Leukemia (2007) 21, 868–876

www.nature.com/leu

REVIEW

A tumor suppressor and oncogene: the WT1 story

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The Wilms' tumor 1 (WT1) gene encodes a transcription factor important for normal cellular development and cell survival. The initial discovery of WT1 as the causative gene in an autosomal-recessive condition identified it as a tumor suppressor gene whose mutations are associated with urogenital disease and the development of kidney tumors. However, this view is not in keeping with the frequent finding of wild-type, full-length WT1 in human leukemia, breast cancer and several other cancers including the majority of Wilms' tumors. Rather, these observations suggest that in those conditions, WT1 has an oncogenic role in tumor formation. In this review, we explore the literature supporting both views of WT1 in human cancer and in particular human leukemias. To understand the mechanism by which WT1 can do this, we will also examine its functional activity as a transcription factor and the influence of protein partners on its dual behavior.
Leukemia (2007) **21,** 868–876. doi:10.1038/sj.leu.2404624;
published online 15 March 2007
Keywords: Wilms' tumor 1; leukemia; oncogene; tumor suppressor

Introduction

Cancer is a proliferative disease characterized by abnormal cells with altered growth properties and growth factor dependencies. A major property of cancer cells is their ability to escape the hindrance of anti-proliferative signals and undergo cellular expansion exploiting pre-existing and novel growth pathways. Possible mechanisms that can explain the uncontrolled growth of cancer cells are the loss of function of tumor suppressors and/or the activation of oncogenes. Although these are opposite functions that would be intuitively mutually exclusive for a single protein, evidence is emerging that one protein can exhibit both properties under different cellular conditions. An example of this is the oncogene Myc.[1] In this review, we argue that the Wilms' tumor 1 (WT1) protein has a similar dual behavior depending upon the cell type in which it is expressed. We will discuss the transcriptional activity of WT1 and provide evidence that WT1 can behave both as a tumor suppressor or an oncogene in the development of the malignant state.

Normal expression of WT1

WT1 is an important regulatory molecule involved in cell growth and development. It is expressed in a tissue specific manner. In the developing embryo, WT1 expression is found primarily in the urogenital system (reviewed by[2,3]). In adult tissues, WT1 expression is found in the urogenital system, the central nervous system and in tissues involved in hematopoiesis, including the bone marrow and lymph nodes.[2]

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Received 28 September 2006; revised 23 January 2007; accepted 24 January 2007; published online 15 March 2007

Structure of WT1

The *WT1* gene, first cloned in 1990, is located at chromosome 11p13.[4] The gene encodes for 10 exons and generates a 3 kb mRNA (Figure 1). Although several transcriptional modifications can occur, there are two predominant alternative splicing events. These include splicing of exon 5 (17 amino acids), and of a stretch of nine nucleotides (three amino acids, lysine, threoine, and serine (KTS)) in the 3′ end of exon 9. Alternative splicing of these two sites gives rise to four different protein isoforms designated A, B, C and D, or (−/−), (+/−), (−/+) and (+/+), representing the presence or absence of exon 5 and KTS insert, respectively.[5] Under normal physiological conditions, the expression of KTS(+)/KTS(−) ratio is maintained at approximately 2:1.[5] In addition to these major alternatively spliced forms, a N-terminally truncated WT1 referred to as AWT1 has recently been described. This arises as a result of gene transcription initiating within a promoter in intron 1.[6]

The N-terminal domain of WT1 is comprised of proline–glutamine-rich sequences and is involved in RNA and protein interactions (Figure 1).[2] This domain is critical for the transcriptional regulatory function of WT1, as deletion studies reveal both transcriptional repression and activation domains. The C-terminal domain of WT1 is composed of four Krüpple-like $Cysteine_2$-$Histidine_2$ zinc fingers, which permit binding to target DNA sequences but are also involved in RNA and protein interactions. The AWT1 isoform differs from WT1 in that it lacks the first 147 amino acids that contain the repression domain.

WT1, the transcription factor

As suggested by the presence of zinc fingers in the C-terminal half of the protein, WT1 has been found to be a potent transcriptional regulator. Among the targets are genes important for cellular growth and metabolism, including extracellular matrix components, growth factors and other transcription factors (Table 1).

