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Expression of heparanase and angiopoietin-2 in patients with endometriosis

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Abstract

Objective: The objective was to investigate the expression of heparanase (Hpa) and angiopoietin-2 (Ang-2) in endometriosis.
Study design: In ectopic and eutopic endometrium of patients undergoing laparoscopy for endometriosis ($n = 86$) and in normal endometrium of patients undergoing laparoscopic tubal ligation or hysteroscopic resection because of uterus septus ($n = 30$), we determined Hpa and Ang-2 gene expression by RT-PCR. To support the mRNA data, the expression of Hpa and Ang-2 protein was measured by Western blot analysis. Finally, Hpa and Ang-2 in these tissues was localized by immunohistochemical staining.
Result(s): The positive rate of Hpa and Ang-2 mRNA in ectopic and eutopic endometrium in the study group was significantly higher than that in normal endometrium in the control group. In the study group, ectopic and eutopic endometrium expressed a higher positive rate of Hpa and Ang-2 protein, whereas in the control group, normal endometrium expressed a lower positive rate of Hpa and Ang-2 protein. In eutopic and ectopic endometrium, there was balanced expression between Hpa and Ang-2. Both Hpa and Ang-2 showed a balanced expression between eutopic and ectopic endometrium. In ectopic endometrium, strong staining for Hpa and Ang-2 was observed both in epithelial cells and in stromal cells, but in eutopic endometrium, Hpa and Ang-2 were mainly expressed in epithelial cells.
Conclusion: The higher expression of Hpa and Ang-2 in ectopic and eutopic endometrium may play an important role in the pathogenesis and development of endometriosis.

Keywords: Heparanase; Angiopoietin-2; Invasion; Angiogenesis; Endometriosis

1. Introduction

Endometriosis is a disease in which the endometrial tissue (the uterine lining, glands and connective tissue) invades other places, such as ovaries, fallopian tubes, bladder and intestines, uterine wall, the lining of the pelvis and the deeper muscle layers of the uterus. The true incidence of this disease is not really known, but it is thought that 10–15% of all women of reproductive age will develop endometriosis. What is more, this disease is becoming more and more common. Among the patients with endometriosis, 80% of women have pain before and during menstrual periods and 40% have infertility. All of these symptoms affect female life and health badly. However, until recently, there have not been any treatments being offered that were aimed at ridding women of this disease, but only some aimed at offering respite. Thus, it is becoming very important for us to find a type of medication that can cure endometriosis radically.

In recent years, it has been gradually realized that endometriosis has some characteristics that are similar to those of malignancies, such as the abilities to invade and metastasize [1–4]. At the same time, it has also been found that cells can obtain this kind of characteristic by decomposing extracellular matrix (ECM) and basement membrane (BM) [5–7].

Currently, most researchers have only focused their studies on endometriosis on some enzymes that were used to degrade proteins in the extracellular matrix (ECM) and

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basement membrane (BM), such as matrix metalloproteinases (MMPs) [8–12] and urokinase-type plasminogen activators (u-PAs) [12–14]. For many reasons, heparanase (Hpa), which was used to degrade heparan sulfate proteoglycans (HSPGs), was neglected by many researchers. Since the Hpa gene and its promoter were cloned and identified successfully, a lot of studies have proved that many kinds of malignant cells could produce or excrete Hpa. Simultaneously, they also have discovered that the expression of Hpa was associated with the transformation, metastasis and angiogenesis of malignant cells [15–23]. However, we have found no comprehensive reports on the relationship between Hpa and endometriosis until now.

The same thing also happened with angiopoietin-2 (Ang-2). It has been generally accepted that the establishment of new blood supplies played a key part in the progression of endometriosis [24–27] and many pivotal angiogenesis stimulators have been proven to be relative to endometriosis, such as basic fibroblast growth factor (bFGF) [28] and vascular endothelial growth factor (VEGF) [29]. But as far as the relationship between Ang-2 and endometriosis is concerned, we have not found any reports either.

