

WT1 Immunoreactivity in Uterine Papillary Serous Carcinomas Is Different From Ovarian Serous Carcinomas

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Abstract

WT1 diffusely stains most ovarian serous carcinomas; reactivity of uterine papillary serous carcinomas has not been evaluated. We studied WT1 expression in 13 International Federation of Gynecology and Obstetrics stage 1 and 5 stage 3 or 4 uterine papillary serous carcinomas without ovarian metastases and compared their reactivity with the WT1 staining of 30 ovarian serous carcinomas. WT1 reactivity was evaluated with the C19 and 6F-H2 antibody clones. All 18 uterine papillary serous carcinomas were nonreactive for WT1. The nonovarian metastases of the 5 high-stage uterine papillary serous carcinomas also were nonreactive for WT1. In contrast, 29 (97%) of 30 ovarian serous carcinomas were reactive for WT1. WT1 reactivity in an unknown primary serous carcinoma would suggest it is from a nonuterine site. The mechanisms underlying these findings are unknown. They raise the possibility of genetic differences between the 2 morphologically similar neoplasms.

Serous carcinoma of the peritoneum and female genital tract is considered a single neoplastic process that can originate from different sites, including ovary and endometrium. This may not be biologically accurate. The less favorable response to cisplatin-based chemotherapy of uterine papillary serous carcinomas compared with ovarian serous carcinomas raises the possibility of molecular differences between the 2 neoplasms despite their morphologic similarity.¹

The Wilms tumor gene (*WT1*) is a tumor-suppressor gene located on chromosome 11p13.² The nuclei of most ovarian and primary peritoneal serous carcinomas and mesotheliomas express WT1, which can be detected immunohistochemically with commercially available antibodies.³⁻⁷ Characterization of the pattern and extent of WT1 expression in uterine papillary serous carcinomas is unknown.

We evaluated WT1 staining in 18 uterine papillary serous carcinomas and compared their reactivity with those of 30 primary ovarian serous carcinomas.

Materials and Methods

Thirteen patients with International Federation of Gynecology and Obstetrics (FIGO) stage 1 primary uterine papillary serous carcinomas were identified retrospectively from the surgical pathology files of William Beaumont Hospital, Royal Oak, MI, for the period January 1993 through December 2000. All were pure serous carcinomas and composed of markedly atypical cells **Image 1** and **Image 2**. All patients underwent complete surgical staging, including a total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, biopsies of the peritoneum and

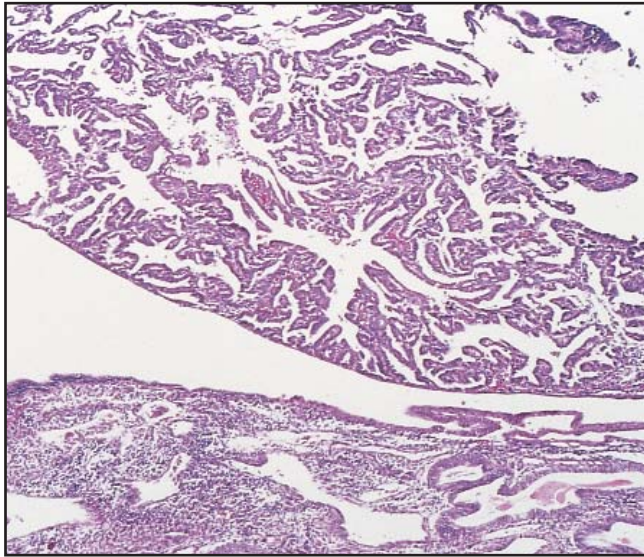


Image 1 Uterine papillary serous carcinoma. A neoplastic frond protrudes into the endometrial cavity. The subjacent endometrium is benign (H&E, $\times 10.5$).

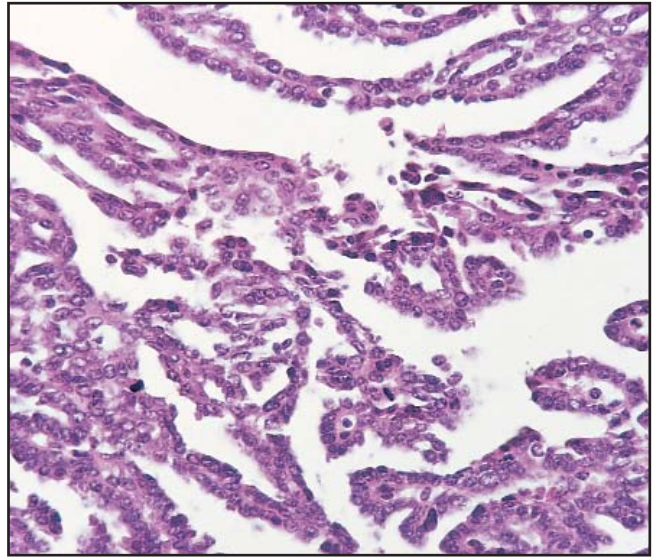


Image 2 Uterine papillary serous carcinoma. Higher magnification of Image 1. The carcinoma is composed of thin papillae that are lined by cytologically high-grade neoplastic cells (H&E, $\times 32$).

