Review Article

Wilms' Tumor Gene 1 in Leukemia: Prognostic or Predictive Biomarker

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Abstract

Wilms' tumor is common in children and is caused by Wilms' tumor gene 1 (*WT1*). The *WT1* mutation has been reported in a variety of hematologic malignancies. Changing expression of miRNA molecules has also been shown to play a role in the development of Wilms' tumor. Considering the fact that *WT1* can be used as a clinical biomarker in leukemia cases, it can be a basis for immunotherapy of leukemia. *WT1* is a gene that can be used as a prognostic biomarker for minimal residual disease, as well as the detection of relapse for clinical remission in leukemia. Furthermore, it can be considered as a predictive biomarker for the treatment of leukemic patients after allogeneic transplantation. This study aimed to review *WT1* expression in leukemia, its involvement in miRNAs expression, as well as its importance in prognosis and treatment of leukemia.

Keywords: *Leukemia, predictive, prognostic, Wilms' tumor 1 gene*

Introduction

Wilms' tumor gene 1 (*WT1*) chromosome (11p13) is essentially a tumor suppressor gene[1] responsible for the development of Wilms' tumor (a type of pediatric kidney cancer), as well as other syndromes such as WAGR (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation) and Denys–Drash syndrome (DDS).[2,3] In contrast to other tumor suppressor genes such as *p53* or *RB1*, the normal expression of *WT1* in adults is limited to a few tissues, including genitourinary system. In bone marrow (BM), the expression of *WT1* in normal primitive progenitor cells is at a low level; however, the overexpression of *WT1* mRNA is observed in several solid tumors and hematologic malignancies such as acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), and myelodysplastic syndromes (MDS).[4,5] Therefore, *WT1* expression can be a universal molecular biomarker in malignant hematopoiesis, and *WT1* mRNA monitoring in peripheral blood (PB) and BM has been reported to determine minimal residual disease (MRD) and relapse in leukemias.[1,4] In addition, some studies have shown that evoking anti-*WT1* immune response using *WT1* peptide vaccine may induce tumor regression in a number of patients.[1]

Although the biological significance of *WT1* expression in leukemia patients has not been elucidated, studies indicate that *WT1* can contribute to the pathogenesis of human leukemia due to its role in the inhibition of transcription, as well as involvement in cellular differentiation and growth arrest. Other studies also suggest that the prognosis in leukemia patients can be inversely associated with the expression level of *WT1*.[4] The purpose of this review is to discuss the role of *WT1* as a detectable tumor biomarker for prognosis and its importance for immunotherapy and prediction of treatment in hematologic disorders.

The Structure and Function of Wilms' Tumor Gene 1

WT1 gene is normally expressed in mesodermal tissues during embryogenesis and encodes a zinc-finger transcription factor.[6] It was first detected as a tumor suppressor gene in patients with WAGR and was subsequently reported as a mutational target in 10%–15% of sporadic cases of Wilms' tumor, as well as in WT1-associated DDS and Frasier syndrome. *WT1* is initially expressed in the cells and tissues progressing toward genitourinary and hematopoietic systems. The expression level of *WT1* is inversely related to differentiation in both systems, and its mutation causes Wilms' tumor, a common type of renal malignancy in children. *WT1* expression

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in hematopoietic system is observed in $CD34^+$ progenitor cells but not in mature leukocytes. In addition, *WT1* is expressed in high levels in acute leukemia cells, and it may also be involved in leukemogenesis. *WT1* could be involved in cell survival, proliferation, and differentiation both as tumor suppressor and oncogene, although its role in normal and malignant hematopoiesis is still unknown. These different functions can be linked to downstream targets, as well as the expression of different *WT1* isoforms. There are four main isoforms of *WT1*, which are a function of two primary splice variants, including the splicing of exon 5 and 9 nucleotides encoding three amino acids between exons 9 and 10 (KTS lysine [K], threonine [T], and serine [S]).[7] *WT1* is first expressed in cells and tissues differentiating into genitourinary and hematopoietic systems.[8,9] *WT1* protein has a proline-glutamine-rich N-terminal transcriptional regulatory domain (exons 1–6) and four C-terminal zinc-finger domains (exons 7–10) that facilitate its binding to DNA. Exons 5 and 9 are subject to alternative splicing, which results in the formation of four different splice isoforms,[10] including $17AA^+/KTS^+$, $17AA^+/KTS^-$, $17AA^-/KTS^+$, and $17AA - KTS^-$, as well as other isoforms.[9] Depending on the expression level, specific isoform, and cellular context, *WT1* protein can act as a transcription activator or repressor.[11] In addition, this protein is essential for cell proliferation and differentiation and is involved in the inhibition of apoptosis along with *p53* and *bcl2*.[12]

