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Correlation between Cyr61 expression and clinicopathologic parameters in adenomyosis

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ABSTRACT

Adenomyosis, a benign invasion of endometrium, is closely related to endometriosis. Cysteine-rich 61 (Cyr61), a protein present in all endometrial tissues and menstrual effluents, is known to be associated with endometriosis. However, its relation to adenomyosis has not been determined thus far. Therefore, here, we aimed to investigate the expression of Cyr61 protein in adenomyosis and determine the correlation between Cyr61 expression and clinicopathologic parameters in patients with adenomyosis. One hundred and twenty patients with histologically diagnosed adenomyosis, who underwent hysterectomy for non-endometrial disease were enrolled in this study. Patients were interviewed using a standard questionnaire consisting of sociodemographic characteristics and reproduction history. The severity of dysmenorrhea and menorrhagia was evaluated using the visual analogue scale (VAS) and pictorial blood-loss assessment chart (PBAC). Samples of serum, endometrial tissue, and peritoneal fluid were collected, and Cyr61 mRNA levels were determined by RT-PCR. The Cyr61 protein levels in endometrial and ectopic lesions were determined by immunohistochemistry and those in serum and peritoneal fluid, by ELISA. We found that expression of Cyr61 was higher in the ectopic endometrium than in the eutopic endometrium. Cyr61 expression in the endometrium was correlated with age, number of natural labors, PBAC score, VAS score, uterine volume, adenomyosis type, and concurrent endometriosis. The Cyr61 protein level in the ascites was higher than that in serum, and no correlation existed between them. Our results suggest that the expression of Cyr61 may be indirectly related to the degree of dysmenorrhea and Cyr61 may be involved in the pathogenesis of adenomyosis.

1. Introduction

Adenomyosis refers to a benign invasion of the endometrium into the uterine myometrium, which leads to an enlarged uterus. The term was first clearly defined in 1972 by Bird et al. (Garcia and Isaacson, 2011; Senturk and Imamoglu, 2015). An adenomyosis is usually surrounded by hyperplastic and hypertrophic myometrium. Despite its malignant biological behavior, the ectopic endometrium appears non-neoplastic under a microscope (Senturk and Imamoglu, 2015). The prevalence of adenomyosis ranges from 5% to 70% worldwide; however, its exact incidence is unknown (Garcia and Isaacson, 2011; Struble et al., 2015). Among symptomatic women with adenomyosis, 50%, 30%, and 20% women have menorrhagia, dysmenorrhea, and metrorrhagia, respectively. Therefore, adenomyosis has an enormous impact on women's health and well-being (Ferenczy, 1998; Bergeron et al., 2006; Leyendecker et al., 2009).

Thus far, the underlying mechanisms of adenomyosis remain unclear (Garcia and Isaacson, 2011). Steroid hormones have been implicated in the pathogenesis of adenomyosis (Benagiano et al.,

Abbreviations: Cyr61, cysteine rich 61; LNG-IUS, levonorgestrel intrauterine system; VAS, visual analogue scale; PBAC, pictorial blood-loss assessment chart; DIE, deep infiltrating endometriosis; OEM, ovarian endometrioma.

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2012). Increased local estrogen levels produced by endometriotic cells may be responsible for hypertrophy in the myometrium and hyperplasia in the endometrium. Further, studies have shown that the use of high-dose progesterone can temporarily induce the regression of adenomyosis (Senturk and Imamoglu, 2015; Benagiano et al., 2012). In recent years, the tissue injury and repair mechanism has been considered a major event in the development of adenomyosis (Leyendecker et al., 2009; Leyendecker and Wildt, 2011). Chronic uterine peristalsis or phases of hyperperistalsis result in microtraumatization at the endomyometrial junction, and tissue injury and inflammation with subsequent healing induce specific physiological process that promote local production of estrogen (Leyendecker et al., 2009). With the increase in the local estrogen levels, peristaltic activity regulated by the ovarian hormone estrogen may be disturbed, leading to continuing hyperperistalsis and self-perpetuating disease (Leyendecker et al., 2009). cysteine-rich 61 (Cyr61, also known as CCN1) is an extracellular matrix-associated protein belonging to the CCN family, which comprises six proteins that have broad roles such as promoting adhesion, migration, and mitosis and regulating proliferation, angiogenesis, tumor growth, and embryogenesis (Brigstock, 2003). In adults, the expression of CCN proteins is related to inflammation and injury repair and has been demonstrated to be a biomarker for breast cancer, ovarian carcinoma, prostate cancer, and rheumatoid arthritis (Lau, 2011; Chen and Lau, 2009). Cyr61 is present in all endometrial tissues, biopsies, and menstrual effluents (Gashaw et al., 2008). In the menstrual cycle, the expression of Cyr61 is significantly higher in the proliferative phase than in the secretory phase, and the highest expression of Cyr61 is noted in menstrual effluents (Gashaw et al., 2008). In addition, deregulated Cyr61 has been correlated with the incidence of endometriosis (Absenger, 2004). Another study in baboons also showed that increased expression of Cyr61 correlated with the development of endometriosis (Gashaw et al., 2006). Despite the fact that adenomyosis was traditionally considered to be closely related to endometriosis, these two diseases are evidently different from each other with regard to their clinical characteristics and pathogenesis (Wang et al., 2009).

