Immunohistochemical study of osteopontin and L-selectin in a rat endometriosis model and in human endometriosis

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Objective: To investigate the localization of the proteins osteopontin (OPN) and L-selectin (SELL).
Design: Retrospective nonrandomized immunohistochemical study in a surgically induced rat model of peritoneal endometriosis and in samples of human endometriotic lesions of ovaries.
Setting: Department of gynecology in a university hospital.
Patient(s): A rat endometriosis model was induced in 10 8-week-old SLC-Sprague-Dawley rats by surgical autotransplantation of uterus. Fourteen premenopausal women with histologically diagnosed endometriosis were selected (mean age, 39 y; range, 25–53 y). Twenty endometriotic specimens were obtained from 14 patients who underwent laparoscopic surgery for enlarged endometriotic cysts.
Intervention(s): Histopathological examination of endometriotic ovarian specimens for OPN and SELL expression by immunohistochemistry.
Main Outcome Measure(s): Demonstration of the immunoreactive staining of OPN and SELL expressions in tissues of a rat endometriosis model and of human endometriosis.
Result(s): In both tissues from a rat endometriosis model and from human endometriosis, OPN was stained more prominently in glandular epithelium than in interstitial space, whereas SELL stained more prominently in interstitial space (macrophages and lymphocytes) than in epithelium. The staining pattern of OPN in ectopic endometriotic lesions was very similar to that in eutopic normal human endometrium in the secretory phase.
Conclusion(s): These results suggested important roles for OPN and SELL in the pathogenesis of endometriosis. (Fertil Steril® 2007;88(Suppl 2):1207–11. ©2007 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, osteopontin, l-selectin, tissue remodeling

The pathogenesis of endometriosis is still poorly defined, although many hypotheses have been proposed (1, 2). In our study published elsewhere, we induced a rat endometriosis model and subjected it to transcriptomics to examine the molecules that are related to endometriosis (unpublished data). Transcriptomics demonstrated up-regulation of the expression of 74 genes. Among them, we focused on two molecules, osteopontin (OPN) and L-selectin (SELL), because these molecules are related to cell adhesion and inflammation, which may be involved in endometriosis.

Osteopontin is a 70-kDa secreted glycosylated phosphoprotein that was originally isolated from bone matrix (3) and has been found in a wide variety of tissues or substances, including milk, urine, kidney, secretory glands, and some tumor tissues (4, 5). It is secreted not only from luminal epithelium but also from interstitial cells such as macrophages and lymphocytes. Osteopontin binds especially to $\alpha v\beta 3$, a kind of integrin, and relates to cell adhesion and migration (6, 7). In the field of gynecology, OPN is expressed in endometrium in the mid and late secretory phases (8). Lessey (9) reported that $\alpha v\beta 3$ expression is reduced in endometrium from women with endometriosis, whereas OPN expression is unaffected. But there are no data on the relation between endometriotic tissue and OPN.

L-Selectin is a 65- to 75-kDa cell adhesion glycoprotein that is a cell surface component and was originally isolated from lymphocytes (10). This molecule plays an important role in lymphocyte adhesion to vascular endothelium at the sites of inflammation, which is immunologically called the *rolling phenomenon*, and then enables lymphocytes to migrate from the blood stream to the interstitial space (11). In the field of gynecology, SELL is related to trophoblast adhesion to the uterine wall, which is the requisite first step of implantation and placentation (12). Kao et al. (13) reported that *N*-acetylglucosamine-6-*O*-sulfotransferase (important in synthesis of SELL ligands) was down-regulated in

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endometrium from women with endometriosis, but there are no data on the relation between endometriotic tissue and SELL, just as with OPN.

We conducted an immunohistochemical study to examine the localization of OPN and SELL on tissues of a rat endometriosis model and of human endometriosis.

MATERIALS AND METHODS

Rat Endometriosis Model

For the induction of an endometriosis model, 10 SLC-Sprague-Dawley rats (8 wk old) were maintained on a 12:12 hour light–dark schedule for 1 week.

