc-finger domains of BCL11B, responsible for DNA-binding, are mutated across different subgroups of T-ALL patients. The majority of the mutations are missense/frameshift mutations, which abrogate the binding of this transcription factor to DNA. B, *ETV6* harbors mutation, especially in the PNT (homodimerization) and ETS (DNA-binding) domains, the majority of which are frameshift mutations. C-E, Frameshift and missense mutations are predominantly present in *RUNX1* (C), *WT1* (D), and *GATA3* (E), which abrogates DNA binding activity [Color figure can be viewed at wileyonlinelibrary.com]

3.3.2 | ETV6

The *ETV6 (or TEL)* gene encodes a transcriptional repressor with two functional domains, a C-terminal DNA-binding domain (ETS domain) and an N-terminal homodimerization domain (PNT domain).[58] The homodimerization of ETV6 is indispensable for its transcriptional repression activity. The repression of target genes by ETV6 is vital in the regulation of cell growth, differentiation, and hematopoiesis.[59,60]

ETV6 is frequently altered in leukemia owing to its participation in various translocations, two of the well-known players being *RUNX1* and *ABL1/2*.[61,62] *ETV6* also undergoes frequent mutation/deletion in 8% of pediatric T-ALL and 14% of adult T-ALL (Figure 2B).[6] In immature T-ALLs, 80% of *ETV6* mutant cases showed concomitant mutations in *NOTCH1*, hinting at a specific interaction between the two oncogenic proteins in the pathogenesis of immature T-ALL.[63] Thus, mutational inactivation of *ETV6* cooperates with oncogenic mutations in other genetic factors like *RUNX1* (8%-10%), *GATA3* (3%-5%), *WT1* (11%-19%), *BCL11B* (9%-16%) to transform preleukemic cells to leukemic cells (Figure 2C-E).[6,52]

4 | EPIGENETIC MUTATIONS IN T-ALL

Besides the genomic alterations in leukemia, the disruption of the epigenomic homeostasis in T cells plays a significant role in the disease progression of T-ALL. In a recent study, T-ALL was found to be among the cancers with the highest frequency of mutations in genes involved in regulating the epigenome.[64] The epigenetic modifications mainly comprise DNA methylation, histone modifications, nucleosome remodeling, and so forth.

4.1 | DNA methylation and demethylation

The DNA methyltransferases DNMT1, DNMT3A, and DNMT3B set up the DNA methylation pattern in humans by covalent addition of methyl groups to cytosine, which is part of the CpG dinucleotides.[65] DNMT3A and DNMT3B are required for de novo methylation and, in cooperation with DNMT1, maintain the methylation marks in the genome.[66] Around 4% to 18% of T-ALL cases harbor mutations in *DNMT3A*, associated with a poor prognosis and shorter disease-free survival[52,67] (Figure 3A and Table 1), with R882H being the most frequent "hotspot" mutation.[68] Whole-exome sequencing identified a high rate of *DNMT3A* mutations (16%) in a high-risk subgroup of adult patients with ETP-ALL[69] making it an ideal prognostic marker. On the contrary, pediatric T-ALL patients harbor infrequent *DNMT3A* mutations (1.4%).[70] In fact, analysis of a large cohort of T-ALL patients using NGS approach correlated *DNMT3A* mutation with older age, immature leukemia, lower remission rates, and increased incidence of relapse.[71]

Therapies targeting the majorly altered/mutated pathways in cancer treatment often undergo clinical failure due to cooperative