Through transfection experiments, it has been found that WT1 can either enhance or repress the expression of specific target genes or constructs. Whether WT1 acts as a repressor or activator can be influenced by the level of WT1 expression, the isoform of WT1 (primarily either +KTS or –KTS), the location of the transcriptional start site in relationship to the WT1 DNA binding site and the cell type in which the experiment was performed.[7–9] For instance, we recently demonstrated that the exogenous expression of WT1 in the human breast cancer cell lines, MDA-MB-468 and MCF-7 and in human leukemic K562 cells, can activate the c-Myc promoter and stimulate cellular proliferation.[10] This is in contrast to studies reported in HeLa cells, where CAT

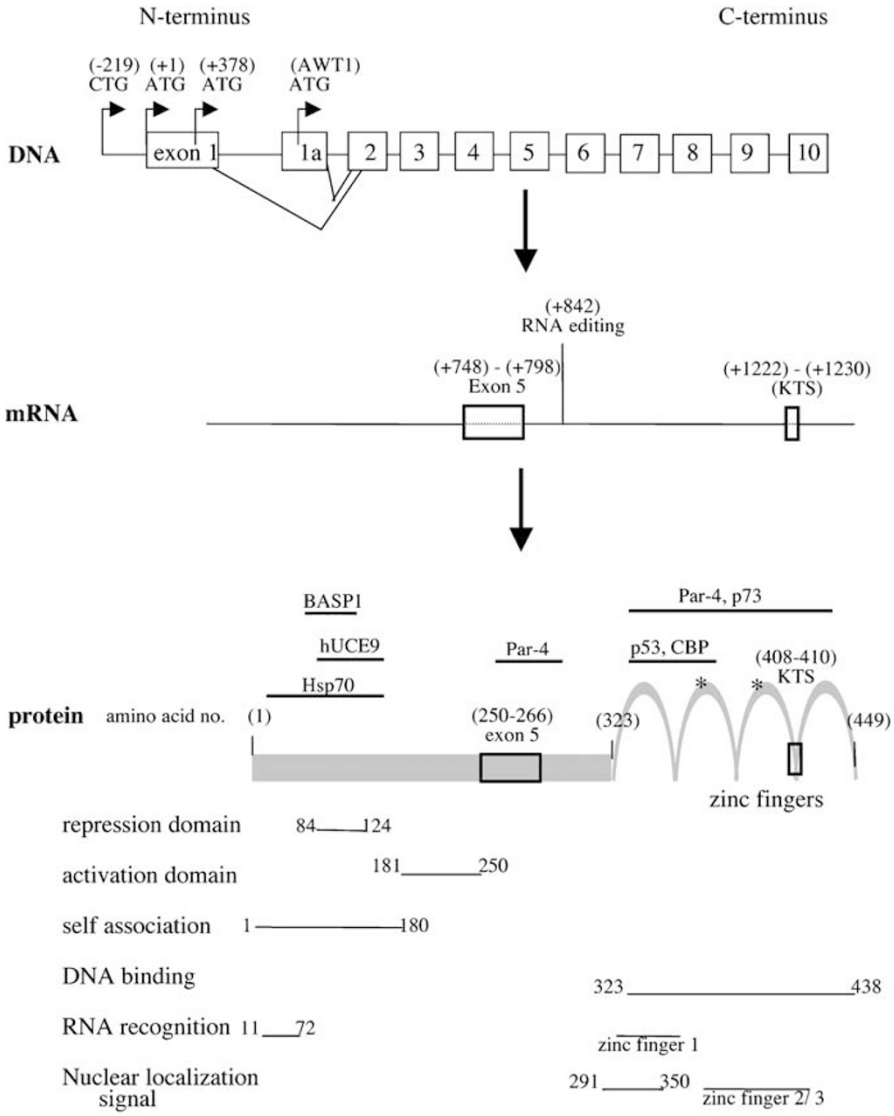

Figure 1 Schematic diagram of the WT1 structure at the DNA (exons only), mRNA and protein level. Several post-translational modifications occur in WT1. WT1 can be translated from four initiation start sites. The most recently reported AWT1 represents a truncated WT1 transcribed from an internal ATG located within the intron between exon 1 and exon 2. At the mRNA level, WT1 RNA is edited at nucleotide position 843 and subject to RNA splicing of exon 5 and of nine nucleotides between exons 9 and 10 yielding the major isoforms of WT1. WT1 protein has several functional domains. N-terminal domain of WT1 is proline–glutamine-rich and contains both a transcriptional repression and activation domain. In addition, WT1 contains self-association and RNA recognition motifs. The C-terminal domain contains four C_2H_2 Krüpple-like zinc-fingers, which in addition to binding DNA and some proteins, can regulate RNA targets and mediate nuclear localization. The numbers indicated in the DNA and RNA represent nucleotide sequence and the numbers indicated in the protein are amino-acid residues. All numbers represent the human WT1 (+/+) isoform. Lines shown above the WT1 protein schematic indicate reported regions of WT1 involved in its interactions with selected regulatory molecules. (*) indicates reported phosphoryaltion sites, Serine 365 and Serince 393, located in zinc-fingers 2 and 3, respectively. Schematic is not drawn to scale.

