Transactivation of the WT1 antisense promoter is unique to the WT1[+/−] isoform

Kim Moorwood*, Ashreena Salpekar, Sarah M. Ivins, Julie Hall, Rachel M. Powlesland, Keith W. Brown, Karim Malik

CLIC research unit, Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK

Received 12 June 1999

Abstract The Wilms' tumour suppressor gene, *WT1*, encodes a zinc finger transcription factor that has been shown to repress a variety of cellular promoters via binding to cognate DNA elements. Our earlier work identified an antisense *WT1* promoter that contains WT1 consensus sites, but is transcriptionally activated by WT1. In this study, we demonstrate that, unlike previous reports of transcriptional regulation by WT1, transactivation of the antisense promoter is unique to a single isoform of WT1. Of the four alternatively spliced isoforms in which exon 5 (at splice I) or amino acid residues KTS (at splice II) are inserted or omitted, only the WT1 isoform containing splice I and omitting splice II (WT1[+/−]) displays transactivation. We demonstrate that transregulation variations observed with WT1 isoforms are not solely attributable to differential DNA binding by [+KTS] or [−KTS] isoforms. Thus, the transactivation of the antisense promoter displays an absolute requirement for exon 5, suggesting that interaction between WT1 and other cellular factors is necessary for this regulatory function.

© 1999 Federation of European Biochemical Societies.

Key words: Wilms' tumour suppressor gene; Transcription factor; Antisense RNA

1. Introduction

WT1 is a tumour suppressor gene associated with Wilms' tumour, an embryonal kidney tumour arising from malignant transformation of renal stem cells. The consequences of germline defects in the WT1 gene are well-illustrated by two congenital syndromes, Denys-Drash syndrome (DDS) and Wilms' tumour, aniridia, genito-urinary abnormalities and mental retardation (WAGR), which present overlapping sets of growth and developmental aberrations and a predisposition to Wilms' tumour. Missense mutations of the *WT1* gene are found in almost all cases of DDS and large deletions at the 11p13 locus result in WAGR. Additionally, up to 10% of sporadic Wilms' tumours also have *WT1* mutations (reviewed in Hastie, 1994 [1]).

WT1 expression is stringently regulated during organogenesis and cell differentiation. In the developing kidney, *WT1* expression is low in the condensing mesenchyme but rises as cells progress towards an immature epithelial cell phenotype. Expression then attenuates as epithelial cells mature [2]. The requirement for *WT1* in normal nephrogenic differentiation is highlighted by urogenital defects in *WT1* null mice [3], which also develop abnormalities of the lung and heart. A role for WT1 in other cell-types is suggested by the decrease of *WT1* expression accompanying chemically induced differentiation of haematopoietic cells [4,5] and the expression of *WT1* in breast tissue [6] and colonic mucosa [7].

The *WT1* gene encodes 52–54 kDa nuclear proteins characteristic of zinc finger transcription factors, with a glutamine-proline-rich *trans*-regulatory domain and a DNA binding carboxy-terminal region [8,9]. The WT1 protein is expressed as four major isoforms arising from two alternative splice sites (I and II) in the gene. Splice I inserts or omits exon 5 (17 amino acids) and splice II occurs within the zinc finger domain, inserting or omitting three amino acids (lysine, threonine and serine, KTS) between zinc fingers 3 and 4 [10]. We refer to the different isoforms according to insertion [+] or omission [−] at splice sites I and II, respectively, so that, for example, isoform [+/−] has an insertion at splice site I, but not at splice site II. The relative abundance of each isoform appears to be fixed both during human kidney development and in Wilms' tumours in a ratio of 8.3 [+/+]:3.8 [−/+]:2.5 [+/−]:1.0 [−/−] [10]. The [−KTS] and [+KTS] WT1 isoforms have different but overlapping DNA binding specificities. WT1[−KTS] has a zinc finger domain more closely resembling that of EGR-1 and, accordingly, interacts with the EGR-1 consensus sequence (CGCCCCCGC) [11], whereas WT1[+KTS] does not. The latter zinc finger domain can, however, bind a broader range of (G+C)-rich DNA elements for which a consensus has not emerged (reviewed in [12]). In addition to these (G+C)-type WT1 binding sites, a (TCC)-repeat motif which is bound by both [+KTS] and [−KTS] isoforms of WT1 has been identified [13]. Both of these isoforms can also bind RNA [14]. Alternative WT1 proteins are also generated by RNA editing [15] and alternative translational initiation [16], giving a total of 16 possible isoforms. The functional distinction between all the WT1 isoforms, however, remains elementary, with [−KTS] isoforms generally considered as transcriptional regulators and [+KTS] isoforms regarded as RNA metabolism co-factors.

Many other genes have been proposed as potential targets for repression by WT1, by virtue of (G+C)-rich WT1 consensus binding sites in their promoters. Some of these genes encode potent mitogens that could contribute to tumourigenesis when derepressed. In transient co-transfection assays, WT1 represses transcription from numerous such promoters, for example *EGR-1, IGF-2, IGF-1-R, C-MYC* and *C-MYB* [11,17–20]. The expression pattern of *WT1* may also be modulated by negative autoregulation via interaction of WT1 protein with the *WT1* 5′ promoter [21,22].

