Am J Blood Res 2020;10(5):151-160
www.AJBlood.us /ISSN:2160-1992/AJBR0117011

Review Article

Molecular update on biology of Wilms Tumor 1 gene and its applications in acute myeloid leukemia

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Received June 27, 2020; Accepted September 16, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Wilms tumor gene 1 (WT1) is an important gene which is involved in growth and development of many organs. It is identified as a tumor suppressor gene in nephroblastoma. However, its role as a tumor oncogene has been highlighted by many studies in haematological as well as non haematological malignant neoplasm. The expression of WT1 on leukemic blast cells sensitised us to explore its impact on neoplastic phenomenon. WT1 is has been found both mutated as well as over expressed in different subsets of acute myeloid leukemia (AML). WT1 is a gene has been used as a biomarker for diagnosis, monitoring of minimal residual disease (MRD) and detection of relapse for molecular remission in AML. It also has potential of being a predictive molecular predictive biomarker for the treatment of leukemic cases after allogeneic transplantation. The WT1 specific expression on blast cells and its interaction with cytotoxic T cell has also been explored for its potential usage WT1 based immunotherapy. Here, we are reviewing molecular updates of WT1 gene and discuss its potential clinical applications as a predictive molecular biomarker for diagnosis, as MRD detection and as immunotherapy in AML.

Keywords: WT1 gene, WT1 mutation, WT1 expression, AML

Introduction

Acute myeloid leukemia (AML) has been a heterogeneous group of disease with various combinations of clinical symptoms, aggressiveness and complications [1]. AML is the second most prevalent type of leukemia diagnosed in adults and children [2]. However, incidence of AML is directly proportional to the increasing age [3]. Several chromosomal rearrangements and mutations have been detected in AML and linked with the diagnosis, pathogenesis, and prognosis of AML [4, 5]. The overall survival is also dependent on genomic profile of mutational burden [6]. In addition to mutations, significant modification at post transcriptional and post-translational level may produce malignant cell changes [7]. WT1 gene is a tumor suppressor gene has been primarily linked with nephroblastoma and now being considered as a tumor oncogene, which plays a critical substantial role in neoplastic process related to hematopoietic malignancies [8].

WT1 gene has dual functioning of acting as tumor suppressor as well oncogenic gene and controls transcription, translation, RNA metabolism at cellular level [9, 10]. WT1 mediated pathway of transcriptional regulation plays an impactful role in normal and malignant hematopoiesis [11]. Overexpression of WT1 specific iso-forms in myeloblast cells of AML with minimal maturation results in the induction of apoptosis and G1 arrest as well [12]. WT1 is a potent transcriptional regulatory molecule that plays a crucial role in the regulation of apoptosis, cell survival, promotes cell proliferation, cell growth, metastasis, differentiation, and normal cellular development [13, 14].

Structure of WT1

The WT1 gene is located on chromosome 11p13 and is 50 kb in length. It consists of 3.2 kb of mRNA produced from total 10 exons. It regulates the expression and encodes a zinc finger transcription factor, which controls cellu-

lar growth and metabolism, including growth factors, extracellular matrix components through binding to GC-rich homologous sequence, and regulates transcription activator or represses expression of specific target genes (ECM) [15, 16]. Mutations of the WT1 gene lead to disorders such as Wilms' tumors or Denys-Drash syndrome [17, 18]. It is implicated in the development of organ systems such as the kidney, retina, spleen, and heart through various signalling pathways [19, 20]. It also promotes epithelial-to-mesenchymal transition (EMT) [21]. WT1 mRNA shows two important splicing regions [22, 23]. These include splicing of exon 5, which encodes 17 amino acids (AA) and another segment of nine nucleotides which code for 3 amino acids such as lysine, threonine, and serine (KTS) on exon 9 [24]. Alternative splice 2 inserts three amino acids-lysine [K], threonine [T], and serine [S] between exons 9 and 10, alter the conserved spacing between zinc fingers 3 and 4, leads to the significant reduction of the DNA binding ability but enhances RNA binding [23, 25]. The alternative splicing of these two positions gives rise to four different protein isoforms: WT1 A (17AA-/KTS-), WT1 B (17AA-/KTS+), WT1 C (17AA+/KTS-), and WT1 D (17AA+/KTS+). KTS-positive isoforms constitute 80% of cellular WT1 [16, 26, 27]. The full-length product encoded by WT1 is a 57 kD protein.

