Assessment of the Utility of PAX8 Immunohistochemical Stain in Diagnosing Endocervical Glandular Lesions

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• **Context.**—PAX8, a member of the paired-box family of genes, is expressed in many tumors of Mullerian origin. However, it is unclear whether PAX8 is a useful marker in diagnosing endocervical glandular lesions because of limited data.

Objective.—To study the expression of PAX8 in endocervical glandular lesions.

Design.—We first studied a cohort of 29 cervical cone biopsies, followed by a second cohort of 17 cases of endocervical adenocarcinoma and 20 cases of uterine endometrioid adenocarcinoma.

Results.—In the first cohort, we found that PAX8 was expressed in 23 of 23 (100%) benign endocervical glandular epithelium, 15 of 16 (94%) adenocarcinoma in situ, and 21 of 26 (81%) invasive endocervical adenocarcinoma specimens. In the second cohort, endocervical adenocarcinomas were positive for PAX8 in 14 of 17 (82%), strongly and diffusely positive for p16 in 14 of 17 (82%), positive for carcinoembryonic antigen in 12 of 17 (71%), positive for vimentin in 2 of 17 (12%), and positive for estrogen receptor in 7 of 17 cases (41%). Uterine endometrioid cancer was positive for PAX8 in 20 of 20 (100%), weakly and/or patchy positive for p16 in 17 of 20 (85%), positive for carcinoembryonic antigen in 2 of 20 (10%), positive for vimentin in 19 of 20 (95%), and positive for estrogen receptor in 20 of 20 cases (100%).

Conclusions.—PAX8 is expressed in the majority of benign, premalignant, and malignant endocervical glandular lesions. The usefulness of PAX8 in differentiating endocervical from endometrial lesions is limited.

**MATERIALS AND METHODS**

**Case Selection**

The first cohort of 29 cervical cone biopsies, with available blocks showing benign endocervix, AIS, and cancer, were retrieved from 2007 through 2011 following a search of the computerized database. This study was approved by the institutional review board.

In addition, a second cohort of 17 cervical cone or hysterectomy specimens with a diagnosis of ECA of usual or endometrioid type and 20 hysterectomy specimens with a diagnosis of uterine endometrioid adenocarcinoma were retrieved from 2007 through 2011 following a search of our computerized database. This study included only uterine adenocarcinoma of endometrioid type, and excluded other high-grade histologic types, such as serous, clear cell, carcinosarcoma, and undifferentiated carcinomas. All slides were reviewed and the diagnosis was confirmed.

**Immunohistochemistry**

Immunohistochemical staining was performed using Ventana BenchMark Autostainer (Ventana Medical System, Tucson, Arizona). Selected paraffin blocks from each case were cut at 5-μm thickness and dried at 60°C for 1 hour. Following deparaffinization, rehydration, and blockage of endogenous peroxidase, slides were treated with antigen retrieval solution followed by incubation with primary antibodies against PAX8 (363A-18, rabbit polyclonal, ready to use, Cell Marque, Rocklin, California), CEA (clone TF3H8-1, ready for use, Cell Marque, Rocklin, California), and estrogen receptor (ER), progesterone receptor (PR), and p16. In addition, human papilloma virus in situ hybridization may also be added to this panel. In our study, we evaluated whether it is helpful to include PAX8 in this IHC panel.
strong nuclear staining in 23 of 23 (100%), 15 of 16 (94%), and invasive cancers demonstrated in 23, 16, and 26 of the total 29 cases, respectively. Overall, benign endocervix, AIS, and invasive cancers demonstrated strong nuclear staining in 23 of 23 (100%), 15 of 16 (94%), and 21 of 26 (81%) cases, respectively (Figure 1, D through F). Of 5 cases with ECA negative for PAX8, the histotypes included endocervical type (n = 2), endometrioid type (n = 1), and poorly differentiated adenocarcinoma (n = 2). In one of the cases of poorly differentiated adenocarcinoma, both in situ and invasive components were identified, and both were negative for PAX8.