*Corresponding author. Fax: (44) (0117) 9287896.
E-mail: k.moorwood@bris.ac.uk

0014-5793/99/$20.00 © 1999 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(99)00944-8

Our previous work identified an antisense *WT1* promoter located in the first intron of the *WT1* gene that was transactivated rather than repressed by WT1 [23]. This led us to hypothesise that activation of the antisense promoter might represent a further feedback loop regulating *WT1* expression. Antisense RNAs encompassing *WT1* exon 1 and the *WIT-1* gene [24] occur in foetal kidney and Wilms' tumours [25,26] and are expressed in parallel with sense *WT1* mRNA [27]. The apparent lack of coding capacity and expression pattern strongly suggest that the antisense transcript may be a regulatory RNA which contributes to the overall control of WT1 protein levels. Indeed, we have recently shown that expression of antisense WT1 exon 1 RNA in 293 cells stably expressing exogenous WT1 upregulates WT1 protein levels [27]. A number of vertebrate genes, including human *N-MYC* [28], *C-MYC* [29], chick type I collagen α1 chain [30] and chick *fgf-2* [31] have cognate endogenous antisense RNAs with potential regulatory roles.

Unlike the numerous in vitro co-transfection studies reporting WT1-mediated promoter repression, our results exhibited WT1-mediated activation of a full-length, natural promoter. The promoters for *EGR-1* [32] and *IGF-2* [33] have been shown to be activated by WT1 in cell-lines lacking wild-type p53, with the transcriptional effect of WT1 reverting to repression in the presence of wild-type p53. However, our previous studies have shown that the *WT1* sense (repressed) and antisense promoters (activated) are reciprocally regulated in the same cells [21,23], suggesting to us that there are other important differences between the mechanisms of WT1-mediated transcriptional activation and repression.

Quantitative perturbations of WT1 expression have been implicated in the pathogenesis of DDS [34], Frasier syndrome [35] and breast cancer [6], the former on the basis of gene dosage and the latter two by altered levels of WT1 isoforms. It therefore remains imperative to categorise factors influencing WT1 gene expression and also the individual roles of the various WT1 isoforms. In this study, we address both questions by investigating the interaction of isoforms with a WT1 regulatory element, namely the antisense promoter. Although previous reports of promoter regulation by WT1 show that activity is independent of alternative splice I (reviewed in [12]), we demonstrate that transactivation of the antisense promoter is unique to the WT1[+/−] isoform and that WT1 binding to (G+C)-rich consensus sites within the antisense promoter alone does not account for activity. The data presented highlight a potential specific role for the WT1[+/−] isoform and a complex control point in normal WT1 gene expression which may be disrupted in disease.

2. Materials and methods

2.1. Promoter-reporter construction and nucleic acids

Plasmid pEWTY contains a 734 bp DNA fragment spanning positions −647–+87 of the human WT1 antisense promoter [23]. This was produced by polymerase chain reaction (PCR) with primers TM-2 and TM-4 (see Fig. 1A) and cloned into the luciferase reporter plasmid pGL2E (Promega), using standard sub-cloning techniques [36]. The WT1 consensus site mutants pE-180m and pE-544m were amplified with their respective mutated primers (see Table 1), followed by overlapping PCR with TM-2 and TM-4. Lastly, the double mutant pE-dm was cloned by combining mutated components from pE-180m and pE-544m. Vectors expressing murine *WT1* cDNAs encoding each of the four WT1 isoforms [+/+, ±, −/+, −/−] from a CMV promoter were kindly supplied by N. Hastie.

293 cells were maintained as previously described [21]. Transient transfections were performed as previously described [23] except that no internal control plasmid was used. Each experiment was repeated in triplicate at least three times. Extracts were prepared and assayed for luciferase activity according to the manufacturer's protocols (Promega).

2.2. Western analysis

Total cellular protein was isolated from transfected 293 cells and analysed by Western blotting as described previously [21].

2.3. Preparation of recombinant WT1 fusion protein and nuclear extracts

WT1 zinc finger-GST fusion protein expression vectors pGEXREV, pGEX-KTS (kind gifts of A. Ward) and pGEXS+KTS (kind gift of M. Little) each contain a 460 bp *Bam*HI fragment encoding residues 313–449 of human *WT1* cDNA. pGEXREV contains the cDNA in reverse orientation. Each was used to transform *Escherichia coli* HB101 from which clonal cultures were grown and then induced with 1 mM IPTG for 4 h. Cells were harvested, washed and protein was isolated using glutathione agarose beads (Sigma) according to the manufacturer's protocol. Nuclear extracts were prepared from confluent monolayers of 293 cells as described by Dent and Latchman [37].

