The value of Wilms tumor susceptibility gene 1 in cytologic preparations as a marker for malignant mesothelioma

ARTICLE *in* CANCER · APRIL 2002

Impact Factor: 4.89 · DOI: 10.1002/cncr.10482 · Source: PubMed

4 AUTHORS, INCLUDING:

Benjamin H Lee
Novartis
55 PUBLICATIONS **4,596** CITATIONS

SEE PROFILE

CANCER
CYTOPATHOLOGY

The Value of Wilms Tumor Susceptibility Gene 1 in Cytologic Preparations as a Marker for Malignant Mesothelioma

Jonathan L. Hecht, M.D., Ph.D.
Benjamin H. Lee, M.D., Ph.D.
Jack L. Pinkus, Ph.D.
Geraldine S. Pinkus, M.D.

Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

BACKGROUND. It has been shown that detection of the Wilms tumor susceptibility gene 1 protein (WT1) has diagnostic utility in the distinction of mesothelioma from adenocarcinoma in tissue sections of pleural tumors. This immunohistochemical study evaluates the effectiveness of WT1 as a marker for malignant mesothelioma in paraffin sections of cell block preparations derived from effusion specimens.
METHODS. The authors evaluated 111 cell blocks for WT1 immunoreactivity, including 14 mesotheliomas and 97 metastatic adenocarcinomas from various sites.
RESULTS. Nuclear reactivity for WT1 was observed in all samples of mesothelioma. However, only 22 of 97 samples (23%) of metastatic adenocarcinoma, nearly all of which were of ovarian origin (91%), exhibited nuclear reactivity for WT1. In 14 other samples (most of pulmonary derivation), WT1 staining restricted to the cytoplasm was observed for some tumor cells and was regarded as nonspecific.
CONCLUSIONS. Based on this staining profile, WT1 represents an effective marker for mesotheliomas in cell block preparations and can aid in its distinction from pulmonary adenocarcinoma. In assessment of effusion specimens with metastatic carcinoma, nuclear reactivity for WT1 is highly suggestive of an ovary primary tumor. ***Cancer (Cancer Cytopathol)*** **2002;96:105–9.**

KEYWORDS: WT1, Wilms tumor, cell block, cytology, body fluids, adenocarcinoma, mesothelioma.

Wilms tumor susceptibility gene 1 is a tumor suppressor gene that initially was identified due to its deletion or mutation in Wilms tumors. Monoclonal antibodies to its protein product, WT1, were developed subsequently, and it was found that they had diagnostic utility not only in the identification of Wilms tumors and desmoplastic small round cell tumors[1,2] but also in the distinction of mesothelioma from adenocarcinoma in pleural tumors.[3,4] This immunohistochemical study evaluates the diagnostic utility of WT1 as a marker for malignant mesothelioma in paraffin sections of cell block preparations derived from effusion specimens.

MATERIALS AND METHODS

A total of 111 cell blocks containing various malignant tumors were identified in the files of the Cytology Division of Brigham and Women's Hospital, Boston, MA. Cell blocks were prepared from pleural fluids (70 blocks), peritoneal fluids (28 blocks), and pericardial fluids (13 blocks) containing tumors of various types. All fluids were diagnosed as *positive for metastatic adenocarcinoma* (97 blocks) or *mesothelioma* (14 blocks). Biopsy documented primary sites of meta-

The current address for Jonathan L. Hecht is Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA, 02215.

Address for reprints: Geraldine S. Pinkus, M.D., Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115; Fax: (617) 713-3044; E-mail: gspinkus@bics.bwh.harvard.edu

Received July 12, 2001; revision received September 11, 2001; accepted September 13, 2001.

static tumors included lung (33 adenocarcinomas), breast (29 adenocarcinomas, including 2 lobular carcinomas), gastrointestinal tract (6 adenocarcinomas), pancreas (2 adenocarcinomas), endometrium (2 adenocarcinomas), ovary (23 adenocarcinomas), prostate (2 adenocarcinomas), and mesothelium (14 adenocarcinomas). Malignant mesotheliomas were all of epithelioid histology. The body cavity fluids were processed into cell blocks using thromboplastin and plasma. The cell blocks were fixed in 10% neutral buffered formalin and embedded in paraffin.