reporter assays showed repression of the Myc promoter by WT1.[11] Another relevant example is the anti-apoptotic gene, Bcl-2. In HeLa and DHL-4 cells, transient co-transfection of WT1 with the Bcl-2 promoter linked to a reporter gene resulted in significant repression of the Bcl-2 promoter.[12] Meanwhile, in the rhabdoid tumor cell line, G401, stable overexpression of WT1 led to increased endogenous Bcl-2 protein.[13] A similar result was observed in K562 cells (MDM, unpublished).

WT1, the tumor suppressor

WT1 was initially discovered as a tumor suppressor in Wilms' tumor (WT), a pediatric kidney malignancy that affects approximately 1:10 000 children. Mutations of WT1 associated with WT are found almost exclusively in the sporadic form of the disease at a low frequency (15%).[14] The abnormalities include large deletions and intragenic mutations (10 and 5% of all WT cases, respectively).[14] However, the majority of WT express wild-type WT1, sometimes to high levels.[3] These findings suggest that WT1 mutations are important only in a small fraction of cases. Furthermore, in WT that express wild-type WT1, it is not known whether the persistent expression of WT1 contributes to the development of the disease or is just a reflection of tumor ontogeny.

In contrast to uncomplicated WT, three related syndromes are associated with heterozygous mutations of WT1. These include

Table 1 Selected transcriptional targets of WT1 and its reported transcriptional effect

Target	*Effect*	*Reference*
Growth factor		
Amphiregulin	Activation	67
Colony stimulating factor-1	Repression	68
Insulin growth factor II	Activation/ repression	69–71
Platelet derived growth factor	Repress/act	72–74
Transforming growth factor-beta	Repression	75
Receptors		
Androgen receptor	Repression	76
Epidermal growth factor receptor	Repression	23,24
Insulin receptor	Repression	23,77
Insulin growth factor – I receptor	Repression	78,79
Retinoic acid receptor alpha	Repression	52
Transcription factor		
c-Myb	Repression	80
c-Myc	Activation/ repression	10,11,81
Cyclin E	Repression	82
Cyclin G1, IGFBP-4	Activation	83
P21	Activation	84
Pax-2	Repression	85
Enzymes		
Human telomerase reverse transcriptase	Repression	86
Ornithine decarboxylase	Repression	87,88
ECM		
E-cadherin	Increased	89
Thrombospondin 1	Repression	90
Syndecan-1	Activation	91
Other		
Wnt-4	Activation	92
Bcl-2	Activation/ repression	11–13
Erythropoietin	Activation	93

Abbreviation: WT1, Wilms' tumor 1.

