# Increased expression of fibroblast growth factor receptor 1 in endometriosis and its correlation with endometriosis-related dysmenorrhea and recurrence

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## ARTICLE INFO

## ABSTRACT

*Objective(s)*: This study aims to identify a critical molecule that potentially participates in endometriosis pathogenesis and characterize its correlation with dysmenorrhea and recurrence.
*Study design*: We utilized a bioinformatics-based strategy to screen for candidate genes and fibroblast growth factor receptor 1(FGFR1) was chosen for further validation. FGFR1 expression was examined in specimens of ectopic and eutopic endometrium obtained from 48 patients with endometriosis and specimens of eutopic endometrium from 26 healthy control subjects using immunohistochemistry and Western blotting. In addition, FGFR shRNA treatment was applied in a nude mice endometriosis model to examine the functional role of FGFR1 in endometriosis formation *in vivo*.
*Results*: FGFR1 was found commonly overexpressed in ectopic endometrium of endometriosis compared with either its eutopic counterpart or endometrium from normal patients ($P < 0.05$). FGFR shRNA treatment impaired endometriosis formation and alleviated endometriosis-related symptoms *in vivo*. FGFR1 expression in ectopic endometrium was correlated with dysmenorrhea severity ($P < 0.05$) and recurrence in endometriosis patients ($P < 0.05$).
*Conclusion(s)*: FGFR1 might be involved in endometriosis development, which could possibly serve as a novel therapeutic target and prognostic marker for this disease.

## Introduction

Endometriosis is a common estrogen-dependent gynecological disorder in females of reproductive age and is characterized by the presence and growth of endometrial tissue outside the uterus [1]. The development of ectopic endometrial tissues suggests intrinsic cellular mechanisms leading to invasion, unrestrained growth, neoangiogenesis, and distant spreading of endometriotic cells [2]. Despite its high prevalence, the etiology and pathogenesis of endometriosis still remain largely unelucidated. Moreover, although hormonal therapy offers an alternative for the treatment of endometriosis, its curative efficacy for endometriosis is often insufficient. Therefore, the development of novel treatment strategies for endometriosis based upon new potential therapeutic targets is imperative.

Previous observations supported the notion that endometriosis might be a "metastasis"-related disease, which offers endometriosis some similarities to cancer diseases although metastasis does not really exist in endometriosis. However, the underlying mechanisms are still unclear [3,4]. Fibroblast growth factor receptor (FGFR) family is part of a group of growth factor receptor tyrosine kinases (RTK) able to induce several cellular processes including cell proliferation, angiogenesis, inhibition of apoptosis, and cell migration [5]. FGFR1, as a member of fibroblast growth factor receptors, is known to drive an epithelial-to-mesenchymal transition (EMT) of primitive streak-localized epiblast cells into mesoderm cells. During EMT, epithelial cells lose their polarity, augment their motility, and begin to express mesenchymal markers, such as vimentin, becoming "mesenchymal-like". This process has also been closely linked to cancer progression and

metastasis. Previously, aberrant FGFR1 expression has been implicated in the progression of a variety of tumor cell types including prostate cancer, colorectal cancer and non-small lung cancer [5]. However, the contribution of FGFR1 to the pathogenesis of benign disorders like endometriosis is still unclear. Thus, our study attempted to identify a critical molecule that might participate in endometriosis development.

## Materials and methods

### Bioinformatics analysis

Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI) was searched to identify candidate genes responsible for endometriosis development. Three GEO datasets analyzing endometriosis were identified, including GSE5108, GSE6364, and GSE7305 [6–8]. These three datasets were compared for overlapped genes differentially-expressed between endometriosis and control. Two criteria were applied: (a) significantly differentially-regulated ($P < 0.05$) between endometriosis and control identified by GEO, (b) significantly differentially-expressed by at least 2-fold ($P < 0.05$). Since endometriosis might be a "metastasis"-related disease similar to cancer although metastasis does not really exist, we further searched eligible genes against KEGG pathway database, Gene Ontology and metastasis-related published studies to identify genes most closely associated with metastasis. These "metastasis" genes were subsequently analyzed in STRING software, an open source web-based tool with established and predicted protein interactions, and visualized protein-protein interaction networks [9]. Those key nodes with over two interactive nodes demonstrated by STRING pathway analysis were selected as potential candidate genes responsible for the pathogenesis of endometriosis.