Immunoperoxidase studies for WT1 were performed manually on 5-μm paraffin sections of the cell blocks after heat-induced epitope retrieval (0.001 M ethylenediamine tetraacetic acid, pH 8.0). Deparaffinized sections were placed in a container of preheated retrieval solution, steamed for 30 minutes (Black and Decker steamer, model HS80), then cooled for 20 minutes, washed under running water, placed in distilled water, and treated with methanolic peroxide (5 parts methanol to 1 part 3% hydrogen peroxide) for 20 minutes. Slides were then washed under running water, placed in distilled water, then placed in 0.05 M Tris buffer, pH 7.6, supplemented with 3% porcine serum. Slides were then incubated for 1 hour with monoclonal antibody specific for WT1 (clone 6F-H2; Dako Corporation, Carpinteria, CA) at a dilution of 1:100, washed, and incubated with horseradish peroxidase-labeled polymer conjugated to goat antimouse immunoglobulin antibodies (Envision + detection system, K4006; Dako Corporation). Antibody localization was effected using a peroxidase reaction with DAB+ as the chromogen (Envision + detection system). Staining intensity was enhanced with DAB enhancer (Zymed Laboratories Inc., San Francisco, CA). Slides were counterstained with methyl green solution or hematoxylin, dehydrated, and coverslipped. Only nuclear staining was regarded as a positive result. The intensity of staining was graded on a 0–3+ scale. A positive control slide of WT1 positive Wilms tumor was included in each run. Control slides substituting Tris buffer for primary antibody were run as negative controls.

RESULTS

Immunoreactivity for WT1 was determined for neoplastic cells in a total of 97 samples of metastatic carcinoma and 14 samples of malignant mesothelioma in cell blocks of pleural, peritoneal, or pericardial fluids. Results of these studies are summarized in Table 1. All tumors that were interpreted as positive exhibited nuclear reactivity in the vast majority of neoplastic cells.

All body fluids containing malignant mesothelioma were reactive for WT1 and exhibited a nuclear staining pattern (Fig. 1). In these samples, both the frankly malignant groups of tumor cells as well as the more benign appearing mesothelial cells were reactive. Of 97 samples of metastatic adenocarcinoma, neoplastic cells in 22 samples (23%) exhibited nuclear reactivity for WT1. However, nearly all reactive samples (91%) represented ovarian metastases (Fig. 2). Of the 23 ovarian tumors evaluated, 20 tumors (87%) were WT1 positive. Primary ovarian tumors in these samples included 19 papillary serous cystadenocarcinomas (18 of 19 positive), 2 mixed serous and endometrioid tumors (both positive), and 2 clear cell carcinomas (both negative). Mucinous ovarian tumors metastatic to body fluids were not included in the study, because none could be found. Two of 29 cell blocks containing metastatic breast carcinomas were WT1 positive. Nuclear staining intensity was strong in the majority of positive tumors (Table 2), and the majority of tumor cells were reactive. The remaining samples with metastatic tumor were regarded as negative, including 33 tumors of pulmonary derivation. Cytoplasmic staining only (Fig. 3) was present in a subset of adenocarcinomas (14 tumors: 9 lung, 2 breast, 1 endometrium, 1 esophagus, and 1 gallbladder) and was regarded as a negative result. This staining may be related to the detection of a non-WT1 cross-reacting epitope, because Tris control slides of these tumors uniformly were negative. Nuclear staining was not seen in any of the latter slides. Cytoplasmic staining was not observed for any of the mesotheliomas. Tris control slides were negative for all WT1 positive tumors with nuclear reactivity in neoplastic cells.

TABLE 1
Wilms Tumor Susceptibility Gene 1 Protein Immunoreactivity in 111 Cell Blocks Containing Mesotheliomas and Adenocarcinomas of Various Types

Tumor type	No. of cell blocks	No. immunoreactive (%)[a]
Mesothelioma	14	14 (100)
Adenocarcinoma		
Lung	33	0[b]
Breast	29	2 (7)
Endometrium	2	0
Ovary	23	20 (91)
Pancreas	2	0
Prostate	2	0
Stomach	3	0
Gallbladder	1	0
Esophagus	2	0

[a] Nuclear reactivity.

[b] Cytoplasmic reactivity was found in nine cell blocks (see text).

