Endometriosis is a benign complication caused by the growth of the endometrial tissue in other places, including the ovaries and fallopian tubes. The real prevalence of endometriosis in the general population is not known precisely because a definitive diagnosis of the disease is possible only through invasive laparoscopic surgery; however, it is estimated that about 10% of women are probably affected by this disease.

Studies show that about 25–50% of infertile women have endometriosis, and 30–50% of women with this disease are infertile.

The exact pathogenic mechanism of endometriosis is unknown; however, it is a multi-factorial disease in which genetic and environmental factors are involved. Numerous studies have examined genetic differences between affected and healthy women. The goal of our study was to evaluate the expression level of Rap1GAP and EPAC1 genes as two important RapGAPs (GTPase-activating proteins) and RapGEFs (guanine nucleotide exchange factors), respectively, in endometriosis tissues and normal endometrium tissues.

**Abstract**

**Background:** Endometriosis is a female reproductive system disease in which the endometrial tissue is found in other women’s organs. Various factors are effective in the development of endometriosis, and because of the interaction of genetics and environmental factors, this disease is a multi-factorial disease. MAPK/ERK and PI3K/Akt/mTOR pathways are activated by growth factors and steroid hormones and are known as two important pathways involved in the processes of growth, proliferation, and survival of endometriosis cells. Raps, monomeric GTPase of the Ras family, are able to activate these pathways independent of Ras. The goal of our study was to evaluate the expression level of Rap1GAP and EPAC1 genes 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. Fifteen ectopic and 15 eutopic samples were taken from women with endometriosis using laparoscopic surgery. The expression of EPAC1 and Rap1GAP genes was investigated by the real-time polymerase chain reaction technique, and the results were analyzed by one-way ANOVA test.

**Results:** EPAC1 upregulated significantly in ectopic tissues compared to eutopic and control tissues. Rap1GAP expression was lower in ectopic tissues compared to control and eutopic tissues.

**Conclusions:** Based on these results, it may be concluded that changes in the expression of the Rap1GAP and Epca1 genes may play a role in the pathways involved in the pathogenesis, displacement, and migration of endometriosis cells.

**Keywords:** Endometriosis, EPAC1, gene expression, Rap1GAP

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

The exact pathogenic mechanism of endometriosis is unknown; however, it is a multi-factorial disease in which genetic and environmental factors are involved. Numerous studies have examined genetic differences between affected and healthy women.
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.[14-16]

EPAC1 and Rap1GAP can alter signaling pathways associated with proliferation, migration, and apoptosis by affecting the activity of Rap and Ras proteins.[7,8] EPAC1, as a cAMP-activated GEF, can activate members of the Rap families and thereby can change the expression of MAPK pathway target genes.[9,10] EPAC1 also activates the Akt pathway by Rap1 activation and then prevents apoptosis in cancer cells through activation of some anti-apoptotic proteins.[11]

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

Accurate information on the expression of EPAC1 and Rap1GAP genes in endometriosis is not available. This study aimed to investigate the expression of these genes in endometriosis tissues as well as normal endometrial tissues to find their possible 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 or any lesion suggesting endometriosis referred to Shahid Sadoughi Hospital Yazd for reasons such as pelvic pain and fallopian tube blockage between April 2019 and March 2020. All women participating in this study were from the Iranian population living in Yazd. To maintain mRNA stability, the tissues were kept in RNA stabilizer solution (RNA later, Yekta Tajhiz Azma, Iran) at ≈-80°C. The inclusion criteria in the two groups (women with endometriosis and without it) were as follows: the age range of the women in this study was 24–45 years. Samples were taken from all subjects in the study during the proliferative phase of the menstrual cycle; none of the women had taken any hormonal drugs in the 3 months before sampling, and no benign tumors or fibroids had been observed in any of the subjects. Also, endometrial cell changes such as fibroids and carcinoma and receiving hormonal medication in the last trimester were our exclusion criteria for both groups. All samples (cases and controls) were approved by the pathology laboratory for histological examination, and samples without contamination with other cell types were selected for examination.