WT, sporadic aniridia, genitourinary abnormalities and mental retardation (WAGR) Denys–Drash Syndrome (DDS) and Frasier Syndrome (reviewed by[14]). The majority of WT1 mutations in DDS are point mutations in zinc finger 2 or 3, which results in truncated proteins or amino-acid substitutions that can act in a dominant negative manner by inhibiting the function of the wild-type protein expressed from the remaining allele.[15,16] Frasier syndrome is primarily caused by point mutations at the second splice donor site in intron 9, resulting in the loss of the KTS(−) isoform in the mutated allele and offsetting the KTS(+):KTS(−) ratio.[17]

The ability of WT1 to induce growth suppression and suppress tumorigenicity in mice also highlights its role as a tumor suppressor.[18–20] For example, ectopic expression of WT1 in kidney-derived RM1 cells results in a reduced number of colonies in cell culture.[21] The stable introduction of the WT1 −/+ isoform into G401, a kidney-derived tumor cell line that does not express endogenous WT1, alters cellular morphology and reduces tumor formation in athymic nude mice.[22] The mechanism may be through cell death, as expression of WT1 in Hep2B and osteosarcoma cell lines, Saos-2 and U20S, can alter signaling pathways and induce apoptosis.[23,24] First, WT1 can directly regulate the apoptotic pathway through induction of proapoptotic genes such as BAK.[25] Also, WT1 can downregulate growth factor receptors such as the epidermal growth factor receptor (EGFR) and the insulin receptor, altering the balance of survival signals towards death.[23]

WT1 in leukemia, a candidate oncogene

In recent years, the study of WT1's involvement in malignant cells has unexpectedly revealed a potential role for WT1 as an oncogene. The prime evidence supporting this is the overexpression of wild-type WT1 in a variety of human cancers of both hematological and non-hematological origin (Table 2). The most studied of these is leukemia where there is a growing body of evidence demonstrating WT1's biological and clinical importance in cell survival, differentiation and proliferation. In this section, we provide a comprehensive review of WT1 in leukemia as a tumor suppressor and oncogene and discuss its likely role in leukemogenesis. To begin, we will describe WT1 in normal blood cell development.

WT1 in normal hematopoiesis

In normal human bone marrow, WT1 is expressed at extremely low levels and is confined to the primitive $CD34^+$ population of cells.[26,27] To ascertain the effect of WT1 on these early cell populations, studies have been carried out in mouse and human cells with different results. Gene knockout of WT1 in mice is embryonically lethal; however, this is not due to a hematopoietic defect as assessed by bone marrow and peripheral blood morphology. This is further confirmed by the finding that fetal liver cells from WT1 deficient animals can reconstitute the hematopoietic system of irradiated adult mice thus indicating that these cells are capable of the full range of proliferation and differentiation.[28,29] However, when chimeric mice are generated from ES cells lacking WT1, the WT1 null ES cells do not compete with wild-type WT1 expressing cells in contributing to bone marrow and blood cell products.[28] This suggests that WT1 plays a role in the self-renewal of early murine hematopoietic cells. This is further supported by studies of Nishida *et al.*[30] who forced the expression of WT1 in hematopoietic progenitor cells by placing WT1 downstream of the *tec* promoter. This resulted in increased bone marrow cellularity and increased numbers of BFU-E, CFU-GM and CFU-GEMM; production of mature cells was normal in these mice. It is of note that these mice did not develop spontaneous leukemia. However, when the bone marrow was infected with a virus carrying AML1-ETO, the animals developed leukemia. The authors conclude that in this model, WT1 serves to maintain the self-renewal of cells, whereas the 'second hit' blocks differentiation, resulting in the development of leukemia.

In human hematopoietic cells, WT1 appears to behave as a tumor suppressor gene as the overexpression of WT1 in early human bone marrow cells leads to growth arrest and reduced colony formation.[31,32] This effect is due to the zinc fingers, as deletion of this domain abolishes the effect.[33] These results indicate the need for caution and qualification, when drawing conclusions as to the role of WT1.