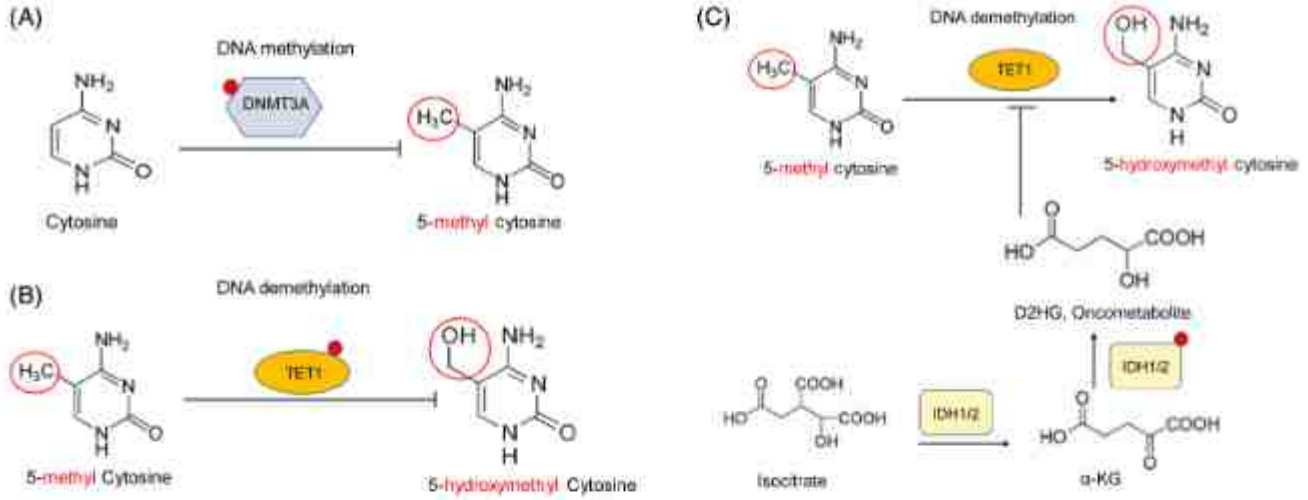

FIGURE 3 Schematic representation of genomic alteration in the epigenetic regulators found in T-ALL. A, *DNMT3A* is responsible for DNA methylation of cytosines, which are part of CpG dinucleotides. Mutation in *DNMT3A* leads to loss of methyltransferase activity and subsequent hypomethylation of CpG islands. B, The TET enzymes catalyze the conversion of methylated cytosine to 5-hydroxymethyl cytosine, which promotes DNA demethylation. *TET1* mutations blocks this action, leading to silencing of active genes, for example, tumor suppressors. C, Mutations in *IDH1/2* inhibit the activity of TET enzymes via the production of the oncometabolite 2-hydroxyglutarate (D2HG). Red circles indicate point mutations [Color figure can be viewed at wileyonlinelibrary.com]

oncogenic lesions from other genes. One such example is the failure of targeted therapies against NOTCH1-driven T-ALL due to recurrent mutagenic events in *DNMT3A*. Kramer et al showed that in mouse models, Dnmt3a loss-of-function cooperates with Notch1 gain-of-function to drive an aggressive T-ALL.[72]

Mutations disrupt the balance in the epigenetic homeostasis in *TET* enzymes, which catalyze the conversion of methylated cytosine to 5-hydroxymethyl-cytosine, promoting DNA demethylation.[73] *TET1* mutations have been reported in 6% to 14% of T-ALLs (Figure 3B).[74] Strikingly, the interaction between inactivated *TET2* and *DNMT3A*^{R882H} alters the global DNA methylation landscape, compromising the function of several tumor suppressors in the mouse model of T-ALL.[75] TET enzymes require 2-oxoglutarate for its function; one of the sources is isocitrate dehydrogenases (IDH) that catalyzes the decarboxylation of isocitrate to 2-oxoglutarate.[76] In fact, a mutation in *IDH1* and *IDH2* reported in 2% to 10% of early immature adult T-ALL subgroup confers a worse clinical outcome (Figure 3C).[10,63,77] Subsequent gain-of-function activity of the enzyme leads to NADPH-dependent reduction of α-ketoglutarate (α-KG) to the oncometabolite, 2-hydroxyglutarate.[77] However, the implications of such cooperative oncogenic mutations in T-ALL disease progression need to be explored further.

4.2 | Posttranslational histone modifications

The histones undergo a multitude of posttranslational modifications, including lysine acetylation, serine, and threonine phosphorylation, lysine and arginine methylation, lysine ubiquitinylation, SUMOylation, and so forth. HATs (histone acetyltransferase) and HDACs (histone deacetylases) regulate the acetylation and deacetylation of core histone proteins and nonhistone proteins like MYB, E2F1, TP53, and RB1, respectively, thereby triggering gene transcription on receiving the proper cue.[78] However, this balance is disrupted when the epigenetic regulators undergo somatic mutation during cancer initiation. In pediatric and adult T-ALL, the histone acetyltransferase *EP300* undergoes mutation in ~2% of the cases whereas *HDAC5* and *HDAC7* are mutated in about 1% and 4% cases, respectively.[74] Mice with double knockout of *Hdac1/2* develop a pathological diseased state characterized by an enlarged spleen and thymus. Infiltration of immature T cells into the lymphoid and nonlymphoid tissues in the knockout mice leads to neoplastic transformation and chromosomal instability.[79]

