Evaluation of Rap1GAP and Epac1 Gene Expression in Endometriosis

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Abstract

Objective: Endometriosis is a female reproductive system disease in which endometrial tissue are found in other women organs. Various factors are effective in the development of endometriosis and due to the interaction of genetics and environmental factors, this disease is a multifactorial disease. MAPK/ERK and PI3K/Akt/mTOR pathways are activated by growth factors and steroid hormones and known as two important pathways involved in the processes of growth, proliferation and survival of endometriosis cells. Raps, monomeric GTPase of Ras family, are able to activate these pathways independently of Ras. The goal of our study was to evaluated the expression level of Rap1GAP and Epac1 gene, as two important RapGAPs (GTPase-activating proteins) and RapGEFs (guanine nucleotide exchange factors) respectively, in endometriosis tissues and normal endometrium tissues.

Materials and Methods: In this study, 15 samples of women without signs of endometriosis were taken as control samples, 15 ectopic and 15 eutopic samples were taken from women with endometriosis using laparoscopic surgery. The expression of Epac1 and Rap1GAP genes was investigated by Real-time PCR technique and results were analysis by One-Way ANOVA test.

Results: Epac1 upregulated significantly in ectopic tissues compared to eutopic and control tissues (Their P-value were <0.0001). Rap1GAP expression was lower in ectopic tissues compared to control samples (P-value was 0.003) and eutopic tissues (P-value was 0.001).

Conclusion: Based on these results, it may be concluded that changes in the expression of the Rap1GAP and Epac1 genes may play role in the pathways involved in the pathogenesis, displacement, and migration of endometriosis cells.

Introduction

Endometriosis is a benign complication caused by the growth of endometrial tissue in other places, including the ovaries and fallopian tubes (1). The real prevalence of endometriosis in the general population is not known precisely because definitive diagnosis of the disease is possible only through invasive laparoscopic surgery, however, is estimated that about 10% of women are probably affected by this disease (2). Studies show that about 25 to 50 percent of infertile women have endometriosis, and 30 to 50 percent of women with this disease are infertile (3).

The exact pathogenic mechanism of endometriosis is unknown; however, it has been shown to be a multifactorial disease in which genetic and environmental factors are involved. Numerous studies have examined genetic differences between infected and healthy individuals, as well as between endometriosis and healthy tissues. They show that changes in some signaling pathways are involved in the development and progression of this disease, including the MAPK and PI3K/Akt pathways (4-7).

Epac1 and Rap1GEF can alter signaling pathways associated with proliferation, migration and apoptosis by affecting the activity of Rap and Ras proteins (8, 9). Epac1, as a cAMP-activated GEF, can activate

members of the Rap families, thereby can change the expression of MAPK pathway target genes (10). Epac1 also activates Akt pathway by Rap1 activation then prevents apoptosis in cancer cells through activation some anti-apoptotic proteins (11).

Rap1GAP is known as a tumor suppressor in various cancers such as thyroid, breast cancers (11, 12). Rap1GAP affects the downstream pathways by activating the endogenous GTPase activity of Rap1 (13). Reduced Rap1GAP gene expression increased cell proliferation and decreases apoptosis through MAPK and Akt pathways in cancer cell lines (14, 15).

Accurate information on the expression of Epac1 and Rap1GAP genes in endometriosis is not available, then the aim of this study was to investigate the expression of these genes in endometriosis tissues as well as normal endometrial tissues in order to find their effect on endometriosis.

Materials And Methods

In this case-control study, we used 15 samples of ectopic and eutopic endometriosis tissues of women with endometriosis and 15 samples of normal endometrial tissues of women who had no signs of endometriosis and referred to Shahid Sadoughi Hospital Yazd for reasons such as pelvic pain and fallopian tubes blockage between April 2019 and March 2020 and their tissues stored in the biobank of the Yazd Reproductive Biology Research Center. In order to maintain mRNA stability, the tissues were kept in RNA stabilizer

solution (RNA later, Yekta Tajhiz Azma, Iran) at -80 ° C. The age range of the women in this study was 24 to 45 years. Samples were taken from all subjects in the study during the proliferative phase of the menstrual cycle and none of the women had taken any hormonal drugs in the three months before sampling, and no benign tumors or fibroids had been observed in any of the subjects.

