American Journal of Pathology, Vol. 140, No. 5, May 1992

Rapid Communication

Expression of the 11p13 Wilms' Tumor Gene, *WT1*, Correlates with Histologic Category of Wilms' Tumor

William L. Gerald, T. Stokes Gramling, Donald A. Sens, and A. Julian Garvin

From the Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina

Homozygous inactivation of* WT1, *a Wilms' tumor gene located on chromosome 11 at p13, is believed to predispose to Wilms' tumor and therefore may be a common occurrence in this cancer. The expression of this gene in primary Wilms' tumors was examined by northern and quantitative RNA slot blot analysis and compared with clinical, histologic, and molecular features of each case. The characteristic 3.2 kb RNA was readily detected in most primary tumors although there was marked variation in the level of* WT1*-specific transcripts. No abnormal-sized RNA products were detected and expression of* WT1 *was not coordinated with that of several other oncofetal genes: N-*myc, *insulinlike growth factor 2 (IGF-2), and c-*myc. *The relative abundance of* WT1*-specific RNA did correlate with the histologic category of Wilms' tumor such that those tumors with heterologous differentiation have, in general, lower relative levels of* WT1 *transcripts than those tumors without heterologous differentiation. These results further establish the heterogeneity of Wilms' tumor with respect to the expression of tumor-associated oncofetal genes and suggest a relationship between* WT1 *expression and cellular differentiation. (Am J Pathol 1992, 140:1031–1037)

Wilms' tumor is a pediatric cancer that resembles and is believed to originate from nephrogenic tissue. Most cases are sporadic and unilateral, however, bilateral and familial cases occur leading to the hypothesis that Wilms' tumor, like retinoblastoma, belongs in the category of cancers that develops on the basis of homozygous loss of function of a tumor suppressor gene.[1,2] However, several lines of evidence suggest that Wilms' tumorigenesis may be more complex. 1) At the genetic level, some patients with familial WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation) show constitutional chromosomal deletions of 11p13 and some sporadic Wilms' tumors show loss of heterozygosity at loci within this same chromosomal band.[3–8] Other sporadic tumors, and patients with Wilms' tumors arising in the context of the Beckwith-Wiedemann syndrome, show loss of allelic heterozygosity at 11p15 not at 11p13.[9–11] In addition, analyses of familial Wilms' tumor show no linkage to either of these loci, suggesting that several genes participate in the development of Wilms' tumors.[12–14] 2) Morphologically, Wilms' tumors are often associated with a proposed precursor lesion consisting of incompletely differentiated nephrogenic tissue (nephrogenic rests), implying that Wilms' tumorigenesis is the result of progression through multiple steps.[15,16] 3) Finally, the prevailing theories of oncogenesis suggest that failure of repression or reactivation of proto-oncogenes and growth factors that play key roles in growth and development allows continued cell proliferation and leads to the subsequent development of malignancy. In the case of Wilms' tumors, the N-*myc* proto-oncogene and IGF-2 are frequently expressed at high levels just as they are in developing kidneys.[17–24] The significance of these events regarding oncogenesis is not established, but the continued expression of oncofetal genes may contribute to tumorigenesis or, on the other hand, it may reflect the normal pattern of expression in the fetal tissues that make up the tumor.

Supported by grants from the United States Public Health Service CA 37887, The Cancer Research Foundation of America and the ACS IN-175-A.

Accepted for publication February 19, 1992.

Address reprint requests to Dr. William L. Gerald, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

The exact molecular events involved and their specific contribution to Wilms' tumorigenesis are unknown, however, the study of individual candidate Wilms' tumor genes will help unravel the series of steps leading to the development of this cancer. A gene that maps to band p13 of human chromosome 11 and is preferentially expressed in the fetal kidney has been isolated and is occasionally deleted in Wilms' tumor samples.[25–27] This gene, designated *WT1,* is proposed to play a key role in kidney development and the genesis of Wilms' tumors. Sequence analysis suggests that the encoded protein product is a zinc-fingered, DNA-binding protein, possibly a transcription factor. Little is known of the regulation of expression of this gene in Wilms' tumors or of its role in various aspects of Wilms' tumor biology. In an effort to gain a better understanding of the relationship between the *WT1* gene and Wilms' tumors, we have explored the expression of this 11p13 Wilms' tumor gene and other oncofetal genes in a series of Wilms' tumors. Our results suggest that *WT1* expression may be related to cell differentiation, and therefore, loss of this gene function may contribute to a specific step in the series of events leading to development of Wilms' tumors.

