Communication

The Wilms' Tumor Gene Product, WT1, Represses Transcription of the Platelet-derived Growth Factor A-chain Gene*

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The Wilms' tumor locus on chromosome 11p13 contains a tumor suppressor gene, \textit{wtt}, which encodes a DNA binding protein (WT1) with four zinc fingers and a glutamine-proline-rich N-terminus and which functions as a repressor of transcription. The platelet-derived growth factor (PDGF) A-chain gene encodes a potent growth factor, which is expressed in high levels in a number of tumor cell lines. We initiated a search for WT1 target genes and now report that WT1 strikingly represses transcription of the PDGF A-chain gene in transient transfection assays and that the WT1 protein interacts directly with a highly G+C-rich region of the PDGF A-chain promoter in gel mobility shift assays. The results suggest that WT1 may function to repress expression of the PDGF A-chain gene and that loss of this or related repressor activities may contribute to the abnormal growth of Wilms' tumors.

Wilms' tumor (WT) is an embryonal cell malignancy of kidney, which occurs both in familial and in sporadic forms (1). The association of Wilms' tumor with aniridia, mental retardation, and urogenital abnormalities (the WAGR syndrome) (2, 3) allowed mapping of a potential tumor suppressor locus on chromosome 11p13 (4). The Wilms' tumor locus contains a tumor suppressor gene, \textit{wtt}, which encodes a protein with four zinc finger domains and a characteristic glutamine- and proline-rich N-terminus. These structural motifs are associated with sequence-specific DNA binding transcriptional regulation and respectively (4, 5). The WT1 protein recognizes DNA sequences containing the core element, 5'-CGCGGGGCCG-3' (6), a sequence similar to the consensus sequence recognized by the **-1 gene product, EGR-1, also called NGFI-A, Zif-268, Krox-24, and TIS8 (8) (reviewed in Ref. 9). The WT1 protein functions as a transcriptional repressor when bound to the EGR-1 consensus sequence (7).

The PDGF A-chain gene encodes a potent mitogen and chemoattractant for cells of mesenchymal origins. It is selectively expressed in high levels in a number of transformed cell lines and may function to promote the growth of transformed cells (reviewed in Ref. 10). In order to pursue mechanisms by which expression of the PDGF A-chain gene may be regulated and a possible role of the PDGF A-chain in transformed cell growth, we isolated, sequenced, and analyzed the human PDGF A-chain gene promoter region (11). A DNA sequence upstream of the TATA box is extremely G+C-rich and is required for efficient expression of the PDGF A-chain gene (12). We now show that the \textit{wtt} gene product binds directly to this highly G+C-rich region and that expression of the PDGF A-chain gene is dramatically reduced by co-expression of the \textit{wtt} gene.

**MATERIALS AND METHODS**

\textbf{Plasmid Constructions—} Restriction endonucleases and DNA ligases were obtained from Bethesda Research Laboratories. The expression vectors used contained the full-length protein coding regions of the human \textit{wtt} or murine **-1 genes, the cytomegalovirus immediate-early promoter, and the SV-40 polyadenylation signal (7). The reporter vector 840PDGF contains the DNA sequences -446 to +388 relative to the transcription start site of the PDGF A-chain gene promoter fused upstream of Basic CAT (Promega) (Fig. 1). Clone \textit{A34PDGF} contains a 34-bp deletion from -34 to -72 (Fig. 1) and was prepared as described elsewhere (12).

\textbf{Cell Cultures, DNA Transfections, and CAT Assays—} NIH3T3 fibroblasts or human embryonic kidney 293 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum. The cells were plated at a density of 1 × 10⁵ cells/100-mm dish 24 h prior to transfection and then transfected by calcium phosphate co-precipitation (13) with 840PDGF (2.5 mg), \textit{A34PDGF} (2.5 mg), or expression plasmids (5 or 15 mg) and a **-galactosidase expression plasmid (1 mg) to establish transfection efficiency. The expression vector added in each transfection was kept constant by addition of CMV vector alone to a total of 20 mg. Forty-eight hours after transfection, cell extracts were prepared, aliquots were normalized for transfection efficiency by assaying **-galactosidase activity (14), and CAT activity was determined (15).