WT1 in leukemia

WT1 as a tumor supressor in leukemia. Two main findings support WT1 as a tumor suppressor in acute myeloid leukemia (AML). First, in one study approximately 10–15% of cases (4/36 patient samples) had mutations of WT1 in leukemic

Table 2 Expression of wild-type WT1 in various human cancers

Type of cancer	*Frequency of detection*	*Method of detection*	*Reference*
Astrocytic tumors	23/25	RT-PCR, IH	94
Bone and soft-tissue sarcomas	28/36	Q-RT-PCR, IH, Seq	95
Brain tumor	23/26	IH	96
Breast cancer	27/31	RT-PCR, Northern	97
Colorectal adenocarcinoma	20/28	Q-RT-PCR	98
De novo lung cancer	41/46 54/56	IH, Seq Q-RT-PCR, IH, Seq	99
Desmoid tumors	5/5	RT-PCR, Northern, Western, IH, Seq	100
Esophageal squamous cell carcinoma	12/12 36/38	Q-RT-PCR IH	101
Head and neck squamous carcinoma	42/56	Q-RT-PCR, Seq	102
Leukemia	30/59 68/86	Northern RT-PCR, Northern	37,38
Malignant methelioma	54/56 50/67 nuc	IH, Seq IH	103,104
Melanocytes	7/9	Northern, RT-PCR	105
Neuronal tumors: brain	16/36	RT-PCR, Seq	106
Neuroblastomas	6/18	RT-PCR, Seq	106
Ovarian carcinomas			
Serous	28/28	IH	107
Endometrial	34/130 10/29	Tissue microarray, IH IH	108,107
Epithelial	78/100	IH	109
Pancreatic ductual adenocarcinoma	30/40	IH, Western	110
Primary thyroid cancer	33/34	Q-RT-PCR, Western, Seq	111
Renal cell carcinoma	4/5	Northern, IH	112

Abbreviations: IH, immunohistochemistry; Q-RT-PCR, quantitative real-time polymerase chain reaction; Seq, sequencing; RT-PCR, real-time polymerase chain reaction; Northern, Northern blotting; Western, Western blotting; Nuc, nuclear staining.

cell DNA.[34] In that study, of the four patients carrying WT1 mutations, five different point mutations were found. These included four nucleotide insertions in exons 1 or 7, and one nonsense mutation in exon 9. These mutations are predicted to yield truncated or modified WT1 protein. Unfortunately, in the published manuscript, there was no assessment of WT1 RNA or protein in cells containing WT1 mutations. Thus, it is not known if these mutations contributed to the development of the disease and the behavior of the cells.

Second, very low levels of WT1 RNA can be found in some forms of AML. We assessed the expression of WT1 in the Valk *et al*.[35] AML microarray expression data set of 285 AML patients and found that the majority of patients have high levels of WT1. However, some subgroups, such as cluster 5, have low to absent levels of WT1. In these patients, high levels of WT1 expression may not be well tolerated. This possibility is supported by the growth inhibiting effect of WT1. For example, when the WT1 KTS(+) isoform is expressed in a WT1-negative human myeloblastic leukemic cell line, M1, decreased tumor formation is observed when the cells are injected into immunocompromised mice.[18] This is consistent with a tumor suppressor function of WT1 in some forms of AML.

WT1 as an oncogene in leukemia

WT1 is overexpressed in leukemia. As mentioned above, WT1 is highly expressed in the bone marrow or peripheral blood of a variety of leukemias in comparison to normal bone marrow and normal progenitor cells. The gene has been wild type in sequence when tested.[36,37] In general, the expression of WT1 varies between and within different forms of human leukemia. In chronic myelogenous leukemia, WT1 levels are usually low in the chronic phase but frequently are seen to increase as patients move into the accelerated and blast crisis phase of the disease.[37,38] In the acute leukemias, increased levels of WT1 can be found in both acute lymphoblastic and myeloblastic leukemia. Combining the results of several studies, WT1 RNA levels, as assessed by RT-PCR and Northern blotting (earlier studies), were elevated in a total of 354 of 476 (74%) AML patient samples.[36–41] For acute lymphoblastic leukemia, WT1 RNA was increased in 86 of 131 (66%) patient samples.[36–38,42] High levels of WT1 were also seen in some forms of myelodysplastic syndromes (MDS).[43] In MDS, increased WT1 expression is associated with higher blast counts and portends an early progression to AML.

