Value of PAX8 and WT1 Immunostaining in Confirming the Ovarian Origin of Metastatic Carcinoma in Serous Effusion Specimens

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Key Words: PAX8; WT1; Ovarian; Metastatic; Effusion

Abstract

We evaluated the detection rates of PAX8 and WT1 immunostaining in 68 (45 as cell blocks, 23 as smears) serous effusion specimens that had a cytologic diagnosis of metastatic carcinoma of ovarian origin. Of the cases, 58 (85%) were positive for PAX8, 56 (82%) were positive for WT1, and 64 (94%) were immunoreactive with either or both markers. Detection rates of PAX8 and WT1 were 85% (44/52) and 92% (48/52), respectively, for metastatic serous carcinoma and 100% (5/5) and 20% (1/5), respectively, for metastatic clear cell carcinoma. Detection rates using cell blocks and smears were 91% and 78%, respectively, with PAX8 and 82% and 83%, respectively, with WT1. We concluded that PAX8 and WT1 had comparable overall detection rates in confirming ovarian origin of malignant effusion. The combination of both markers substantially improved the detection rate. Cell blocks and smears can be used for staining, but a cell block is preferred for PAX8 staining.

Ovarian cancer is the fifth most common cancer among women in developed countries and the leading cause of death in women with gynecologic malignancies. Because of the vague and nonspecific nature of the initial symptoms, ovarian cancers are often at an advanced stage by the time they are diagnosed, and, thus, the overall survival rate remains poor. Accurate diagnosis of metastatic ovarian cancer is important for proper tumor staging and timely treatment and, therefore, has prognostic importance.

Most ovarian cancers (>85%) arise from surface epithelium of the ovary, although studies also showed evidence that ovarian carcinoma may originate in other pelvic organs; the common tumor types include serous (the most common histologic subtype), clear cell, endometrioid, mucinous, transitional, and undifferentiated carcinomas. Ovarian carcinoma often metastasizes to serous cavities, resulting in malignant peritoneal and/or pleural effusion. However, diagnosing metastatic ovarian carcinoma in serous effusion solely on the basis of cytologic features can be challenging because the tumor cells can cytologically resemble reactive mesothelial cells or metastatic adenocarcinomas from other primary sites. A battery of immunostaining markers that recognize mesothelial cells and adenocarcinoma cells is often needed to facilitate a differential diagnosis. For example, calretinin, cytokeratin 5/6, thrombomodulin, and D2-40 are the markers commonly used to identify cells of mesothelial origin, whereas MOC-31, B72.3, Ber-EP4, and Leu-M1 are the markers frequently used to identify adenocarcinomas. Once a diagnosis of metastatic carcinoma is established, tissue-specific markers are often selected, based on clinical history, to identify or confirm the primary site of the metastasis.
Wilms tumor gene product (WT1) is a marker that is positively expressed in approximately 90% of primary ovarian carcinomas, particularly in the serous subtype, and has been used to distinguish carcinoma of ovarian origin from carcinoma with other primary sites. However, WT1 expression can also be detected in benign and malignant mesothelial cells, which can potentially cause difficulty in the interpretation of effusion specimens.

Recently, paired box gene 8 (PAX8) was found to be frequently expressed in primary epithelial ovarian carcinoma. PAX8 is a crucial transcription factor for organogenesis of the thyroid gland, kidney, and müllerian system, and PAX8 also regulates WT1 expression. In a study of primary epithelial ovarian carcinomas by Nonaka et al, PAX8 expression was found in 87% of carcinomas overall and in 96% of serous carcinomas (the most common subtype of ovarian carcinoma). However, to our knowledge, only 3 studies have investigated the role of PAX8 immunostaining in the diagnosis of metastatic ovarian carcinoma in serous effusion specimens, and each of those studies involved relatively few cases.

In the present study, we evaluated the value of PAX8 and WT1 immunostaining in confirming the ovarian origin of metastatic carcinoma in 68 serous effusion specimens. We also examined the effect that sample type (ie, cell block or smear) had on the detection rate of each marker.

