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Immunohistochemical Expression of Wilms' Tumor 1 Protein in Nephroblastoma

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Background. Approximately 10% of nephroblastomas (Wilms' tumors) carry mutations in the Wilms' tumor 1 (WT1) gene. Recently, a WT1 antibody raised against N-terminal 1-181 amino acids of human WT1 became commercially available for immunohistochemical use on paraffin-embedded tissue. The aim of this study was to investigate the diagnostic and prognostic value of WT1 N-terminal antibody in nephroblastomas.
Methods. Twenty-five patients with nephroblastoma were studied. Four clear cell sarcomas of the kidney (CCSK) and 15 neuroblastomas were included for comparative study. WT1 immunostaining was performed on paraffin material using the WT1(6F-H2) antibody. The patients were staged according to the National Wilms' Tumor Study (NWTS) staging system.
Results. Eleven tumors (44%) showed blastemal nuclear staining with or without epithelial nuclear staining. Three of the 13 low-stage tumors (stages I and II) showed WT1 blastemal nuclear staining, while 8 of the 12 high stage (stage III and IV) tumors revealed blastemal nuclear staining. The blastemal nuclear expressions of WT1 were statistically significantly correlated with clinical stage (p = 0.036). All the neuroblastomas and CCSK showed no nuclear immunoreactivity.
Conclusions. The presence of WT1 nuclear immunoreactivity may be helpful to distinguish blastemal predominant nephroblastomas from CCSK and neuroblastomas.

Nephroblastoma [Wilms' tumor (WT)] is one of the most common solid tumors in children. Nephroblastoma is thought to arise from mesenchymal blastema cells that fail to differentiate into metanephric structures but continue to proliferate.[1] The first genetic locus was found in patients with the Wilms-Aniridia genital anomaly-retardation syndrome (WAGR).[2] The gene located at chromosome 11p13 was cloned and designated WT1 in 1990.[3]

WT1 is expressed during all stages of kidney development, while in the mature nephron, WT1 protein expression is restricted to the podocytes.[4] It has also been demonstrated in the mesothelial cells and in stem cells bearing the CD34+ phenotype.[5] The WT1 protein was first classified as a tumor suppressor gene. An activator or oncogenic behavior may be acquired by mutations. It is now recognized that WT1 is mutated in about 10% of nephroblastomas.[4] The WT1 gene has also been observed in hematological malignancies,[6] mesothelial-derived neoplasm,[7] breast cancer,[8] genitourinary tumors[9] and small round blue cell tumors.[10]

The WT1 gene encodes a protein with 4 zinc fingers of the Kruppel-type in the C-terminal region required in tissue differentiation and proliferation.[11] The N-terminal half contains a large proline-glutamine-rich domain important for inhibition of transcriptional activation.[12] Recently, a WT1 antibody raised against the N-terminal 1-181 amino acids of human WT1 became commercially available for immunohistochemical staining on paraffin-embedded tissue. Limited reports in the literature about the utility of this antibody in nephroblastomas[13-15] prompted us to investigate the diagnostic and prognostic value of WT1 N-terminal antibody in nephroblastomas.

METHODS

Twenty-nine patients were treated for nephroblastomas

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during 1988 to 2000 at Mackay Memorial Hospital. Two cases were excluded owing to insufficient clinical data or histopathologic material. Two cases were reclassified as CCSK and excluded. Clinical data, including age at diagnosis, sex, treatment modality and outcome were reviewed. These patients were staged according to the National Wilms' Tumor Study (NWTS) staging system.[16] The treatment protocol included surgery, chemotherapy and radiotherapy. Four CCSK and 15 neuroblastomas were included for comparative study. All the pathologic slides were reviewed by the first author.

Formalin-fixed and paraffin-embedded specimens were processed according to standard avidin-biotin method and stained with WT1 antibody (clone 6F-H2; Dakopatts, Denmark) by an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA). The WT1 antibody was a mouse monoclonal antibody raised against the N-terminal 1-181 amino acids of human WT1. The antibody was diluted at 1:100 with Dako antibody diluent. The antigen retrieval protocol was in citrate buffer solution for 5 minutes in a 121 °C autoclave. Normal kidney tissue was used as a positive control. The specimens were regarded as positive when the percentage of positive cells was more than 10%.

Statistical method

The relationship among WT1 expression and clinical stage was compared by Fisher Exact Test. $p < 0.05$ was considered to be statistically significant.