Elevated levels of WT1 in leukemia are associated with poor prognosis following standard chemotherapy treatment. Consistent with the cell survival and oncogenic roles of the WT1 protein, increased WT1 levels have prognostic significance and are associated with a poor response to therapy.[42,44] In one study, blast cells from 139 AML patients less than 60 years old treated with a typical chemotherapy regimen were assessed for WT1 transcript levels. The probability of 3-year overall survival was 59% in patients with low WT1 levels and 21% in patients with high WT1 levels ($P=0.046$).[45] However, these studies are in contrast with the observations made by Schmid *et al*.[41] where in 125 *de novo* AML patients, WT1 RNA levels did not correlate with disease-free survival or clinical remission. This discrepancy may be due to differences in methodology in measuring WT1 and differences in the patient populations treated or in the intensity of the regimens used.

In a more recent study, WT1 was evaluated along with the proto-oncogene Bcl-2, as prognostic markers of AML treatment outcome.[46] In patients less than 60 years old treated with standard chemotherapy, high expression of both WT1 and Bcl-2 identified a group of patients with significantly poor overall survival ($P=0.0029$), and high incidence of relapse ($P<0.03$). In patients over 60 years old, WT1 and Bcl-2 levels as prognostic markers did not provide the same prognostic information. This may be due to different disease biology in older AML patients or due to the reduced intensity of chemotherapy often used in treating older patients. In general there is an association between the levels of WT1 and Bcl-2, which is in keeping with the observation that WT1 can increase the expression of Bcl-2 (see, WT1, the transcription factor).[13] However, there are also

cases where WT 1 levels are high and Bcl-2 is low, and vice versa. These observations suggest that in some cases the high expression of WT1 and Bcl-2 are linked, whereas in other cases these are independent events.

WT1 is necessary for cell survival and differentiation. The association of WT1 with a poor prognosis suggests that it contributes to the disease phenotype. This has been tested using several methods including antisense oligonucleotides and RNA interference to knockdown endogenous WT1 in highly expressing AML cell lines and primary AML samples. In most cases, loss of WT1 was associated with decreased growth of the leukemic cells and rapid induction of apoptosis.[47,48] These observations strongly support a role of WT1 maintaining the survival of AML cells. Conversely, overexpression of WT1 in K562 cells protected against cell death caused by treatment with the chemotherapy drugs, etoposide and doxorubicin.[48] In these cells, WT1 repressed expression of the proapoptotic gene Bak, resulting in cell survival. However, only the WT1 +exon5 isoform protected the cells whereas WT1 −exon 5 expressing cells remained sensitive to chemotherapy drugs.

The effect of WT1 on cellular differentiation of leukemic cells has been intensely investigated. In culture, it has been observed that induction of differentiation of several leukemic cell lines such as HL60, K562 and NB4 with DMSO, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and all-*trans*-retinoic acid (ATRA), respectively, is associated with a decrease in WT1 mRNA.[49–51] The relative lack of responsiveness of non-acute promyelocytic leukemic cells to retinoic acid treatment may be related to repression of RARα by WT1.[52] A subgroup of patients can be found in the Valk *et al.*[35] data that express low levels of WT1 and increased expression of RARα. It is of interest to learn if these patients would benefit by the addition of retinoid to their therapy.

In U937 cells, WT1 appears to play a role in blocking differentiation, as enforced expression of WT1 inhibits ATRA or vitamin D3 monocytic differentiation.[53] In contrast, forced WT1 expression in K562 cells does not block hemin- or TPA-induced differentiation.[54] This discrepancy is likely due to differences in gene expression between the two cell lines, further emphasizing the contextual effect of WT1 on cell behavior.

WT1 and its Interacting Partners

The above description of WT1 portrays a gene with Janus-like characteristics. The differences in the transcriptional activity of WT1 may be explained by modifications of WT1 and protein–protein interactions that occur in different cellular contexts. Several proteins have been found to associate with and regulate WT1 *in vitro* and *in vivo* (Table 3). One such protein is the tumor suppressor p53, which can associate with the first two zinc-finger domains of WT1. When WT1 is coexpressed with wild-type p53 in p53-null Saos-2 cells, there is an increase in the stability and half-life of p53.[55] Consequently, following UV radiation, the cells enter G1 cell cycle arrest but do not undergo p53-mediated apoptosis, thus allowing the cells to tolerate increased p53 levels and to escape UV-induced cell death.

p53 can also influence the activity of WT1. Idelman *et al.*[56] investigated the impact of p53 on WT1's ability to regulate the insulin growth factor 1 receptor (IGF-1R). In those experiments, forced expression of the WT1 KTS(−) isoform significantly repressed the IGF-1R promoter only in p53 containing Balb/c-3T3 and HCT116+/+ cells but not in their complementary p53 null 10^1 fibroblasts and HCT116−/− cells.