WT1 is involved in the regulation of cell growth and differentiation. The nuclear and transcriptional activity of *WT1* has been observed to increase in most cases of acute leukemia. The expression level of *WT1* is associated with the presence, persistence, or reappearance of leukemic hematopoiesis, which could raise it as a strong prognostic factor.[8]

Expression and Alternation of Wilms' Tumor Gene 1 in Leukemia

Acute myeloid leukemia

AML forms a class of myeloid disorders characterized by increasing number of myeloid-derived blast cells, cell differentiation arrest, and accumulation of undifferentiated cells in BM.[13,14] AML is a heterogeneous disease clinically and genetically, which accounts for 15%–20% of leukemia types in children. The majority of AML patients harbor at least a chromosomal abnormality in BM at diagnosis. However, 40%–49% of adult patients and 25% of children with AML are devoid of any chromosomal abnormality, which is known as cytogenetically normal AML cases (CN-AML) of predominantly adult patients.[8,15] Multiple genetic abnormalities have been observed in this group of patients with a prognostic value. These abnormalities include mutations in *FLT3*, *NPM1*, *CEBPA*, and *MLL* genes, as well as the aberrant expression of *BAALC*, *ERG*, and *MN1* genes.[15] Identification of these markers may be effective to predict the response of patients to treatment and their survival rate.[16]

WT1 is an essential molecular marker in biology and treatment in this group of patients.[2] *WT1* mutation is known to be associated with increased proliferation of AML blast cells, as well as poor prognosis in patients through increasing expression of *ERG* and *BAALC* genes.[17] *WT1* may act as a tumor suppressor gene or an oncogene.[2] However, the expression of different *WT1* isoforms and their target genes may vary in different cells. *WT1* expression has been reported in 70%–100% of AML patients, and its mutation is seen in 10%–15% of adults with AML. Mutation and increased expression of *WT1* can interfere with survival and relapse rates. In addition, the assessment of *WT1* expression is a marker of MRD after treatment.[16] The overexpression of *WT1* has been reported in AML subtypes, which is independent of *WT1* mutation that occurs in 10% of cases. Studies on the role of *WT1* expression as a marker of MRD show that *WT1* has a low expression level in normal BM, which is increased in the majority of AML patients at diagnosis, returning to baseline following effective treatment and increasing in cases of relapse.[18] *WT1* gene mutations in exons 7 and 9 occur in 9%–13% of AML patients,[19] which are reported to be associated with lower complete remission, higher relapse rates, shorter disease-free survival (DFS), and overall survival (OS) but have no prognostic value compared with wild-type *WT1 (WT1* wt).[20]

It has been recognized that the polymorphism of *WT1* gene, as well as its mutation, can be considered as a potential prognostic factor in AML subtypes, but this has not been confirmed by all the studies. In this regard, rs16754 WT1 gene polymorphism in AML patients undergoing chemotherapy caused an increase in OS and DFS, while complete remission in these patients was not affected by *WT1*.[21] In addition, SNP rs16754 in exon 7 of *WT1* gene in CN-AML patients was associated with shorter survival. Therefore, *WT1* gene mutations can be considered to predict the poor prognosis in CN-AML patients.[22] Since *WT1* mutations and polymorphisms can be used as prognostic biomarkers in AML patients,[21,22] it is suggested to assess *WT*1 along with other prognostic biomarkers in these patients [Table 1].

Acute lymphoblastic leukemia

This is the most common childhood malignancy characterized by monoclonal expansion of immature T and B lymphoid precursors in the BM, PB, and organs of the body.[23,24] Increased expression of *WT1* has been reported in the majority of these patients. For example, Tosello *et al*. reported that the prevalence of *WT1* mutations in adult and pediatric T-lineage ALL patients was approximately 12 and 13%, respectively, and the majority of their mutations were similar to AML patients (frameshift mutations

Table 1: Clinical relevance of WT1 expression in leukemia

Type of leukemia	WT1 expression	Clinical relevance of WT1 expression	Ref.
Adult AML	Up Lower frequency/level of expression in FAB-M5-M7	Prognostic marker in relapsed phase Coexpression with bcl-2 for prognosis of groups with high value for DFS As MRD biomarker	[41 42]
Pediatric AML	Up	No prognostic significance	[43]
ALL	Up	Prognostic marker in relapsed phase, MRD	[27, 44]
MDS	Up	Minor histocompatibility antigens for adoptive immunotherapy	[42]
NHL	Significantly lower or even undetectable	-	[44]
CLL	Lack of expression	-	[45]
CML	Overexpression in blast crisis phase	Therapeutic target in Ph+patients	[35, 46, 47]
MM	Normal expression in newly diagnosed patients	-	[40]

AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia, MDS: Myelodysplastic syndromes, NHL: Non-Hodgkin's lymphoma, CLL: Chronic lymphocytic leukemia, CML: Chronic myeloid leukemia, MM: multiple myeloma, MRD: minimal residual disease, DFS: Disease-free survival

within exon 7).[5,25] Furthermore, Chiusa *et al*. studied ALL patients and showed that contrary to previous reports concerning increased *WT1* gene expression in 44%–86% of ALL patients, *WT1* expression was increased in almost all adults with ALL. In addition, this study showed that the level of *WT1* expression correlated with the survival of patients and could be considered a prognostic factor in these patients.[19] Studies have shown that the expression levels of *WT1* are significantly higher in children with T-ALL compared to children with B-cell precursor-ALL but are lower than its normal levels in BM of these patients. Furthermore, in relapsed childhood ALL cases, abnormally high or low expression levels of *WT1* have been observed.[26] Therefore, in general, it can be stated that the expression levels of *WT1* in children with ALL are highly variable and lower than its expression levels in patients with AML and adult ALL. The varying expression of *WT1* may indicate that *WT1* expression in these patients cannot be regarded as a useful marker for MRD, but the very low expression of it in children with ALL compared to its normal physiological expression levels in BM can raise *WT1* as a prognostic factor of relapse in children with ALL.[27] In addition to cytogenetically normal cases of ALL, the variation of *WT1* expression in cytogenetically abnormal ALL subtypes has also been examined. Evidence suggests that high levels of *WT1* expression in patients with MLL-AF4 and B-cell receptor (BCR)-ABL p190 translocation can be considered as a prognostic biomarker in ALL patients harboring these two translocations.[5]

Myelodysplastic syndromes

MDS is a clonal disorder characterized by the abnormal maturation and differentiation of myeloid precursors and increased resistance to proapoptotic signals.[28,29] Studies indicate that *WT1* is a likely tumor suppressor gene and a tumor marker for leukemic or preleukemic MDS blast cells. In this regard, it has been found that the expression levels of *WT1* are indicative of MDS progression, in such a way that *WT*1 expression is increased with disease progress.[30] In addition, pancytopenia is among clinical complications developing following hematologic and nonhematologic diseases. The high expression of *WT1* is a prognostic biomarker of pancytopenia for MDS and even AML among other hematologic malignancies.[31] Clinical evidence has indicated that a single dose of WT1 vaccination in patients with leukemia due to MDS is effective in the reduction of leukemic blast cells in addition to increasing *WT1*-specific cytotoxic T-lymphocytes. Thus, according to clinical evidence, *WT1* peptide can be a basis for immunotherapy of MDS patients.[32]

Chronic myeloid leukemia

This is a clonal stem cell disorder associated with increasing number of myeloid cells in BM and their accumulation in PB. Reciprocal translocation between chromosomes 9 and 22 (known as Philadelphia chromosome) that generates the BCR-ABL fusion protein is typical of this type of leukemia.[33] Although the expression of *WT1* is increased in CML, no significant correlation has been observed between *WT1* with BCR-ABL in newly diagnosed CML patients. Therefore, *WT1* has not been suggested as a marker for monitoring of CML patients.[34] However, other studies have shown that despite the overexpression of *WT1* mRNA and protein in CML cell line, the use of *WT1* inhibitors could inhibit the proliferation of CML cells in cell cycle and reduce the transcription of *WT1* and *P210* genes. It could be argued that the *WT1* gene is a therapeutic target for Ph^+ leukemia patients.[35]

Other

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in middle age that is associated with an abnormal proliferation of lymphocytes and their resistance to apoptosis.[36] 60%–65% of CLL cases harbor a mutation in the variable region of immunoglobulin heavy chain (IGHV), which can alter BCR affinity to antigen, but 35%–40% of CLL cases lack IGHV mutations.[37,38] Mutation in IGHV gene is suggested as a marker of favorable prognosis, while the unmutated CLL (U-CLL) is more aggressive than the mutant case (M-CLL).[37,39]

Monitoring of patients with leukemia, especially multiple myeloma (MM), during and after therapy is of particular importance to assess MRD to determine the effectiveness of treatment. Despite the fact that the expression levels of *WT1* as a marker of MRD are increased in MDS patients, evidence suggests that the expression of WT1 mRNA is normal in newly diagnosed MM patients. Therefore, it can be concluded that *WT1* is not a prognostic biomarker in MM patients [Table 1].[40]

