Immunohistochemical detection of heparanase-1 expression in eutopic and ectopic endometrium from women with endometriosis

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Objective: To investigate the expression of heparanase-1, an endoglycosidase that degrades heparan sulfate proteoglycans, in eutopic and ectopic endometrial tissues from women with endometriosis.
Design: An immunohistochemical study.
Setting: Academic research laboratory and a private infertility clinic affiliated with a university medical center.
Patient(s): Premenopausal women undergoing laparoscopy for endometriosis.
Intervention(s): None.
Main Outcome Measure(s): Expression of heparanase-1 analyzed by immunohistochemical staining in 91 eutopic and 14 ectopic endometrial specimens.
Result(s): We found that 17% (4/24) of the eutopic endometrial specimens in the early proliferative phase and 32% (9/28) of the samples in the midproliferative phase were heparanase-1 positive. However, ≥80% of eutopic endometrial specimens at late proliferate phase and at luteal phase were heparanase-1 positive. Twelve of 14 ectopic endometriotic specimens stained heparanase-1 positive. Comparison of heparanase-1 expression in paired eutopic and ectopic endometrial tissues revealed that 5 of 6 ectopic specimens in the early proliferative phase were heparanase-1 positive, whereas only 1 eutopic specimen was heparanase-1 positive. In comparison with our recent study of heparanase-1 expression in normal endometrium, we found that there was no significant difference in heparanase-1 expression in the eutopic endometrium from women with or without endometriosis.
Conclusion(s): Heparanase-1 was differentially expressed in the eutopic endometrium in the different menstrual phases. Heparanase-1 was highly expressed in the ectopic endometriotic lesions regardless of their menstrual phases, suggesting that the local environment is responsible for increased heparanase-1 expression. (Fertil Steril® 2007;88:1304–10. ©2007 by American Society for Reproductive Medicine.)

Key Words: Heparanase-1, endometrium, endometriosis

Regrowth and tissue remodeling of the endometrium in a normal, unfertile menstrual cycle are coordinated destructive and reconstructive processes, manifested by constant histologic and cytologic changes in both endometrial epithelium and stroma (1, 2). The main driving forces of these changes are the ovarian hormones, estrogen (E) and progesterone (P) (1, 2). In the absence of blastocyst implantation, progesterone (P) production ceases in the late secretory phase, followed by breakdown and collapse of the functionalis layer of the endometrium at menstruation. The shedding of the endometrium is mediated by increased expression and activation of the matrix metalloproteinases, mainly matrix metalloproteinase-3, matrix metalloproteinase-1, and matrix metalloproteinase-9 (1, 2), which degrade the protein components of the endometrial extracellular matrix. These enzymes are regulated by steroid hormones, in particular P, which normally suppresses the expression of these matrix metalloproteinases in the early secretory and midsecretory phases (1, 2). The decline of circulating P levels and its receptor expression in the endometrium in the late secretory phase leads to increased matrix metalloproteinase expression, subsequently leading to the degradation of the extracellular matrix and initiation of menstruation.

At the time of menstruation, most shed endometrial fragments are expelled with the menstrual flow, but some are refluxed through the fallopian tubes into the peritoneal cavity. The ectopic endometrial tissues normally undergo necrosis or apoptosis and are further cleared by macrophages in healthy women. The endometrial fragments that cannot be cleared become ectopic lesions that attach to the peritoneal surface by expressing adhesion molecules and become vascularized

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by angiogenesis (3–7). Though the pathogenesis of endometriosis is poorly understood, ample evidence indicates that E_2 plays a critical role (see review by Bulun et al. [8]). Inhibitors of E_2 biosynthesis such as anastrozole and letrozole are effective in treating endometriosis in both postmenopausal and premenopausal women (8).

Heparanase-1 is an endoglycosidase that specifically degrades heparan sulfate proteoglycans (9–12). Heparanase-1 expression is up-regulated in a variety of malignancies and plays an important role in tumor angiogenesis and metastasis. Heparanase-1 stimulates tumor angiogenesis by releasing growth factors such as fibroblast growth factors and vascular endothelial cell growth factors stored (13–20) in the extracellular matrix and inducing the expression of endothelial cell growth factors (21) and cyclooxygenase-2 (22). Heparanase-1 promotes tumor metastasis by degrading heparan sulfate proteoglycans in the extracellular matrix and basement membrane, allowing tumor cells to spread to a distant site (9, 10, 13, 17, 19, 23).