Rna Extraction And Qrt-pcr

RNA total was extracted from 50 mg tissue samples by RNA X-plus Solution (CinnaGen, Iran) and the quality of the extracted RNA was measured by a nanodrop spectrophotometer. To investigate the expression level of genes in this study, cDNA was synthesized by reverse transcription reaction by cDNA synthesis kit (Yekta Tajhiz Azma, Iran) according to its instructions. In order to perform Real-time quantitative PCR reactions, SYBR Green master mix (Yekta Tajhiz Azma, Iran) was used and according to the instructions, the reactions were performed in a Rotor Gene-Q device (Qiagen, Germany) device. GAPDH gene was utilized as the internal control for Rap1GAP and Epac1 genes normalization. Primer's sequences are shown in Table 1.

Table 1
Oligonucleotide primers

Gene	Primer sequence
Rap1GAP	Forward: 5- <i>GCAC T TCTCGGC</i> <i>GGAGCA T T</i> - 3 Reverse: 5- <i>TGACATCATGGTATGT</i> C
Epac1	Forward: 5-CATGAGGGAGATGATTTTGG-3 Reverse: 5-GCCTCCACATCCTTGATGATA-3
GAPDH	Forward: 5-CAAGAGCACAAGAGGAAGAGAGAG-3 Reverse: 5-TCTACATGGCAACTGTGAGGAG-3

Statistical analysis

Epac1 and Rap1GAP genes expression levels in three groups of ectopic, eutopic and control were measured using the $2^{-\Delta\Delta C_t}$ method. In order to perform statistical analyzes and compare the expression of Epac1 and Rap1GAP genes in ectopic and eutopic endometriosis and normal endometrial tissue, SPSS software (version 26) and One-Way ANOVA with post-hoc Tukey's HSD test were used. P-value < 0.05 was considered statistically significant and the plots were designed by GraphPad Prism 8.

Results

Rap1GAP expression

Rap1GAP expression level was evaluated by qPCR in endometriosis tissues as compared to the normal group. The analysis of data in eutopic and ectopic tissues of endometriosis patients as well as normal tissue indicated that Rap1GAP expression was not significantly different between eutopic endometriosis tissues and normal endometrium and its expression was approximately equal in them (P-value = 0.958). According to the results, Rap1GAP expression level in ectopic tissues was significantly reduced compared to control tissues (P-value = 0.003) and eutopic tissues (P-value = 0.001) (Figure 1A).

Epac1 Gene Expression

Epac1 gene expression was also evaluated by qPCR in endometriosis tissue compared to the healthy group. Our findings demonstrated a significant increase in the expression level of the Epac1 gene in ectopic tissues of endometriosis patients as compared to the eutopic tissues and normal control tissues of healthy individuals (Their P-value were <0.0001). There was not a significant difference in Epac1 gene expression in patients' eutopic tissues and normal endometrium of healthy women (P-value = 0.714) (Figure 1B).

Discussion

Rap1, as a small GTPase, is one of the Ras family genes (16). Rap1 binds to both GDP and GTP and the change between the two states describes it as a molecular switch (17). Rap1 involved in various signaling pathways, including the MAPK/ERK and PI3K/AKT signaling pathways, and by affecting this signaling pathways, it can change the rate of cell proliferation, migration, and cell invasion. The regulation of RAP1 activity in the cells is done by two class of proteins. GEFs (guanine nucleotide exchange factors), like Epac1 and PDZ-GEFs, help to change the bound nucleotide to Rap1 easier and this protein can rebound to more abundant GTP in the cells then increase its activation (18). GAP (GTPase-activating proteins), like Rap1GAP and SPA-1, increases the natural GTPase activity of Rap1 then decreases its activation (19). Many studies have shown that changes in the activity of the MAPK and PI3K/AKT signaling pathway are associated with endometriosis then change in Rap1 and its regulators such as GAPs and GEFs may have role in the pathogenesis of endometriosis.

