Role of TLX1/HOX11 in transcriptional activation of genes relevant to Leukemogenesis

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***Abstract*-T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive malignant neoplasm of the T-cells. T-cells a type of white blood cells originates in bone marrow and develop in thymus. T-ALL accounts for ∼20% of all cases of ALL. It is aggressive in nature and progresses very quickly. Although, causative factors of T-ALL are not much explored, few reports suggest that TLX1 is found inappropriately expressed in leukemic cases and associated with leukemogenesis. *TLX1* encoding the transcription factor T-cell leukaemia homeobox protein 1. Chromosomal translocation is associated with the leukemic transformation of T-cells and 30% of total T-ALL cases are found to be positive for *TLX1* translocation (10:14). Intrigued by this, we are intended to understand the possible role of 10:14 chromosomal translocation and thus the *TLX1* activation and TLX1 induced malignancies complex intracellular changes in genes like NOTCH1, PTPN2, WT1, BCL11b, PTEN, PHF6 genes are altered which are involved in tumour cell survival and progression. This TF regulates a number of genes by binding to the promoter region in the regulatory region of the target gene.**

***Keywords*- T-cell acute lymphoblastic leukaemia (T-ALL), *TLX1/HO11*gene, TLX1-T-cell leukaemia homeobox protein 1,**

I. INTRODUCTION

T-cell leukemia is a kind of blood cancer. The *TLX1* gene, which was formerly known as *HOX11* in humans, encodes a transcription factor known as homeobox protein 1. TLX1- T-cell leukaemia homeobox protein 1 is a member of the NKL subfamily of HOX genes, which controls cell proliferation and differentiation throughout proper spleen and nervous system development. It regulates retinoic acid signaling. TLX1-null mice are devoid of spleens but otherwise healthy. The DNA-binding homeodomain of the TLX1 protein has been conserved throughout evolution, and its physiological expression is typically confined to embryonic development. Although TLX1 is not typically produced in the hematopoietic system (T-cell lineage), genetic disorders such as chromosomal translocations cause the proteins to be inappropriately expressed. The malignant transformation of growing T-cells causes T-cell acute lymphoblastic leukemia (T-ALL), an aggressive hematologic malignancy. In 5 percent to 10% of paediatric T-ALL cases and up to 30% of adult T-ALL cases, the TLX1 transcription factor oncogene translocation t (10;14) (q24; q11) occurs. The leukemogenicity potential of TLX1 was first tested in a bone marrow transplantation experiment model in which murine hematopoietic stem and progenitor cells (HSPCs) were retrovirally transduced with TLX1 and then transferred into lethally irradiated recipients. Two transplanted mice out of twelve mice developed T-ALL. TLX1 overexpression may not be sufficient for T-cell transformation rather TLX1 and NOTCH1 functionally interact to promote the leukemic phenotype. Continuous TLX1 expression is required for leukemic maintenance and its knockdown in T-ALL leads to massive apoptosis of leukemic cells. It was supposed that TLX1 blocks thymocyte differentiation at the DN stage. Time-course analysis of TLX1-dependent thymocyte arrest reveals that TLX1 blocks human thymocyte development prior to the DP stage of differentiation. These pre-T-cells are non-functional and over proliferate without differentiating into T-cells and circulating in a large amount in the blood. Leukemogenesis results from decreased cell death and increased proliferation of undifferentiated TLX1 expressing thymocytes. The lack of anti-apoptotic factors in this stage explains the high responsiveness to chemotherapy and the associated excellent outcome. In TLX1 induced malignancies complex intracellular changes in genes

like NOTCH1, PTPN2, WT1, BCL11b, PTEN, PHF6 genes are altered which are involved in tumor cell survival and progression. This TF regulates a number of genes by binding to the promoter region in the regulatory region of the target gene.

II. TLX1- T-CELL LEUKAEMIA HOMEOBOX PROTEIN 1

This gene codes for a nuclear transcription factor that is part of the NK-linked or NK-like (NKL) homeobox gene family. It was previously known as *HOX11*. In humans, the gene is found on chromosome 10 at 10q24.31 on the long arm of the chromosome. Id-3195 is a gene that has been identified. The T-cell leukemia homeobox 1 protein is 330 amino acids long. The homeodomain is the only DNA binding domain in this protein.