**RNA extraction and cDNA synthesis**

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 using a nanodrop spectrophotometer. To investigate the expression level of genes in this study, cDNA was synthesized by reverse transcription reaction using a cDNA synthesis kit (Yekta Tajhiz Azma, Iran). For this purpose, 1 µg of total RNA was mixed with 1 µl random hexamer primer and brought to 13.5 µl by DEPC-treated water. After mixing, the material was incubated at 70°C for 5 minutes. In the next step, 4 µl 5x first-strand buffer, 1 µl dNTPs (10 mM each), 0.5 µl RNasin (40 U/µl), and 1 µl M-MLV reverse transcriptase were added to the mixture and incubated for 60 minutes at 37°C. In the last step, it was incubated for 5 minutes at 70°C and then kept at -20°C.

**qRT-PCR**

In order to perform real-time quantitative polymerase chain reaction (PCR) reactions, the SYBR Green master mix (Yekta Tajhiz Azma, Iran) was used and the reactions were performed in a Rotor Gene-Q device (Qiagen, Germany) device. The thermal conditions of the reaction were 95°C for 15 min, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 30 s. Also, a final extension of 72°C for 5 min was performed. Our final mixing volume was 13 µl, including 1 µl cDNA, 0.5 µl forward primer, 0.5 µl reverse primer, and 6.25 µl of 2 × QPCR master mix. DNase-RNase-free water was added to reach a final volume of 13 µl. The GAPDH gene was selected as the internal control for Rap1GAP and EPAC1 gene normalization. The primer’s sequences are shown in Table 1.

**Statistical analysis**

EPAC1 and Rap1GAP gene expression levels in the three groups of ectopic, eutopic, and control were measured using the 2ΔΔCT method. To perform statistical analyses and compare the expression of EPAC1 and Rap1GAP genes in ectopic and eutopic endometriosis and normal endometrial tissues, SPSS software (version 26) and one-way ANOVA with post-hoc Tukey’s HSD test (for gene expression analysis) and T-test (for analysis of age) were used. P value < 0.05 was considered statistically significant, and the plots were designed by GraphPad Prism 8.

**Results**

The Rap1GAP expression level was evaluated by qPCR in endometriosis tissues as compared to the normal group. The analysis of data in ectopic and eutopic tissues of endometriosis patients as well as normal tissues indicated that Rap1GAP expression was not significantly different between eutopic endometriosis tissues and the normal endometrium and its expression was approximately equal in

| Table 1: Oligonucleotide primers |
|--------------------------------|
| **Gene**       | **Primer sequence** |
|----------------|---------------------|
| Rap1GAP        | Forward: 5’-GCACCTTCTCGGCAAGGAGCAATTF-3’ |
|                | Reverse: 5’-TGCACATCATGATCAGTGCGGACTCACT-3’ |
| EPAC1          | Forward: 5’-CATGAGGGAGATGATTTTGGA-3’ |
|                | Reverse: 5’-GCCTCCACATCCTCCCTTGGATGTA-3’ |
| GAPDH          | Forward: 5’-CAAGAGCACAAGAGGAGGAGAGGAGGAG-3’ |
|                | Reverse: 5’-CTACATGCGCAACTGTTGGAGGAG-3’ |
them (P-value = 0.958). According to the results, the 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 and Table 2].

EPAC1 gene expression was also evaluated by qPCR in endometriosis tissues 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 values were 0.000008 and 0.000096, respectively). There was not a significant difference in EPAC1 gene expression in patients’ eutopic tissues and the normal endometrium of healthy women (P-value = 0.714) [Figure 1b and Table 2].

There was no significant difference between the mean ages of the two groups participating in this study, patients with endometriosis and those without endometriosis (P-value = 0.3852) [Table 3].