Details have been described elsewhere (14) of the surgical technique of autotransplanting 5 mm × 5 mm of uterine tissue. We used the same technique with minor modifications (15). Uterine tissue was autotransplanted to bilateral peritoneum. Five rats were chosen as an endometriosis model, and the other five rats were chosen as a control model without autotransplanting of uterine tissue. The induced endometrioses were obtained from five anesthetized endometriosis-model rats, 7 days after uterine autotransplantation. Eutopic endometrium was obtained from five anesthetized control model rats. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. After resection, all samples were fixed in 10% buffered formalin and embedded in paraffin for immunohistological examination.

Tissue Samples of Human Endometriosis

Twenty endometriotic lesions were obtained from the premenopausal Japanese women (mean age, 39 y; range, 25–53 y), who underwent laparoscopic cystectomy for enlarged chocolate cysts (n = 20). Collection of human materials for this study was approved by the Committee for the Protection of Human Subjects at the Jichi Medical School. Samples from five patients who underwent hysterectomy as a result of uterine cervical cancer with carcinoma in situ were chosen as a control section of human eutopic endometrium. Three of them were in proliferative phase, and the other two specimens were in secretary phase. Operative findings confirmed no endometriosis in a control group.

Immunohistochemistry

Immunohistochemistry for OPN and SELL was performed on a rat endometriosis model and on tissues of human endometriosis to study the localization of these proteins. Eutopic human endometrial tissues without endometriosis in the proliferative and secretory phase were used as control tissues. The anti-OPN antibody (1B20; American Research Products, Inc., Belmont, MA) is a monoclonal mouse IgG_1 antibody that has been purified (protein A chromatography) from hybridoma supernatant against the synthetic peptide of the C-terminus of OPN of human origin. The anti-SELL antibody (lam1-116; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) is a monoclonal mouse IgG_{2a} antibody that is raised against the lectin domain of SELL of mouse origin.

Paraffin-embedded sections were deparaffinized in xylene and rehydrated in ethanol. The antigen retrieval method was performed only for the SELL antibody, by microwaving in citrate buffer (0.1 M citrate acid and 0.1 M natrium citrate, pH 6.0; microwave, 500 W for 5 min). Endogenous peroxidase activity was quenched with 3% hydrogen peroxidase for 5 minutes.

Each primary antibody was serially diluted in a solution of phosphate-buffered saline containing 1% bovine serum albumin and 0.02% sodium azide. Tissue sections were incubated with primary antibody for 1 hour at room temperature at the following dilutions: 1B20, 1:5,000, and lam1-116, 1:100. Negative control sections were treated without primary antibodies but with nonimmune serum and secondary antibody. Subsequently, sections were washed with phosphate-buffered saline and incubated with secondary antibody (anti-mouse and anti-rabbit: Dako, Kyoto, Japan) for 45 minutes at room temperature. After rinsing with phosphate-buffered saline, the immunoreactive antigen was visualized by using liquid 3,3′-diaminobenzidine as the chromogen (Dako). Slides were counterstained by using hematoxylin, followed by dehydration in a graded series of ethanols and clearing in xylene.

All slides were evaluated by experienced gynecologists (H.F., S.N.) who were blind to the study manipulations. The evaluation of the staining intensities was divided into three ranges: − (<5%), + (5∼50%), and ++ (>50%). Table 1 shows the list of the samples in the study.

RESULTS

Pathologic Features of Autotransplanted Peritoneal Lesions of Rat Endometriosis Model

Seven days after uterine autotransplantation, hematoxylin staining showed that the uterine implants had grown into ellipsoidal cystic structures that were composed of endometriotic cysts, stroma, and fibrotic interstitium, with some inflammatory cells. Endometriotic cysts were detected in all peritoneal lesions (n = 5) in the rat endometriosis model. Fibrotic change also was detected surrounding these cysts.

Pathologic Features of Human Endometriotic Tissue

Hematoxylin staining showed endometriotic cysts with epithelial cells and stroma. In the interstitial area around endometriotic cysts, fibrosis and smooth muscle metaplasia were detected with some inflammatory cells, including lymphocytes and macrophages.

Immunostaining Pattern of OPN

No immunostaining was detected in either epithelial or interstitial cells in rat eutopic endometrium. In the examination of a rat endometriosis model, intense immunostaining was