Similarly, the significance of transcriptional gene repression mediated by the Polycomb repressive complex 2 (PRC2) was elucidated following the *loss of function* mutations of the core components of this complex (*EZH2*, *SUZ12*, and *EED*) in both pediatric[80] and adult T-ALL.[81] The PRC2 component, *EZH2*, along with *SUZ12*, mediates gene silencing by catalyzing di- and tri-methylation of Lys 27 on histone H3 (H3K27me2/3).[82] Mutations in the SET domain of *EZH2*, responsible for its N-methyltransferase activity[80] leads to spontaneous development of T-ALL in mice carrying Cre-mediated deletion of the SET domain of *EZH2*.[83] Therefore, a loss of tumor-suppressive function of EZH2 can account for its low expression and frequent alteration.[83] A study by Ntziachristos et al showed that loss of function of PRC2 components leads to T-cell malignancy initiation, which progresses into T-ALL when concomitant *NOTCH1* mutation triggers the eviction of the PRC2 complex near NOTCH1 target genes, leading to loss of H3K27me3 mark and activation of a host of genes with oncogenic potential.[81]

Counteracting the activity of the PRC2 complex is the histone H3K27me3 demethylase, an ubiquitously transcribed X (UTX) chromosome tetratricopeptide repeat protein (*KDM6A*).[84] Exome sequencing of a cohort of adult T-ALL patients and cell lines identified somatic mutations (~4.5%) in the *UTX/KDM6A* gene.[85] UTX was identified as the first X-linked tumor suppressor in T-ALL which escapes chromosome X-inactivation in female T-ALL patients, thus, explaining the skewed gender distribution of inactivating somatic mutations.[86] Loss of *Utx* accelerates leukemia onset in a Notch1-driven T-ALL mouse model.[87] Surprisingly, in TAL1-positive T-ALL, it plays an oncogenic role by promoting TAL1-specific leukemic gene expression program.[88] This subtype-specific differential role of UTX makes it a potential candidate for epigenetic drug targeting. In fact, inhibition of UTX with the H3K27 demethylase inhibitor GSK-J4 selectively eliminates TAL1-positive leukemic cells in both cell culture and xenograft models of patient-derived leukemia.[88]

In contrast, the H3K27 histone demethylase, JumonjiD3 (JMJD3) (or KDM6B) is required for the seeding and maintenance of T-ALL through interaction with NOTCH1. Genetic ablation of Jmjd3 in T-ALL diminishes leukemic cells in the peripheral blood, and minimizes the infiltration of leukemic cells into the spleen and liver.[89]

Along with histone methyltransferase and demethylases, inactivating mutations in the deubiquitylating enzyme, USP7 (Ubiquitin-specific-processing protease 7) destabilizes the tumor suppressors, p53, DNMT1, PTEN and histone H2B.[64,90] The mutations reside mostly at the binding interface between the catalytic domain of USP7 and ubiquitin, thereby interfering with the deubiquitinase activity of the enzyme.[64] However, the somatic loss of function mutations was reported only in 8% of pediatric T-ALL and rarely in adult T-ALL cases.[64] Nevertheless, malignant transformation of immature T cells due to loss of a deubiquitylating enzyme warrants further investigation.

4.3 | PHF6: An epigenetic regulator?

The role of HATs, HDACs, PRC complex, and so forth as epigenetic modifiers have been well documented in leukemia. However, growing evidence suggests the role of plant homeodomain finger 6 (*PHF6*) gene, initially discovered as the causal gene behind the X-linked disorder, Borjeson-Forssman-Lehmann syndrome, as an epigenetic modifier.[91] PHD domain-containing proteins are known to recognize methylation marks on histone, implicating the role of PHF6 in chromatin structure reorganization.[92]

A study by Vlierberghe et al showed that the *PHF6* gene harbors inactivating mutations and deletions in 16% to 19% of pediatric and 30% to 38% of adult primary T-ALL cases (Figure S4).[93] Loss of function of this tumor suppressor gene was significantly associated with leukemogenesis driven by aberrant expression of the transcription factor oncogenes, *TLX1* and *TLX3*.[93] Thus, PHF6 can be called a new X-linked tumor suppressor in T-ALL, inactivation of which leads to aberrant expression of oncogenic transcription factors in leukemia.[93]

