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Decreased expression of FOXA2 promotes eutopic endometrial cell proliferation and migration in patients with endometriosis

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KEY MESSAGE

This research in a cell model using endometrial stromal cells isolated from endometrial tissue showed that decreased expression of FOXA2 promotes the proliferation and migration of eutopic endometrial cells in patients with endometriosis. These findings suggest that FOXA2 might be a progesterone-induced gene, which may provide a new clue for endometriosis treatment.

A B S T R A C T

Endometriosis is characterized by eutopic endometrial cell 'metastasis' to ectopic foci. FOXA2 is a member of the forkhead transcription factor family, which may participate in transcriptional regulation in endometrial cells and contribute to the aetiology of endometriosis. This study investigated the roles played by FOXA2 in eutopic endometrium using endometriosis samples. Western blotting showed that the relative expression of FOXA2 was significantly reduced in eutopic endometrium from patients with endometriosis (n = 14) compared with endometriosis-free controls (n = 16) (0.69 ± 0.07 versus 1.24 ± 0.06, $P < 0.05$). To mimic eutopic endometrium of endometriosis, primary eutopic endometrial stromal cells (ESC) of controls were harvested and transfected with FOXA2 siRNA. MTT assay showed that cell viability of ESC with transfected FOXA2 siRNA increased significantly, whereas the apoptosis rate decreased as indicated by flow cytometry experiments (both $P < 0.05$). Wound healing assays revealed that transfection of FOXA2 siRNA promoted ESC migration. Moreover, real-time PCR analysis showed progesterone-induced FOXA2 expression in ESC under physiological conditions. In conclusion, these findings indicate that FOXA2 might be a progesterone-induced gene, which may participate in the 'metastatic' process of eutopic endometrium to ectopic loci in patients with endometriosis.

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Introduction

Endometriosis is a common condition in women, characterized by the presence of uterine endometrial glands and stroma outside the uterine cavity. Although endometriosis has been considered as a benign gynaecological disease, it possesses malignant tumour behaviours, including tissue invasion, distant organ spreading and angiogenesis (Siufi Neto et al., 2014). Several classical theories have been proposed (Vercellini et al., 2014) to explain these biological behaviours, including retrograde menstruation, coelomic metaplasia and induction theory. Among them, the retrograde menstruation theory has been widely accepted, but the definitive mechanism remains unknown.

Altered expression of some genes has been observed in eutopic endometrium from women with endometriosis, which might contribute to the ectopic endometrial implantation (Timologou et al., 2016). Identifying these key genes would provide new insights into the regulation of ectopic endometrial migration. The FOXA family consists of FOXA1 (Hnf3α), FOXA2 (Hnf3β) and FOXA3 (Hnf3γ) (Kaestner, 2000), all containing a winged helix DNA-binding domain with high homology (Friedman and Kaestner, 2006). This family plays an important role in multiple stages of mammalian life. It has been shown that FOXA2 is essential for differentiation of endoderm-derived organs at the embryonic stage, such as liver and pancreas. Additionally, FOXA2 participates in the regulation of glucose metabolism and homeostasis in adults (Banasik et al., 2012; Kaestner, 2000). It has been shown that mutation of FOXA2 in mice resulted in a phenotype lacking definitive node and notochord, leading to embryonic lethality (Dufort et al., 1998). In adult mice, FOXA2 was specifically expressed in the glandular epithelium of uterine endometrium (Besnard et al., 2004).