diaphragm, lymph node dissections, and pelvic washings. The ovaries were completely submitted and histologically evaluated in all cases.

Five cases of FIGO stage 3 or 4 primary uterine papillary serous carcinomas without ovarian metastases after complete histologic evaluation of the ovaries were identified in the surgical pathology files of William Beaumont Hospital during the same time period. All patients underwent complete surgical staging. The sites of extrauterine involvement by carcinoma were lymph nodes (4 cases), pelvic peritoneum (3 cases), and bowel serosa (3 cases).

Thirty ovarian serous carcinomas with no involvement of the uterus that were accessioned during the same period were selected randomly from the files of William Beaumont Hospital. All patients also underwent complete surgical staging. The uterine serosa, endometrium, and cervix were free of carcinoma in all cases. The carcinomas were pure serous and composed of markedly atypical cells. Four of 30 ovarian serous carcinomas were included in a previous study.⁷

A representative tissue block of primary carcinoma containing ample neoplasm was selected from each case for immunohistochemical study. A tissue block from the metastasis also was selected from each of the 5 cases of metastatic uterine papillary serous carcinoma.

Consecutive sections, 3 μ m thick, were cut from a representative block of carcinoma, and each section was placed on a charged slide. Sections were deparaffinized using sequential immersions into 2 xylene baths, 3 baths of

decreasing alcohol concentrations, and 2 water baths, followed by a 1-minute wash in water. Slides dedicated to the C19 clone of the WT1 antibody (1:400 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) were immersed in citrate buffer (pH 6.0). Slides dedicated to the 6F-H2 clone of the WT1 antibody (1:200 dilution; DAKO, Carpinteria, CA) and p53 antibody (DO-7 clone, 1:200 dilution; DAKO) were immersed in EDTA (pH 7.0). Both buffer containers then were placed into a commercial vegetable steamer at 95°C for 25 minutes. The slides were allowed to cool on the counter, remaining immersed in the heated EDTA buffer-filled containers for 5 minutes, followed by a 2-minute rinse with water while remaining in the containers. The slides were transferred into tris(hydroxymethyl)aminomethane-filled containers (pH 7.0) and allowed to undergo an additional 10 minutes of cooling on the countertop. They then were transferred to a commercial immunohistochemical autostainer (DAKO) and were first washed with buffer, followed by a hydrogen peroxide incubation. The latter was rinsed off, and the primary antibody was applied. The primary antibody was incubated over the sections for 20 minutes at room temperature. After the primary antibody was washed off, the components of the Envision-plus (DAKO) detection system were applied, including an antimouse polymer, 2 distilled-water washes, and a final incubation in diaminobenzidine for 4 minutes. Sections were counterstained with hematoxylin and coverslipped.

A positive control slide containing known cytokeratin-reactive tissues was stained with each batch of simultaneously

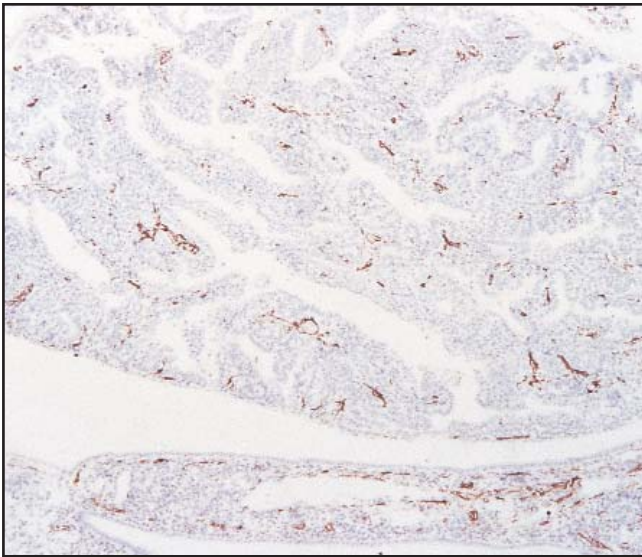


Image 3 Uterine papillary serous carcinoma from Image 1. There is no WT1 reactivity (hematoxylin counterstain, $\times 10.5$).