In summary, along with these epigenetic regulators, mutation in several other histone methylases, demethylases, acetylases, such as SETD2, MLL complex, NSD2 disrupt the epigenetic equilibrium in T-ALL.[74]

5 | MECHANISTIC INSIGHT INTO THE GENESIS OF MUTATION IN T-ALL

The deleterious point mutations in genes associated with DNA repair, signaling networks, transcription factors, epigenetic regulators, and so forth initiates a chain of events that activates the oncogenic programs in T-ALL. A recent study indicated the formation of non-B DNA structures as a major determinant of mutagenesis, which increases the likelihood of recurrent somatic mutations in the genome.[94] Several reports suggest that one of the major causes of genomic instability is the occurrence of non-B DNA structures in the genome.[95-98] Formation of noncanonical structures like G-quadruplexes, triplexes, cruciform are favored in several genes due to the abundance of repetitive sequences in the eukaryotes.[99-102] Interestingly, such altered structures in the genome have been mapped to regions of patient chromosomal breakpoints in the majority of genes deregulated in lymphoid cancers, for example, *BCL2* major breakpoint region associated with t (14;18) translocation in follicular lymphomas.[95,98,103-105] Similarly, G-quadruplex motifs have been reported in *c-MYC*, *c-KIT*, *VEGF*, *HIF1α*, and *KRAS*, associated with different types of chromosomal aberrations.[95,106-110] In addition to G-quadruplex, Palindromic AT Rich Repeat (PATRR) and cruciform DNA structure have also been implicated in t(11;22).[111]

t(10;14) translocation, associated with 5% to 10% of T-ALL cases, involves a reciprocal translocation between chromosomes 10 and 14, wherein the *HOX11* gene juxtaposes with the TCR locus, resulting in altered expression of *HOX11* in T cells.[97,112] The presence of two G-quadruplex motifs, capable of folding into G4 structure, flanking either side of *HOX11* breakpoint regions suggests a potential role of G-quadruplex formation and increased frequency of chromosomal translocation.[97,103]

Additionally, misrecognition of certain chromosomal fragile sites, LMO2 (t(11;14) (p13; q11)), Ttg-1 (t (11;14) (p15; q11)), and Hox11 (t (10;14) (q24; q11)) by the RAG complex serves as one of the major determining factors behind the frequency of these translocations in T-ALL.[113] Analysis of several human translocation breakpoints at non-Ig genes, including *BCL2*, *BCL1*, and *E2A* reveals an additional mechanism of genetic rearrangement around the breakpoint regions in several leukemias and lymphomas.[114] The breakpoints showed clustering around CpG sites, which was more prominent in pro-/pre-B stages and drastically reduced in other mature cell types. The increased frequency of translocation around the "CpG hotspot" region can be explained by the sequential action of AID and RAG, wherein demethylation of CpG regions by AID generates T:G mismatches, prone to cleavage by RAG endonuclease, before repair can be completed.[114] This double-strand break event leads to frequent translocations observed in B cell lymphomas and leukemia. Besides, AID is also capable of generating point mutations and other genomic aberrations by binding to single-stranded