**DISCUSSION**

Rap1, as a small GTPase, is one of the Ras family genes. Rap1 binds to both GDP and GTP, and the change between the two states describes it as a molecular switch. Rap1 is involved in various signaling pathways, including the MAPK/ERK and PI3K/AKT signaling pathways, and by affecting these signaling pathways, it can change the rate of cell proliferation, migration, and cell invasion. The regulation of RAP1 activity in the cells is performed by two classes of proteins. GEFs (guanine nucleotide exchange factors), such as EPAC1 and PDZ-GEFs, help to change the bound nucleotide to Rap1 easier, and this protein can re-bind to more abundant GTP in the cells and then increase its activation. GAP (GTPase-activating proteins), such as Rap1Gap and SPA-1, increases the natural GTPase activity of Rap1 and then decreases its activation. Many studies have shown that changes in the activity of the MAPK and PI3K/AKT signaling pathways are associated with endometriosis, and changes in Rap1 and its regulators such as GAPs and GEFs may have a 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 gastric cancers. 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 human umbilical vein endothelial cells (HUVECs). In 2003, Kao LC et al. examined the expression profile of genes in endometriosis tissues of patients by using the micro-array method and concluded that the expression of the Rap1GAP gene in endometriosis tissues is reduced compared to normal endometrial tissues. By examining the expression of the Rap1GAP mRNA level in ectopic and eutopic tissues and comparing them with the expression of this gene in normal endometrial tissues, it seems that decreased expression of this gene is observed in endometriosis eutopic tissues, which may be related to the role of this gene in regulating the pathways involved in endometriosis cell migration and displacement in this disease.

EPAC1, as a GEF enabled by cAMP, can replace GDP with GTP in Raps and activate them. EPAC1 is activated by growth factors through GPCRs (G-protein-coupled receptors). Studies have shown that increased expression of this gene in various cancers such as ovarian and breast can increase proliferation, migration, and cell invasion. 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. It has been shown that knockdown of 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

![Figure 1: Comparison of EPAC1 and Rap1GAP gene expression with GAPDH gene expression in the ectopic, eutopic, and control tissues. (a) The ratio of Rap1GAP to GAPDH and (b) the ratio of gene expression of EPAC1 to GAPDH. One-way ANOVA with post-hoc Tukey’s HSD test was used.](image-url)

| Groups | Mean±SEM |
|--------|----------|
|       | Rap1GAP  | EPAC1  |
| Control | 1.220±0.1757 | 1.207±0.2145 |
| Eutopic | 1.293±0.2568 | 0.876±0.1141 |
| Ectopic | 0.305±0.07725 | 3.165±0.4548 |

**Table 3: Demographic data of the participants**

| Groups | Patients* | Age (yr)** |
|--------|-----------|------------|
| Case (Ectopic and Eutopic) | 15 | 33.53±1.2 |
| Control | 15 | 35.27±1.5 |

* Data are presented as total numbers of each variable. **Data are presented as mean±SEM.
cancer cell lines reduces the expression of target genes in this pathway, including cyclin D1 and CDK4, and causes the cell to remain in the G1 phase of the cell cycle. These results showed that increasing the expression of the EPAC1 gene in ovarian cancer cells increases cell proliferation and survival and reduces cell apoptosis.[25]

Because there was no study on the expression of the 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 mRNA in the endometriosis. In this study, it was found that the expression of the EPAC1 gene in ectopic tissues has increased compared to eutopic tissues and the normal endometrium. These results may indicate that increased expression of the 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 the Rap1GAP gene, as one of the factors that inhibit 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 down-regulated in ectopic tissues. On the other hand, the 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 the normal endometrium. These results suggest that increased EPAC1 expression and decreased expression of Rap1GAP in ectopic tissues may activate pathways involved in the progression and migration of endometriosis cells to other organs. Given that various factors can play a 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.

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**Ethics approval and consent to participate**