Function of WT1

The N-terminal terminal transactivation domain of WT1 is composed of proline, glutamic acid, serine, and glycine-rich sequences [28, 29]. This N-terminal domain is relevant for transcriptional regulatory function of WT1, such as transcriptional repression [30]. The C-terminal of WT1 is composed of four zinc-fingers moieties, each of which has two cysteine and two histidine [31]. This zinc finger permits binding of target DNA sequences, regulating gene transcription such as RNA and protein interactions. Thus truncated WT1 might exhibit oncogenic properties [32].

WT1 in normal haematopoiesis

Differential expression of WT1 isoforms may support isoform-specific differentiation in hematopoiesis and leukemogenesis [33]. The KTS insert significantly increases the flexibility of protein [34] by limiting related binding sites at the major groove in DNA. KTS2 isoforms may repress or activate transcription in normal hematopoietic cells [35]. KTS2 isoforms is arrests cells in G1 phase and induces myelomonocytic differentiation of CD34 positive hematopoietic progenitor cells [36]. The KTS forms also co-localize preferentially with already available ubiquitous transcription factors. In contrast, most of KTS(+) isoforms are found in a speckled pattern and co-localize with small nuclear ribonucleoprotein particles (snRNPs), suggesting a role in site specific splicing [37]. WT1 KTS(+) does not influences p21 expression however it promotes EMT, specially within solid neoplastic diseases [38]. However, WT1 KTS(-) increases p21 expression and cell proliferation with diminishing reproductive potency and G2 arrest [39]. The WT1 KTS(-) isoform more strongly enhances CD95L mediated cell death in T-cell acute lymphoblastic leukemia (T-ALL) [40]. The major WT1 subtypes have inhibitory functions, e.g., WT1 KTS [+], WT1 KTS(-) can inhibit the expression of the apoptotic genes such as p53, Bak, Bax or caspase-9, also induces expression of transcription factor BCL2; thus promoting an anti-apoptotic effect. One study on targeted transgenic murine model reported that WT1 was not found in long term-hematopoietic stem cells. Deletion of WT1 among young and adult mice resulted in death of animals in time frame of approximately 10 days with following causes such as glomerulosclerosis, atrophy of pancreas, and diminished extramedullary hematopoiesis [41].

WT1 in AML

The impact of WT1 gene mutation on AML was first identified in 1994 by King-Underwood and his colleagues on its possible role of drug resistance [42]. Recurrent somatic mutations in WT1 appear to occur in approximately 6-15% of newly diagnosed cases of AML [41]. These include deletions, insertions, and base substitutions mutations, primarily targeting exon 7 and 9 [43-48]. However, the vast majority of mutations resulting in loss-of-function, and expression of truncated proteins perform in a dominant-negative manner, which may contribute to a myeloid differentiation block present in AML blasts [46].