RESULTS

Clinicopathological findings

Twenty-five children were studied, 12 males and 13 females, age ranged from 4 months to 19 years at diagnosis. The median age at surgery was 4.4 years. The most common chief complaints were abdominal mass or abdominal distension (20/25). Four patients had hematuria, and 1 had abdominal pain. Three patients had the tumors ruptured. None of the cases showed nephroblastoma-predisposing syndromes such as WAGR, Beckwith-Wiedemann, or Denys-Drash syndromes.

One patient had synchronous bilateral nephroblastomas. The tumor stage was I in 10, II in 2, III in 10, IV in 2, and V in 1 (The tumor in each kidney of this patient was substaged as stage I). The follow-up period ranged from 3 to 15 years (mean 7.1 years). Two patients died from their tumors.

All the nephroblastomas revealed no anaplasia. The composition of most of the tumors was triphasic (22/25). Three tumors were predominantly blastemal type. Skeletal muscle was the most common heterologous stromal cell type.

WT1 expression in nephroblastoma tissue

The uninvolved kidney showed a very intense nuclear staining of glomerular podocytes for WT1 (Fig. 1A). The tubules were negatively stained. Nuclear immunoreactivity of various intensity was observed in blastemal and epithelial elements of the nephroblastomas (Fig. 1B). Eleven tumors (44%) showed blastemal nuclear staining with or without epithelial nuclear staining. The strongest staining was in the neoplastic glomerular component (Fig. 1C). All the tumors showed cytoplasmic stain in the stromal cells. The heterologous rhabdomyoblasts showed strong positivity in a cytoplasmic pattern. The endothelial cells of blood vessels show obvious cytoplasmic staining.

Three of the 13 (23%) low-stage (stages I and II) tumors showed WT1 blastemal nuclear staining, while 8 of the 12 (67%) high-stage (stages III and IV) tumors revealed blastemal nuclear staining. The blastemal nuclear expression of WT1 was statistically significantly correlated with clinical stage ($p = 0.036$).

WT1 expression in neuroblastomas

All the fifteen cases showed no nuclear staining. Three cases revealed cytoplasmic staining in the Schwannian stroma. Four cases had cytoplasmic staining in the cells toward ganglion differentiation and occasionally in undifferentiated neuroblastic cells.

WT1 expression in clear cell sarcoma of the kidney

All the 4 cases revealed no nuclear staining (Fig. 1D). One case showed focal cytoplasmic staining.