Under these conditions, we analyzed the expression of Hpa and Ang-2 mRNA and protein expression levels in ectopic and eutopic endometrium of endometriosis using the reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting respectively, on the other hand, the localizations of these two molecules were demonstrated by immunohistochemistry. At same time, we included patients with normal endometrium as the control group.

2. Materials and methods

2.1. Participants

All tissues came from those patients who had surgical treatment from February 2004 to July 2005 and they had also been confirmed by pathologic diagnosis.

2.1.1. Study group

Of 86 patients whose average age at the time of surgery was 36.8 ± 4.2 years (range 22–45 years), 50 were in the proliferative phase and 36 patients were in the secretory phase. According to the Revised American Fertility Society Classification of endometriosis (R-AFS) in 1985, we found that there were 25 patients in phases I–II and had 61 patients in phases III–IV.

2.1.2. Control group

Twenty-six patients undergoing laparoscopic tubal ligation or hysteroscopic resection because of uterus septus, who did not have endometriosis, served as the control group. Their average age at the time of surgery was 34.2 ± 3.6 years (range 25–38 years), 16 patients were in the proliferative phase and 10 patients were in the secretory phase.

None of patients in these two groups had received preoperative hormone treatment for 3 months and none of them had any endocrinopathies. The phase of the menstrual cycle was confirmed by histopathological diagnoses of the patients' endometria. All tissues were obtained after operation. A fragment of each tissue was fixed in Bouin's solution, embedded in paraffin and sectioned (4 μm) for standard immunohistochemistry. The remainder was immediately frozen in liquid nitrogen and stored at −80 °C for RNA and protein isolation. Informed consent was obtained from each participant before surgery and the Ethical Board of the Central South University approved all procedures.

2.2. Reverse transcriptase polymerase chain reaction

2.2.1. Collection of eutopic and ectopic endometrium and RNA preparation

Based on the guanidinium thiocyanate–phenol–chloroform method, total RNA from the tissue was extracted using the commercially available TRIzol reagent (Gibco:BRL). In brief, after being taken out of refrigeration, tissues were milled, lysed in 1 ml of TRIzol and left at room temperature for 5–10 min. Subsequently, 0.2 ml of chloroform was added, followed by vigorous agitation for 15 s. The sample mixture was kept at room temperature for 2–3 min and then centrifuged at 12,000 × *g* for 15 min at 4 °C. The aqueous phase was incubated with 0.5 ml of isopropanol for 10 min at room temperature and then centrifuged at 12,000 × *g* for 10 min at 4 °C. The precipitated RNA was washed with 75% ethanol and dissolved in 0.1% DEPC-treated water. RNA concentrations were measured spectrophotometrically (1_OD260_40 mg/ml of RNA).

2.2.2. Method of RT-PCR

The expression levels of Hpa, Ang-2 and glyceraldehyde-3-phosphodehydrogenase (GAPDH) gene were determined by reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was reverse transcribed in 30 ml of a solution containing 1 (g of total RNA Random Hexamer Primers, 5× Reaction buffer, RiboLock RNase Inhibitor, 10 mM dNTP Mix, Revert Aid M-MuLV RT and DEPC-treated Water (Fermentas, Burlington, Ontario, Canada). Polymerase chain reaction (PCR) was then performed to compare the expression of Hpa and Ang-2 mRNA in ectopic and eutopic endometrium of endometriosis with that in normal endometrium of the control group. Based on published DNA sequences of human Hpa and Ang-2 gene, primers for Hpa [30] (sense, HPU-355 5′-TTCGATCCCAAGAAGGAATCAAC-3′, antisense, HPL-229 5′-GTAGTGATGCCATGTAACTGAATC-3′), primers for Ang-2 [31] (sense, 5′-GGACAATTATTCAGCGACGTG-3′, antisense, 5′-GAGCGAATAGCCTGAGCCTT-3′). Primers for GAPDH (sense, 5′-GCTGGCGCTGAGT ACGTCGT-3′, antisense, 5′-TGGGTGTCGCTGTTGAAGTC-3′). PCR was performed using a MJR PCR System (MJ Research Corporation, Waltham, MA, USA). The following conditions were applied to Hpa PCR