WT1 mutations are usually denoted by loss-of-normal function. Mutational analysis of a large

Table 1. WT1 gene Immunotherapy studies

Year	Research Group	Trial no clinicaltrials.gov/show/	Inclusion criteria	Clinical Outcome	Ref no
2018	Maslak et al.	NCT01266083	AML in CR	Stimulated specific immune response	[57]
2018	Nakata et al.	UMIN000015870	AML in CR	Molecular CR maintained for 3.5 years	[58]
2018	Liu et al.	NCT01842139	AML in CR	WT1 vaccine with Montanide induced CD8 response	[59]
2017	Anguille et al.	NCT00965224	AML in CR	Delay in relapse was seen in vaccine group	[60]
2017	Kobayashi et al.	NCT2001440920	AML in CR	Reduction in WT mRNA transcripts	[61]
2015	Brayer et al.	NCT00665002	AML in CR	Vaccinations well tolerated. Relapse-free survival >1 year	[62]
2011	Rezvani et al.	NCT00488592	AML in CR & MDS	CD8 response detected in all evaluable patients	[63]
2010	Maslak et al.	NCT00398138	AML in CR	Promising disease-free and overall survival	[64]
2008	Rezvani et al.	NCT00433745	AML in CR & MDS	Reduced in WT1 mRNA expression	[65]
2004	Mailander et al.	NCT00153582	Recurrent AML	Vaccine induced CR in recurrent AML absence of toxicity	[66]

CR, complete remission; AML, acute myeloid leukemia; MDS, Myelodysplastic Syndrome.

cohort of AML cases demonstrated that mutations of WT1 are mutually exclusive with ten-eleven translocation methylcytosine dioxygenase 2 (TET2), isocitrate dehydrogenase 1 (IDH1), isocitrate dehydrogenase 2 (IDH2), or CCAAT enhancer-binding protein alpha [CEBPA] mutations [47, 48]. Rampal et al. revealed that TET2 catalyzes the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) [49]. TET2 loss-of-function mutations and IDH1/2 mutations result in inhibition of the DNA demethylation pathways with an accumulation of 5-mC and a decrease of 5-hmC. WT1 mutations attenuate the TET2 function; a reduction in WT1 would potentially reduce TET2 activity in AML. Several studies confirmed that similar epigenetic alterations characterize WT1-mutant AMLs, as found in TET2 and IDH1/2 mutant AML. WT1-mutant AMLs presented a global reduction in 5-hydroxymethylcytosine (5-hmC) levels and interruption of gene-regulatory interfaces required for normal hematopoiesis. WT1 with TET2 as a cofactor, transcriptionally regulates maternally expressed gene 3 (MEG3) expression. MEG3 induces GO/G1-phase & apoptosis and decreases cell proliferation by regulating p53 expression. In WT1 or TET2 mutated AML cell lines, the lncRNA of maternally expressed gene 3 (MEG3) is significantly downregulated [50].

Subsequently, numerous studies emphasized possible impact of WT1 gene expression irrespective of their mutational status as an independent factor. Various researches have established relationship between WT1 gene expression and AML. There has been a most recent study by Liu et al. [51] from china where WT expression was studied on 195 odd cases of AML and established as marker of MRD. Rautenberg et al. [52] studied WT gene expression on post allogeneic transplant cases of AML & MDS to utilize it as marker for prediction of relapse. Nomedede et al. [53] proved it as marker of prognostic marker among 585 cases of AML in European region. A study from china [54] proved WT1 gene expression as an independent prognostic marker in cases of acute leukemia. There have been two independent studies [55, 56] from Italy where WT1 gene expression has been evaluated as marker for risk stratification and long term progression respectively.

Therapeutic implications of WT1 in AML

There has been a trial to attempt to use peptide based vaccines against WT1 especially in cases where given overexpression has been documented. Numerous studies on WT immunotherapy have been carried till date using a variety of different vaccination strategies, for example (HLA-restricted versus non HLA-restricted peptides). Clinically meaningful responses have been reported in several trials in both AML and MDS cases, with associated increases in WT1-specific T-cell frequencies (**Table 1**) [57-66]. The expression of WT1 on normal tissues and its role in normal haematopoiesis has been an issue of probable possibility of autoimmune phenomenon. However, there have been no reports till date [49]. This approach has, thus demonstrated clinical efficacy but still requires further large-scale evaluation. Another

alternative approach has been development of monoclonal antibodies that may recognizes a peptide fragment of WT1 complexed with HLA-A0201. This antibody demonstrated efficacy in a NOD/SCID mouse xenotransplanted with human leukemias [49].