DISCUSSION

The present study was carried out to investigate

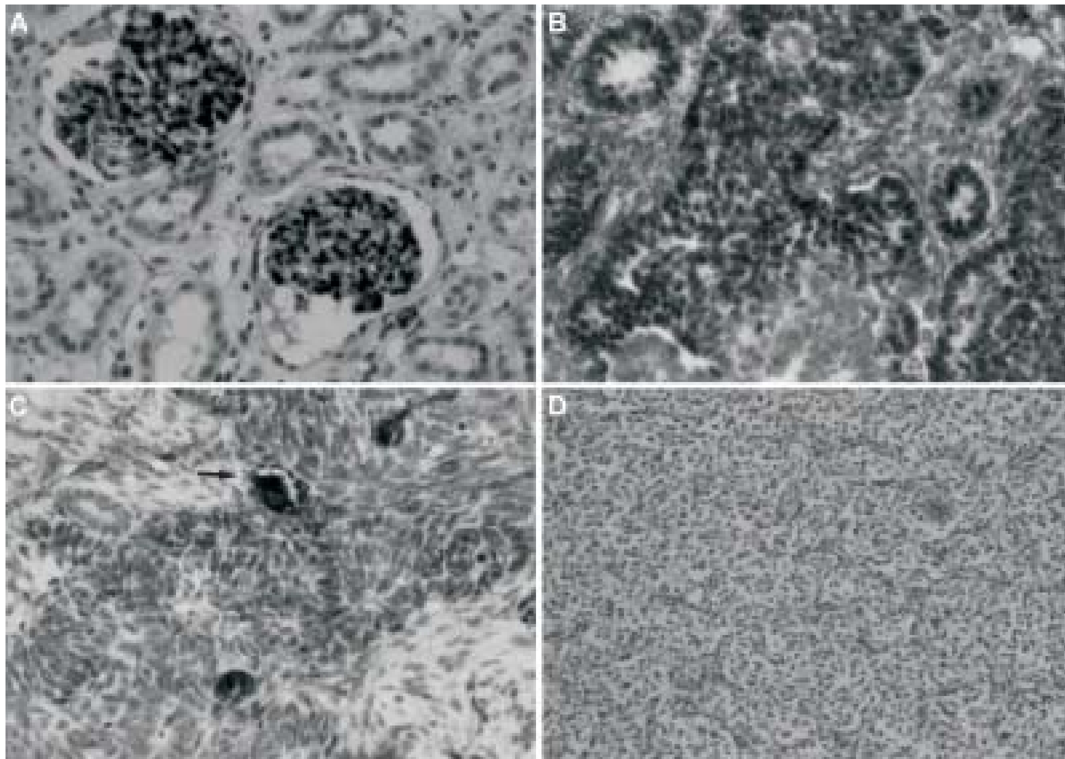

Fig. 1. WT1 antibody immunohistochemistry. (**A**) Normal kidney positive podocytes (400X); (**B**) Nephroblastoma with epithelial and blastemal nuclear positivity (400X); (**C**) Glomerular differentiation(arrow) in nephroblastomas with strong nuclear staining (200X); (**D**) CCSK with negative staining (100X).

whether the expression of WT1(6F-H2) protein had a diagnostic and prognostic value in nephroblastomas, using paraffin-embedded tissue sections. Our results showed that 11 tumors (44%) had blastemal nuclear staining with or without epithelial nuclear staining. All the tumors showed cytoplasmic staining in the stromal cells. The results are similar to that of Carpentieri's and Barnoud's studies using the same antibody WT1(F-6).[10,13] However, the results are slightly different from Ramani's study using WT(C-19).[14] That study showed higher rate of blastemal or epithelial nuclear immunoreactivity and weak or negative stromal cell cytoplasmic staining. The discrepancy may be explained by the difference of the antibodies used.

Beckwith and Palmer separated nephroblastomas into favorable and unfavorable subtypes.[17] The latter were subdivided into anaplastic nephroblastomas and sarcomatous nephroblastomas. The sarcomatous nephroblastomas, CCSK and malignant rhabdoid tumor are now recognized as neoplasms distinct from nephroblastomas. Two cases of nephroblastomas in our files were reclassified as CCSK and excluded.

Nephroblastoma must be distinguished from other pediatric renal primary or metastatic tumors. The most frequently encountered differential diagnoses include CCSK and neuroblastoma. Rhabdoid tumor is not a frequent source of diagnostic confusion.[18] CCSK are not uncommonly misinterpreted as blastemal predominant nephroblastomas. Distinguishing these 2 tumors is very important, since the treatment and the outcome are different. The 4 clear cell sarcomas in our study and 1 case in the reported literature show no WT1 nuclear staining.[9] All the 15 neuroblastomas showed no nuclear staining. The presence of WT1 nuclear immunoreactivity may be helpful to distinguish blastemal predominant nephroblastomas from CCSK or neuroblastoma. The limited sampling of CCSK preclude a definite conclusion. Further study on more cases is warranted.

Cytoplasmic WT1 immunoreactivity seems to be more nonspecific than nuclear staining. Cytoplasmic WT1 staining in our series could be seen in the stromal cells and rhabdomyoblasts of the nephroblastomas,

Schwannian stromal cells and a few tumor cells of neuroblastoma, occasional tumor cells of CCSK and endothelial cells of non-neoplastic blood vessels of most tumors. Cytoplasmatic WT1 staining was also reported in Carpentieri and Ramani's studies.[13,14] In the latter study, weak cytoplasmic staining could be seen in some tubular elements of nephroblastomas. In Carpentieri's study, the cytoplasmic pattern was seen in 75% of nephroblastomas and was almost exclusively stromal and weak.[13] Strong cytoplasmic staining was seen in the heterologous (muscle) element of nephroblastomas and most rhabdomyosarcomas. The cytoplasmic detection of WT1 may be explained that many transcription factors are synthesized and reside in the cytoplasm in an inactive form. Activation of these factors by phosphorylation may be an essential mechanism for nuclear translocation from the cytoplasm.[13]

