

endometriosis, or may indeed result from a completely different pathogenesis. To explore this question, we decided to compare immunohistochemical staining patterns in noncystic and cystic endometriosis lesions.

Design: Case controlled study.

Materials/Methods: Formalin fixed, paraffin-embedded sections from noncystic endometriosis lesions and ovarian endometriotic cysts, were immunostained with anti bcl-2, anti-p53, anti matrix metalloproteinase IX, anti collagen VI, and anti-PTEN using the streptavidin-biotin method. Fisher's exact test was used for statistical comparison.

Results: Neither endometriosis lesions nor endometriotic cysts stained with anti-p53. Anti bcl-2 stained 100% (30/30) of endometriosis lesions compared to only 23% (7/30) of endometriotic cysts ($p < 0.0001$) and anti matrix metalloproteinase IX stained 85% (23/27) of endometriosis lesions and only 39% (14/36) of endometriotic cysts ($p = 0.0003$). Anti collagen VI however, stained only 6% (2/35) of endometriosis lesions and 75% (21/28) of endometriotic cysts ($p < 0.0001$). While anti-PTEN stained all endometriosis lesions (10/10) and all endometriotic cysts (16/16), the staining patterns differed. Staining in endometriosis lesions tended to be diffuse and predominantly cytoplasmic, but in endometriotic cysts was segmental (14/16), and intranuclear (5/16 cases).

Conclusions: This is the first comparative immunohistochemical study showing that endometriotic cysts are different from endometriosis lesions, with relative overexpression of collagen VI, under-expression of bcl-2 and matrix metalloproteinase IX, and differing expression of PTEN.

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3:45 P.M.

O-230

Heparanase-1 expression in endometrium during menstrual cycle in healthy control women and in women with endometriosis. Jianchi Ding, Xiulong Xu, Jikun Shen, Donald P. Braun, Nasir Rana, W. Paul Dmowski. Institute for the Study and Treatment of Endometriosis, Oak Brook, IL; Institute for the Study and Treatment of Endometriosis and Rush Medical Coll, Oak Brook, IL; Institute for the Study and Treatment of Endometriosis and Medical Coll of Ohio, Toledo, OH.

Objective: Endometriosis is the most common cause of the female infertility in the United States. Previous studies have demonstrated that some matrix metalloproteinases, which function to degrade the basement membrane (BM) and the extracellular matrix (ECM) by proteolysis, are frequently dysregulated in the endometriosis patients. Heparanase-1 (HPR1) is an endoglycosidase that specifically degrades the heparan sulfate (HS) moiety of proteoglycans, a chief component of the BM and ECM. HPR1 plays an important role in angiogenesis and tumor metastasis. In this study we investigated the expression of HPR1 in uterine endometrium in control women during menstrual cycle and its expression in women with endometriosis, and whether HPR1 expression resulted in the degradation of HS in the BM of the endometrial glands.

Design: Endometrial sections were examined for HPR1 expression using immunohistochemical staining. HS deposition was analyzed by immunofluorescence staining with an HS-specific mAb.

Materials/Methods: Forty-nine paraffin blocks of uterine tissues were retrieved from the pathology laboratory. Of these, 28 were laparoscopically positive and 21 were negative for endometriosis. Tissue sections were made and were immunohistochemically stained for HPR1 expression with rabbit anti-HPR1 serum. HS deposition in the BM of the endometrial glands was determined by immunofluorescence staining with an HS-specific mAb.

Results: Six of 21 (28.6%) normal endometrial tissue specimens scored HPR1 positive, compared to 13 of 28 (46.4%) HPR1 positive in the endometrial tissues from women with endometriosis. When endometrial phase was taken into consideration, we found that 2/15 (13.3%) samples tested positive at the proliferative phase and 4/6 (66.7%) were positive at the secretory phase in controls. In women with endometriosis, three times more samples (9/23, 39.1%) tested positive during the proliferative phase when compared with controls, with a similar proportion of samples (4/5, 80%) testing positive during the secretory phase. HS deposition in basement membrane of the endometrial glands was negatively correlated with the HPR1 expression, suggesting that lack of HS deposition is due to functional expression of HPR1.