Remarkably, heparanase-1 is also highly expressed in villous trophoblasts of both the human and bovine placenta (24–27). It is presumed that heparanase-1 expression may assist trophoblastic cell invasion into the endometrium and promote angiogenesis. A recent study by Kodama et al. (28) showed that heparanase-1 is differentially expressed in the eutopic endometrium in the different phases of the menstrual cycle from women without endometriosis. Consistent with these observations, our recent study showed that heparanase-1 expression is prominent in normal endometrium in the late proliferative phase and luteal phase (29). We further showed that E can induce heparanase-1 expression and heparan sulfate degradation in the endometrial cells (29).

Our present study seeks to determine whether heparanase-1 expression in the eutopic endometrium from women with endometriosis is different from that in women without endometriosis, and whether heparanase-1 expression is increased in the endometriotic implants, compared with their eutopic counterparts.

MATERIALS AND METHODS

Tissues

After approval by the Rush University Institutional Review Board, paraffin blocks of eutopic endometrial specimens from 91 women (mean age ± SD, 32.2 ± 5.7 years; median age, 34 years; range, 21–47 years) with endometriosis were retrieved from the pathology laboratory repository. Among these 91 patients, 14 patients had ectopic endometriotic specimens. All women underwent laparoscopy by the senior author as part of an infertility evaluation. No hormonal medications were used during the cycle. At the laparoscopy, the pelvic organs were examined for the presence and extent of endometriosis. Endometriosis was staged according to the revised American Fertility Society classification (30). Women with pelvic diseases other than endometriosis and adhesions were not included in the study. During the laparoscopic procedure, samples of the uterine and ectopic endometrium were obtained from the uterine fundus with the Novak's curette. Part of each specimen was fixed immediately in 4% formaldehyde and transferred to a pathology laboratory. Tissues were embedded in paraffin within 48 hours. A heparanase-1–positive pancreatic adenocarcinoma was sectioned and included as a positive control for immunohistochemical staining.

Immunohistochemical Analysis

Tissue sections were dewaxed with xylene and rehydrated. Slides were heat inactivated in 10 mmol/L sodium citrate (pH 6.0) in a microwave for 3 minutes. Cooled slides were rinsed with phosphate-buffered saline solution (PBS) and then incubated with 1% H_2O_2 in methanol for 30 minutes at room temperature. Sections were blocked with 10% normal goat serum in PBS for 30 minutes at room temperature followed by 1 hour incubation with an anti-heparanase-1 rabbit serum (1:200 dilution) in PBS with 1% normal goat serum. The specificity of this anti-heparanase-1 antiserum has been verified in previous studies (31, 32) by Western blot test and has been used in immunohistochemical analysis of heparanase-1 expression in several types of cancers (32–34). Slides were washed and then incubated with goat anti-rabbit antibody–biotin conjugate (PharMingen, San Diego, CA) diluted at 1:300 in PBS/5% human serum and 5% normal goat serum. Streptavidin–horseradish peroxidase conjugate (Zymed, San Francisco, CA) diluted at 1:200 in PBS with 5% normal human serum and 5% normal goat serum was added and incubated for 45 minutes at room temperature. Color development was done with diaminobenzidine substrate (Sigma, St. Louis, MO) followed by diaminobenzidine enhancer (Vector Laboratories, Burlingame, CA). Slides were counterstained with Mayer's hematoxylin for 2 minutes, dehydrated, and mounted. Heparanase-1 expression was graded in a blinded fashion by three investigators in this study (J. S., P. G., and X. X.). Negative heparanase-1 staining was defined as no heparanase-1 signal at all in either the stromal or glandular cells (−) or with weak signal in <20% of cells in either the stromal or glandular cells (±). Positive heparanase-1 staining was defined as moderate signal intensity in 20% to 60% of the stromal or glandular cells (+), strong heparanase-1 signal intensity in >60% of either the stromal or glandular cells (++), or strong signal intensity in >90% of the stromal and glandular cells.

Statistical Analysis

Fisher's exact test was conducted by using the SigmaStat3 software (Richmond, CA) to analyze the difference of heparanase-1 expression in various menstrual phases, the difference of heparanase-1 expression in the endometrium from women with and without endometriosis, and the difference in the eutopic and ectopic endometrium. A P value $<.05$ was considered statistically significant.