

## Review Article: Wilms' Tumor 1 Gene (WT1) Clinical and Therapeutic Implications

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**Abstract:** Wilms' tumor gene 1 (WT1) is an embryonic zinc-finger transcription factor. WT1 protein is overexpressed in a variety of hematological malignancies and solid tumors. Previous reports have suggested that whether the WT1 gene acts as a tumor suppressor gene or an oncogene might depend on the differences in the interactions of the WT1 protein with other regulatory proteins. In the present review, we will discuss the role of WT1 gene in the process of cancer progression and give an overview on its expression in different tumors. We will also provide information about WT1 gene as a potential attractive target for therapeutic strategies in the treatment of cancer.

**Key words:** WT1 • Tumerigenesis • Cancer Therapy

### INTRODUCTION

Wilms' tumor gene 1 (WT1) is an embryonic zinc-finger transcription factor, which was originally identified as a tumor suppressor gene inactivated in Wilms' tumors [1]. WT1 was discovered upon analysis of commonly deleted regions on the short arm of chromosome 11 in children with the WAGR syndrome, a syndrome characterized by the association of Wilms' tumor (W), aniridia (A), genitourinary malformations (G) and mental retardation (R) [2]. It was later found that WT1 germ line mutations were associated with Denys-Drash syndrome and Frasier syndrome, syndromes that lack abnormalities of the eye (Aniridia) and CNS (mental retardation), but have severe Genitourinary malformations and Wilms' tumor [3]. In normal tissue, WT1 is expressed during embryogenesis where it plays a pivotal role in the development of the urogenital tract. It is expressed in cells that undergo a mesenchymal-epithelial transition, like those found in the kidney [4]. In adults, it is expressed at very low levels in the kidney, ovary, testis, spleen and normal hematopoietic progenitor cells [5]. Previous studies have shown that the WT1 protein is a predominately nuclear protein. It has four Krüppel-type C2H2 zinc fingers, indicating that it acts as a transcriptional regulator [6]. WT1 has also been implicated in post-transcriptional regulation, including RNA-splicing. As a result of alternative splicing, alternative translational initiation sites and RNA editing,

the WT1 gene can encode for as many as 36 different proteins [7]. The four main isoforms are generated by alternative splicing, which causes the inclusion or exclusion of the 17 amino acid sequence coding for exon 5 or the three amino acid (lysine, threonine and serine: KTS) region in exon 9 (From this point on, the different isoforms will be designated by “±17a.a.” in combination with “±KTS.”) [8] The KTS+ isoforms represent 80% of the transcripts. These isoforms have different DNA-binding and transactivation activities and different nuclear localizations, which indicates that they have different functions. WT1 binds to the promoter of its target genes [9] (Figure 1).

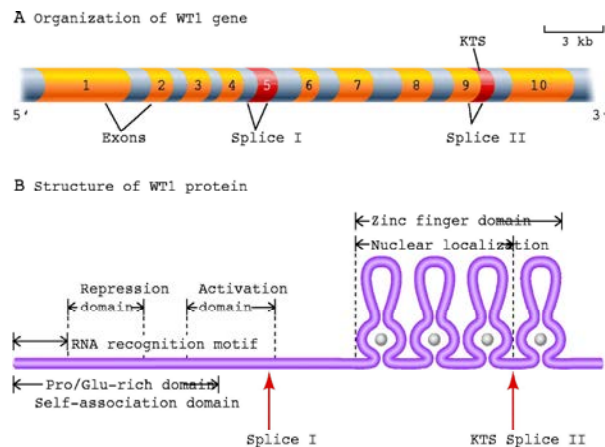


Fig. 1: Organization of the Wilms' tumor1 (WT1) locus and basic structure of the WT1 proteins [10]

WT1 is an extremely complex gene capable of exerting significant influence on cell biology. *In vitro* studies have shown that the target genes potentially regulated, most often negatively, by WT1 include genes that code for transcription factors such as PAX2, PAX8, as well as for growth factors or their receptors: IGF2, IGFR, PDGF-A, TGF- $\beta$ , EGFR [11]. WT1 also acts at the post-transcriptional level and can bind to RNA of the IGF-2 gene [12]. Additionally, WT1 has a putative role in splicing. Furthermore, WT1 has been co-immunoprecipitated with numerous other proteins including tumor protein p53 (p53), prostate apoptosis response protein 4 (par-4) and tumor protein p73 (p73). Many of these binding partners can be used to modulate WT1's transcriptional activities. Furthermore, WT1 is involved in male sex determination through targeting SRY (Sex-determining region Y) protein, also known as Testis-determining factor (TDF) [13]. It was reported that WT1 protein shuttled continuously between the nucleus and the cytoplasm and therefore cytoplasmic WT1 protein was associated with ribosomes and actively translating polysomes. WT1 might be a multifunctional component of protein complexes that regulate three steps of gene expression: transcriptional control, RNA processing and translational regulation [14]. All the formerly mentioned functions of WT1 gene indicate its vital role in the cellular growth and differentiation.