### Comparison of PAX8 Expression in ECA Versus Uterine Endometrioid Adenocarcinoma

The 20 patients with UEC ranged in age from 41 to 85 years (mean, 67 years; median, 72 years) and their tumors included International Federation of Gynecology and Obstetrics grade 1 (n = 15), grade 2 (n = 3), and grade 3 (n = 2). The 17 patients with ECA ranged in age from 30 to 72 years (mean, 46 years; median, 45 years).

The results of immunohistochemical staining for PAX8, p16, CEA, vimentin, and ER are summarized in Table 2. Figure 2 shows a case of ECA (Figure 2, A) that demonstrated nuclear positivity for PAX8 (Figure 2, B), nuclear and cytoplasmic positivity for p16 (Figure 2, C), and cytoplasmic positivity for CEA (Figure 2, D). The tumor cells were negative for vimentin (Figure 2, E) and ER (Figure 2, F). A case of endometrial adenocarcinoma (Figure 2, G)

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### Statistical Analysis

Statistical analyses were performed with SPSS 19.0 (IBM, Armonk, New York). We used χ² tests to compare the IHC results of different groups. P-values less than .05 were considered statistically significant.

### RESULTS

#### PAX8 Expression in Benign Endocervix, AIS, and Invasive ECA

Of 29 patients with ECA or AIS, the histotypes included endocervical type or usual type (n = 17), endometrioid type (n = 7), and poorly differentiated adenocarcinoma (n = 5). Benign endocervix (Figure 1, A and D), AIS (Figure 1, B and E), and ECA (Figure 1, C and F) were present for evaluation in 23, 16, and 26 of the total 29 cases, respectively. Overall, benign endocervix, AIS, and invasive cancers demonstrated strong nuclear staining in 23 of 23 (100%), 15 of 16 (94%), and 21 of 26 (81%) cases, respectively (Figure 1, D through F). Of 5 cases with ECA negative for PAX8, the histotypes included endocervical type (n = 2), endometrioid type (n = 1), and poorly differentiated adenocarcinoma (n = 2). In one of the cases of poorly differentiated adenocarcinoma, both in situ and invasive components were identified, and both were negative for PAX8.

### Table 1. Summary of Results of PAX8 Expression by Immunohistochemistry in Endocervical Adenocarcinoma (ECA) and Adenocarcinoma In Situ (AIS)

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Cases, No. and Type</th>
<th>PAX8 Positive, No./Total (%)</th>
<th>PAX8 Antibody Dilution</th>
<th>Criteria for Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacha et al,1 2011</td>
<td>6 ECA</td>
<td>5/6 (83)</td>
<td>Rabbit polyclonal, Biocare Medical, Concord, California</td>
<td>1:500</td>
</tr>
<tr>
<td>Tong et al,2 2011</td>
<td>5 ECA</td>
<td>0/5 (0)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:100</td>
</tr>
<tr>
<td>Heidarpour et al,4 2014</td>
<td>2 ECA</td>
<td>0/2 (0)</td>
<td>Mouse polyclonal, Ab53490, Abcam, Cambridge, United Kingdom</td>
<td>1:200</td>
</tr>
<tr>
<td>Laury et al,5 2011</td>
<td>5 AIS</td>
<td>5/5 (100)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:800</td>
</tr>
<tr>
<td>Shukla et al,6 2013</td>
<td>66 AIS</td>
<td>64/66 (97)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:200</td>
</tr>
<tr>
<td>Goyal et al,7 2014</td>
<td>15 ECA</td>
<td>13/15 (87)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:200</td>
</tr>
<tr>
<td>Danialan et al,8 2013</td>
<td>8 AIS</td>
<td>Moderate faint</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:100</td>
</tr>
<tr>
<td>Yemelyanova et al,9 2014</td>
<td>21 ECA</td>
<td>18/21 (86)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>1:400</td>
</tr>
<tr>
<td>Current study</td>
<td>16 AIS</td>
<td>1/16 (6.25)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td>Ready to use</td>
</tr>
<tr>
<td></td>
<td>43 ECA</td>
<td>35/43 (81)</td>
<td>Rabbit polyclonal, Proteintech, Chicago, Illinois</td>
<td></td>
</tr>
</tbody>
</table>

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*Scores of extent (0, no staining; 1, 25% cells positive; 2, 50% cells positive; 3, >50% cells positive) and intensity of immunohistochemical staining (0, negative; 1, weak; 2, moderate; 3, strong) were combined. A combined score of >2 was considered positive.