To date, the level of Cyr61 has not been specifically investigated in adenomyosis. Therefore, in this study, we aimed to detect the expression of Cyr61 in the endometrium, serum, and peritoneal fluid of patients with adenomyosis and to analyze the possible correlation between Cyr61 expression and various clinicopathological parameters.

2. Materials and methods

2.1. Participants

This study was approved by the Institutional Review Board of the International Peace Maternity and Child Health Hospital, Shanghai, China. Written informed consent was obtained from each participant before recruitment. The participants were assured that their tissue samples would be used only for the purpose of this study and that all their information would be kept strictly confidential. All biopsy specimens were collected according to the guidelines of the Declaration of Helsinki.

From February 2014 to October 2015, women diagnosed with adenomyosis, who were treated by hysterectomy at the International Peace Maternity and Child Health Hospital were included in this study. The diagnosis was confirmed histologically. Pathologists diagnosed adenomyosis and classified it as diffuse or focal type according to previous literature (Bazot et al., 2001). Only patients with adenomyosis who returned a completed standard questionnaire were enrolled. Patients with endometrial pathology and malignancy, pelvic inflammatory disease, other infectious diseases, pregnancy, immunologic diseases, cancer, and liver and renal diseases were excluded. Participants with regular menstrual cycles (28–32 days), aged 30–53 years, were not administered any hormonal therapy including oral contraceptive pills, progestins, gonadotropin-releasing hormone agonist, or levonorgestrel intrauterine system (LNG-IUS) within 3 months before surgery. Before the hysterectomy, participants were interviewed personally using a standard questionnaire on their sociodemographic characteristics and reproductive history, and the visual analogue scale (VAS) and the pictorial blood-loss assessment chart (PBAC) were used to assess the severity of dysmenorrhea and menorrhagia, respectively. After the surgery, two surgeons who operated the patients completed a post-operative questionnaire on lesion assessment; samples taken; and visual detection of pelvic inflammation, exudates, or adhesions (Brunham et al., 2015). In addition, several clinicopathological characteristics confirmed by pathological diagnosis were recorded. Two pathologists were responsible for classifying the menstrual stage according to Noyes' report (Noyes et al., 1975).

2.2. Tissue collection

Samples of eutopic endometrium (n = 110) and ectopic endometrium (n = 108) were obtained after removal of the uterus during surgery. All the ectopic endometrium samples were taken from the ectopic lesions located in the myometrium. Each fresh tissue was divided into two parts. One part was snap frozen in liquid nitrogen, and stored at −80 °C until processed. We extracted RNA within 3 days of obtaining the samples. The other part of the tissue was embedded in 10% formaldehyde for 24–72 h for immunohistochemical analysis.

After excluding peritoneal fluid contaminated by blood and its paired peripheral blood, 30 samples of peripheral blood and an equal number of peritoneal fluid samples were collected within 24 h before and during surgery, respectively. Peripheral blood and peritoneal fluid were collected in anti-coagulative tubes and 15-ml centrifuge tubes, respectively, and were stored in the refrigerator at 4 °C. Blood and peritoneal fluid were processed within 2 h of obtaining the samples. Subsequently, the blood and peritoneal fluid were centrifuged at 3000 rpm for 10 min, and the supernatant was frozen in liquid nitrogen and reserved (Rahmioglu et al., 2014).

2.3. Pain evaluation and menorrhagia evaluation

2.3.1. Pain evaluation

The severity of dysmenorrhea was evaluated using the VAS system, which consists of a 10-cm horizontal line with two extremes (0, no pain; 10, the worst pain imaginable) (Peveler et al., 1996). Patients were asked to mark a spot on the line to indicate the average severity of dysmenorrhea in the last 6 months. The distance between the mark and the left extreme was measured in millimeters (score, 0–100) (Peveler et al., 1996; Bourdel et al., 2014). The severity of dysmenorrhea was scaled according to the VAS scores as follows: score of 1–50 represents mild pain, 51–80 represents moderate pain, and 81–100 represents severe pain (Sheng et al., 2009).

Dysmenorrhea was classified as primary dysmenorrhea when painful menstruations occurred within 1 year after menarche and as secondary dysmenorrhea, when the painful menstruations occurred later in life (Lafay Pillet et al., 2014).

2.3.2. Menorrhagia evaluation

Menorrhagia was defined as excessive menstrual blood loss of >80 mL per period (Protheroe, 2004). The periodic blood loss was assessed using PBAC, a semi-objective visual assessment tool (Goshtasebi et al., 2015). Every patient was asked to enter on the