**WT1 and Tumorigenesis:** Although *WT1* was originally identified as a tumor suppressor gene, WT1 protein is overexpressed in a variety of hematological malignancies and solid tumors [15]. Previous reports have suggested that whether the WT1 gene acts as a tumor suppressor gene or an oncogene might depend on the differences in the interactions of the WT1 protein with other regulatory proteins. Several WT1 target genes have been identified that are likely to play important roles in neoplastic transformation regardless of tissue type. Among these are genes involved in proliferation and cell cycle regulation, growth factors and their receptors and apoptosis [16]. For example, cyclin E and p21 are both key molecules and play antagonistic roles in the regulation of the cell cycle progression from the G1 into the S phase. Some reports showed that WT1 suppressed the expression of cyclin E and promoted that of p21 [17]. Bcl-2 is up-regulated by WT1 in some cell types but not in others. This function would be expected synergistically slow progress through the cell cycle. On the other hand, it is reported that WT1 protein resulted in up-regulation of the expression levels of epidermal growth factor receptor (EGFR), which participates in the tumorigenesis of some malignancies

[18]. Furthermore, WT1 + KTS isoforms have been shown to have a role in gliomagenesis by inhibition of p53-mediated apoptosis [19]. WT1 is aberrantly overexpressed in 60 to 87% of mammary cancers [15]. A case of malignant mesothelioma containing a homozygous point mutation in WT1 was reported [20]. WT1 expression was readily detected in renal cell carcinoma cell lines and was also detected in a single oncocytoma specimen. Because oncocytomas are benign tumors, this finding raises the possibility that WT1 expression might be an early step in renal tumorigenesis, or that it may simply be a marker for a common cell of origin for these 2 tumor types [21]. WT1 expression has been demonstrated in serous epithelial ovarian tumors and in serous adenocarcinomas of the ovary. This gene is also expressed, however, in ovarian surface epithelium, the lining of inclusion cysts of the ovary and fallopian tubular epithelium. There was no significant survival difference between patients with low or high WT1 expression levels in these ovarian tumors [22]. The expression of WT1 in lung cancer has prognostic effects. Oji *et al.* [23] found that high level of WT1 IgG antibody expression in lung cancer is associated with a worse prognosis. Amin *et al.* [24], reported WT1 expression in malignant mesothelioma cell lines and in primary tissue specimens. The Wilms' tumor 1 (WT1) gene is known to be overexpressed in hepatocellular carcinoma (HCC) and to upregulate tumor growth and oncogenic potential. WT1 over expression in tumor tissues of patients was associated with increased tumor size and with a short doubling time of HCC, indicating that WT1 is associated with tumor growth. However, the mechanism by which WT1 modulates liver cells to increase oncogenic potential and tumor growth remains unknown [25]. WT1 expression has not been shown to be an independent prognostic factor in women with uterine cancer [26]. WT1 was found to be expressed in gastric carcinoma, colon cancer and melanoma [27, 28] and is involved in a translocation associated with Desmoplastic Small Round Cell Tumor (DSRCT) [15]. Only few studies have investigated the role of WT1 in primary glioma. Prior studies have documented that neuroepithelial tumors exhibit *de novo* WT1 expression, which increases with the grade of malignancy [29]. Although WT1 expression in normal brain tissue is isolated to endothelial cells, however it has been proved that it is related to the angiogenesis in glial neoplasms [30, 31]. In the past, WT1 protein was thought to be predominantly located in the nucleus, because its function was control of transcription and RNA processing [32]. However, it has been shown that WT1 protein is located in the cytoplasm of tumor cells [33].

**Estimation of WT1 Expression in Tissues:** RT-PCR (Real Time –PCR) is widely employed for determination of WT1 expression [28]. However, the results of RT-PCR are not always correlated with protein expression and this method is not suitable for tumors that consist of cells of various types, including malignant and non-malignant cells, because the results are dependent on the proportion of malignant to normal cells. Therefore, immunohistochemical analysis of WT1 expression in solid tumors may be a better option than RT-PCR [34].

**WT1 Role in Cancer Therapy:** The introduction of a WT1-based vaccine in clinical trials was first described by Oka *et al.* [29]. Sugiyama *et al.* [35], reported that the growth of WT1 protein expression in leukemia and solid tumor cells was inhibited by treatment with WT1 antisense oligomers. It is well known that tumor-specific CD8 + cytotoxic T lymphocytes (CTLs) constitute the most important effectors for antitumor responses and recognize peptides derived from endogenous proteins presented on the cell surface in association with MHC class I molecules. Sugiyama *et al.* [36], successfully synthesized MHC class I-binding peptides and demonstrated that the WT1 peptides induced peptide specific CTLs as a result of *in vivo* immunization of mice with the peptide. They produced HLA-A\*2402-or HLA-A\*0201-restricted 9-mer WT1 peptides and showed that the stimulation of peripheral blood mononuclear cells with the WT1 peptides elicited WT1-specific CTLs in an *in vitro* human system. Another possible mechanism of action of a WT1-based vaccination could lie in its suppression of WT1's inactivation of tumor suppressor gene p53 (TP53). This hypothesis was initially tested in p53-null Saos-2 osteosarcoma cells and it was revealed that WT1 inhibits p53-mediated apoptosis, which is normally induced by chemotherapy, radiation and overexpression of wild-type p53 [37]. Clark *et al.* [19], continued to investigate this relationship between WT1 and p53 in glioblastoma by examining the effect of WT1 expression and WT1 silencing on p53-mediated cell death and response to radiotherapy. Results showed that WT1 silencing led to glioblastoma cells becoming susceptible to radiation induced death, suggesting a potential target to improve responses to radiotherapy. Vaccination with WT1 epitopes was evident to be safe, feasible and potentially able to mediate sustained immune responses in patient with Myelodysplastic syndrome/ Acute myeloid leukaemia (MDS/AML) [37].

## CONCLUSIONS

Although originally isolated as a tumor suppressor gene, WT1 appears to have a more complex role in oncogenesis. WT1 expression may have some prognostic value in some neoplasms. It has been proved to be an optimal target for immunotherapeutic approaches to the treatment of tumors that overexpress this transcription factor.

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