Letters to the Editor

CORRESPONDENCE RE: CARPENTIERI DF, NICHOLS K, CHOU PM, MATHEWS 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(10):1080–6.

To the Editor: We read with interest Carpentieri *et al.'s* (1) recently reported experience with a commercial WT1 antibody on a variety of pediatric small round blue cell tumors (SRBCT) using the mouse monoclonal antibody (clone: 6F-H2, DAKO) against the *N*-terminal amino acids 1–181 of the human WT1 protein, and we would like to share our experience.

We constructed tissue micro-arrays (TMA) with 24 Wilms' tumors. Each one was sampled eight times in different histologic areas to be sure that the three different components of the tumors were sampled (epithelial, blastemal, and stromal), as it was the normal renal tissue and the nephrogenic rests.

All cases were sporadic Wilms' tumors, and the archive material was up to 5 years old. In each case, tissue sections stained with hematoxylin and eosin were assessed to examine the morphological features and select the study areas.

The TMA tissue sections were stained by immunohistochemistry using the same monoclonal antibody anti-human WT1 (clone: 6F-H2, DAKO) at a dilution of 1:50. Twenty cases were reliable for this study.

As Carpentieri *et al.* mention, our results showed high cytoplasmatic positivity in all of the cases where the stromal component had rhabdomyoblastic differentiation (5/24). Two of them were stage V (bilateral). When we analyzed the nuclear positivity within the different components of the Wilms' tumors, there was a nuclear positivity in the blastemal component in 13/20 cases, in the epithelial component 5/20, and in the mesenchymal component 11/20. Among the 13 cases with nuclear positivity in the blastemal component, 12 had not received preoperative chemotherapy; and among the negative cases ($n = 7$), only one did not receive preoperative chemotherapy ($P < 0.001$).

The presence of high levels of WT1 expression in the epithelial and blastemal components of Wilms' tumors has been classically demonstrated, whereas stromal elements were found to be expressed at very low levels (2). The nuclear localization of the protein encoded by the WT1 gene in embryonic and adult tissues also has been demonstrated (3). In addition to specific nuclear immunoreactivity, antibodies to WT1 have been reported to stain the cytoplasm of the desmoplastic stroma of some carcinoma specimens (4, 5) and of blood vessels and connective tissue (6). These results have been interpreted as cross-reactivity with an epitope unrelated to WT1.

Our results reinforce Carpentieri's hypothesis that there is a role for WT1 in the pathogenesis of tumors with rhabdomyomatous differentiation and that the cytoplasmatic positivity for WT1 has to be interpreted as a special expression of this gene.

As we had significantly more cases with nuclear positivity in the blastemal component within the patients that did not receive preoperative chemotherapy, our results also suggest that WT1 plays a role in the chemotherapy activity on the blastemal component of the Wilms' tumors.

Studies of the nature of mutations and the gene expression profile of such cases are now being done to understand this process.

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In reply: We appreciate Dr. Sredni *et al.'s* comments and support regarding our recent observations on the expression of WT1 in pediatric tumors with myogenic differentiation. Her data also suggest an important association between the pattern of staining and prognostic implications.

Molecular analysis is rapidly expanding our knowledge of Wilms' tumor and pointing to new diagnostic and therapeutic targets (1, 2). Tissue microarray is becoming an important tool in this analysis, as long as standards of tissue processing and antigen retrieval (3–5) are adopted for useful insights and statistically significant conclusions.

The importance of a standard methodology is stressed by a recent report (6) describing a negative immunohistochemical expression of WT1 proteins with the same antibody (6F-H2) in Wilms' tumors with a myogenic phenotype. In contrast to our report, their experiment used a higher dilution (1:200) and pepsin retrieval.

Interestingly, the same report noted a common point mutation in the zinc finger 3 of the WT1 protein necessary for DNA binding. The latter suggesting that the atypical cytoplasmic expression noted in our analysis and by Dr. Sredni may not be secondary to cross-reactivity with an epitope unrelated to WT1.

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