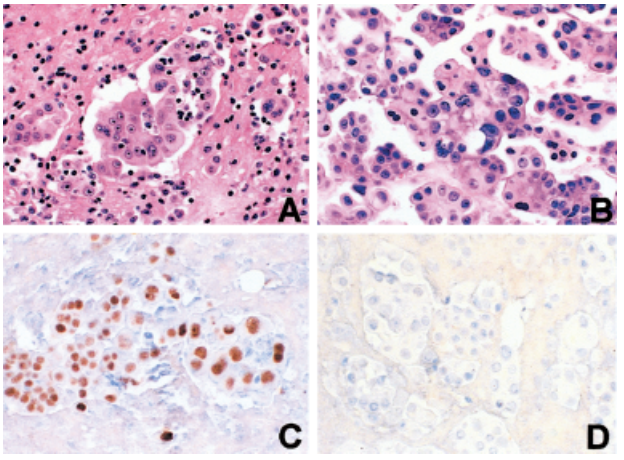

FIGURE 1. Hematoxylin and eosin-stained cell blocks of mesothelioma (A) and metastatic adenocarcinoma (B). Almost all neoplastic cells exhibited nuclear reactivity for Wilms tumor susceptibility gene 1 protein (WT1) in sections of mesothelioma (C) (immunoperoxidase study, methyl green counterstain). The background lymphocytes and histiocytes were nonreactive. The majority of nonovarian metastatic carcinomas were nonreactive for WT1, as noted in a cell block with metastatic pulmonary adenocarcinoma (D) (immunoperoxidase study, hematoxylin counterstain; original magnification, ×400 in A, C, and D; ×600 in B).

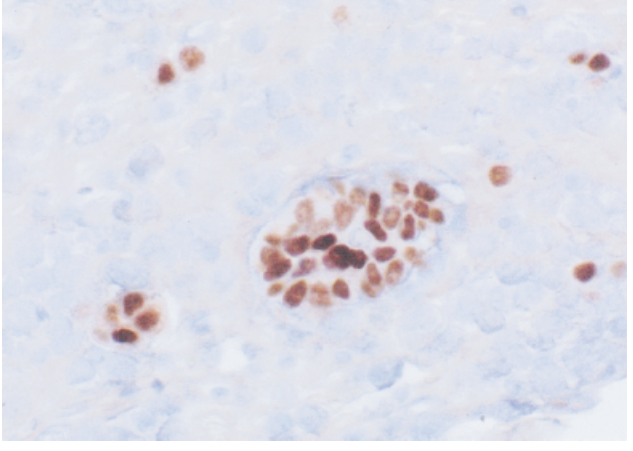

FIGURE 2. Ovarian adenocarcinoma with strong nuclear reactivity for Wilms tumor susceptibility gene 1 protein. Note that the benign mesothelial cells also were reactive and served as a positive internal control (immunoperoxidase study, methyl green counterstain; original magnification, ×400).

Nuclear immunoreactivity for WT1 was apparent for benign mesothelial cells in 54 adenocarcinomas. In some tumors, staining of numerous mesothelial cells created difficulty in identifying tumor cells, particularly if small numbers of tumor cells were present. In other tumors, WT1 positive mesothelial cells were present, but interpretation was not compromised (Fig. 4A). In tumors that contained only a few benign appearing mesothelial cells, interpretation was straightforward. In four instances, cell blocks contained numerous mesothelial cells that were positive for WT1, creating great difficulty in accurate interpretation (Fig. 4B). In these slides, detection of tumor cells was facilitated either by using a hematoxylin counterstain (rather than methyl green) or by performing an additional immunostain for calretinin to verify the identification of the mesothelial cells.

TABLE 2
Staining Intensity for Wilms Tumor Susceptibility Gene 1 Protein in Cell Blocks with Malignant Mesothelioma and Metastatic Ovarian Carcinoma

		Staining intensity			
Tumor type	No. of cell blocks	0	1+	2+	3+
Ovary	23	3	2	1	17
Mesothelioma	14	0	1	5	8