The interaction of specific WT1 domains with auxiliary proteins is an important aspect of WT1 regulation. Specifically, the prostate apoptosis response factor, Par-4, has been shown to be a regulator of WT1 activity that depends on which domain of WT1 it interacts with. Initially, Par-4 was found through a yeast two-hybrid screen with the WT1 A isoform as bait.[57] The C-terminus leucine zipper region of Par-4 interacted with WT1's zinc-finger domain. In transient transfection assays in HEK293 cells, Par-4 suppressed WT1's ability to activate the expression of DNA constructs containing WT1 binding sites linked to a CAT

Table 3 Selected interacting partners of WT1

Gene	*Effect*	*WT1 region required for binding (amino-acid residue)*	*Evidence of interaction*	*Reference*
Signal transducers and activators of transcription 3 (STAT3)	Synergistic, activator of survival function	1–281	Yeast two-hybrid, IP	113
Brain acidic soluble protein 1 (BASP1)	Repressor	71–101	Affinity chroma-tography IP	114
Creb binding protein (CBP)	Activator	Zinc-finger 1 and 2	IP	115
P53	Repressor	Zinc-finger 1 and 2	IP	116
	Repressor		IP	56
Par4	Repressor	zinc-finger 1–4	yeast two-hybrid, IP	57
	Activator	245–297	protein affinity, IP	117
P73	Repressor	zinc-finger 1–4	IP	118
Bone marrow Zinc finger 2	Repressor	zinc-finger 1–4	Affinity chroma-tography, IP	119
Ciao-1	Repressor	zinc-finger 1–4	yeast two-hybrid, IP	120
Hsp70	Activator	6–180	IP	121
E1B 55K	Repressor	zinc-finger 1 and 2	IP	122
Human ubiquitin conjugating enzyme 9 (hUCE9)	Repressor	85–179	yeast two-hybrid, IP	123
U2AF65	RNA processing	zinc-finger 1–4	yeast two-hybrid, IP	124
Orphan nuclear receptor SF-1	Synergistic activation	ND	yeast two-hybrid, IP	125
Four-and-half LIM-domain FHL2	Activator	182–298	yeast two-hybrid, GST pull-down, IP	126
WT1-associating protein	ND	c-terminus KTS(−)	yeast two-hybrid, GST pull-down, IP	127
WT1	Repressor	N-terminus	yeast two-hybrid, GST pull-down	128

Abbreviations: IP, co-immunoprecipitation; ND, not determined.

reporter. In those settings, Par-4 repressed WT1's transactivation activity.

In another set of experiments, Richards *et al.* showed that the introduction of WT1 +/− but not WT1 −/− could protect 293 cells against cell death induced by UV irradiation or etoposide. The authors suggested that this was due to an interaction between Par-4 and the 17 amino-acid insert of WT1, as Par-4 expression was enhanced in the treated cells. However, the authors did not show that Par-4 was associated with full length WT1 in the treated cells, nor did they explore the status of p53 in these cells. As the interaction between WT1 and p53 has been shown to protect against the toxic effects of p53, this possibility needs to be ruled out, before attributing an antiapoptotic role to Par-4 in association with WT1.

In the context of AML, inspection of the Valk *et al.*[35] data set shows that Par-4 is high in normal CD34+ cells, but low to absent in the majority of WT1-expressing AML samples. This may explain in part, how AML cells can withstand high-level expression of WT1. It is yet to be determined how Par-4 is silenced in those cases.