Impact of Wilms' Tumor Gene 1 on miRNAs Expression

miRNAs form a group of small noncoding RNA molecules inhibiting their target mRNAs by binding the 3-UTR region. Studies have shown that miRNAs play an important role in the development of WT1, as well as their involvement in the kidney development.[48] The oncogenic role of *WT1* in leukemogenesis has been observed, as well as its increased expression during leukemia. However, some studies have shown that miRNAs such as miR-125a can regulate and suppresses WT1 expression by the 3'UTR of WT1 mRNA in myeloproliferative disease.[49] Table below shows different roles of miRNA molecules and their altered expressions in WT1 [Table 2].

Wilms' Tumor Gene 1 as a Predictive Biomarker in Leukemia

Several leukemias cannot be cured with available treatments, including MDS and acute leukemias. In addition, the efficiency of donor leukocyte infusion in leukemia patients is restricted for reasons such as myelosuppression and graft-versus-host disease; therefore, the need for new therapeutic strategies arises in this context.[42] Leukemic patients who cannot respond to treatment receive allogeneic hematopoietic stem cell transplantation (HSCT). Although the prognosis of leukemic patients with HSCT have been improved, relapse remains as a major challenge in these patients.[54]

*WT*1 is among the genes found to be applicable as a prognostic marker to detect relapse in clinical remission in AML and ALL cases.[55] *WT1* is an interesting target in the field of immunotherapy in leukemia. Immunological activity of *WT1*, as well as its specific antibodies and cytotoxic T-lymphocyte responses have been detected in different hematologic malignancies.[56] Targeting *WT1* in acute leukemias for immunotherapy has been suggested in some studies due to its high concentrations in leukemic cells. On the other hand, the use of dendritic cells and the generation of *WT1*-specific CTL have been shown effective in the development of immune cells.[57]

Studies have shown that *WT1* peptide vaccine has been effective in leukemia patients, and clinical improvement has been observed in different types of patients with hematological malignancies.[58] *WT1* is an active immunogenic antigen, and IgG and IgM antibodies for *WT1* have been found in leukemic patients.[59] In allogeneic HSCT, graft-versus-leukemia (GVL) can occur, in which the leukemia cells can be removed by donor T-cells. Therefore, the GVL effect may be increased with *WT1* peptide vaccine.[54] *WT1*-derived CD8 T-cell epitopes have been investigated in some studies. HLA-A0201-restricted peptide 124-134, HLA-A24-restricted peptide 235–243, *WT1*-235 (CMTWNQMNL), and its analog (CYTWNQMNL) peptides can cause CTLs to lyse WT1þ leukemic cells in a HLA-A24-restricted manner.[60,61] *WT1*-derived CD4 epitopes also have been found to be able to bind HLA-DR types in some studies. Peptide 124–138, 247–261, 332–347, and 337–347 bind to HLA-DR53, DRB1*0401, HLA-DR53, HLA-DRB1*0405, and HLA-DP5, respectively.[62,63]

In conclusion, *WT1* peptide vaccine treatment can be considered as a favorable tool to improve leukemic patients with new peptides and their analogs under study so that *WT1* could be suggested as a predictive biomarker for usefulness of treatment after allogeneic HSCT.

Conclusions and Future Perspectives

A number of cytogenetic abnormalities observed in leukemia have been detected in *WT1*, including chromosome 22 deletion. Perhaps, these abnormalities are indicative of a good or poor prognosis in leukemia in case of further investigations given their favorable or poor prognosis in *WT1*.[64] On the other hand, preventing relapse after allogeneic HSCT with *WT1* peptide vaccine is an interesting treatment strategy in the future, which can introduce *WT1* as a predictive biomarker for leukemic patients; however, further studies are required to achieve this goal.

Table 2: miRNAs expression changes in Wilm's tumor

Involvement of miR in disease	Upregulated	Downregulated	Ref.
Tumor progression	miR-17.5p, miR-18a, miR-19b, miR-92, miR-20a, miR-224, miR-125a	miR-192, miR-194, miR-215, miR-200c and miR-141, miR-126	[49, 50]
Tumor relapse and mortality	Lin28B	-	[51]
Diagnosis	miR-10a-5p, miR-30b-3p, 34a-3p, miR-106b-3p, miR-141-5p, miR-577, miR-877-5p	-	[52]
Prognostic marker of early metastasis	miR-106b	-	[53]

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Conflicts of interest

There are no conflicts of interest.

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