Estrogen-mediated activation of fibroblasts and its effects on the fibroid cell proliferation

NING LUO, QIYU GUAN, LIHUA ZHENG, XIAOYAN QU, HONG DAI, and ZHONGPING CHENG

SHANGHAI, CHINA

In this study, we explored the role of estrogen-mediated activation of stromal fibroblasts in the pathogenesis of uterine fibroid in patients. We isolated uterine fibroids and surrounding smooth muscle from patients and separated fibroblasts using immunomagnetic beads. We also measured the expression levels of estrogen receptors in fibroblasts and examined cell proliferation, expressions of fibroblast activation protein (FAP), extracellular matrix (ECM) (fibronectin, laminin, collagen I), growth factors (transforming growth factor-β, insulin-like growth factor-1), and cell proliferation pathway stimulated by estrogen. We also silenced the expression of FAP by RNA interference and analyzed the expression levels of these markers before and after E2 stimulation. Finally, we also investigated the effect of activated fibroblast supernatant on cell proliferation of fibroblasts, smooth muscle cells, and fibroid cells. We found that fibroblasts in uterine fibroid were activated, and the expression levels of estrogen receptors from fibroid cells were higher than those from smooth muscle cells. After estrogen stimulation, the proliferation activity of fibroblast was enhanced, and the expression of FAP, ECM, and growth factors was increased; the signaling pathway involved in cell proliferation was also activated. Interestingly, the activated fibroblast supernatant stimulation can promote cell proliferation. Silencing of FAP expression could inhibit the E2-mediated biological effects. In conclusion, estrogen promotes proliferation of uterine fibroids through the activation of fibroblasts, thus, activated fibroblasts may play an important role in the pathogenesis of uterine fibroids, which could be targeted in future for the treatment of uterine fibroid. (Translational Research 2014;163:232–241)

Abbreviations: BSA = bovine serum albumin; ECM = extracellular matrix; ELISA = enzyme-linked immunosorbent assay; ERα = estrogen receptor α; ERβ = estrogen receptor β; FAP = fibroblast activation protein; HBSS = Hanks balanced salt solution; IGF = insulin-like growth factor; MIT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS = phosphate buffered saline; PCR = polymerase chain reaction; SMC = smooth muscle cell; TAF = tumor-associated fibroblasts; TGF = transforming growth factor; UFC = uterine fibroid cell

Uterine fibroids are the most common benign tumors affecting the female reproductive system.[1] Clinical observations show that uterine fibroids are hormone-dependent. Uterine fibroids rarely appear before menarche and regress after menopause.[2,3] Studies have shown that estrogen plays an important role in the development of fibroid tumors, but its underlying mechanisms are poorly understood.

From the Department of Obstetrics and Gynecology, Yang-Pu Center Hospital, Shanghai, China.

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Reprint requests: ZhongPing Cheng, Department of Obstetrics and Gynecology, Yang-Pu Center Hospital, 450 Tengyue Rd, Shanghai, China; e-mail: mdcheng18@263.net.

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AT A GLANCE COMMENTARY

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Background

Fibroblast is a main component of uterine fibroids, however, its role in the pathogenesis of uterine fibroid is still unclear. Whether fibroblast is activated and what is the underlying molecular mechanism had not been defined yet, thus, this study investigated the role of fibroblast in patient tissue, which could be a direct evidence for fibroblast in the pathogenesis of uterine fibroid.

Translational Significance

This study demonstrates that stromal-activated fibroblast plays an important role in the pathogenesis of uterine fibroid, which could be targeted in the treatment of uterine fibroid in future.

The surrounding stromal cells, various secreted growth factors, and the signal transduction between cells have been recognized as important factors in tumor growth, and these factors provide a complex tumor microenvironment, which promote the growth and invasion of tumor cells.[4] Under normal circumstances, fibroblasts are in a quiescent state but can be activated by injury, inflammatory, and tumor state.[5] It has been shown that about 80% of fibroblasts in tumor tissue keep a constant activation status.[6] Tumor tissue fibroblasts are also known as tumor-associated fibroblasts (TAF), and activated fibroblasts are involved in tumor development.

Uterine fibroid tissue is mainly composed of fibroid cells, fibroblasts, as well as a large number of extracellular matrix (ECM) components. Fibroblasts provide nutritional support and survival framework for fibroid cells, and ECM is primarily secreted from fibroblasts.[7] Previous studies on the pathogenesis of uterine fibroids have mainly focused on the differentiation and proliferation of fibroid cells. However, the histologic features of fibroid tissue suggest that fibroblasts may play an important role in the generation of uterine fibroids.

The importance of activated fibroblasts in tumor pathologic process and estrogen in the pathogenesis of uterine fibroids suggest that fibroblasts may play an important role in estrogen-mediated pathogenesis of uterine fibroids. In this study, we isolated fibroblasts from tumor tissues of patients, and monitored their activation levels. We first used estrogen to stimulate fibroblasts, and compared the cell activation and proliferation and the expression of molecules involved in signaling pathways before and after stimulation. We then suppressed fibroblast activation protein (FAP) expression using RNA interference techniques and monitored the effect of estrogen on TAF activation to explore the mechanism of estrogen-mediated activation of stromal fibroblasts in fibroid cell proliferation.

METHODS

Reagents. Antibodies for phosphorylated and total v-akt murine thymoma viral oncogene homolog (p-AKT and AKT), phosphorylated and total extracellular signal-regulated kinases 1/2 (p-ERK1/2 and ERK1/2), phosphorylated and total mitogen-activated protein kinase kinase 1 (p-MEK and MEK), phosphorylated and total FBJ murine osteosarcoma viral oncogene homolog (p-c-fos and c-fos) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from cell signaling technologies, and antibodies for CD90, FAP, ERα, ERβ, fibronectin, and laminin were obtained from Abcam. Secondary antibodies of goat anti-rabbit Alexa Fluor 555, goat anti-mouse FITC, goat anti-rabbit HRP, and goat anti-mouse HRP were purchased from Beyotime Institute of Technology, China and enzyme-linked immunosorbent assay (ELISA) kits for transforming growth factor (TGF)-β, insulin-like growth factor (IGF)-1, and collagen 1 from Abcam. Estrogen (β-Estradiol, E2) and progesterone were obtained from Sigma Aldrich.

Patients and samples. The research was carried out according to the principles of the Declaration of Helsinki, and informed consent was obtained from all patients. This study was approved by the ethics committee of the Yang-Pu Hospital, Shanghai, China. Patients were randomly selected from the Department of Obstetrics and Gynecology, Yang-Pu Center Hospital between May 2011 and July 2011, and 10 uterine fibroid cases were studied. The removal criteria included patients who were subsequently diagnosed with uterine adenomyosis and patients with a history of coronary artery disease, hypertension, or hematologic disorders. Fibroid and myometrial tissue were obtained by laparoscopy combined with myomectomy from patients before they were treated for symptomatic fibroid disease using the uterine artery occlusion (UAO) technique. After fibroid and myometrial tissue were surgically removed, part of fresh tissue specimens $1.5 \times 1.0 \times 1.0$ cm in size were immersed in Hanks balanced salt solution (HBSS) buffer (100 U/mL penicillin, 100 μg/mL streptomycin added), and another part of the tissue was confirmed by histologic examination of the specimens removed.

Primary cell isolation and culture. Primary fibroid and myometrial cells were isolated and cultured as described.[8] Briefly, after fibroid and adjacent myometrial tissues were surgically removed, the