Materials and Methods

Tissue Samples

Samples of tumor and adjacent normal tissue were obtained from surgical specimens received in the Department of Pathology and Laboratory Medicine at the Medical University of South Carolina and through the National Cancer Institute Cooperative Human Tissue Network. The tissue was snapfrozen and stored at −80°C. In all cases, the diagnosis was confirmed by histologic examination. Tumors were subcategorized into those with differentiated heterologous elements, and those without, by histologic examination of available slides and review of surgical pathology and consultation reports.

RNA Isolation and Analysis

RNA was prepared from frozen tissue by the acid guanidinium, phenol/chloroform method of Chomczynski and Sacchi.[28] RNA was denatured with glyoxal and electrophoresed in 1.2% agarose gels.[29] Northern and slot blot analyses were performed using Zetaprobe nylon membranes (BioRad, Richmond, CA) and alkaline transfer according to the manufacturer's instructions. Probes used in this study were: the 1.8 kb *Eco*RI fragment of *WT33* for *WT1;*[25] the 1 kb *Eco*RI-*Bam*HI fragment of pNB-1 for N-*myc*[30]; the 0.8 kb *Pst*I fragment of phigf2 for IGF-2;[31] the c-*myc* third exon probe from Oncor (Gaithersburg, MD); a 40-mer antisense oligonucleotide probe for 28S ribosomal RNA (Oncogene Science, Manhasset, NY); and a 0.5 kb *Eco*RI fragment for β-actin (a gift from Dr. Ted Gansler, Emory University, Altanta, GA).

Double-stranded DNA fragments used as probes were radiolabeled with ^{32}P-dCTP by the random primer method[32] using a kit from Promega (Madison, WI). Oligonucleotide probes were end-labeled with ^{32}P-ATP using a 5′ end-labeling kit from New England Nuclear (Boston, MA).

Results

Expression of WT1 in Primary Wilms' Tumors

The primary tumors used in this study were unselected and represent a spectrum of Wilms' tumors. Patient demographics and tumor attributes are listed in Table 1. Age ranged from 1 to 10 years. Two of the primary tumors showed anaplasia as defined by the National Wilms' Tumor Study Group and patients presenting in all four stages are represented. None of the patients for whom clinical information is available exhibited features associated with the WAGR, Denys-Drash, or Beckwith-Weidemann syndromes, and only one (case #7) presented with bilateral Wilms' tumors.

Primary Wilms' tumors were evaluated for expression of the 11p13 Wilms' tumor gene, *WT1.* Deletions and loss of allelic heterozygosity in this chromosomal region have been detected in Wilms' tumors, and it might be expected that the structure or function of *WT1* is commonly altered in this malignancy. Ten micrograms of total RNA from primary tumors and adjacent normal kidney were analyzed by Northern blot analysis using ^{32}P-labeled probes. Of the 27 primary Wilms' tumors shown in Figure 1, 19 showed expression of the characteristic 3.2 kb *WT1* RNA by Northern blot analysis. The transcript was not detected, or present at low levels, in total RNA isolated from normal kidney adjacent to tumors. An altered *WT1* RNA species was not detected in any of the tumors. It, therefore, appears that most Wilms' tumors have at least one *WT1* allele that is structurally, grossly intact, and transcriptionally active. This analysis does not, however, rule out the possibility of small mutations within the gene that do not significantly affect transcript size.

An amazingly large variation in the level of *WT1* expression exists among primary Wilms' tumors with approximately a 100-fold difference apparent by slot-blot hybridization of serially diluted total RNA and densitometric analysis (Figure 2). This wide variation in the level of