III. CONTRIBUTION OF *TLX1* IN T-ALL (T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA)

T-cell acute lymphoblastic leukaemia (T-ALL) is a deadly hematologic malignancy caused by the malignant transformation of T-cells while they are still growing and not differentiated enough to support immune system. In 5% to 10% of paediatric T-ALL patients and up to 30% of adult T-ALL cases, the TLX1 transcription factor oncogene is the target of translocation t (10;14) (q24; q11).

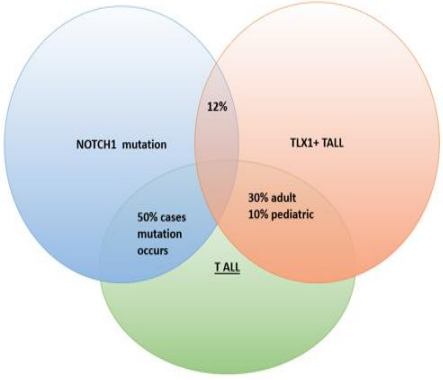

Figure no:1 Contribution of *TLX1* in T-ALL condition

IV. ACTIVATION OF *TLX1* IN T-CELL LEUKEMOGENESIS

T-cell leukaemia homeobox 1 (*TLX1*, also known as *HOX11)* promotes cell fate determination and organ growth by acting downstream of the genetic cascade controlling spleen development. The hematopoietic system does not typically express TLX1 (T-cell lineage or B-cell lineage). TLX1 has no role in adult life, but when triggered by chromosomal translocations in T-cell acute lymphoblastic leukaemia, it causes malignant transformation of T-cells (T-ALL). In the T-cell receptor loci, the entire *TLX1* gene translocated under the influence of strong enhancers. T-ALL patients with a t (10;14) (q24q11) translocation, in which the TLX1 gene is juxtaposed to the Ddelta2, Ddelta3, or Jdelta1 gene segments of the TCRdelta receptor at locus 14q11.2, have been found. The most important factor is how chromosomal breaks appear inside the cells. Ionizing radiation, reactive free radicals, pathologic activity by nuclear enzymes, failed topoisomerase processes, and mechanical stress are all examples of universal causes that affect all living cells and inside lymphoid cells as well. The RAG complex's physiologic activities, as well as activation-induced deaminase (AID), lead to translocations in lymphoid cells. *HOX11* gene translocation with the TCR promoter is considered to be caused by errors in the normal V(D)J rearrangement mechanism during T-cell development. Indeed, investigations of certain t (10;14) (q24; q11) breakpoints found heptamer and nonamer-like sequences in the DNA flanking the 10q24 breakpoints, which typically signal V(D)J recombination. [4] Some studies hint to the presence of G-quadruplex structures near the *HOX11* breakpoint area, which might explain the translocation's fragility. [5] By inhibiting T-cell differentiation before the beta selection stage, defects in the normal gene rearrangement process resulted in abnormal expression of the TLX1 transcription factor inside cells and promote the formation of blasts.

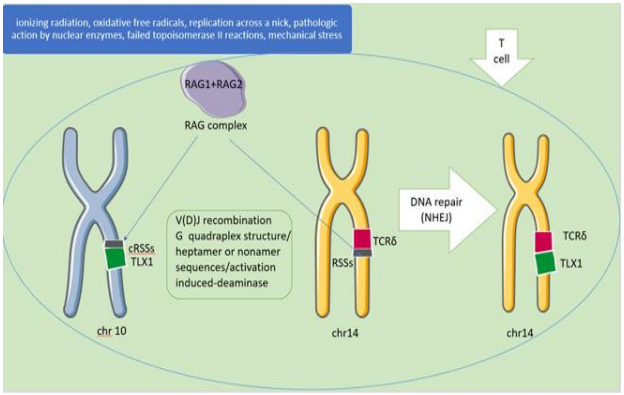

Figure no:2 - Activation of *TLX1* in T-cell leukemogenesis (Most possible mechanisms by

which the translocation t (10;14) (q24; q11) occurs inside the T-cell and the intact *TLX1* gene translocated under the influence of strong enhancers in the TCRδ receptor, when external and internal stress imposes on cells (such as ionic radiation, oxidative stress, nick or pathologic action by nuclear enzymes). RAG (Recombination activating gene) enzyme mediated breaks occur inside the chromosome, generally on RSSs (recombination signal sequences) or sometimes on cRSSs (cryptic Recombination signal sequences) and again re-join by the DNA repair mechanism i.e.-NHEJ-NeighbourHood End Joining)