2.4. Electromobility shift assay (EMSA)

Oligonucleotides were annealed in 1 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, by heating to 80°C and then cooling slowly. 1.75 pmol double strand oligonucleotide was end-labelled using 0.37 MBq [γ-^{32}P]ATP (3000 Ci/mmol, NEN ^{32}P) and T4 polynucleotide kinase (Immunogen International) after which the probe was diluted to 0.175 pmol/μl with TE and then stored at −20°C. Fusion protein quantified by a Bio-Rad protein assay was mixed with 1μg poly-dI/poly-dC in 20 μl total volume of buffer P (1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris, pH 7.5, 4% glycerol) and pre-incubated on ice for 15 min. After addition of 1 μl diluted probe, incubation continued for 20–30 min. Reactions were loaded onto a 5% non-denaturing polyacrylamide gel (80:1 acrylamide:bis-acrylamide, 0.5×TBE) and run at 100 V for 2 h. Gels were dried and autoradiographed with Hyperfilm MP (Amersham International).

3. Results

3.1. Transactivation of the antisense promoter is specific for the WT1[+/−] isoform

Our previous studies demonstrating WT1-mediated transactivation of the antisense promoter utilised a 293 cell-line derivative stably transfected with a human WT1[+/−] expressing construct [23]. In this study, we assessed the efficacy of transactivation by other WT1 isoforms by co-transfection of the antisense promoter with mouse WT1 isoforms constitutively expressed by a CMV promoter. The native promoter plasmid employed, pEWTY, is depicted in Fig. 1A and included 87 bp downstream of the previously determined transcriptional start site to ensure that transactivation was not a consequence of deleting cryptic binding sites 3′ of the transcriptional start site.

In marked contrast to the *WT1* sense promoter which is auto-repressed by all WT1 isoforms [22], transient co-transfection assays in 293 cells revealed that the [+/−] isoform transactivated the antisense promoter to a level of approximately 3.5-fold over controls. At similar levels of expression (shown in Fig. 1C), three of the four WT1 isoforms, [−/−], [−/+] and [+/+], had no significant effect on the antisense promoter above control levels found in the absence of WT1 (Fig. 1B). The level of transactivation of the antisense promoter is quantitatively similar to that previously demonstrated for 293 derivatives stably expressing exogenous human WT1[+/−] [23], indicating that the specificity of transactivation is not a consequence of species variation or choice of