Conclusions: To our knowledge, this is the first study demonstrating the

expression of HPR1 in human uterine endometrium. HPR1 expression showed cyclic changes during the menstrual cycle, indicating HPR1 may be involved in the regulation of endometrial tissue remodeling and cyclicality. Our results also indicate that immunohistochemically detectable HPR1 is functional since its expression was negatively correlated with HS detection in basement membrane of endometrial glands. The expression of HPR1 may also be upregulated in women with endometriosis compared to controls during the proliferative phase of the menstrual cycle. Such changes might be related to enhanced angiogenic or metastatic behaviors in subsets of cells from women with endometriosis.

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O-231

Intense focal expression of de novo synthesized haptoglobin by subluminal endometrial stroma correlates with the window of implantation in women with endometriosis. Kathy L. Sharpe-Timms, Randy L. Zimmer, Emily A. Ricke. Univ of Missouri-Columbia, Columbia, MO.

Objective: A uniquely glycosylated form of haptoglobin is synthesized and secreted by the stroma of endometriotic lesions and the eutopic endometrium from women with endometriosis. This endometriosis-associated haptoglobin, stimulated by inflammatory cytokines and growth factors, has novel immunomodulatory and angiogenic properties that could affect the process of embryo implantation. The objective of this research was to correlate aberrant haptoglobin expression and localization and altered fertility in women with endometriosis.

Design: Randomly designed, prospective study of haptoglobin in the subluminal epithelium during the menstrual cycle.

Materials/Methods: Women of reproductive age presenting to the MU Ob/Gyn physicians for diagnosis or treatment of endometriosis (nulliparous, infertile, $n = 19$) or surgical sterilization (multiparous controls, $n = 12$) were enrolled. Age and race were similar between the groups. Exclusion criteria included use of steroid modulating medications, uterine fibroids, endometrial hyperplasia or cancer, endometrial tissues histologically out of phase, or menstrual tissues. Endometrial biopsies were obtained from informed consenting women with and without endometriosis during the proliferative and secretory stages of the menstrual cycle. Tissues were formalin fixed, paraffin embedded and sectioned at 5 microns. Cycle day was determined by a combination of last menstrual period and histological evaluation. Haptoglobin gene expression was evaluated by non-isotopic in situ hybridization and protein localization was assessed immunohistochemically with an antibody validated to detect endometriosis associated haptoglobin. Additional immunostaining was performed on adjacent sections using antibodies against vimentin, CD 68 (macrophage) and CD 45 (pan leukocyte) to define the cellular site of haptoglobin localization. Computer assisted image analysis was used to evaluate the intensity of haptoglobin gene expression and protein localization. Chi Square Analyses, Spearman's Rank Order Correlation and Regression Analyses were used to evaluate the patterns and intensity of haptoglobin expression and localization.

Results: Focal sites of endometriotic haptoglobin were observed in the stroma immediately beneath the luminal epithelium in women with endometriosis, primarily during the secretory phase of the cycle (proliferative $n = 2/7$, 28.5%; secretory $n = 10/12$, 83.3%). The intensity of the subluminal haptoglobin expression increased ($p = 0.001$) and was correlated with the window of embryo implantation (days 19 to 22; $p = 0.019$). Subluminal endometriotic haptoglobin was not detected in women without endometriosis, regardless of the stage of the cycle.

Conclusions: Intense, focalized subluminal localization of endometriotic haptoglobin in the endometrial stroma during the window of implantation in women with endometriosis suggests that this uniquely glycosylated protein with immunomodulatory and angiogenic properties may contribute to the reduced fertility associated with this disorder. Endometrial epithelial cytokines and growth factors may act as paracrine factors and regulate this highly focal haptoglobin production.

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