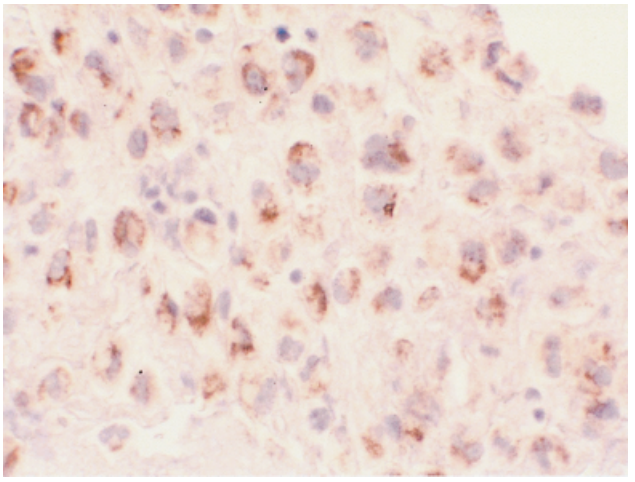

FIGURE 3. A cell block with metastatic pulmonary adenocarcinoma. Granular cytoplasmic staining for Wilms tumor susceptibility gene 1 protein was observed in some metastatic adenocarcinomas, including 9 of 33 tumors of pulmonary derivation, as noted in this cell block. This finding was regarded as a nonspecific staining pattern (immunoperoxidase study, methyl green counterstain; original magnification, ×400).

DISCUSSION

WT1 is a tumor suppressor gene that was identified initially as contributing to the development of Wilms tumors due to deletion or mutation.[5] WT1 gene product is expressed preferentially in the urogenital system and in mesoderm-derived tissues.[6] The use of WT1 reactivity in the diagnosis of mesothelioma in biopsy tissue sections has been reviewed recently and reportedly has a sensitivity of approximately 75% for detecting mesothelioma.[3,4] Although reactivity has been re-

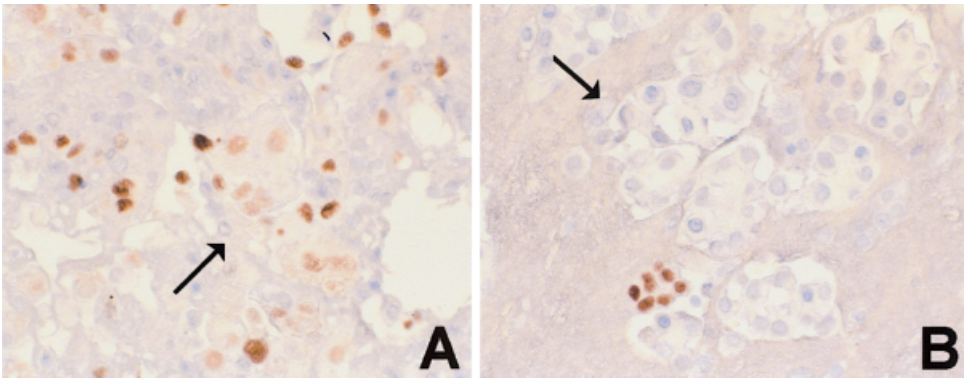

FIGURE 4. Four cell blocks with metastatic adenocarcinoma contained abundant background benign mesothelial cells, which were reactive for Wilms tumor susceptibility gene 1 protein (WT1). In two of these blocks, tumor cells also exhibited nuclear staining for WT1, as noted in a sample of metastatic ovarian carcinoma (A) (methyl green counterstain; original magnification, ×400). In the other two blocks, tumor cells were nonreactive, as seen in a sample of metastatic breast carcinoma (B) (hematoxylin counterstain; original magnification, ×400). Arrows indicate tumor cells. Definitive interpretations in difficult samples were accomplished either by counterstaining with hematoxylin or by performing an immunohistochemical study for calretinin to identify the mesothelial cells.

ported in single instances of renal carcinoma and melanoma, it is typically negative in metastatic adenocarcinomas.[3] The exception is ovarian papillary serous carcinoma,[4] for which WT1 reactivity has been reported in up to 83% of tumors. Our results for cell block preparations support the high incidence of WT1 reactivity for these ovarian tumors.

The current study examined the utility of WT1 as a diagnostic discriminant for tumors in cytologic material from a broad range of primary sites. Staining for WT1 was positive in all (100%) of the malignant mesotheliomas, with 93% showing strong staining (2–3+). Only 23% of adenocarcinomas were positive, and the majority (91%) were nonmucinous ovarian tumors in origin. Because ovarian tumors do not typically metastasize to the pleura, the specificity of WT1 staining for mesothelial cells in pleural fluids (ovarian tumors excluded) is high.

The effectiveness of our immunohistochemical procedure was excellent given the variability in tumor cell preservation in the cytologic preparations. Reactivity of background benign mesothelial cells was prominent in a subset of tumors and posed some difficulty in interpretation. This problem could be obviated readily by using a hematoxylin counterstain to facilitate morphologic identification of tumors or by performing an additional calretinin study to verify the identity of mesothelial cells.

