Expression of oct-4 and c-kit antigens in endometriosis

The objective of this study was to test the expression of the oct-4 and c-kit, both markers of stem cells, in the ectopic endometrial tissue of endometriotic lesions of women with severe endometriosis. Our findings show that ectopic epithelial cells express oct-4 and c-kit and this suggests that the ectopic endometrium in endometriosis has a stem cell origin and could explain the possible progression to ovarian cancer. (Fertil Steril 2011;95:1171–3. ©2011 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, stem cells, oct-4, c-kit

Endometriosis affects women during their reproductive age and is often associated with infertility. It is characterized by the presence of the endometrial tissue outside of the uterine lumen (1, 2) and it is generally accepted that tubal reflux during menstrual shedding may lead to the implantation of endometrial cells in the pelvic zone, with the subsequent development of the disease.

The risk of ovarian carcinoma in patients with endometriosis has been noted, especially in cases of clear cell cancer, which is supposed to develop from endometriotic implants in the ovary (3–5). Several investigators have suggested that endometriosis is a precancer disease, in which endometrial ectopic cells may be cells with cancer-like characteristics that differentiate into neoplastic cells (6, 7).

Oct-4 is a transcription factor, molecular marker for pluripotent cells and plays an essential role in maintaining the undifferentiated state needed for cell pluripotency (8). It is well known that oct-4 is expressed in embryonic stem cells, germ cells, and in the embryo at various stages of development. This embryonic transcriptional regulator is expressed in several cancers such as osteosarcoma, prostate cancer, cervix carcinoma, and lung cancer (9). Oct-4 has been found in the epithelial cells of normal endometrium (10–15).

The c-kit is a proto-oncogene that encodes for a tyrosine kinase receptor, of which the ligand is the so-called stem cell factor (14). Changes in the expression of the proto-oncogene c-kit are associated with aggressive behavior of both benign and malignant tumors, but there are few data on c-kit expression in endometriosis (16).

In the present study we analyzed the concurrent expression of the oct-4 and c-kit antigen in the eutopic and ectopic endometrium

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3,3’-diaminobenzidine was used as a chromogen. Counterstaining was performed with Meyer’s hematoxylin. The positive controls were endometrial tissue that showed expression for oct-4 and c-kit. Negative controls were performed by replacing the primary antibody with mouse immunoglobulin at the same concentration as the primary antibody. A semiquantitative analysis of specific staining was performed using the Histochemical SCORE (HSCORE) system, according to McCarty et al. (19), to score the immunohistochemistry slides and perform statistical analysis. The HSCORE was calculated using the following equation: 

$$HSCORE = \sum P(i+1)$$

where i is the intensity of staining with a value of 1, 2, or 3 (weak, strong, or very strong) and Pi is the percentage of stained cells for each intensity, varying from 0%–100%. For all samples, 10 microscopic fields were counted by two of the authors independently in each slide.

The intraobserver and interobserver coefficient of variation (CV) were 3.4% and 4.2%, respectively. Three slides from each sample were checked for both antigens and each observer was blinded as to the sample. The slides were numbered progressively by a technician, who reported on a separate worksheet the number of the slide and the corresponding name of the patient or control. The slides were numbered progressively as appropriate.

In patients with endometriosis-associated ovarian cancer, benign-appearing ovarian masses are typically present several years before the diagnosis of the cancer. A slightly elevated CA-125 level is also typically present many years before the diagnosis in these patients (21). Ovarian endometrioma could be viewed as a neoplastic process, particularly in perimenopausal women. Understanding the mechanisms of the development of endometriosis and elucidating its pathogenesis and pathophysiology are intrinsic to the prevention of endometriosis-associated ovarian cancer and the search for effective therapies.

Therefore, the expression of these antigens may also show the presence of a subset of stem-like cells inside the ectopic epithelial cells of endometriosis lesions, which may have self-renewing characteristics and being the reservoir cells that allow the disease to remain active (22–24). The oct-4 and c-kit antigens are expressed in the stem cells, and their expression in the ectopic epithelial cells is also circumstantial evidence that endometriosis may be due to a subset of mesenchymal stem cells that proliferate and differentiate in the endometriotic cells with self-renewing capacity. Recently some evidence has been reported that endometriosis has its origins from mesenchymal stem cells, such as the presence of endometriotic cells with Y chromosome in women transplanted with stem cells for leukemia.

Our results support a previous study (8) and demonstrated that the increased expression of oct-4 and c-kit indicate the presence of stem-like cells. These cells can induce the development of disease and the progression to ovarian cancer.

### Table 1A

<table>
<thead>
<tr>
<th>Results for oct-4.</th>
<th>% Positive</th>
<th>Positive</th>
<th>H SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopic endometrium</td>
<td>32.3 ± 7.8</td>
<td>++</td>
<td>98.5 ± 24.7</td>
</tr>
<tr>
<td>Eutopic endometrium</td>
<td>3.2 ± 1.1</td>
<td>+ –</td>
<td>12.3 ± 2.8</td>
</tr>
<tr>
<td>Epithelial cells of controls</td>
<td>2.8 ± 0.9</td>
<td>+ –</td>
<td>10.5 ± 3.1</td>
</tr>
</tbody>
</table>

### Table 1B

<table>
<thead>
<tr>
<th>Results for c-kit.</th>
<th>% Positive</th>
<th>Positive</th>
<th>H SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopic endometrium</td>
<td>58.6 ± 17.1</td>
<td>++</td>
<td>128.5 ± 24.7</td>
</tr>
<tr>
<td>Eutopic endometrium</td>
<td>21.2 ± 10.1</td>
<td>+</td>
<td>62.3 ± 21.8</td>
</tr>
<tr>
<td>Epithelial cells of controls</td>
<td>22.7 ± 9.9</td>
<td>+</td>
<td>59.5 ± 25.3</td>
</tr>
</tbody>
</table>

Statistical analysis was performed with the SPSS statistical package 16 (Chicago, IL), using the Mann-Whitney sum rank test as appropriate. \(P<.05\) was considered statistically significant.
REFERENCES