### Patients and tissue samples

Eutopic endometrium used for Western blot analysis was obtained from 26 patients undergoing hysterectomy for benign indications other than endometriosis (like leiomyoma) as control, and ectopic endometrium was obtained from endometriosis patients at the Gynecological Department of West China Second Hospital of Sichuan University (Chengdu, China) from 2009 to 2014. None of the patients had received any preoperative hormonal therapy prior to surgery. All these samples were obtained by experienced gynecologists and gynecological surgeons and examined by experienced pathologists who confirmed the diagnosis of disease samples.

Paraffin-embedded eutopic endometrial and paired ectopic endometrial specimens were obtained from 48 patients who underwent surgical resections in the same hospital from 2009 to 2014. They were further subjected to immunohistochemical analysis of identified proteins for validation and subsequent follow-up were carried out for these 48 patients. This study was approved by the Institutional Ethics Committee of Sichuan University. Informed consents were obtained from all patients prior to analysis.

### Laser capture microdissection (LCM) and Western blotting

All lesions were microdissected from 12-μm-thick frozen sections using LCM system (Leica, Germany). Normal endometrial tissues were dissected to obtain negative control proteins. Western blotting analysis was conducted as previously described [10]. The primary antibody was rabbit anti-FGFR1 (Santa Cruz Biotechnology). β-Actin was used as an internal control.

### Immunohistochemistry

Immunohistochemistry (IHC) was performed as described previously [10]. The primary antibodies included rabbit anti-FGFR1 (Santa Cruz Biotechnology) and rabbit anti-Ki-67 (Santa Cruz Biotechnology). Slides were evaluated by two independent pathologists in a double-blinded manner. Any discrepancy between the two evaluators was resolved by reevaluation and careful discussion until agreement was reached.

### Animal model of endometriosis

The guidelines for animal care were approved by the Institutional Animal Care and Use Committee of Sichuan University (Chengdu, Sichuan, People's Republic of China). Endometriosis lesions were acquired from premenopausal women with endometriosis at West China Second Hospital, Sichuan University. In vivo endometriosis nude mice model was surgically induced as described previously [11]. In particular, each mouse received an intraperitoneal injection of PBS containing a suspension of five human endometriotic tissue fragments with a size of about 10 mm³ per mouse into the ventral midline and subcutaneous injection of 0.5 μg of 17β-estradiol was performed on days 1 and 2 to facilitate the implantation of endometriotic lesions. Mice were next assigned randomly to one of the following groups (5 per group): (a) PBS, 100 μl of PBS; (b) Lipo, Lipofectamine 2000 (Invitrogen) at 62.5 μg/100 μl of PBS; (c) shFGFR1, FGFR1 shRNA at 25 μg/100 μl of PBS. Intraperitoneal treatment was initiated 5 days after inoculation. Mice received therapy, three times a week and were sacrificed at 21 days postinoculation. Intraperitoneal endometrial nodules were resected and measured immediately to assess the treatment efficacy as previously described [10].

### Pain assessment and follow-up of endometriosis patients

Pain assessment of endometriosis patients was conducted as described elsewhere [12]. The 48 patients with endometriosis were further followed up for recurrence for 30 months after surgery. The recurrence of endometriosis was defined as: (a) the presence of ovarian cysts of 3 cm in diameter, along with characteristic echoes as detected by transvaginal ultrasonography for two consecutive menstrual cycles, coupled with or without the recurrence of dysmenorrhea or pelvic pain requiring medical intervention, or as (b) the presence of de novo endometriosis as confirmed by histology following a second surgery. We also included endometrial tissue samples from 26 women with other benign gynecological diseases as controls and none of them had endometriosis or adenomyosis.

### Statistics

Data are presented as mean ± SD of three independent experiments unless otherwise indicated. GraphPad Prism (GraphPad Software Inc., CA, USA) was used for data analysis. The comparison of distributions of continuous variables was made using the Wilcoxon test or Kruskal–Wallis test. Jonckheere–Terpstra trend test was utilized to test for trend of FGFR1 immunoreactivity in women reporting more severe dysmenorrhea as described previously [13]. The correlation between FGFR1 staining scores and dysmenorrhea scores was analyzed using Pearson's $\chi^2$ test. Comparisons between two groups were performed with the Student's t test, and differences among multiple groups were evaluated by one-way ANOVA analysis. Differences were considered statistically significant at $P < 0.05$.

## Results

### Bioinformatics identification of FGFR1 as a candidate gene for endometriosis development

A total of 493 genes significantly differentially-regulated by at least 2-fold ($P < 0.05$) and overlapped in three datasets were