Other mechanisms for altered WT1 function

In addition to protein–protein interactions, other mechanisms that can affect the function of WT1 include alternate splicing, usage of alternate promoters and post-translational modification of WT1. As already mentioned, there are four major isoforms of WT1 that vary in their ability to affect gene expression. The usage of the promoter just upstream of exon 1a as well as the use of an alternate initiation ATG within WT1 exon 1 causes deletion of the repression domain, thus changing WT1 from a repressor to a transactivator.[58] Finally, Ye *et al.*[60] and Sakamoto *et al.* [59] have shown that Ser 365 and 393 in the second and third zinc-fingers of WT1 can be phosphorylated by PKA or C. These modifications block the ability of WT1 to bind to DNA and hence affect transcription. Of note, phosphorylation of WT1 also results in cytoplasmic retention and impaired nuclear translocation. These findings represent only a few of the ways in which WT1's activities can be altered. The existence of other phosphorylation sites or types of modifications such as acetylation, that might affect WT1's function, is to be discovered.

Concluding remarks

Since the initial discovery of WT1 as a tumor suppressor gene in a small proportion of WT, a role of WT1 as an oncogene has emerged and has been the focus of this review. To summarize, the conflicting views of WT1 as a tumor suppressor or oncogene are clearly context specific. For example, overexpression of WT1 in normal human hematopoietic stem cells leads to growth arrest. Meanwhile, non-mutant WT1 is highly expressed in human AML and its reduction by a variety of means is associated with cell death. Some conditions such as the presence or absence of regulatory protein partners may account for the variable behavior of WT1. For example, Par-4 interacts with WT1 to induce cell death; the silencing of Par-4 in AML is thus important for the oncogenic behavior of WT1. Furthermore, the finding that the interaction of p53 with WT1 alters the proapoptotic behavior of both proteins may allow us to understand the importance of the persistent expression of wild-type p53 and WT1 in AML cells.[61] This can also explain the rapid induction of cell death owing to inhibiting either p53 or WT1 in such cells. The proapoptotic effect of WT1 may also be affected by growth factor signaling as it has been shown that EGFR expression can block WT1-induced cell death.[24] Along the same lines, it is interesting to note that FGFR1 and WT1 are expressed at high levels in advanced forms of prostate cancer, but not in less progressed forms of that disease.[62] Engagement of signaling pathways may also be important for high level expression of WT1 in AML, as cases with Flt-3 internal tandem duplications have among the highest levels of WT1 Valk *et al.*[35]

Based on the above, it is clear that WT1 is playing an oncogenic role in AML and other forms of cancer. However, it is not known whether WT1 is involved in disease initiation or progression. The studies in prostate cancer suggest that WT1 is important in disease progression. Such a role is also likely in some forms of AML. A number of groups have used the levels of WT1 in remission bone marrows as a means of assessing the quality of a remission and to predict impending relapse. In general, these studies have found that rising levels of WT1 in bone marrow samples predict disease recurrence.[63] In these cases, aberrant WT1 expression may be an initiating event or an event that is absolutely required for transformation. However, somewhat unexpected was the observation that despite an initial WT1-positive disease, 15–20% of patients were WT1 negative at relapse.[64] This can be explained in several ways. First, the relapsed disease is entirely different from the initial disease. Second, a stem cell acquired a first hit, and the subsequent activation of WT1 in that cell resulted in full-blown leukemia. This progressed population of cells was eliminated by chemotherapy but cells with the initiating lesion persisted. The acquisition of a non-WT1 second event complements the first hit causing relapse. The ability to distinguish whether WT1 is an initiating or a secondary event has important clinical impact as treatment programs such as immunotherapy or inhibition of WT1 in AML stem cells is developed.[65] Finally, it is necessary to be sure that the negative result is not a technical artifact owing to the development of a point mutation within a primer binding site.[66]

Future studies of WT1 in AML should address questions such as how is WT1 activated in AML cells? Is the activation of WT1 an early or late event in the development of a particular leukemia? And what is the cellular milieu that allows a proapoptotic protein such as WT1 to be expressed, as this knowledge may make it possible to revert WT1 from an oncogene to an executioner.

Acknowledgements

We acknowledge Armando Poeppl for critical readings of the manuscript. This work was supported by a grant from the NCI of Canada and the Terry Fox Foundation.

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