Table 1. *Features of Primary Wilms' Tumors*

Case	Age	Sex	Stage	Heterologous differentiation
1	2	F	I	Present
2*	3	M	IV	Present
3	4	F	III	Absent
4	5	F	IV	Absent
5	1	F	I	Absent
6	1	F	NK	Absent
7	10	M	NK	Absent
8	3	M	NK	NK
9	3	M	NK	NK
10	NK	NK	NK	NK
11	1	F	NK	Absent
12	5	M	NK	Absent
13	1	M	NK	NK
14	NK	NK	NK	NK
15	NK	NK	NK	NK
16	1	NK	NK	NK
17	NK	NK	NK	Absent
18	NK	NK	NK	Present
19	1	NK	I	NK
20	3	M	III	Present
21	3	NK	I	NK
22	3	F	I	Present
23	3	F	IV	Absent
24	8	NK	NK	Absent
25	NK	NK	NK	NK
26	NK	NK	NK	NK
27	NK	NK	NK	Absent
28	2	M	IV	Present
29	2	F	NK	Present
30	4	F	II	Absent
31	8	F	II	Present
32	2	F	II	NK
33	5	M	III	Absent
34	1	M	NK	NK
35	2	NK	I	Present
36*	4	F	III	Absent
37	4	F	I	Present
38	2	F	I	Absent
39	5	F	I	Present
40	9	NK	NK	NK
41	1	M	NK	Present

* Denotes anaplastic WT.
NK = not known.

WT1 expression among primary Wilms' tumors led to an analysis of clinical, histologic, and molecular features that could possibly correlate with *WT1* expression.

Comparison of Oncofetal Gene Expression and Level of WT1*-specific RNA*

It has previously been noted that Wilms' tumors often exhibit high levels of expression of some oncofetal genes that are normally active in developing kidney. In particular, N-*myc* and IGF-2 transcripts are frequently present and c-*myc* transcripts are occasionally found. The *WT1*-encoded protein sequence is homologous to transcription factors, and the expression of these oncofetal genes may be influenced by the regulatory activity of the *WT1* gene product. To evaluate the relationship between oncofetal genes and the level of expression of *WT1*, Northern analysis was performed with probes for N-*myc*, IGF-2 and c-*myc*, using the same primary tumors analyzed for *WT1* transcripts. From visual inspection of autoradiograms (Figure 1), many Wilms' tumors contained increased levels of N-*myc* mRNA, and all but two showed greatly enhanced expression of IGF-2, as compared with normal kidney. The level of expression for these two genes varied considerably among the primary tumors studied. On the other hand, c-*myc* expression was significantly elevated in just three cases with only one case showing high level expression. The sizes of hybridizing bands are in agreement with published sizes of mRNA corresponding to each gene and no abnormal size transcripts were detected. An obvious relationship between