*The extent (S) and intensity (Int) of immunohistochemical staining were scored as follows: S0, no immunoreactivity; S1, up to 10% of cells showing immunoreactivity; S2, between 10% and 50% of cells showing immunoreactivity (moderate); S3, >50% of cells showing immunoreactivity (diffuse); Int0, no staining; Int1, mild intensity; Int2, moderate intensity; Int3, strong intensity.

*Scores of extent (negative, 0%; 1+, 1%–25%; 2+, 25%–50%; 3+, 51%–75%; 4+, 76%–100% positive cells) and intensity of immunohistochemical staining (0, no staining; 1, weak; 2, moderate; 3, strong) were multiplied to get an immunohistochemical composite score.
demonstrated nuclear positivity for PAX8 (Figure 2, H), cytoplasmic positivity for vimentin (Figure 2, K), and nuclear positivity for ER (Figure 2, L). Rare cells were positive for p16 (Figure 2, I). The tumor cells were negative for CEA (Figure 2, J). Statistically significant differences of p16, CEA, vimentin, and ER immunostains were found between these 2 groups ($P < .001$), but no statistically significant difference for PAX8 was found ($P = .05$).

**COMMENT**

Our results showed that the majority of benign and malignant endocervical glandular lesions demonstrated positive nuclear staining for PAX8. Earlier studies showed that PAX8 positivity ranges from 97% to 100% in AIS and 0% to 87% in ECA. The discrepancies among various studies may be attributable to several factors; the first is small sample size. Our study included 43 ECA (combined 2 cohorts) and 16 AIS, and is one of the largest studies. Second, there was no clear definition of what was considered a positive stain in the earlier studies (Table 1). We defined 10% or more of cells with positive nuclear staining of any intensity as positive. Third, anti-PAX8 antibodies from different companies or different concentrations of antibodies were used in each study (Table 1).

In a recent study, Danialan et al suggested that PAX8, together with IMP3 (insulin-like growth factor-II mRNA-binding protein 3), may be used in differentiating benign endocervical glandular lesions from malignant lesions. They reported that PAX8 expression was strong and diffuse in benign conditions, and became focal and weaker in AIS and ECA. Our study showed a similar trend of loss of PAX8 expression in AIS and adenocarcinoma. The problem of their study was poor reproducibility of interpreting staining intensity. It may not be cost-effective to routinely perform IHC for the purpose of differentiating precancers and cancers from their benign mimics, because the latter can be excluded using morphology in the majority of cases.

Differentiating ECA from UEC can sometimes be challenging because of a significant degree of morphologic

**Table 2. Results of Immunohistochemical Staining of Endocervical Carcinoma (ECA) Versus Uterine Endometrioid Carcinoma (UEC)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Result</th>
<th>ECA, No./Total (%)</th>
<th>UEC, No./Total (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX8</td>
<td>Positive</td>
<td>14/17 (82.4)</td>
<td>20/20 (100)</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3/17 (17.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>p16</td>
<td>Strong/diffuse</td>
<td>14/17 (82)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weak/patchy</td>
<td>3/17 (18)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3/17 (18)</td>
<td>17/20 (85)</td>
<td></td>
</tr>
<tr>
<td>CEA</td>
<td>Positive</td>
<td>12/17 (71)</td>
<td>2/20 (10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>5/17 (29)</td>
<td>18/20 (90)</td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td>Positive</td>
<td>2/17 (12)</td>
<td>19/20 (95)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>15/17 (88)</td>
<td>1/20 (5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ER</td>
<td>Positive</td>
<td>7/17 (41)</td>
<td>20/20 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10/17 (59)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CEA, carcinoembryonic antigen; ER, estrogen receptor.
overlap, especially in biopsy or curettage material. The current IHC panel includes CEA, vimentin, ER/PR, and p16; however, none of these markers are specific when used alone. Yemelyanova et al recently reported that the majority of ECAs expressed PAX8 at significantly lower levels when compared with endometrial adenocarcinomas, suggesting its diagnostic value. Our result did not find a significant difference in PAX8 expression level in UEC versus ECA by IHC. As mentioned, poor reproducibility of interpreting staining intensity limits its value for differential diagnosis. Therefore, the usefulness of PAX8 in differentiating endocervical from endometrial lesions is limited. Furthermore, our data supported that the immunohistochemical panel that includes CEA, vimentin, estrogen, and ER/PR is useful in differentiating UEC from ECA.