Rap1GAP is known in various cancers as a tumor suppressor and its expression has been reduced in thyroid and pancreatic cancers (20, 21). Decreased Rap1GAP expression can alter the expression of the MAPK and PI3K/Akt pathways target genes, resulting in increased cell growth and migration and proliferation in breast cancer and human umbilical vein endothelial cells (HUVECs) (15, 22). In 2003, Kap LC et al. examined the expression profile of genes in endometriosis tissue of patients by using the microarray method and concluded that the expression of Rap1GAP gene in endometriosis tissue is reduced compared to normal endometrial tissue (23). Kaam et al. investigated promoter methylation and expression of some genes such as Rap1GAP in eutopic endometriosis tissue and normal endometrial tissue. They did not find a significant differences between the two tissues in promoter methylation or gene expression (24). Our results in eutopic and control tissues were in line with the study of Kaam et al. By examining the expression of Rap1GAP gene in ectopic and eutopic tissues and comparing them with the expression of this gene in normal endometrial tissue, it seems that decreased expression of this gene observed in endometriosis ectopic tissues, which may be related to the role of this gene in regulating the pathways involved in endometriosis cells migration and displacement in this disease.

Epac1, as a GEF that enabled by cAMP is able to replace GDP with GTP in Raps and activate them. Epac1 is activated by growth factors through GPCRs (G-protein-coupled receptors) (25, 26). Studies have shown that increased expression of this gene in various cancers such as ovarian and breast can increase proliferation, migration and cell invasion (27, 28). The study of prostate cancer cell lines has shown that increasing Epac1 expression through activating MAPK and Akt signaling pathways and ultimately activating mTOR increases cell survival and cell proliferation (10). It has been shown that knockdown Epac1 by siRNA in ovarian cancer cell lines can reduce the activity of the PI3K/Akt signaling pathway so that by decreasing the expression of Epac1, the amount of p-Akt is reduced. Decreasing the amount of p-Akt in ovarian cancer cell lines reduces the expression of target genes in this pathway, including cyclin D1 and CDK4, and causes the cell to remain in G1 phase of the cell cycle. These results showed that increasing the expression of Epac1 gene in ovarian cancer cells increases cell proliferation and survival and reduces cell apoptosis (28).

Since there was no study on the expression of Epac1 gene in endometriosis tissues and according to previous studies that have pointed to the role of MAPK and PI3K/Akt pathways in the incidence of endometriosis, in this study we evaluated the expression level of Epac1 gene in the endometriosis. In this study, it was found that the expression of Epac1 gene in ectopic tissues has increased compared to eutopic tissues and normal endometrium. These results may indicate that increased expression of Epac1 gene could play a role in endometriosis progression, migration and displacement of endometriosis cells during endometriosis.

Conclusion

The results of this study showed that the expression of Rap1GAP gene, as one of the proteins that inhibits the activity of MAPK and Akt signaling pathways through Raps proteins, did not show any significant difference in normal endometrial and eutopic tissues, however its expression was downregulated in ectopic tissues. On the other hand, Epac1 gene, which is one of the activators of Raps proteins, shows increased expression only in ectopic tissues of patients with endometriosis compared to eutopic tissues of patients and normal endometrium. These results suggest that increased Epac1 expression and decreased expression of Rap1GAP in ectopic tissues may activate pathways involved in progression and migration of endometriosis cells to other organs. Given that various factors can play role in the activity of MAPK and Akt pathways involved in the development of endometriosis, it is suggested that other genes involved in these pathways and epigenetic factors affecting the activity of these pathways be investigated in future studies.

Abbreviations

RapGAPs
GTPase-activating proteins
RapGEFs
guanine nucleotide exchange factors

Declarations

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Competing interests

All authors declare that they have no Conflicts of interest/Competing interests regarding this paper.

Authors' contributions

All authors have read and approved the manuscript.

M.D, G.Y: performing the main steps of essay and writing the manuscript.

R.S and A.K: Collecting the samples and helping to perform RNA extraction and qPCR.

F.S, M.V: Analysis of results and doing statistical tests.

Consent to participate

Written informed consent was obtained from each participant before the collection of tissue samples

Ethics approval

The Institutional Ethics Committee of the Shahid Sadoughi University of Medical Sciences of Yazd approved the study design (IR.SSU.MEDICINE.REC.1399.258).

Consent for publication

Not Applicable

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Figures

Figure 1

Comparison of Epac1 and Rap1GAP genes expression with GAPDH gene expression in three ectopic, eutopic and control tissues. The ratio of Rap1GAP to GAPDH (A) and the ratio of gene expression of Epac1 to GAPDH (B).