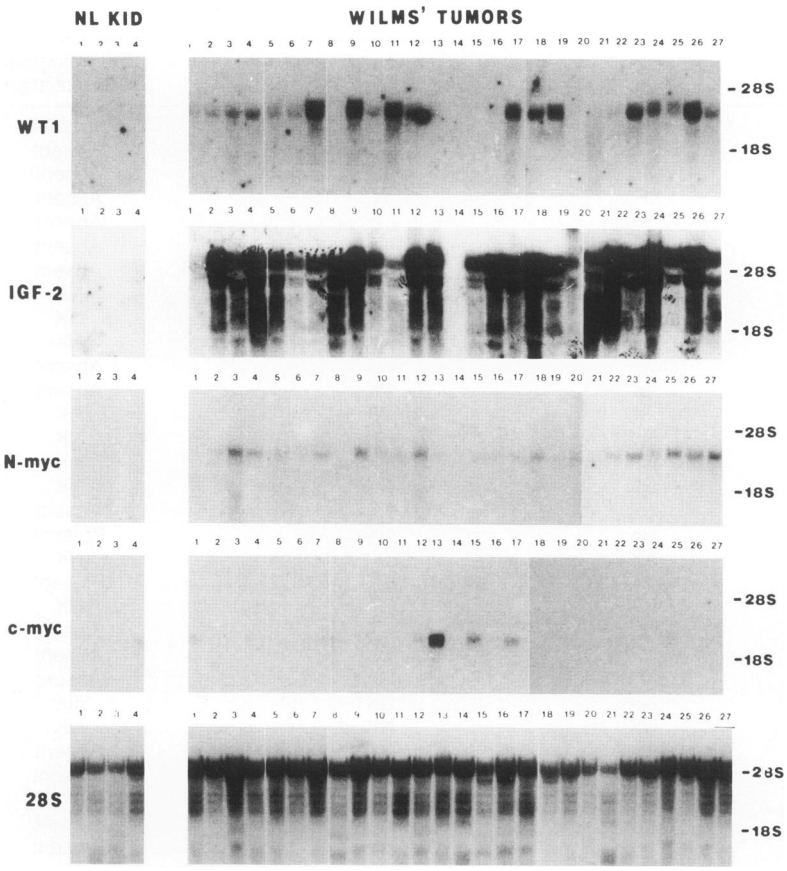

Figure 1. *Northern blot analysis of primary Wilms' tumors and normal kidney. Ten micrograms of total RNA isolated from either normal kidney adjacent to tumors (NL KID 1-5) or Wilms' tumors (1-27) were electrophoresed in each lane and hybridized with ^{32}P-labelled probes for the genes indicated at the left of each panel. The tumors analyzed in this figure are a subset of those studied by quantitative slot blot analysis in Figure 2. Exposure times were: 2 days for* WT1; *overnight for IGF-2; 3 days for N-*myc *and c-*myc; *and 6 hours for 28S ribosomal RNA. Numbers at the top of the figure correspond to the case number in Table 1.*

the steady-state level of transcripts for these genes and the level of *WT1* RNA was not evident by this analysis.

Correlation Between Tumor Differentiation and WT1 *Expression*

Various parameters of each case were examined for correlation with the level of *WT1* expression as determined by slot-blot analysis. No statistically significant relationship was detected beween *WT1* expression and clinical stage of presentation, patient age, or the presence of anaplasia. Previous work, however, had indicated that *WT1* expression is most prominent in specific cell types and may play a role in cell differentiation.[33,41] Based on this observation, we evaluated the association between histologic features of the primary tumors and the level of *WT1* RNA. Beckwith and coworkers have suggested that Wilms' tumors can be divided into two categories based on histologic features.[15] Tumors with heterologous stromal elements (striated muscle, cartilage, and bone) and a prominent stromal component are associated with the WAGR syndrome and the presence of intralobar nephrogenic rests. Those that do not have heterologous elements, and are composed primarily of blastema with epithelial differentiation, are associated with the Beckwith-Wiedemann syndrome and perilobar nephrogenic rests. This might suggest that these two groups represent Wilms' tumors with different biologic characteristics and possibly different mechanisms of tumorigenesis. After review of available slides and surgical pathology reports, 12 of the cases in our study were found to contain heterologous tissue elements and 15 cases did not. Those cases with heterologous elements and stromal predominance showed a lower level of *WT1* RNA than the group of tumors that did not have heterologous elements and were blastema-rich (Figure 3). A significant difference ($P = 0.008$) occurred between the mean level of relative *WT1* RNA for the two groups although exceptions to the general trend existed for both categories.

Discussion

Earlier epidemiologic and statistical analyses suggested that the development of some tumors, such as Wilms' tumor and retinoblastoma, is due to inactivation of both alleles of a single tumor suppressor gene. Recent data strongly support this concept in the case of retinoblastoma, but the events contributing to Wilms' tumorigenesis