On the other hand, PAX8 may be useful in differentiating tumors of non-Müllerian origin from those of Müllerian origin. However, our data showed loss of expression of PAX8 in a small number of endocervical AIS (1 of 16, 6%) and ECA (8 of 43, 19%). Thus, pathologists should be aware of this pitfall when using PAX8 immunohistochemical stain to rule out tumors of Müllerian origin.

Figure 2. A case of endocervical adenocarcinoma (A) demonstrated nuclear positivity for PAX8 (B), nuclear and cytoplasmic positivity for p16 (C), and cytoplasmic positivity for carcinoembryonic antigen (CEA) (D). The tumor cells were negative for vimentin (E) and estrogen receptor (ER) (F). A case of endometrial adenocarcinoma (G) demonstrated nuclear positivity for PAX8 (H), cytoplasmic positivity for vimentin (K), and nuclear positivity for ER (L). Rare cells were positive for p16 (I). The tumor cells were negative for CEA (J) (hematoxylin-eosin, original magnification ×100 [A and G]; original magnification ×100 [B through F and H through L]).
It is worth mentioning that although secondary tumors of the cervix and endocervix are uncommon, they must be considered in the differential diagnosis. The most common primary tumors to metastasize to the cervix are from endometrium and ovary; rarely, metastases are from gastrointestinal tract, breast, and kidney. Therefore, metastatic tumors should always be in the differential diagnosis if the tumor cells demonstrate negative nuclear staining for PAX8. PAX8 is usually expressed in Müllerian tumors and not expressed in many non-Müllerian tumors, including breast, colon, and gastroesophageal cancers.\(^1,3\)

Even though PAX8 can also be expressed in kidney, thyroid, and thymus neoplasms, usually these tumors can be differentiated from Müllerian tumors based on morphologic features.\(^1\)

During the formation of the reproductive system, para-mesonephric ducts (also called Müllerian ducts) differentiate into the oviduct, uterus, cervix, and upper one-third of the vagina.\(^12\) This may explain why endocervix expresses PAX8, similar to other organs of Müllerian origin. Interestingly, PAX8 has also been reported to be expressed in endocervicosis in lymph node, which is a Müllerian inclusion of mucinous endocervical type.\(^13\) In addition, recent reports have shown that PAX8 is expressed in lesions of Wolffian/mesonephric origin, including mesonephric remnants, mesonephric hyperplasia, mesonephric adenocarcinoma, and carcinosarcoma.\(^7,8,14,15\) This supports that PAX8 is expressed in tumors of both Müllerian and Wolffian origins.

In summary, PAX8 is expressed in the majority of benign, premalignant, and malignant endocervical glandular lesions. The usefulness of PAX8 in differentiating endocervical from endometrial lesions is limited. Because a small percentage of endocervical neoplasms are negative for PAX8, pathologists should be aware of this pitfall when using PAX8 immunohistochemical stain to rule out tumors of Müllerian origin.

### References


### CAP16 Abstract Program Submission Dates Announced

Abstract and case study submissions to the College of American Pathologists (CAP) 2016 Abstract Program will be accepted beginning on Friday, January 8 through 5 p.m. Central time, March 11, 2016.

Accepted submissions will appear on the *Archives of Pathology & Laboratory Medicine* Web site as a Web-only supplement to the September 2016 issue. The CAP16 meeting will be held from September 25 to 28 in Las Vegas, Nevada.

Visit the CAP16 Web site (<www.cap.org/cap16>) for additional abstract program information as it becomes available.