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 of women affected by endometriosis throughout the menstrual cycle. This was to evaluate whether in endometriotics lesions there are undifferentiated cells with cancer-like characteristics.

Endometrial tissue specimens were obtained from 33 women who underwent laparoscopic surgery for severe endometriosis according to the revised criteria of the American Society of Reproductive Medicine (17). The surgical procedures were carried out in the Department of Obstetrics and Gynecology of the S. Andrea Hospital, Rome, Italy, from September 2008 through May 2009. Patients were counseled about the nature of the study and gave written informed consent. Institutional Review Board approval was obtained.

Samples were obtained from the endometrium of the uterus, ovarian endometriomas, and peritoneal implants. A total of 33 eutopic endometria, 30 ovarian endometriomas, 10 peritoneal implants, and 5 adhesions were collected from patients with endomtriosis. Furthermore, endometria of 68 healthy women in different phases of the menstrual cycle, obtained during hysteroscopy procedures, were used as controls.

Biopsy samples were fixed in 10% neutral-buffered formalin at 4°C overnight and were subsequently paraffin embedded. Before performing immunohistochemistry, sections of the tissues were stained with hematoxylin- eosin (H & E) to select tissues with ectopic epthelial cells. Serial sections of the same selected samples, 5-μm thick, were used for immunohistochemistry. Commercially available monoclonal antibodies (mAb) were used for the detection of oct-4 and c-kit (Santa Cruz, Santa Cruz, CA). Immunohistochemistry was performed according to a previously described method (18, 19). Briefly, tissue sections were dewaxed and rehydrated conventionally and the quenching of endogenous peroxidase was achieved by incubation with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature. All tissue sections were exposed to a nonimmune block with normal horse serum for 30 minutes at room temperature. Incubations with the first antibody were carried out at 4°C overnight with a dilution of 1:100 for the monoclonal mouse anti-human Fas-L and with a dilution of 1:50 for the monoclonal mouse anti-human Fas antigen. Thereafter tissue sections were labeled with an avidin-biotin-peroxidase detection system Vectastain (Vector Laboratories, Burlington, VT). Each step was followed by meticulous washing with phosphate-buffered saline (PBS). Finally,

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TABLE 1A

Results for oct-4.

	% Positive	Positive	H SCORE
Ectopic endometrium	32.3 ± 7.8	++	98.5 ± 24.7
Eutopic endometrium	3.2 ± 1.1	+ −	12.3 ± 2.8
Epithelial cells of controls	2.8 ± 0.9	+ −	10.5 ± 3.1

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TABLE 1B

Results for c-kit.

	% Positive	Positive	H SCORE
Ectopic endometrium	58.6 ± 17.1	++	128.5 ± 24.7
Eutopic endometrium	21.2 ± 10.1	+	62.3 ± 21.8
Epithelial cells of controls	22.7 ± 9.9	+	59.5 ± 25.3

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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 = ΣPi(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. Only after the slide was analyzed by the two different observers and scored for the HSCORE, each value was reported on the worksheet against the corresponding name of the patient for each slide number. The HSCORE analysis was performed separately for the component of endometrium, glandular and stromal cells, and for the ectopic tissue (each observer performed four different HSCOREs for each slide).

In control tissues the epithelial cells of eutopic endometrium showed a 3.2% nuclear staining for oct-4 and a 3.8% staining in the stromal cells. In the eutopic endometria of women with endometriosis a similar pattern of staining was observed, with 3.5% and 3.6% in epithelial and stromal cells, respectively, without any statistically significant difference. Ectopic epithelial cells showed nuclear staining for oct-4 in all samples with 32.3%, both in ovarian and peritoneal lesions, with a statistically significant difference of $P<.001$. The stromal cells were not stained for oct-4. Results are shown in Table 1A.

The epithelial cells of eutopic endometrium in control tissues showed the staining for c-kit antigen on the cellular membranes in 21.2%, whereas the stromal cells were mostly negative. In the eutopic endometria of women with endometriosis a similar pattern of staining was observed, with 22.7% of epithelial cells stained and no staining in the stromal cells without any statistically significant difference. Ectopic epithelial cells (58.6%) were stained for c-kit in all samples both in ovarian and peritoneal lesions, with a statistically significant difference of $P<.001$. The stromal cells were not stained for c-kit. Results are shown in Table 1B.

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.

In the present study we have shown that the ectopic endometrial cells express the antigens oct-4 and c-kit, which are generally considered markers of undifferentiated cells. The expression of these antigens in ectopic epithelial cells showed that these cells, at least in part, may represent a cell subset with neoplastic potential, and consequently, they may develop into cancerous cells. Recent evidence suggests that a subset of cells within a tumor have "stem-like" characteristics. These tumor-initiating cells, distinct from nonmalignant stem cells, show low proliferative rates, high self-renewing capacity, propensity to differentiate into actively proliferating tumor cells, resistance to chemotherapy or radiation, and they are often characterized by an elevated expression of the stem cell surface marker CD133 (20).

In ectopic endometrial cells the expression of oct-4 and c-kit may be associated with similar mechanisms. There is further circumstantial evidence that women with endometriosis may be at risk for ovarian cancer.

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.

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