The distinction between malignant epithelioid mesotheliomas and adenocarcinomas, particularly of lung origin, in body cavity fluids requires a panel of immunomarkers that typically includes antibodies reactive for adenocarcinoma or mesothelioma. The optimal panel of markers for fluids appears to vary by institution, but includes HBME-1, thrombomodulin, MOC-31, Ber-EP4, and CD15 (Leu-M1)[7] as well as calretinin.[8–11] Ordonez has reviewed the use of these and other markers on tissue sections.[12]

Because WT1 is expressed in mesotheliomas, it has been used to distinguish epithelioid type mesotheliomas from adenocarcinomas in pleural biopsies. Our studies demonstrate that WT1 also can play a part in this distinction as part of a panel of immunostains applied to cytologic (cell block) material. In light of a recent report of focal, aberrant calretinin staining in primary and metastatic sarcomas of the pleura,[13] WT1 is especially helpful, because it represents another positive marker for mesothelial cells. At our institution, we recently added WT1 to the routine antibody panel to differentiate pulmonary adenocarcinoma from mesothelioma in tissues and fluids. The panel includes TTF-1, CD15 (Leu-M1), carcinoembryonic antigen, calretinin, and WT1.

CONCLUSIONS

WT1 is a sensitive marker for malignant mesothelioma and may be a useful diagnostic discriminant in its distinction from adenocarcinoma, particularly in disease of pulmonary derivation. For metastatic adenocarcinoma, strong nuclear reactivity for WT1 was observed nearly exclusively (91%) for neoplasms of ovarian origin, particularly papillary serous cystadenocarcinomas, providing a helpful marker for the identification of this tumor type.

REFERENCES

1. Hill DA, Pfeifer JD, Marley EF, et al. WT1 staining reliably differentiates desmoplastic small round cell tumor from Ewing sarcoma/primitive neuroectodermal tumor. An immunohistochemical and molecular diagnostic study. *Am J Clin Pathol.* 2000;114(3):345–353.

2. Ramani P, Cowell JK. The expression pattern of Wilms' tumour gene (WT1) product in normal tissues and paediatric renal tumours. *J Pathol.* 1996;179(2):162–168.
3. Kumar-Singh S, Segers K, Rodeck U, et al. WT1 mutation in malignant mesothelioma and WT1 immunoreactivity in relation to p53 and growth factor receptor expression, cell-type transition, and prognosis. *J Pathol.* 1997;181(1):67–74.
4. Ordonez NG. Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and nonpulmonary adenocarcinoma. *Am J Surg Pathol.* 2000;24(4):598–606.
5. Call KM, Glaser T, Ito CY, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell.* 1990;60(3):509–520.
6. Hastie ND. Wilms' tumour gene and function. *Curr Opin Genet Dev.* 1993;3(3):408–413.
7. Lozano MD, Panizo A, Toledo GR, Sola JJ, Pardo-Mindan J. Immunocytochemistry in the differential diagnosis of serous effusions: a comparative evaluation of eight monoclonal antibodies in Papanicolaou stained smears. *Cancer (Cancer Cytopathol).* 2001;93(1):68–72.
8. Wieczorek TJ, Krane JF. Diagnostic utility of calretinin immunohistochemistry in cytologic cell block preparations. *Cancer (Cancer Cytopathol).* 2000;90(5):312–319.
9. Chhieng DC, Yee H, Schaefer D, et al. Calretinin staining pattern aids in the differentiation of mesothelioma from adenocarcinoma in serous effusions. *Cancer (Cancer Cytopathol).* 2000;90(3):194–200.
10. Kitazume H, Kitamura K, Mukai K, et al. Cytologic differential diagnosis among reactive mesothelial cells, malignant mesothelioma, and adenocarcinoma: utility of combined E-cadherin and calretinin immunostaining. *Cancer (Cancer Cytopathol).* 2000;90(1):55–60.
11. Barberis MC, Faleri M, Veronese S, Casadio C, Viale G. Calretinin. A selective marker of normal and neoplastic mesothelial cells in serous effusions. *Acta Cytol.* 1997;41(6):1757–1761.
12. Ordonez NG. The immunohistochemical diagnosis of epithelial mesothelioma. *Hum Pathol.* 1999;30(3):313–323.
13. Miettinen M, Limon J, Niezabitowski A, Lasota J. Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. *Am J Surg Pathol.* 2001;25(5):610–617.