Prognostic implications of WT1 in AML

The undetectable leukemic stem cells (LSC) on morphology are strong independent factor for future relapse [67]. The monitoring of MRD is of utmost importance to prevent future relapse and improve overall survival. Detectable levels of WT1 expression during follow-up in AML cases are a potential marker for assessment of residual blast populations or even to predict future relapse of AML [68]. However, there are indicators for the involvement of WT1 in malignant events of AML blasts, such as the interactions of WT1 with the proto-oncogene bcl-2 and tumor suppressor gene p53 [69]. Despite advancement in development of new treatments protocols, many cases are refractory to ongoing therapeutic strategies. They have a higher relapse rate, with overall long-term survival of patients signifying below 40% and more than 60% among cases over 65 years of age succumbing to disease within one year of diagnosis [70]. It is assumed that relapses originate from undetectable populations of LSC, which are characterized by a pronounced self-renewal capacity that evade traditional chemotherapy [71]. In vitro killing of tumor cells by WT1-specific CD81 cytotoxic T lymphocytes facilitated the development of a WT1 based vaccine. WT1-specific immunotherapy might be useful to optimize multimodal therapy of haematological malignancies [72, 73]. There are various studies which have correlated the expression of WT1 and data of AML cases. Lovvik et al. observed that in AML cases at primary diagnosis, 66% had more than 20-fold WT1 overexpression in peripheral blood (PB) or bone marrow (BM) (PB 74%; BM 45%) [74]. In another study, Ho et al. revealed that in a group of paediatric AML, a significant difference in event-free survival and leukemia-free survival for cases with high versus low WT1 expression was found [75]. Nomdedeu et al. reported that de novo AML patients with high-level WT1 expression with 3-year overall survival (OS) was only 19%, whereas patients with low-level WT1 expression 3-year overall survival (OS) was about 64% [54]. Brieger et al. showed in his study of 52 AML cases WT1 gene was overexpressed in 41/52 (79%) patients at the time of first diagnosis. The majority of the 14 patients lost WT1 expressions that were studied in CR, where as in 4 cases reappearance of WT1 expression estimated before relapse [76]. In one of the study with background knowledge of WT1 and Bcl2 controlling apoptosis, WT1 expression and proto-oncogene Bcl-2 was estimated simultaneously as prognostic markers of AML treatment outcome. The study showed that increased WT1 and Bcl-2 expression was associated with reduced rate of continuing complete remission and increased deaths among AML cases with age less than 60 years [77].

Application of WT1 for detection of minimal residual disease

WT1 is a potential marker for detection of MRD in AML. The identification of MRD has led to substantial improvements in early recognition of recurrence of AML [78]. Many studies have determined that the MRD assessment provided evidence to stratify high-risk AML patients better and give significant insight into the effectiveness of treatment. Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) serves as an effective treatment strategy for high-risk patients with AML in CR [79]. WT1 expression affects prognosis of Allo-HSCT in AML [80]. Furthermore, increased WT1 expression was associated more with higher rate of relapse compared, with cases under remission were constantly associated with low levels [81]. There are studies which have showed association between expression of WT1 gene and chances of relapse after allogeneic stem cell transplant (Allo-HSCT). Ogawa et al. found that higher WT1 levels after Allo-SCT were associated with increased chances of relapse. There was constant doubling time in WT exponential expression in sust of cases having relapse. The significance of WT1 log reduction after induction chemotherapy to be an independent predictor of relapse [82]. Weisser et al. observed that higher than 2 log decline in WT1 transcript levels from the beginning of chemotherapy was correlated with a significantly improved Overall Survival (OS) and event-free survival (EFS). After induction chemotherapy, decreased WT1 gene expression in AML patients conferred a more favourable prognosis and correlated with high-