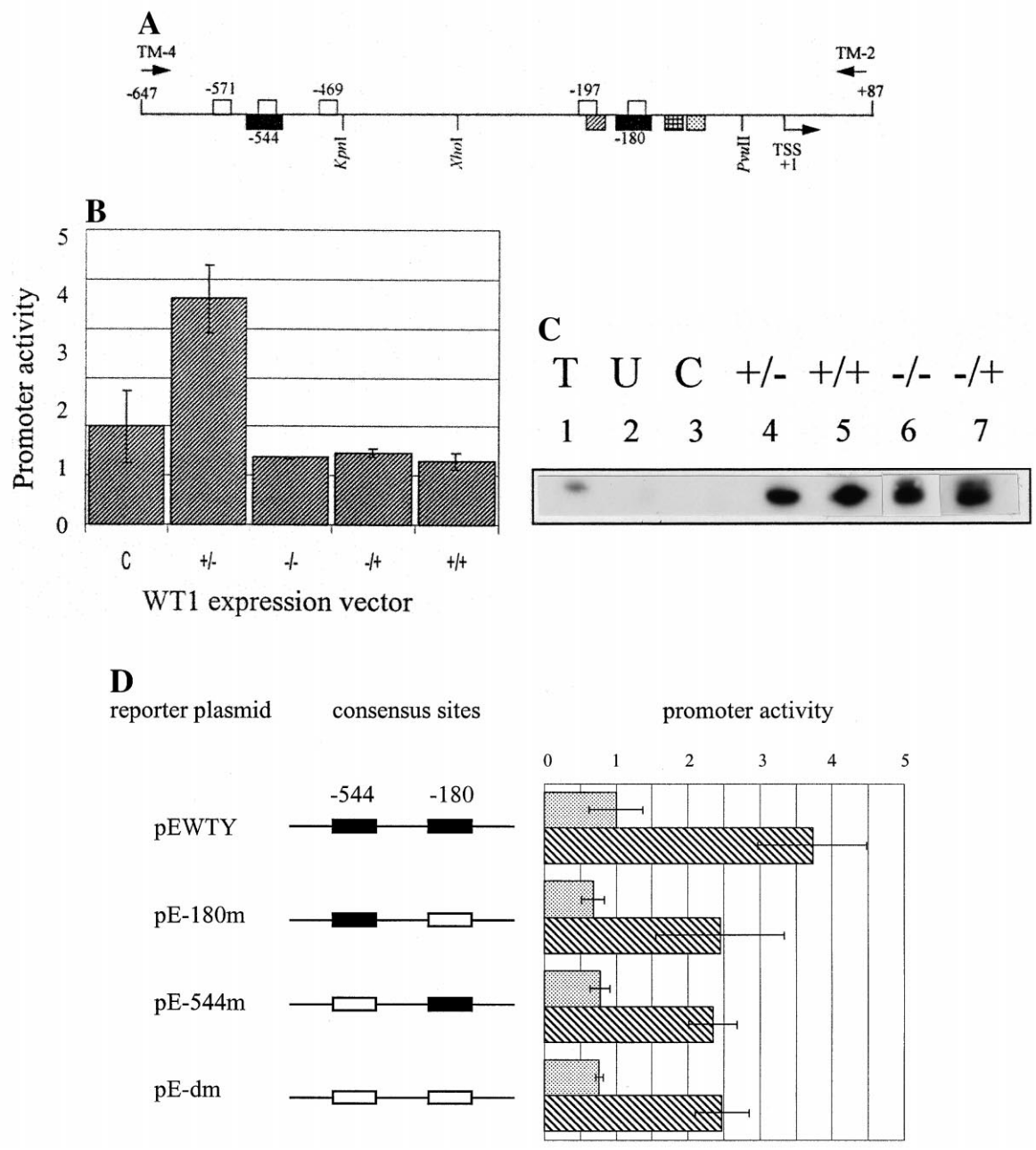

Fig. 1. Activation of the WT1 antisense promoter by WT1 isoforms. (A) Schematic representation of the WT1 antisense promoter in pEWTY. Putative binding sites for WT1 (closed box), Sp1 (open box), Ap1 (hatched box), Ap2 (cross-hatched box) and Pax-2 (stippled box) are shown. Restriction sites, the transcription start site (TSS) and primers TM-4 and TM-2, used for amplifying the fragment, are indicated. Numbers are relative to the TSS (+1). (B) Luciferase activities of antisense promoter/luciferase reporter plasmids co-transfected with each WT1 isoform. 293 cells were co-transfected with pEWTY and the expression vectors: WT1[+/+], WT1[+/−], WT1[−/+], WT1[−/−] or an empty RcCMV cassette as control (C). The luciferase values obtained are expressed as a proportion of the activity found using RcCMV. Results are mean ± S.E.M. of at least three experiments performed in triplicate. (C) Western analysis of WT1 isoforms proteins from 293 cells transfected with each WT1 expression vector (±, +/+, −/+ and −/−) or empty cassette as control (C) were analysed by Western blotting, probed with anti-WT1 (C-19) antibody. Untransfected cells (U) and T5A1 cells constitutively expressing WT1[+/−] from an uninduced metallothionein promoter (T) are shown as negative and positive controls, respectively. (D) Luciferase activities of mutated antisense promoter/luciferase reporter plasmids co-transfected with WT1[+/−]. Mutated derivatives of pEWTY, pE-180m, pE-544m and pE-dm were constructed as described in Section 2. The scheme indicates whether the consensus sites at −544 and −180 are wild-type (closed box) or mutant (open box) in each plasmid. Luciferase activities are shown for each plasmid co-transfected into 293 cells with WT1 isoform expression plasmid WT1[+/−] (hatched bars) or empty cassette as control, RcCMV (stippled bars). Luciferase values obtained were expressed as a proportion of the activity found using pEWTY and RcCMV. Results are mean ± S.E.M. of at least three experiments performed in triplicate.

expression vector. Amongst the plethora of transfection studies of transcriptional regulation by WT1, this is the first report of regulation by a single specific isoform. The necessity for WT1[−KTS] suggested that binding to classical EGR-type consensus sequences was, as expected, a key determinant for transactivation. More surprisingly, however, alternatively spliced exon 5, previously shown to be essential for repression of the *PDGF-A* promoter [38], is obligatory for transactivation of the antisense promoter.

3.2. DNA binding requirements for transactivation

Our transfection studies show a precise requirement for splice I inclusion and splice II exclusion for WT1 antisense promoter transactivation. Splice II is located in the DNA binding zinc finger domain of WT1, so we therefore assessed the effect of mutating two WT1 consensus binding sites within the promoter [23] and the binding of WT1 zinc finger fusion proteins to these sites.

Derivatives of the full-length antisense promoter-reporter plasmid pEWTY were constructed by PCR-based mutagenesis. Mutations were generated in either one or both of the WT1 sites while retaining putative Sp1 core binding sites (GGGCGG at −180 and GGGCCG at −544). Sequences of each WT1 site and its mutated derivatives were confirmed by DNA sequencing and are displayed in Table 1. We co-transfected 293 cells with pCMV-WT1[+/−] or RcCMV control plasmid and antisense reporter constructs carrying either wild-type (pEWTY) or mutated WT1 sites (pE-180m, pE-544m and pE-dm, representing mutations at sites −180, −544 and both sites, respectively).

Table 1
Sequences of wild-type and mutated WT1 sites in the antisense promoter

Location	Allele	Sequence
−544	wt	5′-GCTTCGCGG**GGGCC**GGGTGCTC
−544	m	5′-GCTTCAAAA**GGGCCG**CATGCTC
−180	wt	5′-AAGAGAG**GGGCGG**GCATCGG
−180	m	5′-AAGAGAAAA**GGGCGG**CAAT

Nucleotide sequences are given showing potential WT1 binding sites (underlined) and Sp1 boxes (bold) in the antisense promoter. Oligonucleotides corresponding to the mutated alleles (m) were used to create reporter plasmids carrying mutations at one or both WT1 sites as described in Section 2. Complementary oligonucleotides were annealed to oligonucleotides of the above sequences to provide wild-type (wt) and mutated (m) probes as described in Section 2.