V. MECHANISM OF ACTION OF TLX1 AND ITS TARGETS

For a long time, homeodomain proteins (homeoproteins) have been known to be potent transcriptional regulators. Depending on the physiological environment and its interactions with transcriptional cofactors, TLX1 may serve as both an activator and a repressor of gene transcription at the molecular level. The protein's quaternary structure interacts as a monomer or a homo/heterodimer. During spleen development, TLX1 regulates cell fate determination and organ growth. This protein is also involved in neuronal cell fate determination. The TLX1-dependent regulation of retinoic acid (RA) metabolism is important for spleen organogenesis since it controls many RA metabolism genes. TLX1 cooperates with the AP-1 family of transcription factors to control gene expression by binding to the regulatory regions of RA-associated genes through the AP-1 site. [7] Because the in vitro DNA binding sites of homeoproteins are short sequences that are widely distributed throughout the genome and also interact with chromatin-modifying enzymes. For instance, it was reported that the T-cell acute lymphoblastic leukaemia (T-ALL) causing homeoprotein TLX1 suppresses the PP1/PP2A serine/threonine phosphatases which regulates numerous dephosphorylation reactions (I. Riz and R.G. Hawley, Oncogene 2005) and more recently discovered that TLX1 also regulates the activity of the histone/transcription factor acetyltransferase CBP (CREB binding protein). (I. Riz et al., Oncogene2007). Due to downregulation of mitotic checkpoint genes in TLX1 pre-leukemic cells, scientist discovered that TLX1 tumor cells had faulty mitotic checkpoint activation. In TLX+ T-ALL patients, TLX1 hijacks T-cell differentiation and causes an early cortical maturation block. TCR rearrangement is inhibited by TLX1 binding to the ETS1 (Protein C-ets-1 protein by ETS1 gene) transcription factor and suppressing TCR enhancer activity. [11]

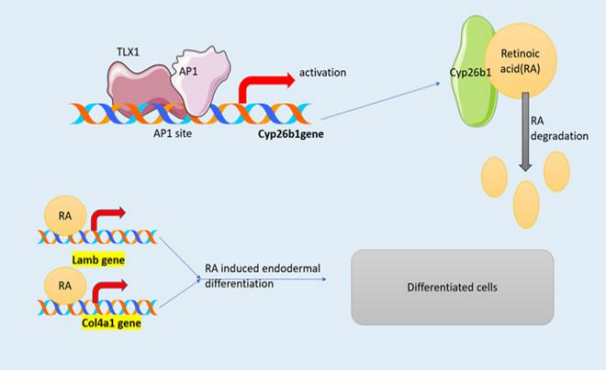

Figure no:3 -The role of TLX1 in retinoic acid signaling during endodermal differentiation in embryonic stage.

VI. TLX1 DRIVEN TRANSCRIPTIONAL ACTIVATION OF OTHER IMPORTANT GENES

This transcription factor solely not sufficient for the development of T-cell pathogenesis and persistent TLX1 expression is required for tumor maintenance. There are various specific genetic alterations present in TLX1 induced leukaemia which are generally not present in the non TLX1 induced T-ALL. There are various cooperative mutations present in the TLX1 induced T-ALL tumors. presence of recurrent numerical and structural chromosomal alterations in these tumors. Various disease models are used to understand the genetic interaction of the TLX1 with other genes in leukaemia and to study about the basic mechanism of oncogenic transformation of genes and disease progression.

During TLX1-induced leukemogenesis, the Notch signaling pathway is triggered, most often via spontaneous mutation. This unplanned occurrence helps blasts survive leukemia. The NOTCH1 signaling pathway is involved in the aetiology of T-ALL in more than half of the patients. As a requirement for complete leukemic transformation, establish a high genetic pressure for obtaining

activating NOTCH1 mutations. Overexpression of TLX1 alone may not be enough to cause T-cell leukemic transformation; rather, TLX1 and NOTCH1 combine to enhance the leukemic phenotype. The use of GSI (gamma secretase inhibitor) to suppress NOTCH has just a minor impact on leukemia development. [1]

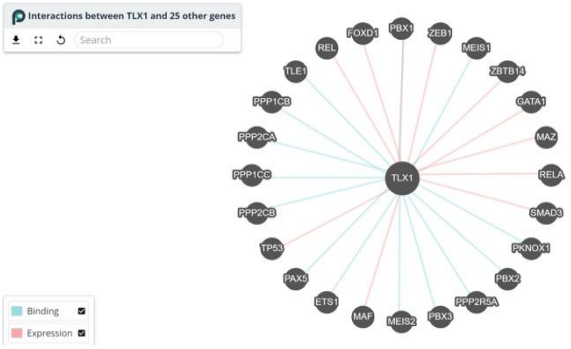

Figure:4- Pathways common database from TLX1 gene cards.