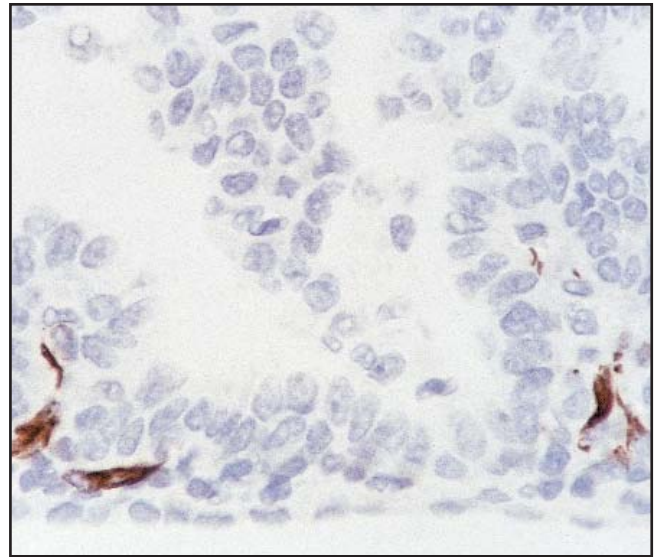


Image 4 Uterine papillary serous carcinoma from Image 1. There is no WT1 reactivity in the nuclei of the neoplastic cells. Endothelial cells are reactive and serve as a positive internal control (hematoxylin counterstain, $\times 160$).

stained slides. The percentage of reactive invasive adenocarcinoma cells was quantified as 0%, fewer than 5%, 5% to 25%, 26% to 50%, 51% to 75%, or more than 75%.

Two WT1 antibodies were used in the study. The polyclonal C-19 antibody was directed toward a region of the carboxyl terminus of the protein, and the monoclonal 6F-H2 antibody was directed toward a region of the amino terminus of the protein. All carcinomas were stained with p53, which served as a control mechanism to confirm that the neoplasms included in the study were of the serous type.

Results

All 18 uterine papillary serous carcinomas were nonreactive with both WT1 antibodies **Image 3** and **Image 4**. The 5 metastases also were nonreactive. Endothelial cells, an internal positive control, had cytoplasmic staining in all cases. All 18 carcinomas had diffuse, strong nuclear p53 reactivity **Image 5**.

Twenty-nine (97%) of 30 ovarian serous carcinomas were reactive for WT1 **Image 6**. The 6F-H2 antibody produced stronger homogeneous reactivity than did the C-19 clone, but the percentage of reactive cells was similar. One neoplasm was nonreactive with both WT1 antibodies. Four (13%) of 30 ovarian carcinomas had WT1 nuclear reactivity that was only of weak intensity, and the other 25 (83%) had reactivity of moderate or strong intensity **Table 1**. None of the 29 reactive carcinomas had staining in

fewer than 5% of the cell nuclei. Two carcinomas (7%) had WT1 nuclear reactivity in 5% to 25% of the neoplastic cells, 5 carcinomas (17%) had nuclear reactivity in 26% to 50% of the neoplastic cells, 12 carcinomas (40%) had nuclear reactivity in 51% to 75% of the neoplastic cells, and 10 carcinomas (33%) had nuclear reactivity in more than 75% of the cells. Twenty-five (83%) of 30 ovarian carcinomas were reactive for p53.

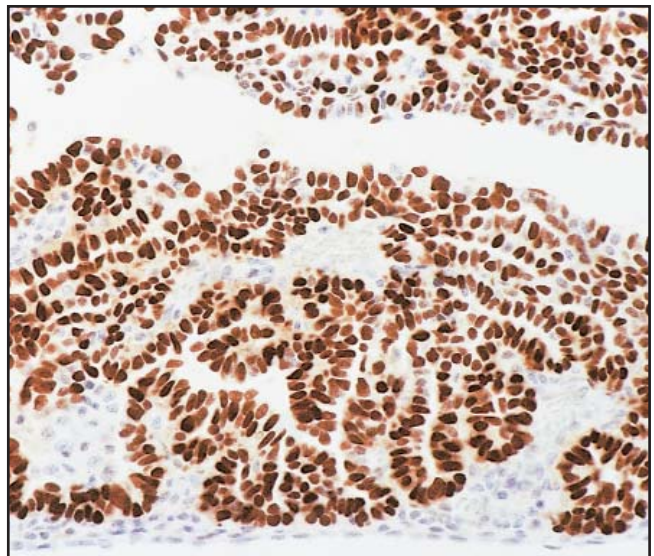


Image 5 Uterine papillary serous carcinoma from Image 1. There is strong, diffuse nuclear p53 reactivity in the neoplastic cells (hematoxylin counterstain, $\times 18$).

