Terminal Deoxynucleotidyl Transferase in type B Acute Lymphoblastic Leukemia

Abstract

Terminal deoxynucleotidyl transferase (TdT or DNTT) is a nuclear enzyme whose expression is restricted to normal tissue (thymus and bone marrow) and to lymphoid precursors of the B and T cell. TdT catalyzes the addition of deoxynucleotides independently of the template in the 3'-terminal hydroxyl end of the oligonucleotide primers. In addition, it plays a crucial role in the insertion of N regions during the rearrangement of immunoglobulin genes and receptor T and B cells (TCR and BCR) at the DJ binding sites and variable (V) diversity (D) binding (J). This mechanism of the diversity of the binding is essential for the development of a repertoire of immunoglobulin's and B and T cell receptors. TdT is expressed in malignant lymphoblastic tumors of precursors, including precursors of B and T cells. In this review, we describe the biological importance of TdT in acute lymphoblastic leukemia of type B cells.

Keywords: Terminal deoxynucleotidyl transferase, rearrangement of immunoglobulin genes and acute lymphoblastic leukemia of type B cells.

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Functions of TdT

The ability of the immune system to respond to the wide range of potential pathogens that infect us during the development of life depends on the diverse repertoire of antigenic receptors expressed by B and T lymphocytes [1, 2]. The great diversity of antigenic receptors is largely generated part by the recombination process V D J, in which DNA elements are randomly linked to form the variable domains of the antigenic receptor genes [3]. Generation of the functional heavy chain (H) and light (L) genes from immunoglobulin's through V D J recombination occurs in a gradual process during the development of B cells in the primary lymphoid organs such bone marrow and the spleen. Among the key enzyme in this process is the terminal deoxynucleotidyl transferase enzyme (TdT or DNTT) [4].

The gene from TdT is located on chromosome 10q23-q25, This gene is a member of the X-type DNA polymerase family that codes for a 58-kDa DNA polymerase independent of the template that catalyzes the addition of deoxynucleotides to the 3'-hydroxyl terminus of the oligonucleotide primers [5, 6].

TdT is responsible for the addition of random nucleotides in the junction (N region) of the heavy chain of rearranged Ig, during recombination of V D J in the maturation of B and T cells, playing an important role in the development and variations of antigenic receptors in the immune system. Transcriptionally TdT is regulated by transcription factors such as AP-1, as well as through recombinant gene expression activators (RAG). TdT activity can also be regulated at the post-transcriptional level by phosphorylation. Another group of proteins that also regulate the TdT activity is known as TdT interaction factors (TdiF1) [7-11].
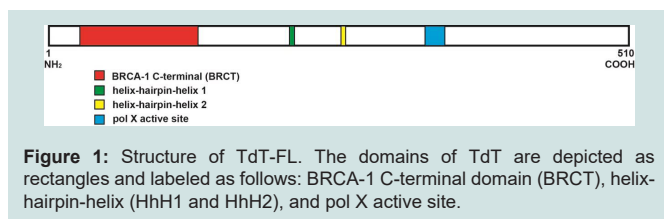
Since that the cloning trials from TdT were initiated, there was an increase in knowledge about properties and functions under normal and pathological conditions of TdT, In humans, the expression of TdT is restricted in lymphoid precursors of lineage T and B, and in hematological cancers. TdT isoforms are slightly more complicated as each have three alternative splice variants designated as TdTS (short), TdTL1 (long) and TdTL2 (long) [12,13]. There is evidence of possible human isoforms of TdT (hTdT) derived from the genomic sequences of hTdT, which led to the identification of the short isoform (hTdTS), as well as the mature long transcripts that contain exon XII (hTdTL1) and another that includes the exon VII (hTdTL2) in lymphoid cells [14].

Normal B and T lymphocytes express exclusively hTdTS and hTdTL2, whereas expression of hTdTL1 appears to be restricted to transformed lymphoid cell lines [15].

The newly discovered hTdT isoforms should be considered in the future examination of human leukemias [16].

The structure-function analysis of the murine TdT protein was also performed to determine the functions of the structural motifs that have been implicated in protein-protein and DNA-protein interactions. In this analysis was demonstrated that the N-terminal portion of TdT, including the C-terminal BRCA-1 domain (BRCT), is not required for TdT activity, although the BRCT domain contributes to the activity of adding N-nucleotide [17].

The second helix-hairpin-helix domain of TdT, but not the first, is required for this activity. The deletion analysis also suggested that the complete C-terminal region of TdT is necessary for the addition of

Figure 1: Structure of TdT-FL. The domains of TdT are depicted as rectangles and labeled as follows: BRCA-1 C-terminal domain (BRCT), helix-hairpin-helix (HhH1 and HhH2), and pol X active site.

N-nucleotide *in vivo* Homology among members of the pol X family is not limited to their catalytic center (amino acid position 332 to 349). Additional domains shared by several of these polymerases include BRCA-1 C-terminal (BRCT) domains as well as helix-hairpin-helix (HhH) domains, The BRCT domain is a phosphopeptide binding motif that mediates protein-protein interactions and is commonly found in proteins involved in DNA recombination and repair, such as BRCA-1, XRCC4, and DNA ligase IV (amino acid position 26 to 130) [18-20]. (Fig, 1).