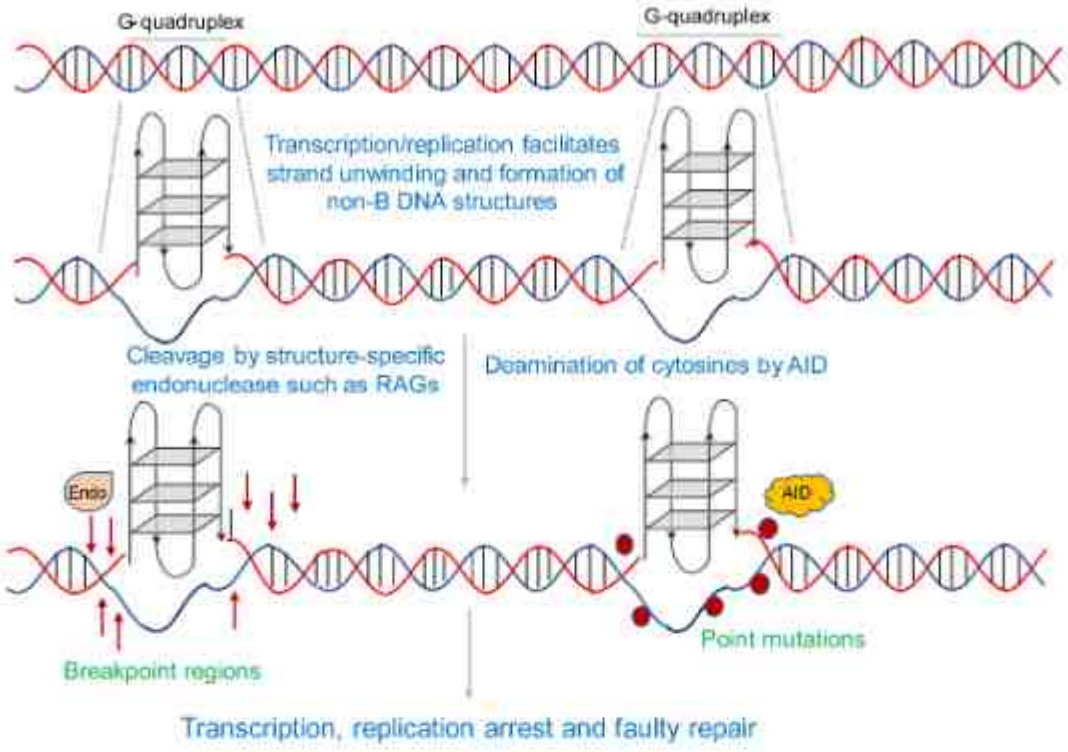

FIGURE 4 The plausible mechanism behind the introduction of genomic lesions in leukemia/lymphoma. Inside the cell, unwinding of DNA takes place during transcription/ replication/ recombination, and so forth facilitating the formation of different non-B DNA structures like G-quadruplex, triplex, cruciform, and so forth. The formation of such noncanonical structures in the genome makes it prone to cleavage by structure-specific endonuclease like RAGs or deamination by deaminases like AID. This results in genetic mutations, single-strand, and double-stranded breaks, which ultimately culminates into fragile breakpoint regions. Activation of faulty DNA repair pathways further contributes to the disease biology and pathogenesis of leukemia/lymphoma. Arrow heads indicate breakpoint regions, while red circles indicate point mutations [Color figure can be viewed at wileyonlinelibrary.com]

regions of non-Ig genes for example, *p53*, *c-MYC*, *KRAS*, *SMAD4*, *BCL6* in different types of cancer.[115-118] However, more studies are required in this direction to elucidate the mechanism of generation of point mutations in T-ALL (Figure 4).

6 | CONCLUSION

The genetic and epigenetic aberrations that accumulate in the genome play a significant role in altering the genetics of childhood and adult T-ALL. Point mutations, specifically the missense and frameshift mutations, can lead to loss of functionality or gain of the oncogenic activity of different genes. This sets the stage for a series of different oncogenic events transforming normal T cells to leukemic cells. While the role of genomic lesions behind the pathogenesis of T-ALL is quite well studied, alteration of the chromatin landscape due to inactivation of histone methylases, demethylases, ubiquitinases, such as *DNMTs*, *KDM6A/B*, *USP7*, and so forth warrants further investigation. Decoding the mutation spectrum of these genes in different subtypes of T-ALL would pave the way for the development of "new-age" epigenetic drugs leading to a better treatment outcome. One of the plausible mechanisms behind the generation of these mutations is the formation of non-B DNA structures in the vicinity of the breakpoint regions.[95,98,105] Likewise, an in-depth analysis of the breakpoint regions is imperative to decipher the seeding events behind the initiation of the deleterious genetic rearrangements in T-ALL.

7 | FUTURE DIRECTIONS

Mutagenic events contribute significantly to the disease biology of hematological and nonhematological malignancies. However, in leukemia and lymphoma, both chromosomal translocations and driver mutations are the leading players behind altering its genetic and epigenetic profile. While the molecular mechanism behind chromosomal translocations is well explored, the potential reasons behind the induction of driver mutations in T-ALL needs more attention. This is particularly important in epigenetic regulators, mutations in which are capable of altering the chromatin landscape, organization, and transcriptional status of cancer-related genes. Besides, driver genes harboring a distinct mutation pattern can also serve as biomarkers in leukemia. Genetic and epigenetic biomarkers can predict disease prognosis, treatment outcome, and cancer initiation.[119] Thus, identifying the driver mutations in putative genes involved in leukemogenesis and determining the molecular mechanism behind their origin is pivotal for the development of targeted therapies in leukemia.

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CONFLICT OF INTEREST

Authors disclose that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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