As shown in Fig. 1D, pEWTY was activated approximately 3.5-fold by WT1 co-transfection. Antisense promoter constructs with mutations in one or both WT1 consensus sites were activated to a similar degree as pEWTY by WT1 (between 3- and 3.7-fold), demonstrating that the WT1 consensus sites at −180 and −544 are not required for transcriptional activation of the antisense promoter. We also performed EMSAs using oligonucleotide probes representing each WT1 consensus and found that neither WT1[−KTS] nor WT1[+KTS] fusion proteins bound the site at −180 (Fig. 2B), confirming

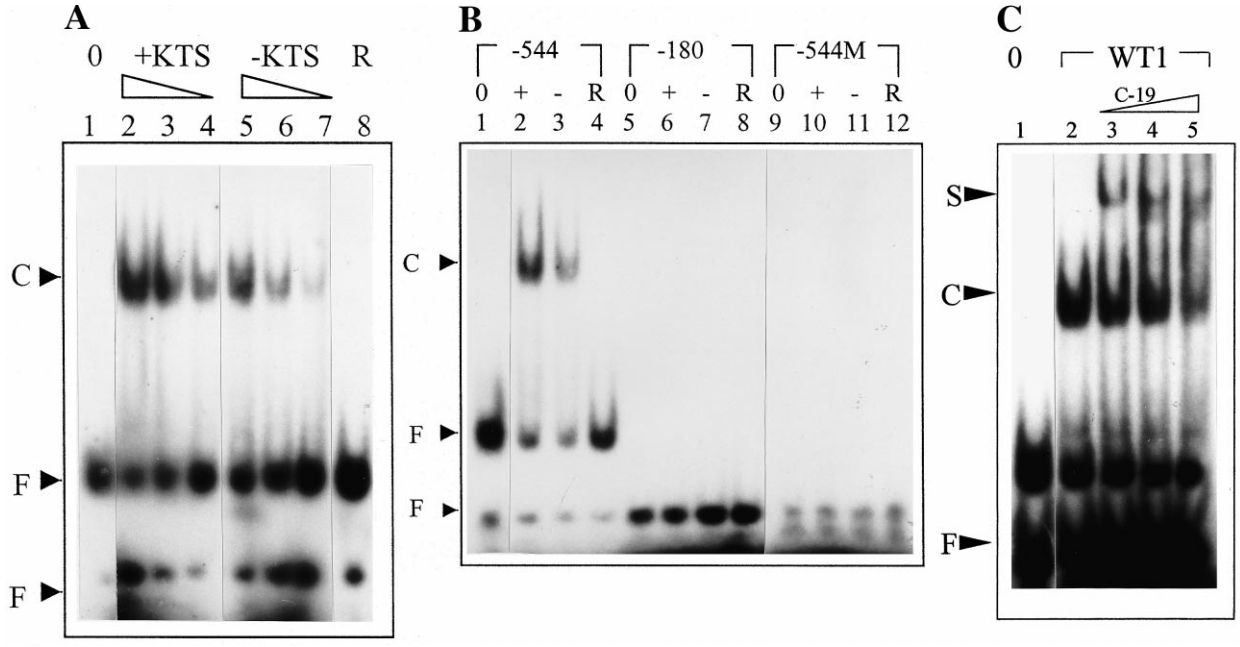

Fig. 2. EMSA of antisense promoter sites −544 and −180 and WT1 zinc finger-GST fusion proteins. (A) Both WTZF[−KTS] and [+KTS] form a complex with site −544. Oligonucleotide probe −544 was labelled with ^{32}P and incubated (as described in Section 2) with WTZF[−KTS] (−), lanes 2–4; WTZF[+KTS] (+), lanes 5–7; no protein (0), lane 1; or reverse expression product (R), lane 8. The triangle represents decreasing concentrations of protein. Lanes 2 and 5, 0.2 μg; lanes 3 and 6, 0.1 μg; lanes 4 and 7, 0.05 μg. F, free probe; C, complex. (B) WTZF does not form a complex with consensus site −180 or a mutated version of site −544. Oligonucleotide probes corresponding to antisense promoter positions −544, −180 and a mutated version of site −544 (sequences shown in Table 1) were used in EMSA. Probes were as follows: lanes 1 and 2, probe −544 (−544); lanes 3–7, probe −180 (−180); lanes 8–10, mutated probe −544 (−544M). Proteins were as follows: lanes 3 and 8, no protein; lanes 2, 7 and 10, 0.7 μg reverse expression product (R); lanes 1 (0.3 μg), 4–6 and 9 (0.7 μg), WTZF[−KTS] (WT1). The triangle represents decreasing protein titres. Lane 4, 0.7 μg; lane 5, 0.5 μg; lane 6, 0.3 μg. F, free probe; C, complex. (C) Antibody to WT1 super-shifts WTZF site −544. EMSA was performed as in A but with a constant amount of WTZF (WT1) (0.3 μg WTZF[−KTS]), lanes 2–5. Lane 1, no protein (0). Increasing titres of antibody are represented by the triangle: lane 3, 0.1 μg; lane 4, 0.5 μg; lane 5, 1.0 μg. F, free probe; C, complex, S, super-shift.

that this is not a target for WT1. Interestingly, site −544 is bound by both fusion proteins in EMSA (Figs. 2A and B) and the mutated site −544 is not (Fig. 2B), even though the corresponding transfection construct, pE-544m, remains transactivatable by WT1[+/−]. This may reflect an uncharacterised context dependency for binding and transactivation.