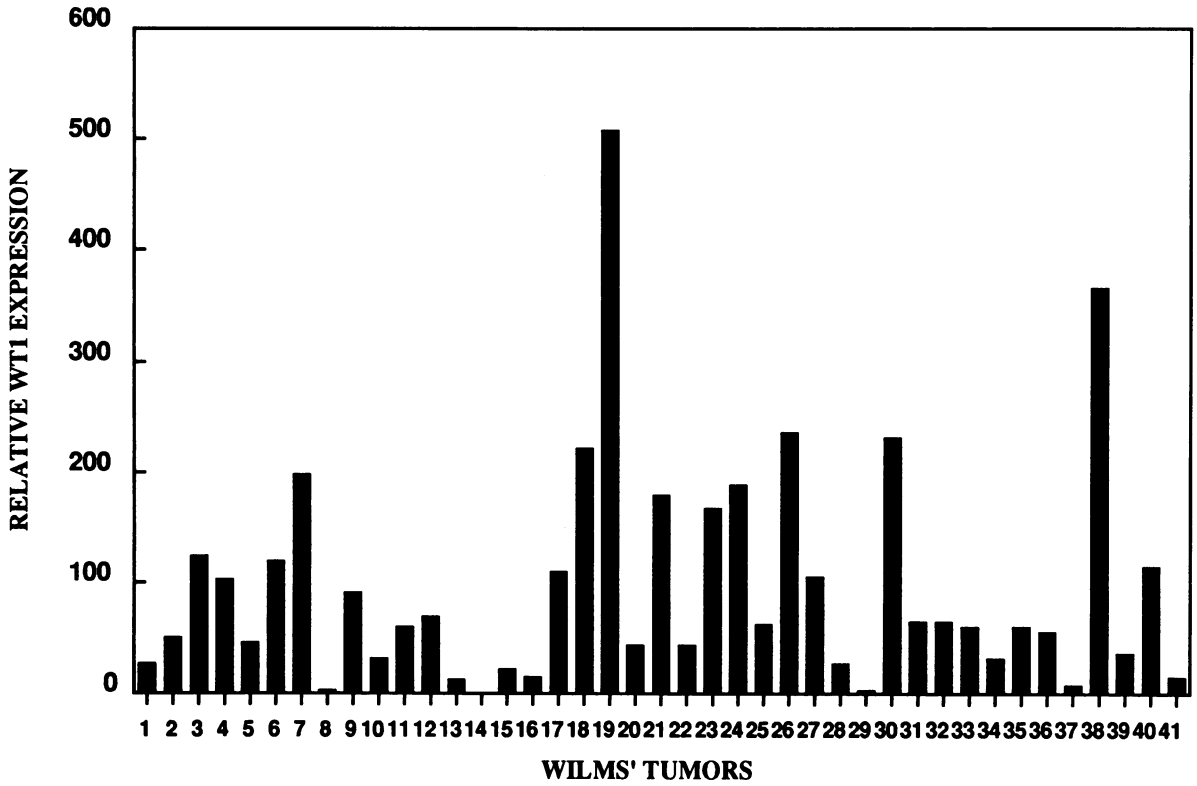

Figure 2. *Bar graph representation of quantitative RNA slot blot analysis. Total RNA was blotted using a slot-blot apparatus and hybridized with ^{32}P-labelled* WT1 *probe. Autoradiograms were subjected to densitometric analysis. Values are expressed as integrated optical density normalized by* β*-actin expression. Numbers at the bottom of the figure correspond to the case number in Table 1.*

appear to be more complex. Two, and possibly more, genetic loci have been implicated based on chromosomal deletion, loss of heterozygosity, and genetic linkage. Benign possible precursor lesions (nephrogenic rests) have been identified suggesting a multistep process of carcinogenesis, and expression of oncofetal and growth factor genes have been detected in most Wilms' tumors examined. The contribution of each of these events to Wilms' tumor biology is not well understood; however, the recent isolation of the 11p13 Wilms' tumor gene, *WT1,* allows investigation of the role of this specific genetic factor.

Our analysis of expression of *WT1* in a spectrum of primary Wilms' tumors demonstrates that grossly normal transcripts are present in most cases, and no abnormal size products were produced in detectable amounts. This implies that at least one structurally intact *WT1* allele is present and transcribed in the majority of Wilms' tumors, although the possibility of mutational events not detected by Northern analysis, but affecting *WT1* protein product function, needs to be excluded.[39,40] A tumorigenic effect due to loss of *WT1* function is tenable for those Wilms' tumors that have low or undetectable levels of *WT1* transcripts, and the rare tumors that have homozygous deletion of *WT1*.[26,34–38]

The complexity of Wilms' tumorigenesis and the extreme variability of *WT1* RNA levels in primary tumors suggests that *WT1* does not necessarily function as a classic tumor suppressor gene but plays an important role in some other aspect of tumor biology. Although the function of *WT1* is still unknown, *in situ* hybridization studies in the developing kidney localize *WT1* transcripts to the condensing blastemal cells, renal vesicle, and maturing renal corpuscle, particularly the podocytes of the glomerular epithelium. *In situ* localization in Wilms' tumors is also restricted to specific cell types, primarily tubular epithelial structures and to a lesser extent blastemal cells, whereas stromal cells are negative.[33,41] These results imply that *WT1* expression is restricted to certain cell types or cells at a specific stage of differentiation. If this is the case, the level of *WT1* expression in neoplasia would be expected to correlate with the relative amount of that specific cell type present. Our results tend to support this concept as does the work of others.[33,35,41] Tumors that exhibit primarily blastemal and epithelial differentiation show higher levels of *WT1* expression than those that are stromal predominant with heterologous differentiation. *WT1* may play an important role at a specific stage of tissue differentiation, and disruption of this event predisposes to development of Wilms' tumors with specific types of cellular components. However, other molecular events, possibly including disruption of other steps in re-