HhH domains are non sequence-specific DNA binding motifs that contact DNA by interactions of peptide backbone nitrogen atoms with phosphate groups of the DNA (HhH1 amino acid position 213 to 218) (HhH2 amino acid position 257 to 261), It has been shown that TdT plays a very important role in the development of B lymphocytes in fetal development since it has been observed that the precursors of B cells from lymph nodes are positive for TdT [21, 22].

Biases in the recombination process and/or cellular selection through Ig receptors are thought to account for the nonrandom nature of the Ig repertoire; however, the details of these selection mechanisms and the relative impact of each type of selection mechanism on the Ig repertoire have not been determined. The Ig H chain repertoire displays two interesting nonrandom characteristics that have been particularly well studied:

1. Unequal usage of D_H reading frames (RF), and

2. Over usage of the V_H81x gene segment [23-25].

However, during neonatal life the immunoglobulin diversity is limited and the absence of TdT expression with the consequent lack of addition not contemplated during the neonatal period, and together with the predominant use of a single D_H reading frame, leads to serious limitations of diversity in the CDR3 region of Ig Heavy chains (H). The repertoire of the neonatal Ig H chain is also characterized by the restricted use of V_H, with predominant expression of certain V_H segments, such as V_H81x, which are rarely evident during adult life. When the expression of TdT is induced in the neonatal repertoire of V_H81xDJ_H, the synthesis of TdT cancels the bias in the reading frame D_H during the fetal/neonatal period through an independent mechanism of the Ig receptor. These findings suggest that the bias of the D_H reading frame during neonatal life is determined to a large extent by homology-directed binding [26].

Also found, was that the synthesis of TdT alters the selection of productively rearranged V_H81xDJ_H alleles in the neonatal spleen through an Ig-dependent mechanism. These results demonstrate that TdT can indirectly influence the Ig repertoire by influencing the selection processes dependent on the receptor and the independent receptor [27]. It has been reported that TdT play a very important role in the ontogeny of B lymphocytes in adults, since in one study a relationship between the expression of TdT with specific markers of lineage B (CD19) was demonstrated, but the expression of CD19 did not, it is a marker of early ontogeny of B lymphocytes, therefore it can be used to CD79a, which is highly specific for B cells and which can also be expressed very early in ontogenesis, In the same way, it was demonstrated that TdT has the same function in the development of B lymphocytes in a murine model because it increases the insertion in the N-region in pre-B cells, It is also known that TdT is involved in the ontogeny of T lymphocytes [16, 28-30].

Tdt in Leukemia

Leukemia is a type of blood cancer, which starts in blood-forming tissue, such as the bone marrow, and causes large numbers of immature blood cells to be produced and enter the bloodstream. Leukemia is subdivided into different subtypes according to cellular maturity (acute or chronic) and cell type (lymphocytic or myeloid). Acute lymphoblastic leukemia (ALL) is a cancer of lymphocytes, a type of white blood cell that is part of the body's immune system. ALL is the most common cancer in children under 18, the great majority of ALL is of type B lineage (75-80%). In studies on TdT in subjects with acute leukemia, an increase in its expression was observed, In 1978, it is described for the first time that there is a higher percentage of TdT expression in leukocytes of B-type ALL than in type T, and in experimentally demonstrating the biological role not only in B cell proliferation [31-34].

In a study in which it was evaluated, the activity of TdT in samples of bone marrow and peripheral blood of patients with various types of leukemia such as acute myeloblastic leukaemia and chronic granulocytic leukaemia, it was reported that there is an increase in the activity of TdT in those patients with ALL. In another study, they conclude that the assay of TdT in the peripheral blood or bone marrow of patients with acute leukemia is of value in differentiating lymphoid (including non-T non-B) from myeloid leukemia [35, 36].

Since then TdT has been used as a diagnostic marker of ALL implementing microscopy methods as main tools; immunofluorescence and immunohistochemistry [37]. But because there are reports of positive TdT cells in acute myeloid leukemias, their diagnostic value has been questioned [38]. An alternative that has been employed is the use of flow cytometry as a method that allows quantitative analysis, since it recognizes differences between ALL and AML. In a study performed on subjects with B-ALL, high levels of TdT was observed, while AML had low levels [39]. For this reason, TdT can be an effective biomarker for classifying leukemias of lymphoid origin, in the same way, it is valuable to define the stages of maturation of leukemias [40].

It has recently been shown that the expression of TdT increases in the presence of different cytosines (IL-2, IL-7, and IL-15) and that inhibiting TdT reduces the expansion of B and T cells and therefore both decreases apoptosis and proliferation [41]. On the other hand, a high number of TdT-positive cells has been reported in inflamed pediatric kidneys in children with lymphoblastic leukemia [42]. It has also been reported that in pediatric patients with ALL who overexpress miR-125b, miR-100, and miR-99a are resistant to treatment with vincristine and that it reduces the expression of 11

genes, including TdT [43].

Conclusion

In spite of the great scientific advance that has extended the knowledge on the process of leukemogenesis, little is known about the molecular events that participate in the development of ALL. TdT is an enzyme that is involved in the ontogeny of B lymphocytes in a normal way. There are also articles that report the altered expression of TdT in type B ALL. TdT expression is currently evaluated through immunohistochemistry, immunofluorescence and flow cytometry. Therefore, an alternative treatment be to inhibit the expression of TdT plus the combination with routine treatments for type B ALL.

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