The reduced mobility band formed in the presence of WTZF-GST is super-shifted when anti-WT1 antibody is included (Fig. 2C), confirming that the reduced mobility of the probe is due to WT1 binding. Although we cannot exclude WT1 binding to as yet uncharacterised sites, our results indicate that the DNA binding capability is clearly not sufficient for transactivation of the antisense promoter. Furthermore, the specific transactivation by WT1[+/−] cannot be attributed solely to differential DNA binding arising from inclusion or omission of splice II, again emphasising the importance of splice I in transactivation of this promoter.

We note that the mutated promoter constructs showed slightly lower basal transcription levels (with RcCMV control plasmid) than pEWTY, but this was not statistically significant ($P > 0.05$, Dunnett's *t*-test). The ratio of expression levels in the presence and absence of WT1 for all four plasmids is, however, similar (between 3.0 and 3.7), showing that the mutated promoter sequences are activated by WT1 to a similar extent as the wild-type promoter.

4. Discussion

The regulation of WT1 protein levels depends upon an increasingly complex series of interactions between a variety of proteins and DNA elements. These include increased expression via a haematopoeitic cell-specific enhancer located 3′ of the gene [39], induction by pax-2 and pax-8 in the developing kidney and urogenital system [40,41] and negative feedback of *WT1* transcription via (G+C)-rich sites in its own promoter [21,22]. These control mechanisms may be further augmented by cellular *WT1* antisense RNAs [24–26] for which we have previously identified a promoter [23] and shown a positive correlation between levels of antisense RNA and WT1 protein expression [27]. Interestingly, although WT1 functions widely as a transcriptional repressor, we found it to activate antisense transcription, indicating that transcriptional regulation by WT1 may be promoter-specific and exerted by more than a single mechanism.

There are few previous reports of a transactivation function for WT1 on natural promoters. Recently, the *SRY* promoter has been shown to be transactivated by WT1[−KTS] with an isoform-specificity attributable to differential DNA binding by [−KTS] and [+KTS] variants to WT1 consensus sites [42]. In contrast to the transactivation of the antisense promoter, the presence or absence of exon 5 at splice I had no effect on transactivation. Similarly, transactivation of the Syndecan-1 promoter is also indiscriminate for WT1 isoforms [43] as are other promoters repressed in *trans* by WT1 (reviewed in [12]). Two further examples of transactivation have been in a cellular background deficient in wild-type p53. In cell-lines lacking wild-type p53, WT1 has been found to activate transcription from promoters with EGR-1 consensus sites and reconstitution of wild-type p53 reduced the level of activation or resulted in repression [32]. Also, the expression of endogenous *IGF-2* in the RM1 Wilms' tumour cell-line, which expresses a mutated *p53* gene, is induced by transfected WT1 [33]. Although we have not investigated the p53 status of 293 cells used in our study, we have previously shown that WT1 represses its own (G+C)-rich promoter in the same cells [21]. This reciprocal regulation of sense and antisense promoters indicates that the differences in their activities cannot be accounted for simply by tissue-specific *trans*-acting factors such as p53, but may also involve the specific *cis*-sequence contexts of the promoters and the assembly of promoter-specific transcriptional complexes.

Previous reports of both promoter repression and activation show a dependence upon binding of WT1 protein to DNA, usually at (G+C)-rich sites. We have found that transactivation of the antisense promoter is independent of two putative binding sites upstream of the transcription start site. The isoform-specificity of antisense promoter transactivation may be attained by interaction of WT1[+/−] with an accessory factor. The N-terminus of WT1 contains poly-proline and glutamine-rich tracts which Madden et al. have demonstrated to repress transcription when fused to a heterologous DNA binding domain [44]. This effect was reported as being much more pronounced in NIH3T3 than 293 cells, implicating interaction of WT1 with other cell-specific factors. Similarly, repression of the *PDGF-A* promoter was released by competition of a DNA binding domain-deleted WT1 protein with the wild-type protein [45], indicating protein-protein interaction. More recently, several WT1 interacting proteins, hubc-9, par-4, ciao-1 and hsp70 [46–49], have been isolated using the yeast two-hybrid system and shown to modulate the transcriptional regulatory abilities of WT1. Although interestingly, some of these bind to the C-terminal zinc finger domain of WT1. These examples add to the abundant evidence to suggest that functions of WT1 can be modulated by interaction with accessory factors. Our results indicate that transactivation of the WT1 antisense promoter is specifically dependent upon the insertion of these 17 amino acids at splice I and we postulate that their function is to allow interaction with other nuclear factors. This would provide a mechanism for cell-specific factors to feed into the complex pathways regulating WT1 levels.