Materials and Methods

We retrospectively searched our institution’s pathology database for cases with a definitive cytologic diagnosis of metastatic ovarian carcinoma in peritoneal or pleural effusion made between January 2002 and January 2010 at The University of Texas M.D. Anderson Cancer Center, Houston. All patients had surgically confirmed primary ovarian carcinoma before collection of effusion specimens but no other primary carcinoma. Cases with a low quantity of tumor cells in the specimens were excluded from this study.

A total of 68 cases (46 cases of peritoneal fluid and 22 cases of pleural fluid) were identified, and each case was from 1 patient. Direct smears or cell blocks were made during routine patient care. Briefly, direct smears were air dried for Diff-Quik staining (Stat Lab, Lewisville, TX) or fixed in modified Carnoy fixative (6:1 ratio of 70% ethanol/glacial acetic acid) for Papanicolaou staining. For making cell blocks, a cell pellet obtained from centrifugation was fixed in a 1:1 mixture of 95% ethanol and 10% formalin and embedded in paraffin.

Immunostaining for PAX8 and WT1 were performed on cell-block sections (n = 45) or, if a cell-block tissue was not available, on Papanicolaou-stained smears (n = 23). Briefly, the cell-block sections were deparaffinized and rehydrated and then treated with 3% hydrogen peroxide in methanol for 5 minutes to block endogenous peroxidase activity. Antigen retrieval was conducted by steaming the slides for 45 minutes in 10 mmol/L citrate buffer, pH 6.0 (for PAX8), or in Tris-EDTA buffer, pH 8.0 (for WT1). Cell-block sections were incubated for 30 minutes with normal nonimmune serum to eliminate nonspecific staining. For Papanicolaou-stained smears, the antigen-retrieval procedure was identical to that used for cell-block sections. Cell-block sections or smears were incubated with primary antibody against PAX8 (dilution 1:100; Protein Tech, Chicago, IL) or with antibody against WT1 (clone 6F-H2, dilution 1:40; DAKO, Carpinteria, CA) for 15 minutes at room temperature. The immunostain was developed using 3,3′-diaminobenzidine tetrahydrochloride as the chromogen. Slides were counterstained with Mayer hematoxylin. Appropriate negative and positive controls were included. Staining for PAX8 and WT1 was evaluated and scored by 2 pathologists (L.Z. and Y.G.). Nuclear staining in more than 5% of the malignant cells was considered a positive result for the marker. For positive cases, the staining intensity was further graded as weak, moderate, or strong.

This study was approved by the M.D. Anderson Institutional Review Board.

Results

All 68 patients had previously confirmed histologic diagnoses of primary ovarian carcinoma, which included 52 serous carcinomas (46 high grade and 6 low grade), 5 clear cell carcinomas, 1 mucinous carcinoma, 1 malignant mixed müllerian tumor, and 9 carcinomas with mixed components. Of the 9 carcinomas with mixed components, 5 had serous and endometrioid carcinoma components; 1 had serous and transitional carcinoma components; 1 had serous, undifferentiated, and transitional carcinoma components; 1 had clear cell and serous carcinoma components; and 1 had transitional and undifferentiated carcinoma components.

Among the 68 effusion specimens with metastatic ovarian carcinoma, immunostaining showed positive results for PAX8 in 58 cases (85%) and positive results for WT1 in 56 cases (82%; Table 1). Immunostaining showed positivity for both markers in 50 cases (74%) and positivity for either or both markers in 64 cases (94%). In 4 cases (6%), stains were negative for both markers. In the 52 cases of metastatic serous carcinoma, PAX8 was detected in 44 cases (85%) and WT1 in 48 cases (92%); 2 cases showed immunoreactivity only for PAX8, and 6 cases showed immunoreactivity only for WT1. Within this subtype, strong staining intensity was seen more frequently with WT1, and moderate and weak staining intensity was seen more often with PAX8 (Table 1). However, differences in staining intensity did not
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### Table 1
Detection Rate and Staining Intensity of PAX8 and WT1 in Metastatic Ovarian Carcinoma in Serous Effusion Specimens