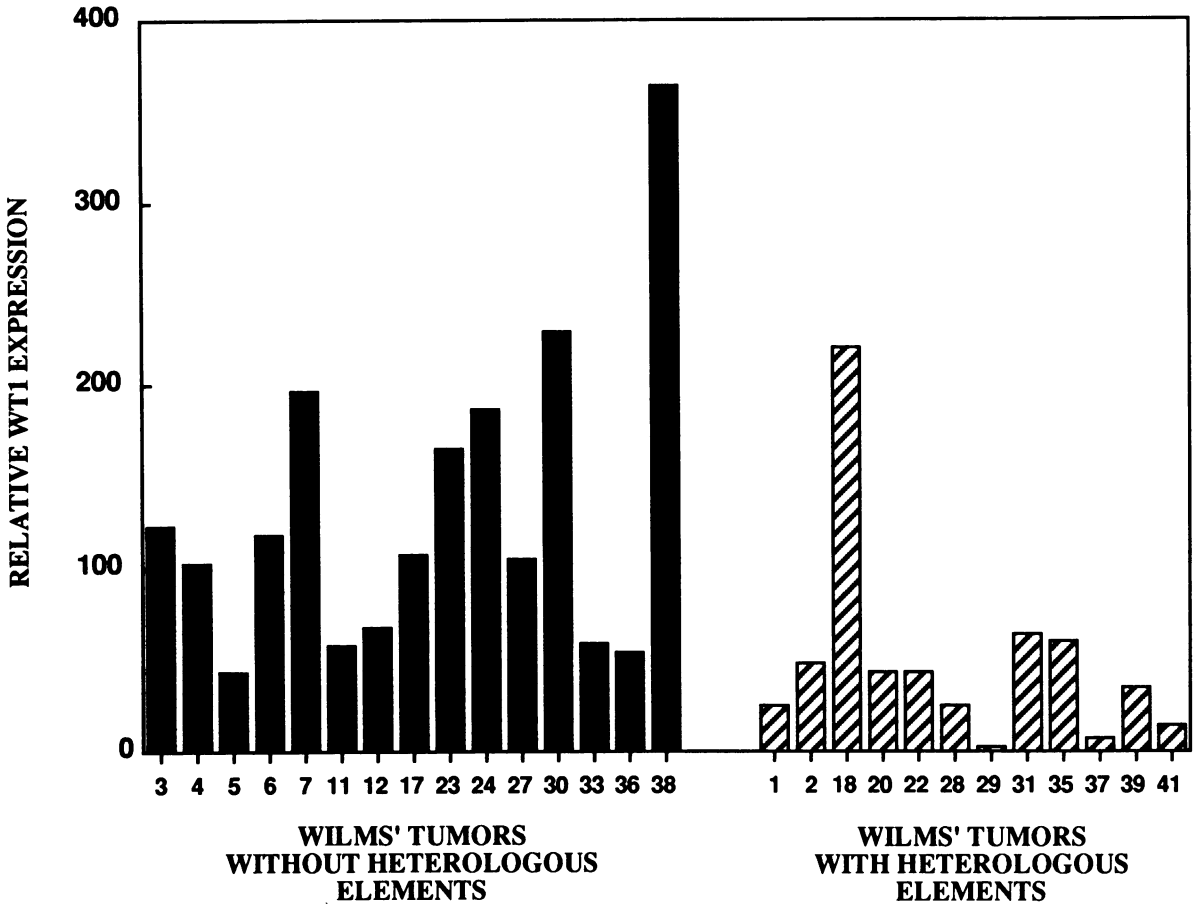

Figure 3. *Comparison of quantitative RNA slot blot analysis for Wilms' tumors with and without heterologous elements hybridized with* WT1 *probe. Values are expressed as integrated optical density normalized by* β*-actin expression. Numbers at the bottom of the figure correspond to the case number in Table 1.*

nal tissue differentiation, may also contribute to the development of Wilms' tumor. In those primary tumors, *WT1* may be expressed as a normal transcript in tumor tissue recapitulating nephrogenic blastema in early stages of differentiation toward epithelial structures.