er Overall Response Rate (ORR), and 2-yr overall survival rates and disease-free survival (DFS) rate were highly significant (*P*-value <0.05) [83]. In an another study, Cilloni et al. demonstrated that less than 2 log decline in WT1 transcripts after induction therapy enhanced the significant risk of relapse in patients with AML [51]. While another research group, Ido et al. showed association between expression levels of WT1 before and after Allo-HSCT and the risk of fatality among AML. In patients with AML who underwent Allo-HSCT after two years, the fatality rate was significantly lower in AML patients who are having low expression levels of WT1 when compared with cases having high expression level of WT1. Furthermore, in the whole cohort of AML patients, WT1 mRNA ≥5000 copies/μg RNA before Allo-HSCT was significantly associated with an increased risk of mortality [84]. These studies are suggesting that the WT1 mRNA level might reflect tumor burden.

Future research on WT immunotherapy in AML

The virtue of being a pan leukemia markers makes WT1 as a potential target for immunotherapy. Both higher expression of WT1 and mutation in WT1 gene is involved in AML has lead to both clinical and preclinical therapeutic strategies in hematological and solid malignancies such as uterine sarcoma [85-87]. In cases where raised WT1 expression has been estimated, WT1 based immunotherapy combined with standard neoadjuvant therapy induces T cell recognition of tumor antigens by vaccination and induces immune response to produce tumor antibodies in breast cancer cases [88]. WT1 peptide vaccination can induce cells of WT1-specific cytotoxic T lymphocytes, prevent relapse, and sustains long-term, complete remission in AML [89]. After WT1 vaccination, most studies showed that the number of granulocytes, lymphocytes, and leukemia blast cells were reduced. Phase I study conducted mice vaccinated with Mycobacterium Bovis bacillus Calmette-Guerin cell-wall skeleton (BCG-CWS) with WT1 peptide survived significantly longer when compared with a non-vaccinated mice. Thus, adjuvant like BCG-CWS may prove to enhance the clinical efficacy of WT1 vaccine for humans [90]. Currently WT1 vaccine based immunotherapy research are in early trials and phase I studies have generated positive inclinations. This has promoted phase II trials to evaluate it further, once phase II clinical trial has been carried out on AML cases. The treatment with WT1 peptide was combined with treatment with granulocyte-macrophage colony-stimulating factor [GM-CSF]. This cytokine is used as an adjuvant and functions as a white blood cell growth factor. The treatment with this vaccine was well-tolerated, blast reduction and hematological improvement were seen in some patients; the results were overall promising [91, 92]. Such in vitro findings have led to design of the human WT1 vaccine. Recently several studies (**Table 1**) have reported the safety and efficacy with favorable results concerning the use of the WT1 peptide vaccine in patients with AML. These results showed that the WT1 vaccine is well tolerated, stimulates a specific immune response, and can improve the prognosis of patients.

Conclusion

The newer advancement in field of molecular genetics and therapeutic researches has drastically improved the overall survival figure for a complex heterogeneous disease such as AML. Our understanding on the impact of mutation and expression of WT1 gene on AML has greatly improved. This has facilitated application of WT1 as as potential biological marker for diagnosis, clinical management, monitoring of therapy, detection of MRD and Immunotherapy. The current article has tried to emphasize the upcoming application of WT1 based interventions in AML. WT1 encodes a transcription factor that plays a regulatory role in normal and malignant haematopoiesis. Frequent monitoring of the WT1 gene expression level during follow-ups in AML patients is useful as a marker for residual blast populations or even to predict the risk of relapse following allogeneic SCT. The current review validates WT1 as promising potential biomarker for AML on the basis of available published medical literature.

Acknowledgements

We are thankful to Science and Engineering Research Board, Department of Science and Technology, Government of India through Grant No. EEQ/2016/000318 for supporting resources in this study.

Disclosure of conflict of interest

None.

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