\textbf{Gel Retardation Assays—} Either 20 or 100 ng of the purified EGR-1, WTT, or SP1 proteins were combined with binding buffer (10 ng Tris-HCl (pH 7.9), 60 mg MgCl₂, 1 mm dihydrotestosterone, 1 mm EDTA, 5% glycerol, 5% sucrose, 1 mm phenylmethylsulfonyl fluoride) and 2 μg of poly(dI-dC) (Boehringer Mannheim). After 5 min at room temperature, 1 μl of 32P-labeled oligonucleotide probe was added, the incubation was continued for 15 min, loading buffer was added, and DNA-protein complexes were separated by denaturing, 5% acrylamide gels by electrophoresis at 100 V for 3-4 h. The running buffer consisted of 45 mM Tris borate (pH 8.3), 45 mM boric acid, and 1 mM EDTA. The gels were dried and analyzed by autoradiography. The oligonucleotides of the PDGF A-chain promoter and their competitors are illustrated in the legend to Fig. 3. The oligonucleotides were labeled by filling the recessed 3' end with avian myeloblastosis virus reverse transcriptase and [α-32P]dCTP (Du Pont-New England Nuclear); specific activities ranged from 0.2 to 1.0 × 10⁶ cpm/pmol DNA. Unlabeled competitor DNAs, when appropriate, were added at the same time as the labeled DNAs.

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1 The abbreviations used are: PDGF, platelet-derived growth factor; bp, base pair(s); CMV, cytomegalovirus; CAT, chloramphenicol acetyltransferase.
RESULTS

The human PDGF A-chain gene promoter contains a highly G+C-rich sequence immediately 5' to the TATA box (Fig. 1B). This sequence includes a 13-bp oligo(dG):oligo(dC) motif and three continuous SP1 binding sites, forming a large "GC box." The GC box also contains two uninterrupted sequences and three continuous SP1 binding sites, forming a large "GC box." The GC box also contains two uninterrupted sequences (Fig. 1B, underlined), which are recognized by the utl1 gene product (6), sequences which also are similar to consensus sequences recognized by the EGR-1 protein (7) (Fig. 1). The mutant Δ34PDGF CAT, which retains 10 of the 13-bp dG:dC motif, one Sp1 binding site (5'-GGGCAG-3'), and no obvious core consensus for WT1 binding (Fig. 1), retains less than 20% of the promoter activity of the PDGF A-chain gene (Fig. 2).

To determine if the WT1 and EGR-1 regulated transcription of the PDGF A-chain gene, expression vectors (Fig. 1A), which contained the full-length coding regions of utl1 (CMV-WT1) and egr-1 (CMV-EGR-1) were co-transfected with 840 PDGF (wild type) CAT or with Δ34PDGF CAT reporter plasmids. CAT activity was then determined in lysates of NIH3T3 fibroblasts co-transfected with 840PDGF CAT plus CMV-EGR-1 or 840PDGF CAT plus CMV-WT1.

Co-transfection of the WT1 expression vector with the intact PDGF A-chain promoter repressed expression of the reporter CAT activity to very low levels whereas the egr-1 gene product reduced reporter CAT activity only slightly (Fig. 2A). A CMV vector containing only the zinc finger region of WT1 (CMV-WTZF) did not repress transcription of 840PDGF CAT (Fig. 2A), consistent with earlier observations that the N-terminal glutamine-proline-rich repression domain is required for this activity (7). Thus, the structural requirements for repression of PDGF A-chain gene by WT1 are similar to what has been previously described (7).

That is, an intact zinc finger domain and N-terminal repression domain of WT1 are required as well as binding sites for WT1 in the promoter region of the target gene. The WT1 gene product does not act as a generalized repressor of all promoters since reporter plasmids lacking WT1 binding sites or containing mutated forms of the WT1 site are not repressed by WT1 (7, 18). Basal activity of Δ34PDGF CAT which lacks two Sp1 sites but which retains G+C-rich residues was ~20% as active as 840 PDGF CAT. Expression of the Δ34PDGF CAT construct was also substantially inhibited by co-expression of the WT1 protein, despite the lack of an obvious core consensus site for WT1. However, the continued ability of WT1 to repress the Δ34PDGF CAT construct can be explained by the observation that the WT1 protein still recognizes a truncated version of the GC box present in Δ34PDGF CAT (see Fig. 3C) but not when the remaining sequences within the GC box are deleted (data not shown).

The influence of the CMV-WT1 and CMV-EGR-1 genes on the expression of 840PDGF CAT was also tested by cotransfection at increasing concentrations of CMV-WT1 and CMV-EGR-1 expression vectors. Even at the lowest concentration of the WT1 expression vector used, 840 PDGF CAT activity was substantially repressed in both NIH3T3 cells and human embryonic kidney-derived 293 cells (Fig. 2B, Table I). Interestingly, co-transfection of egr-1 expression vector with the 840 PDGF CAT plasmid consistently resulted in a small but positive increment of expression of CAT in 293 cells, while in NIH3T3 cells EGR-1 consistently demonstrated a repressor effect on 840PDGF-CAT expression (Table I). This result suggests that the influence of EGR-1 on the PDGF A-chain promoter is cell type-specific and that EGR-1 has the

FIG. 1. Schematic representation of the plasmids used in transient transfection assays. A, expression vectors for use in transient transfection assays contained the full-length protein or zinc finger domain coding regions of human utl1 or murine egr-1, the cytomegalovirus immediate-early promoter, and SV40 polyadenylation signals. B, the reporter vector of PDGF A-chain gene promoter. The locations of the WT1 binding sites and deleted sequence are also shown.