Acknowledgments

The authors thank Karen Latte, Cynthia Gittinger, and Jim Nicholson for manuscript preparation and figures.

References

1. Knudson AG: Mutation and cancer: a statistical study. Proc Natl Acad Sci 1971, 68:820–823
2. Knudson AG, Strong LC: Mutation and cancer: a model for Wilms' tumor of the kidney. J Natl Cancer Inst 1972, 48:313–324
3. Riccardi VM, Sujansky E, Smith AC, Francke U: Chromosomal imbalance in the aniridia-Wilms' tumor association: 11p interstitial deletion: Pediatrics 1978, 61:604–610
4. Francke U, Holmes LB, Atkins L, Riccardi VM: Aniridia-Wilms' tumor association: evidence for specific deletion of 11p13. Cytogenet Cell Genet 1979, 24:185–192
5. Koufos A, Hansen MF, Lampkin BC, Workman ML, Copeland NG, Jenkins NA, Cavenee WK: Loss of alleles on human chromosome 11 during genesis of Wilms' tumour: Nature 1984, 309:170–172
6. Orkin SH, Goldman DS, Sallan SE: Development of homozygosity for chromosome 11p markers in Wilms' tumour: Nature 1984, 309:172–174
7. Reeve AE, Housiaux PJ, Gardner RJ, Chewing WE, Grindley RM, Millow LJ: Loss of a Harvey ras allele in sporadic Wilms' tumour: Nature 1984, 309:174–176
8. Fearon E, Vogelstein B, Feinberg A: Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumors: Nature 1984, 309:176–178
9. Waziri M, Patil S, Hanson J, Bartley SA: Abnormality of chromosome 11 in patients with features of Beckwith-Wiedemann syndrome: J Pediatr 1983, 102:873–876
10. Turleau C, de Grouchy J, Nihoul-Fekete C, Chavin-Colin F, Junien C: Del 11p13/nephroblastoma without aniridia. Hum Genet 1984, 67:455–456
11. Koufos A, Grundy P, Morgan K, Aleck KA, Hadro T, Lampkin BC, Kalbakji A, Cavenee WK: Familial Wiedemann-Beckwith syndrome and a second Wilms' tumor locus map to 11p15.5: Am J Hum Genet 1989, 44:711–719