Overall survival of over 85% of patients with nephroblastomas can now be achieved using combination therapy with chemotherapy, surgery, and in some cases radiotherapy.[19] Our result showed that the blastemal nuclear expression of WT1 were statistically significantly correlated with clinical stage. However, there were only 2 mortalities in our series. We could not compare the relationship of WT1 expression and survival rate. Using the same methods, Ghanem's study of 61 nephroblastomas suggested that WT-1 expression is related to prognosis.[15]

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REFERENCES

1. Machin GA, McCaughey WT. A new precursor lesion of Wilms' tumor (nephroblastoma): intralobar multifocal nephroblastomatosis. *Histopathology* 1984;8:35-53.
2. Riccardi VM, Sujansky E, Smith AC, Francke U. Chromosomal imbalance in the anirida-Wilms' tumor association: 11p interstitial deletion. *Pediatrics* 1978;61:604-10.
3. Bonetta L, Kuehn SE, Huang A, Law DJ, Kalikin LM, Koi M, *et al*. Wilms' tumor locus on 11p13 defined by multiple CpG island-associated transcripts. *Science* 1990;250:994-7.
4. Scharnhorst V, van der Eb AJ, Jochemsen AG. WT1 proteins: functions in growth and differentiation. *Gene* 2001;8:141-61.
5. Baird PN, Simmons PJ. Expression of the Wilms' tumor gene (WT1) in normal hemopoiesis. *Exp Hematol* 1997;25:312-20.
6. Inoue K, Ogawa H, Sonoda Y, Kimura T, Sakabe H, Oka Y, *et al*. Aberrant overexpression of the Wilms' tumor gene (WT1) in human leukemia. *Blood* 1997;89:1405-12.
7. Amin KM, Litzky LA, Smythe WR, Mooney AM, Morris JM, Mews DJ, *et al*. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *Am J Pathol* 1995;146:344-56.
8. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, *et al.* Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res* 2001;61:921-5.
9. Satoh F, Tsutsumi Y, Yokoyama S, Osamura RY. Comparative immunohistochemical analysis of developing kidneys, nephroblastomas and related tumors: considerations on their histogenesis. *Pathol Int* 2000;50:458-71.
10. Barnoud R, Sabourin JC, Pasquier D, Ranchere D, Bailly C, Terrier-Lacombe MJ, *et al*. Immunohistochemical expression of WT1 by desmoplastic small round cell tumor: a comparative study with other small round cell tumors. *Am J Surg Pathol* 2000;24:830-6.
11. Scharnhorst V, van der Eb AJ, Jochemsen AG. Internal translation initiation generates novel WT1 protein isoforms with distinct biological properties. *J Biol Chem* 1999;274:2356-62.
12. Reddy JC, Morris JC, Wang J, English MA, Haber DA, Shi Y, *et al*. WT1-mediated transcriptional activation is inhibited by dominant negative mutant proteins. *J Biol Chem* 1995;270: 10878-84.
13. Carpentieri DF, Nichols K, Chou PM, Matthews M, Pawel B, Huff D. The expression of WT1 in the differentiation of Rhabdomyosarcoma from other pediatric small round blue cell tumors. *Mod Pathol* 2002;15:1080-6.
14. Ramani P, Cowell JK. The expression pattern of Wilms' tumour gene (WT1) in normal tissues and paediatric renal tumours. *J Pathol* 1996;179:162-8.
15. Ghanem MA, van der Kwast TH, Den Hollander JC, Sudaryo MK, Oomen MH, Noordzij, *et al*. Expression and prognostic value of Wilms' tumor 1 and early growth response 1 proteins in nephroblastoma. *Clin Cancer Res* 2000;6:4265-71.
16. D'Angio GJ, Evans AE, Breslow N, Beckwith B, Bishop H, Feigl P, *et al.* The treatment of Wilms' tumor: Results of the national Wilms' tumor study. *Cancer* 1976;38:633-46.
17. Beckwith JB, Palmer NF. Histopathology and prognosis of Wilms' tumors: results from the First National Wilms' Tumor Study. *Cancer* 1978;41:1937-48.

18. Murphy WM, Beckwith JB, Farrow GM. Tumors of the kidney. In: Tumors of the kidney, bladder, and related urinary structures. Atlas of Tumor Pathology. Armed Forces Institute of Pathology, Washington, DC, 1994;34-6.
19. Gordon AM. Wilms' tumor (nephroblastoma). *Curr Opin Urol* 2001;11:567-70.