Our previous study indicated that FOXA2 might be implicated in the aetiology of endometriosis (Yang et al., 2015); however, the detailed mechanism remains unclear. A recent study suggested that FOXA2 was involved in controlling cell cycle progression during endometrial hyperplasia formation (Villacorte et al., 2013). We postulate that decreased expression of FOXA2 might prompt metastasis of endometrial cells by affecting cellular biological functions such as proliferation, apoptosis and migration. It has been shown in a previous study that FOXA2 expression is regulated by progesterone in mouse uterus (Bazer, 2010). Conditional deletion of FOXA2 resulted in impeded uterine gland development, decidualization (Jeong et al., 2010), which was regulated by progesterone. Progesterone resistance in endometriosis patients might be due to a decreased responsiveness of the progesterone receptor, its chaperone immunophilins and deregulated progesterone-regulated genes in eutopic endometrium (Burney et al., 2007). Our previous study confirmed that FOXA2 expression decreased in eutopic and ectopic endometrium compared with normal endometrium by PCR and immunohistochemical staining (Yang et al., 2015). This study focuses on the deregulated expression in eutopic endometrium in endometriosis. We propose that FOXA2 might be a progesterone-regulated gene, which may participate in eutopic endometrium development in endometriosis.

Materials and methods

Sample collection and preparation

Eutopic endometrial tissues were collected from 14 patients with endometriosis that had been both surgically and histologically confirmed in the Department of Gynecology of The Ninth People's Hospital of Chongqing. The enrolled patients without dysmenorrhoea were diagnosed at stage I according to the American Society for Reproductive Medicine Revised Classification of Endometriosis (American Society for Reproductive Medicine, 1997). As controls, endometrial biopsy specimens were collected from 16 healthy women who underwent tubal sterilization and were laparoscopically confirmed to be free of endometriosis. All case and control women were at the secretory stage, displayed normal menstrual cycles and had not received anti-inflammatory or hormonal therapy for 3 months prior to surgery.

The endometrial tissue samples from each group were digested for cell culture, snap frozen and kept below –80°C for subsequent research. The study was approved by the Ethics Committees of The Second Affiliated Hospital of Chongqing Medical University on 26 February 2013 (reference number 2013-045) and The Ninth People's Hospital of Chongqing on 25 February 2013. Informed consent was obtained in writing from all subjects before surgery.

Western blot analysis

Western blot analysis was performed as in previous work (Yin et al., 2013). Briefly, total proteins were extracted by freezing (liquid nitrogen)–thawing in a lysis buffer, vortexed for 2 min and then centrifuged at 12,000 *g* for 15 min. The supernatants were transferred to fresh tubes and the Bradford method was used to measure the total protein content (Bradford, 1976).

Protein samples (100 μg) were subjected to electrophoresis with a discontinuous system consisting of 12% polyacrylamide resolving gels and 8% stacking gels, and then transferred to nitrocellulose membranes (Merck Millipore, USA). Membranes were washed and blocked by 5% skimmed milk in PBS with 0.1% Tween 20 (PBST) for 1 h at room temperature, followed by incubation with primary antibodies, rabbit polyclonal anti-human FOXA2 antibody (Millipore, USA; at 1:300) and monoclonal mouse anti-human glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH; Cell Signaling Technology, USA; at 1:3000) at 4°C overnight. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the loading control and the blank membranes served as the negative control. In a separate experiment, HepG2 hepatoma cells (The Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) (Zhao et al., 2014) and endometrial stromal cells (ESC) from control women were used to demonstrate the positivity of the FOXA2 antibody (**Supplementary Figure S3**). After washing with PBST, membranes were incubated with horseradish peroxidase-conjugated secondary antibody, goat anti-rabbit antibody (Western Biotechnology, China) and goat anti-mouse (Western Biotechnology, China) antibody, at a dilution of 1:5000 at room temperature for 1 h and an enhanced chemiluminescence procedure was used to detect the bound horseradish peroxidase-conjugated secondary antibody. Protein expression levels were obtained by a Chemi-doc image analyser (Bio-Rad; Hercules, CA, USA).

Cell culture and transfection

Primary ESC were obtained from the control group. Endometrial tissue was transported from the site of collection to the laboratory in Hanks' Balanced Salt Solution. The tissue was then minced and digested in Hanks' Balanced Salt Solution containing 1% penicillin, 1% streptomycin, 5% collagenase and 0.5% deoxyribonuclease at 37°C for 30 min with agitation. The dispersed endometrial cells were separated by filtration through a wire sieve (73 mm diameter pore, Sigma). ESC were plated on plastic 12-well plates in DMEM/F12 phenol red medium

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