12. Grundy P, Koufos A, Morgan K, Li FP, Meadows A, Cavenee WK: Familial predisposition to Wilms' tumor does not map to the short arm of chromosome 11: Nature 1988, 336:374–376
13. Huff V, Compton D, Chao L, Strong L, Geiser C, Saunders G: Lack of linkage of familial Wilms' tumor to chromosomal band 11p13: Nature 1988, 336:377–378
14. Schwartz CE, Haber DA, Stanton VP, Strong LC, Skolnick MH, Houseman DE: Familial predisposition to Wilms tumor does not segregate with WT1 gene: Genomics 1991, 10:927–930
15. Beckwith JB, Kiviat NB, Bonadio JF: Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumor: Ped Pathol 1990, 10:1–36
16. Hennigar RA, Othersen HB, Garvin AJ: Clinicopathologic features of nephroblastomatosis: Urology 1989, 33:259–270
17. Reeve AE, Eccles MR, Wilkins RJ, Bell GI, Millow LJ: Expression of insulin-like growth factor-II transcripts in Wilms' tumour: Nature 1985, 317:258–260
18. Scott J, Cowell J, Robertson ME, Priestley LM, Wadey R, Hopkins B, Pritchard J, Bell GI, Rall LB, Graham CF, Knott TJ: Insulin-like growth factor-II gene expression in Wilms' tumour and embryonic tissues: Nature 1985, 317:260–262
19. Nisen PD, Zimmerman KA, Cotter SV, Gilbert F, Alt FW: Enhanced expression of the N-*myc* gene in Wilms' tumors: Cancer Res 1986, 46:6217–6222
20. Haselbacher GK, Irminger J-C, Zapf J, Ziegler WH, Humbel RE: Insulin-like growth factor II in human adrenal pheochromocytomas and Wilms tumors: expression at the mRNA and protein level: Proc Natl Acad Sci USA 1987, 84:1104–1106
21. Shaw APW, Poirier V, Tyler S, Mott M, Berry J, Maitland NJ: Expression of the N-myc oncogene in Wilms' tumor and related tissues: Oncogene 1988, 3:143–149
22. Hirvonen H, Sandberg M, Kalimo H, Hukkanen V, Vuorio E, Salmi TT, Alitalo K: The N-myc proto-oncogene and IGF-II growth factor mRNAs are expressed by distinct cells in human fetal kidney and brain: J Cell Biol 1989, 108:1093–1104
23. Maitland NJ, Brown KW, Poirier V, Shaw APW, Williams J: Molecular and cellular biology of Wilms' tumor: Anticancer Res 1989, 9:1417–1426
24. Brice AL, Cheetham JE, Bolton VN, Hill NCW, Schofield PN: Temporal changes in the expression of the insulin-like growth factor II gene associated with tissue maturation in the human fetus: Development 1989, 106:543–554
25. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE: Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus: Cell 1990, 60:509–520
26. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP: Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping: Nature 1990, 343:774–778
27. Bonetta L, Kuehn SE, Huang A, Law DJ, Kalikin LM, Koi M, Reeve AE, Brownstein BH, Yeger H, Williams BRG, Feinberg AP: Wilms' tumor locus on 11p13 defined by multiple CpG island-associated transcripts: Science 1990, 250:994–997
28. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Anal Biochem 1987, 162:156–159
29. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd edition. Cold Spring Harbor Laboratory Press, 1989, 7.40–7.42
30. Schwab M, Alitalo K, Klempnauer K-H, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J: Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumor. Nature 1983, 305:245–248
31. Bell GI, Merryweather JP, Sanchez-Pescador R, Stempien MM, Priestley L, Scott J, Rall LB: Sequence of a cDNA clone encoding human preproinsulin-like growth factor II: Nature 1984, 310:775–777
32. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983, 132:6–13
33. Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, Bard J, Buckler A, Pelletier J, Housman D, van Heyningen V, Hastie N: The candidate Wilms' tumour gene is involved in genitourinary development: Nature 1990, 346:194–197
34. Lewis WH, Yeger H, Bonetta L, Chan HSL, Kang J, Junien C, Cowell J, Jones C, Dafoe LA: Homozygous deletion of a DNA marker from chromosome 11p13 in sporadic Wilms tumor. Genomics 1988, 3:25–31
35. Rose EA, Glasser T, Jones C, Smith CL, Lewis WH, Call KM, Minden M, Champagne E, Bonetta L, Yeger H, Housman DE: Complete physical map of the WAGR region of 11p13 localizes a candidate Wilms' tumor gene. Cell 1990, 60:495–508
36. Ton CCT, Huff V, Call KM, Cohn S, Strong LC, Housman DE, Saunders GF: Smallest region of overlap in Wilms tumor deletions uniquely implicates an 11p13 zinc finger gene as the disease locus. Genomics 1991, 10:293–297
37. Huang A, Campbell CE, Bonetta L, McAndrews-Hill MS, Chilton-MacNeill S, Coppes MJ, Law DJ, Feinberg AP, Yeger H, Williams BRG: Tissue, developmental, and tumor-specific expression of divergent transcripts in Wilms tumor. Science 1990, 250:991–994
38. Huff V, Miwa H, Haber DA, Call KM, Housman D, Strong LC, Saunders GF: Evidence for WTI as a Wilms tumor (WT) gene: intragenic germinal deletion in bilateral WT. Am J Hum Genet 1991, 48:997–1003
39. Pelletier J, Bruening W, Li FP, Haber DA, Glaser T, Housman DE: WT1 mutations contribute to abnormal genital system development and hereditary Wilms' tumor. Nature 1991, 353:431–434
40. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegal JE, Houghton DC, Junien C, Habib R, Fouser L, Fine RN, Silverman BL, Haber DA, Housman D: Germline mutations in the Wilms' tumor suppressor gene as associated with abnormal urogenital development in Denys-Drash syndrome. Cell 1991, 67:437–447
41. Pritchard-Jones K, Fleming S: Cell types expressing the Wilms' tumour gene (WT1) in Wilms' tumor: implications for tumour histogenesis: Oncogene 1991, 6:2211–2220