<table>
<thead>
<tr>
<th>Surgical Diagnosis of Primary Ovarian Tumor</th>
<th>PAX8</th>
<th>WT1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of Positive Cases</td>
<td>Intensity of Expression*</td>
</tr>
<tr>
<td>Serous carcinoma (n = 52)</td>
<td>44 (85)</td>
<td>8</td>
</tr>
<tr>
<td>Clear cell carcinoma (n = 5)</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous carcinoma (n = 1)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Malignant mixed müllerian tumor (n = 1)</td>
<td>1 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoma, mixed type (n = 9)</td>
<td>8 (89)</td>
<td>1</td>
</tr>
<tr>
<td>Total (n = 68)</td>
<td>58 (85)</td>
<td>9</td>
</tr>
</tbody>
</table>

* Values are given as number of cases.

#### Image 1
WT1 and PAX8 staining in serous effusion specimens. **A.** A metastatic serous carcinoma on a cell-block section is positive for PAX8 with moderate staining intensity (×200). **B.** A metastatic serous carcinoma on a smear is positive for PAX8 with strong staining intensity (×400). **C.** A metastatic serous carcinoma on a cell-block section is positive for WT1 with strong staining intensity (×200). **D.** Benign mesothelial cells on a smear are positive for WT1 staining (×200).
considerably affect interpretation of staining result. In the 5 cases of metastatic clear cell carcinoma, PAX8 was expressed in all cases (100%) and WT1 in only 1 case (20%). In the 9 cases from patients whose primary ovarian carcinoma showed mixed components, the detection rate using PAX8 was 8 (89%) of 9 cases, and using WT1, the detection rate was 6 (67%) of 9 cases. One case of malignant mixed müllerian tumor was detected by both markers, and 1 case of mucinous carcinoma was negative for both markers.

Detection rates on the cell blocks and on smears were 91% and 78%, respectively, with PAX8 and 82% and 83%, respectively, with WT1. Notably, in 21 cases (31%), mesothelial cells also demonstrated immunoreactivity for WT1, and no single case showed immunoreactivity for PAX8.

**Discussion**

Serous cavities are common sites of metastases. Ovarian carcinoma is one of the common primary carcinomas; other common sites of primary carcinoma include lung, breast, endometrium, upper gastrointestinal tract, and colorectal region. Immunostaining is frequently required, in conjunction with cytologic features, to confirm or identify the primary site of malignant effusion and the tumor subtype. PAX8 has recently emerged as a surrogate marker for carcinoma of müllerian origin and seems to be more effective than WT1 for the detection of primary ovarian carcinoma, with detection rates of 87% and 63%, respectively. To date, the value of PAX8 in the identification of metastatic ovarian carcinoma in serous effusion has been reported in only 3 small studies. Tong et al examined 31 cases and found that PAX8 was expressed in 71% of the serous effusion specimens of metastatic ovarian carcinoma. McKnight et al examined 41 cases of metastatic ovarian carcinoma (30 serous effusion specimens and 11 fine-needle aspiration specimens) and reported detection rates of 90% with PAX8 and 93% with WT1. Wiseman et al found PAX8 expression in 94% of 34 serous ovarian carcinomas and in all 8 nonserous ovarian carcinomas studied. In our current series of 68 metastatic ovarian carcinomas in effusion specimens, we found comparable detection rates between PAX8 and WT1, 85% and 82%, respectively, for the entire cohort, and 85% and 92%, respectively, for the serous carcinoma subtype.

It is noteworthy that, in the present study, we found that a combination of PAX8 and WT1 staining substantially increased the overall detection rate of metastatic ovarian carcinoma to 94%. This is attributed to the fact that each marker has a different detection rate for various subtypes of ovarian carcinoma. Our data showed that WT1 was superior to PAX8 in the detection of the serous type of metastatic carcinoma. Conversely, PAX8 seemed more effective than WT1 at detecting the clear cell type of metastasis (100% vs 20%) and carcinoma with mixed components (89% vs 67%). These findings are consistent with previous studies in which PAX8 immunoreactivity was detected in most subtypes of primary ovarian carcinoma, whereas WT1 staining was generally negative in nonserous subtypes of primary ovarian carcinoma.