FIG. 2. Regulation of transcription from the PDGF A-chain CAT reporter plasmids by EGR-1 and WT1. A, calcium phosphate-mediated transfections were performed in murine NIH3T3 fibroblasts and 293 cells. Each dish of cells was transfected with 840 PDGF (2.5 μg) or Δ34 PDGF (2.5 μg), the indicated expression plasmid (5 μg), and a β-galactosidase expression vector (1 μg) as an internal control for transfection efficiency. The total amount of CMV vector in each transfection mixture was kept constant at 20 μg by addition of CMV vector alone. Forty-eight hours after transfection, cell extracts were prepared and aliquots normalized for transfection efficiency via assay of β-galactosidase activity were used for determination of CAT activity. After autoradiographic exposure, the thin layer chromatography plates were scanned and percent conversion values were calculated.
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Activity of the PDGF A-chain CAT chimeric gene co-transfected with different expression plasmids

The activity of the PDGF CAT plasmid (840PDGF) alone was set equal to 1.0, and the activity in different co-transfections was expressed as a percentage with respect to the 840PDGF.

|          | 840-PDGF (2.5 µg) | EGR-1 (5 µg/15 µg) | WT1 (5 µg/15 µg) | WTZF (15 µg) |
|----------|------------------|-------------------|-----------------|-------------|
| 3T3      | 1.0              | 0.77/0.46         | 0.11/0.08       | 0.87        |
| 293      | 1.0              | 1.5/1.9           | 0.24/0.31       | 1.25        |

To further characterize the interaction of the WT1 protein with the GC box of the PDGF A-chain promoter, a 30-bp oligonucleotide (5'−GGGGCGGGGCGGGGCGGCGGGG−3') containing the GC box was synthesized, end-labeled, incubated with purified WT1, SP1, and EGR-1 proteins, and analyzed in gel mobility shift assays (Fig. 3A). A single distinct protein-DNA complex was identified when purified WT1 protein was mixed with the end-labeled probe (Fig. 3A). In a control experiment, a mutated WT1 protein, WT-ZF(delf3), which lacks the third zinc finger domain, did not bind to the GC box (Fig. 3A). EGR-1 protein DNA complexes also were observed.

To test the specificity of WT1/GC box interactions, competition binding assays were done with unlabeled DNA fragments, including the unlabeled probe itself, a fragment with a typical consensus Sp1 binding site (5'−GGGGCGG−3'), and a fragment with a typical consensus EGR-1 binding site (5'−GGGGGCGGG−3'). The unlabeled homologous probe and the EGR-1 consensus sequence were effective competitors with the WT1 protein/GC box interaction whereas the Sp1 sequence did not compete (Fig. 3B). Since WT1 repressed the activity of the ΔA4PDGF CAT plasmid (Fig. 2), we synthesized the oligonucleotide 5'−AGCTTCCGCGGGAGGGCGGGCGGGCGGGGCGGGAC−3', which contains the mutated GC box, and performed gel shift assays (Fig. 3C). Importantly, the WT1 and EGR-1 proteins bound to this sequence to a similar extent as the wild-type GC box used in Fig. 3A. These results suggest that WT1 and EGR-1 recognize the DNA sequence 5'−GGGGGCGGG−3' as well as the canonical core consensus 5'−GGGGGCGGG−3'. Thus, both EGR-1 and WT1 proteins interact with the GC box of the PDGF A-chain promoter in a sequence-specific manner.

**DISCUSSION**

The wt1 gene is a potential tumor suppressor gene whose homozygous inactivation is associated with development of Wilms' tumor (3, 4). The wt1 gene encodes a protein with four zinc fingers and other domains characteristic of transcription factors (4). However, both the normal function(s) of WT1 and the loss of function associated with development of embryonal cell tumors are unknown. The PDGF A-chain gene encodes a potent mitogen that is expressed in very high levels in a number of transformed cells and is highly temporally and spatially regulated during development. The mechanisms which regulate transcription of the PDGF A-chain gene are only beginning to be understood, and its in vivo functions remain to be fully established.