Overall, the Bcl11b gene (B-cell lymphoma/leukaemia 11B) was discovered as a new tumor suppressor gene that was often deleted and altered in T-ALL. TLX1 binds the promoter of the Bcl11b gene and suppresses BCL11B expression, which is important for TLX1-induced transformation of T-cells. BCL11B is a zinc finger transcription factor that is important for T-cell progenitors in the thymus to differentiate and survive. [21]

NUP214-ABL1 oncogene rearrangement- Thisincludes the rearrangement of the NUP214-ABL1 oncogene. The NUP214-ABL1 fusion protein is a constitutively active protein tyrosine kinase that enhances T-lymphoblast proliferation and survival and is present in 6% of individuals with T-cell acute lymphoblastic leukemia. This takes part in kinase signaling, by deregulation of this survival of blasts improved with increased kinase signaling. Increased HOX expression is linked to the NUP214-ABL1 rearrangement as well.[16]

Mutations in the WT1 gene-(Wilms tumor1) tumor suppressor gene found mutated in T-ALL. Frameshift insertions and deletions were the most common mutations, with a few nonsenses point mutations thrown in for good measure. Which encode prematurely shortened WT1 proteins devoid of the C-terminal zinc finger domains. The deregulated transcription factor affects the cell growth, differentiation and apoptosis mechanisms. [17]

PHF6 Inactivating mutations and deletions were found in 16% of pediatric and 38 % of adult primarily in T-ALL samples. This gene product is X-linked plant homeodomain finger 6 (PHF6) protein. PHF6 mutations, in particular, are nearly exclusively detected in T-ALL samples from men. Abnormal expression of the TLX1 gene is linked to leukemias reported well. The presence of an X-linked tumor suppressor in T-ALL suggests a substantial genetic connection between the disease and male subject. [18]

Deletion of the PTPN2 phosphatase- PTPN2 (protein tyrosine phosphatase non-receptor type 2) its role in the regulation of the immune system that functions as a negative regulator of a variety of tyrosine kinases activity. Deletion of PTPN2 was specifically found in T-ALLs with abnormal expression of the TLX1 oncogene had PTPN2 deletion. PTPN2 was also found to be a negative regulator of NUP214-ABL1 kinase activity. [19]

PTEN tumor suppressor gene- In 17% of T-ALL cases, the PTEN (Phosphatase and Tensin homolog) tumor suppressor gene is inactivated. The phosphatase protein product of this gene is engaged in cell cycle regulation, stopping cells from expanding, dividing too quickly and forming cell aggregates. Multiple cell-cycle checkpoints, notably the mitotic spindle checkpoint, are harmed when PTEN activity is lost. [20]

TLX1 on the mitotic machinery or, more likely, subsequent genetic abnormalities found in TLX1-induced malignancies. CHEK1, a crucial player in the normal functioning of the cell-cycle machinery and the mitotic checkpoint, was one of these genes. [1]

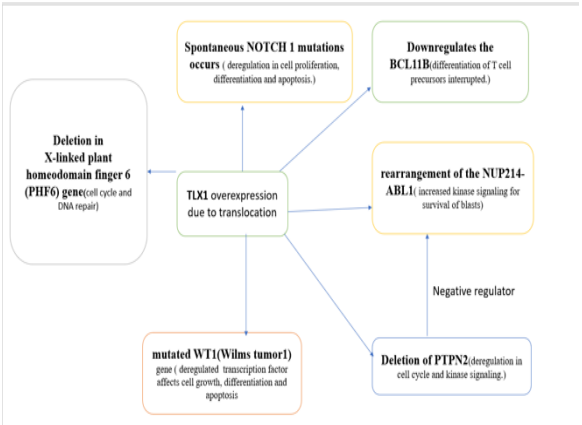

Figure no:5- TLX1 overexpression association with other genes.

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