Specifically, Nonaka et al reported positive PAX8 staining in 96% of serous papillary carcinomas, 89% of endometrioid carcinomas, 100% of clear cell carcinomas, and 8% of mucinous carcinomas; positive expression of WT1 in the corresponding tumor types was 87%, 28%, 0%, and 0%. Therefore, in cases in which only 1 stain can be performed (owing to limited sample availability) to confirm an ovarian origin of malignant effusion, a decision to select PAX8 vs WT1 should be made on the basis of the tumor subtype of the primary ovarian carcinoma, if the history is available.

Differences in staining intensity were observed between PAX8 and WT1 staining, mostly in the serous carcinoma subtype. Strong intensity was more frequently seen with WT1, and moderate and weak staining was found more often with PAX8. However, the intensity difference did not considerably influence interpretation. Cell-block material is generally considered the optimal sample type for immunostaining in cytologic specimens because the fixation and staining procedures used for cell-block sections are similar to the procedures used for histologic tissue samples. In previous studies with PAX8 on serous effusion samples, only cell-block tissue was used. However, in routine practice, it is not uncommon for a smear to be the only sample type available, and staining needs to be considered on the smear. We previously showed that testing for estrogen receptor can be reliably performed on cytologic smears, and smears performed equally as well as cell-block sections in terms of estrogen receptor detection rates. To evaluate whether PAX8 and WT1 staining results might be affected by sample type, we included smears in this study and compared the detection rate between smears and cell blocks. We found that the detection rate using WT1 on both sample types was comparable (82% vs 83%); however, the detection rate using PAX8 on cell blocks was higher than that on smears (91% vs 78%). These results indicate that cell blocks and smears can be used for PAX8 and WT1 staining; however, cell block is the preferred sample type for PAX8 staining.

While our results showed that the use of both PAX8 and WT1 staining improves the overall detection rate of metastatic ovarian carcinoma, previous studies indicated that the combination also increases specificity. Metastatic ovarian carcinoma can have morphologic overlap with metastatic carcinoma from other primary sites. When there is a need to distinguish metastatic carcinoma of an ovarian primary site from those of other primaries, especially in workup of malignant effusion specimens in patients with an unknown
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history of malignancy at initial examination, inclusion of both markers in an immunostaining panel will narrow the list of possible primary sites for differential diagnosis. For example, positive immunoreactivity with both PAX8 and WT1 favors a diagnosis of metastatic ovarian carcinoma, although the immunophenotype can also be seen in rarely occurring primary peritoneal serous carcinoma.13,22,23 Negative immunoreactivity with both PAX8 and WT1 can be seen in metastatic carcinoma from lung, gastric, pancreatic, and colorectal primaries.14,22,23,30,31 Furthermore, the same tumor subtype can arise from different primary sites with no discernible morphologic differences. For example, serous carcinoma and endometrioid carcinoma can arise from the ovary or uterus. Inclusion of both WT1 and PAX8 would help to ascertain the primary site in the metastatic setting because WT1 is positive for ovarian serous carcinoma but is usually negative for uterine serous carcinoma.13,32 PAX8 staining alone is unable to make this distinction because PAX8 is positive in the majority of ovarian and uterine adenocarcinomas and their subtypes.19,22 Finally, we and others observed that mesothelial cells showed immunoreactivity with WT1 but not with PAX8.22,23,33 Therefore, inclusion of PAX8 would overcome the drawback of WT1 and facilitate distinguishing metastatic adenocarcinoma from reactive mesothelial cells, an important but sometimes problematic issue if solely cytomorphologic features are used.

PAX8 and WT1 had comparable overall detection rates for metastatic ovarian carcinoma in serous effusion specimens. PAX8 more readily detected clear cell carcinoma than did WT1, but PAX8 was less effective at detecting serous carcinoma. The combination of PAX8 and WT1 immunostains substantially increased the overall detection rate. Both cell-block sections and smears can be used for PAX8 and WT1 staining, but cell-block tissue is the optimal sample type for PAX8 staining.

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References