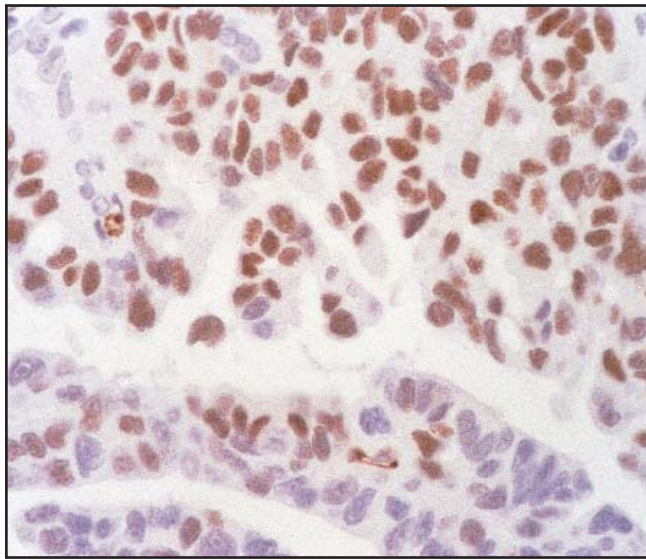


Image 6 Ovarian serous carcinoma. There is extensive WT1 nuclear reactivity (hematoxylin counterstain, $\times 96$).

Table 1
WT1 Immunoreactivity in 30 Ovarian Serous Carcinomas*

Percentage of Stained Cells	Intensity			
	0+	1+	2+	3+
0	1 (3)	0 (0)	0 (0)	0 (0)
<5	0 (0)	0 (0)	0 (0)	0 (0)
5-25	0 (0)	1 (3)	1 (3)	0 (0)
26-50	0 (0)	2 (7)	1 (3)	2 (7)
51-75	0 (0)	1 (3)	3 (10)	8 (27)
>75	0 (0)	0	2 (7)	8 (27)

* Data are given as number (percentage).

Discussion

The 18 uterine papillary serous carcinomas and 5 of their metastases were nonreactive for WT1, whereas 29 (97%) of 30 ovarian serous carcinomas were reactive for WT1. The extent and intensity of WT1 expression in ovarian serous carcinomas in the present study is similar to that reported by Goldstein et al⁷ and other authors.^{3-6,8} The serous papillary nature of the studied tumors was supported by strong diffuse p53 nuclear reactivity of the neoplastic cells.⁹⁻¹²

The molecular mechanisms underlying these observations are unknown. Genetic mutations in uterine and ovarian serous carcinomas have been similar.^{13,14} However, this may be a reflection of the search process rather than global genomic similarities. The striking difference in WT1 nuclear expression between uterine and ovarian serous carcinomas raises the possibility of mutation differences between the 2

neoplasms and focuses attention on the region in the genome to be searched. One possible explanation for these findings is that uterine papillary serous carcinomas express a WT1 isoform that is not identified by the anti-WT1 antibodies used in the present study. Alternative RNA splicing results in 4 WT1 protein isoforms that vary in the arrangement and structure of the 4 carboxy-end zinc fingers.¹⁵⁻¹⁷ We believe this is an unlikely cause of uterine papillary serous carcinoma nonreactivity because both WT1 antibodies used in the present study, the polyclonal carboxyl terminus directed C-19 clone and the monoclonal amino terminus 6F-H2 clone, were nonreactive.

The results have 2 practical implications. First, they suggest that WT1 could help separate or identify the primary site of a serous carcinoma. WT1 nuclear reactivity in the cells of a serous carcinoma would suggest that the neoplasm was not a primary uterine neoplasm. Although the lack of reactivity would seem to be supportive of a uterine primary tumor, we take a cautionary approach to negative immunohistochemical staining results. Second, they serve as a strong reminder that similar-appearing carcinomas of organs that are in proximity or considered to be embryologically related may be biologically distinct entities.

WT1 was nonreactive in all 18 uterine papillary serous carcinomas and reactive in the overwhelming majority of ovarian serous carcinomas. WT1 reactivity in an unknown primary serous carcinoma would suggest it is from a non-uterine site. The mechanisms underlying these findings are unknown. They raise the possibility of genetic differences between the 2 morphologically similar neoplasms.

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