The discovery of isoform-specific functions for WT1, such as we report, is especially pertinent as there is increasing evidence of WT1 isoform imbalance being associated with disease. Aberrant splicing of KTS variants has been reported in Frasier syndrome [35] and WT1 splice variants lacking splice I and/or splice II are increased in breast tumours relative to normal tissue [6]. More recently, a sporadic WT was shown to have a mutation within exon 5, resulting in the production of a tumour-specific WT1 protein with decreased activity relative to the wild-type protein in assays of transcriptional repression and growth inhibition [50]. Further sporadic WTs have also been shown to have altered levels of exon 5 splice forms [51]. Such variations may result in altered expression of downstream WT1 target genes, with pleiotropic consequences for cell growth. Our results further suggest the possibility of a cascade of deregulation arising from inappropriate isoform expression and interaction with WT1 regulatory elements. Clearly, the complete elucidation of WT1 isoform functions will greatly expand our understanding of the developmental processes and diseases in which WT1 may be involved.

Acknowledgements: We wish to thank Nick Hastie for providing the WT1 expression vectors and Andrew Ward and Melissa Little for

providing WT1 zinc finger-GST fusion proteins and helpful advice. The work was supported by the Cancer and Leukaemia in Childhood (CLIC) trust.

References

[1] Hastie, N.D. (1994) Ann. Rev. Genet. 28, 523–558.
[2] Pritchard Jones, K., Fleming, S., Davidson, D., Bickmore, W., Porteous, D., Gosden, C., Bard, J., Buckler, A., Pelletier, J. and Housman, D. (1990) Nature 346, 194–197.
[3] Kreidberg, J.A., Sariola, H., Loring, J.M., Maeda, M., Pelletier, J., Housman, D. and Jaenisch, R. (1993) Cell 74, 679–691.
[4] Phelan, S.A., Lindberg, C. and Call, K.M. (1994) Cell Growth Differ. 5, 677–686.
[5] Sekiya, M., Adachi, M., Hinoda, Y., Imai, K. and Yachi, A. (1994) Blood 83, 1876–1882.
[6] Silberstein, G.B., van Horn, K., Strickland, P., Roberts, C. and Daniel, C.W. (1997) Proc. Natl. Acad. Sci. USA 94, 8132–8137.
[7] Hiltunen, M.O., Koistinaho, J., Alhonen, L., Myohanen, S., Marin, S., Kosma, V.-M., Paakkonen, S. and Janne, J. (1997) Br. J. Cancer 76, 1124–1130.
[8] Call, K.M., Glaser, T., Ito, C.Y., Buckler, A.J., Pelletier, J., Haber, D.A., Rose, E.A., Kral, A., Yeger, H. and Lewis, W.H. (1990) Cell 60, 509–520.
[9] Gessler, M., Poustka, A., Cavenee, W., Neve, R.L., Orkin, S.H. and Bruns, G.A. (1990) Nature 343, 774–778.
[10] Haber, D.A., Sohn, R.L., Buckler, A.J., Pelletier, J., Call, K.M. and Housman, D.E. (1991) Proc. Natl. Acad. Sci. USA 88, 9618–9622.
[11] Rauscher, F.J., Morris, J.F., Tournay, O.E., Cook, D.M. and Curran, T. (1990) Science 250, 1259–1262.
[12] Menke, A.L., Van der Eb, A.J. and Jochemsen, A.G. (1998) Int. Rev. Cytol. 181, 151–212.
[13] Wang, Z.-Y., Qiu, Q.-Q., Enger, K.T. and Deuel, T.F. (1993) Proc. Natl. Acad. Sci. USA 90, 8896–8900.
[14] Caricasole, A., Duarte, A., Larsson, S.H., Hastie, N.D., Little, M., Holmes, G., Todorov, I. and Ward, A. (1996) Proc. Natl. Acad. Sci. USA 93, 7562–7566.
[15] Sharma, P.M., Bowman, M., Madden, S.L., Rauscher, F.J. and Sukumar, S. (1994) Genes Dev. 8, 720–731.
[16] Bruening, W. and Pelletier, J. (1996) J. Biol. Chem. 271, 8646–8654.
[17] Drummond, I.A., Madden, S.L., Rohwer-Nutter, P., Bell, G.I., Sukhatme, V.P. and Rauscher, F.J. (1992) Science 257, 674–678.
[18] Werner, H., Rauscher, F.J., Sukhatme, V.P., Drummond, I.A., Roberts, C.T. and Leroith, D. (1994) J. Biol. Chem. 269, 12577–12582.
[19] Hewitt, S.M., Hamada, S., Mcdonnell, T.J., Rauscher, F.J. and Saunders, G.F. (1995) Cancer Res. 55, 5386–5389.