The data presented here strongly suggest that the WT1 protein binds to the GC box of the PDGF A-chain gene and functions as a potent repressor of expression of the PDGF A-chain gene in transient transfection assays. The EGR-1 protein also recognizes the GC box of the PDGF A-chain gene and functions as both an activator or repressor of transcription depending on the cell line used for assays. These results thus suggest that regulation of function of the PDGF A-chain gene promoter is highly complex and that WT1 is one of the transcription factors that has the potential to strikingly repress its expression in vivo. Since the wt1 gene product functions as a potent repressor of transcription, wt1 expression or its function may be significantly reduced or absent in tumors that contain high levels of PDGF A-chain. One function of tumor suppressor genes may be to directly repress the expression of important growth factor-encoding genes which may act in an autocrine manner during normal development.
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(10, 16, 17). In support of this, we have recently shown that the insulin-like growth factor II gene (IGF-II), a gene which is overexpressed in Wilms' tumors, is also a target for repression by the WT1 protein (18).

It is important to note that the WT1 and EGR-1 proteins are only two of the many transcription factors that may regulate PDGF A-chain gene expression during growth and development. The PDGF A-chain gene is temporally and spatially regulated during growth and development (16). The highly restricted pattern of WT1 expression in developing kidney and urogenital systems (19, 20) suggests that WT1 plays a major role in PDGF A regulation in these organs, yet it also suggests the presence of other negative regulators of PDGF A expression in other tissues and at other times of embryonic development. These factors may be additional WT1-like or EGR-1-like molecules, which recognize the GC box. In this context, it is interesting to note that the EGR-1 protein (which is expressed in many different tissues and cell types (9)) can function as both an activator and repressor of transcription depending on cell type. Identification of additional transcription factors, which negatively regulate PDGF A-chain expression, may lead to the isolation of new tumor suppressor genes which, like WT1, function in a highly tissue-specific manner.

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REFERENCES

1. Matsunaga, E. (1981) Hum. Genet. 57, 231–246
2. Miller, R. W., Fraumeni, J. F., and Manning, M. D. (1964) N. Engl. J. Med. 270, 925–927
3. Riccardi, V. M., Sujaansky, E., Smith, A. C., and Franke, U. (1978) Pediatrics 61, 804–810
4. Call, M. M., Glaser, T., Ito, C. Y., Buckler, A. J., Pelletier, J., Haber, D. A., Rose, E. A., Kral, A., Veger, H., Lewis, W. H., Jones, C., and Housman, P. E. (1990) Cell 60, 509–520
5. Geisler, M., Poustka, A., Caveness, W., Neve, R. L., Ohlin, S. H., and Bruns, G. A. P. (1990) Nature 343, 774–778
6. Rauscher, F. J., III, Morris, J. F., Tournay, O. E., Cook, D. M., and Curran, T. (1990) Science 250, 1257–1262
7. Madden, S. L., Cook, D. M., Morris, J. F., Gaszer, A., Sukhatme, V. P., and Rauscher, F. J., III (1991) Science 253, 1550–1553
8. Herschman, H. R. (1989) Trends Biochem. Sci. 14, 445
9. Sukhatme, V. P. (1990) J. Am. Soc. Nephrol. 1, 859–866
10. Deuel, T. F. (1987) Annu. Rev. Cell Biol. 3, 443–492
11. Takimoto, Y., Wang, Z. Y., Kohler, K., and Deuel, T. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1885–1890
12. Wang, Z., Lin, X.-H., Qiu, Q.-Q., and Deuel, T. F. (1992) J. Biol. Chem. 267, 17022–17031
13. Graham, F. L., and Van der Eb, A. J. (1973) Virology 52, 456–467
14. Spaete, R. R., and Mocarski, E. S. (1980) J. Virol. 36, 136
15. Gorman, G. M., Moffat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1044–1051
16. Yeh, H.-J., Rutt, K. G., Wang, Y.-X., Parks, W. C., Smider, W. D., and Deuel, T. F. (1991) Cell 64, 209–216
17. Bejsek, B. E., Hoffman, R. M., Lipps, D., Li, D. Y., Mitchell, C. A., Majerus, P. W., and Deuel, T. F. (1992) J. Biol. Chem. 267, 3289–3295
18. Drummond, I. A., Madden, S. L., Bowser-Nutter, P., Bell, G. I., Sukhatme, V. P., and Rauscher, F. J., III (1992) Science 257, 674–678
19. Buckler, A. J., Pelletier, J., Haber, D. A., Glaser, T., and Housman, D. Z. (1991) Mol. Cell. Biol. 11, 1707–1712
20. Pelletier, J., Schalling, M., Buckler, A. J., Rogers, A., Haber, D. A., and Housman, D. (1991) Genes & Dev. 5, 1345–1356
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