This study and the whole process of holding it has been done under the supervision of the ethics committee of Shahid Sadoughi University of Medical Science, Yazd, Iran, and informed consent has been obtained from all patients and participants in this study.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Alimi Y, Iwanaga J, Loukas M, Tubbs RS. The clinical anatomy of endometriosis. A review. Cureus 2018;10:e3361.
2. Shafrir AL, Farland L, Shah D, Harris H, Kvaskoff M, Zondervan K, et al. Risk for and consequences of endometriosis: A critical epidemiologic review. Best Pract Res Clin Obstet Gynaecol 2018;51:1-15.
3. Misserer SA, Hankinson SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. Am J Epidemiol 2004;160:784-96.
4. Bora G, Yaba A. The role of mitogen-activated protein kinase signaling pathway in endometriosis. J Obstet Gynecol Res 2021;47:1610-23.
5. Madanes D, Bilotas MA, Bastón JJ, Singla JJ, Meresman GF, Barañao RI, et al. PI3K/AKT pathway is altered in the endometriosis patient's endometrium and presents differences according to severity stage. Gynecol Endocrinol 2020;36:436-40.
6. Yarahmadi G, Dehghanian M, Sandoghsaz RS, Savaei M, Shamsi F. Evaluation of NF1 and RASA1 gene expression in endometriosis. Eur J Obstet Gynecol Reprod Biol 2022;15:100152.
7. Kumar N, Gupta S, Dabrai S, Singh S, Sehrawat S. Role of exchange protein directly activated by cAMP (EPAC1) in breast cancer cell migration and apoptosis. Mol Cell Biochem 2017;430:115-25.
8. Li Q, Teng Y, Wang J, Yu M, Li Y, Zheng H. Rap1 promotes proliferation and migration of vascular smooth muscle cell via the ERK pathway. Pathol Res Pract 2018;214:1045-50.
9. Miza UK, Pizzo SV. Epac1-induced cellular proliferation in prostate cancer cells is mediated by B-Raf/ERK and mTOR signaling cascades. J Cell Biochem 2009;108:998-1011.
10. Kosuru R, Chrzanowska M. Integration of Rap1 and calcium signaling. Int J Mol Sci 2020;21:1616.
11. Lv W, Liu S, Zhang Q, Yi Z, Bao X, Feng Y, et al. Downregulation of epac reduces fibrosis and induces apoptosis through akt signaling in human keloid fibroblasts. J Surg Res 2021;257:306-16.
12. Shah S, Brock EJ, Jackson RM, Ji K, Boerner JL, Sloane BF, et al. Downregulation of Rap1GAP: A switch from DCIS to invasive breast carcinoma via ERK/MAPK activation. Neoplasia 2018;20:951-63.
13. Jaśkiewicz A, Pająk B, Orzechowski A. The many faces of Rap1 pathway. J Cell Mol Med 2019;23:3897-904.
14. Xu M, Zhou J, Zhang Q, Le K, Xi Z, Yi P, et al. MiR-31-21-3p promotes tumor invasion and metastasis by suppressing Rap1GAP in papillary thyroid cancer in vitro. Ann Transl Med 2020;8:1229.
15. Dorn A, Zoellner A, Follo M, Martin S, Weber F, Marks R, et al. Rap1a deficiency modifies cytokine responses and MAPK-signaling in vitro and impairs the in vivo inflammatory response. Cell Immunol 2012;276:187-95.
16. Nussinov R, Jung H, Zhang M, Tsai C-J, Sablina AA. The mystery of Rap1 suppression of oncogenic Ras. Trends Cancer 2020;6:369-79.
17. Jin X, Di X, Wang R, Ma H, Tian C, Zhao M, et al. RBM10 inhibits cell proliferation of lung adenocarcinoma via RAP1/AKT/CREB signalling pathway. J Cell Mol Med 2019;23:3897-904.
18. Looi C-K, Hii L-W, Ngai SC, Leong C-O, Mai C-W. The role of Rap1GAP functions as a tumor suppressor gene and is regulated by DNA methylation in differentiated thyroid cancer. Cytogenet Genome Res 2021;161:227-35.
19. Dehghanian M, Yarahmadi G, Dehghanian M, Sandoghsaz RS, Savaei M, Shamsi F. Evaluation of NF1 and RASA1 gene expression in endometriosis. Eur J Obstet Gynecol Reprod Biol 2022;248:105822.
effects of Rap1GAP overexpression on proliferation and migration of endothelial cells via ERK and Akt pathways. J Huazhong Univ Sci Technol 2011;31:721-7.

23. Kao L, Germeyer A, Tulac S, Lobo S, Yang J, Taylor R, *et al*. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. Endocrinology 2003;144:2870-81.

24. Formoso K, Lezoualc’h F, Mialet-Perez J. Role of EPAC1 Signalosomes in cell fate: Friends or foes? Cells 2020;9:1954.

25. Gao M, Ma Y, Bast RC, Li Y, Wan L, Liu Y, *et al*. Epac1 knockdown inhibits the proliferation of ovarian cancer cells by inactivating AKT/Cyclin D1/CDK4 pathway *in vitro* and *in vivo*. Med Oncol 2016;33:1-10.