[20] McCann, S., Sullivan, J., Guerra, J., Arcinas, M. and Boxer, L.M. (1995) J. Biol. Chem. 270, 23785–23789.
[21] Malik, K.T.A., Poirier, V., Ivins, S.M. and Brown, K.W. (1994) FEBS Lett. 349, 75–78.
[22] Rupprecht, H.D., Drummond, I.A., Madden, S.L., Rauscher, F.J. and Sukhatme, V.P. (1994) J. Biol. Chem. 269, 6198–6206.
[23] Malik, K.T.A., Wallace, J.I., Ivins, S.M. and Brown, K.W. (1995) Oncogene 11, 1589–1595.
[24] Huang, A., Campbell, C.E., Bonetta, L., McAndrews Hill, M.S., Chilton MacNeill, S., Coppes, M.J., Law, D.J., Feinberg, A.P., Yeger, H. and Williams, B.R. (1990) Science 250, 991–994.
[25] Eccles, M.R., Grubb, G., Ogawa, O., Szeto, J. and Reeve, A.E. (1994) Oncogene 9, 2059–2063.
[26] Campbell, C.E., Huang, A., Gurney, A.L., Kessler, P.M., Hewitt, J.A. and Williams, B.R.G. (1994) Oncogene 9, 583–595.
[27] Moorwood, K., Charles, A.K., Salpekar, A., Wallace, J.I., Brown, K.W. and Malik, K. (1998) J. Pathol. 185, 352–359.
[28] Krystal, G.W., Armstrong, B.C. and Battey, J.F. (1990) Mol. Cell. Biol. 10, 4180–4191.
[29] Celano, P., Berchtold, C.M., Kizer, D.L., Weeraratna, A., Nelkin, B.D., Baylin, S.B. and Casero Jr., R.A. (1992) J. Biol. Chem. 267, 15092–15096.
[30] Farrell, C.M. and Lukens, L.N. (1995) J. Biol. Chem. 270, 3400–3408.
[31] Savage, M.P. and Fallon, J.F. (1995) Dev. Dyn. 202, 343–353.
[32] Maheswaran, S., Park, S., Bernard, A., Morris, J.F., Rauscher, F.J., Hill, D.E. and Haber, D.A. (1993) Proc. Natl. Acad. Sci. USA 90, 5100–5104.
[33] Nichols, K.E., Re, G.G., Yan, Y.X., Garvin, A.J. and Haber, D.A. (1995) Cancer Res. 55, 4540–4543.
[34] Hastie, N.D. (1992) Hum. Mol. Genet. 1, 293–295.
[35] Barbaux, S., Niaudet, P., Gubler, M., Grunfeld, J., Jaubert, F., Kuttenn, F., Fekete, C.N., Souleyreau-Therville, N., Thibaud, E., Fellous, M. and McElreavey, K. (1997) Nat. Genet. 17, 467–470.
[36] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Cold Spring Harbor Laboratory Press, New York.
[37] Dent, C.L. and Latchman, D.S. (1993) in: Transcription Factors (Latchman, D.S., Ed.), p. 11, IRL at OUP, Oxford.
[38] Wang, Z.Y., Qiu, Q.Q., Huang, J., Gurrieri, M. and Deuel, T.F. (1995) Oncogene 10, 415–422.
[39] Fraizer, G.C., Wu, Y.J., Hewitt, S.M., Maity, T., Ton, C.C.T., Huff, V. and Saunders, G.F. (1994) J. Biol. Chem. 269, 8892–8900.
[40] Dehbi, M., Ghahremani, M., Lechner, M., Dressler, G. and Pelletier, J. (1996) Oncogene 13, 447–453.
[41] Dehbi, M. and Pelletier, J. (1996) EMBO J. 15, 4297–4306.
[42] Shimamura, R., Fraizer, G.C., Trapman, J., Lau, Y.C. and Saunders, G.F. (1997) Clin. Cancer Res. 3, 2571–2580.
[43] Cook, D.M., Hinkes, M.T., Bernfield, M. and Rauscher, F.J. (1996) Oncogene 13, 1789–1799.
[44] Madden, S.L., Cook, D.M. and Rauscher, F.J. (1993) Oncogene 8, 1713–1720.
[45] Wang, Z.Y., Qiu, Q.Q., Gurrieri, M., Huang, J. and Deuel, T.F. (1995) Oncogene 10, 1243–1247.
[46] Wang, Z.Y., Qiu, Q.Q., Seufert, W., Taguchi, T., Testa, J.R., Whitmore, A., Callen, D.F., Welsh, D., Shenk, T. and Deuel, T.F. (1996) J. Biol. Chem. 271, 24811–24816.
[47] Johnstone, R.W., See, R.H., Sells, S.F., Wang, J., Muthukkumar, S., Englert, C., Haber, D.A., Licht, J.D., Sugrue, S.P., Roberts, T., Rangnekar, V.M. and Shi, Y. (1996) Mol. Cell. Biol. 16, 6945–6956.
[48] Johnstone, R.W., Wang, J., Tommerup, N., Vissing, H., Roberts, T. and Shi, Y. (1998) J. Biol. Chem. 273, 10880–10887.
[49] Maheswaran, S., Englert, C., Zheng, G., Sean Bong Lee Wong, J., Harkin, D.P., Bean, J., Ezzell, R., Garvin, A.J., McCluskey, R.T., Decaprio, J.A. and Haber, D.A. (1998) Genes Dev. 12, 1108–1120.
[50] Guan, L., Liu, J., Xu, Y. and Wang, Z. (1998) Cancer Res. 58, 4180–4184.
[51] Liu, J.-J., Qang, Z.-Y., Deuel, T.F. and Xu, Y.-H. (1999) Biochem. Biophys. Res. Commun. 245, 197–199.