Expression levels of genes involved in lipogenesis and cholesterol synthesis in adenomyosis

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Abstract

Objectives: Adenomyosis is a benign uterine disease that occurs with the invasion of the endometrial gland and stoma into the myometrium. The etiology and molecular pathology of adenomyosis are not yet fully understood. Tissue samples of patients diagnosed with adenomyosis and healthy endometrial tissues were investigated for the lipogenesis and cholesterol synthesis pathways. It was aimed to determine the difference between adenomyosis and healthy endometrial tissues in terms of lipid metabolism and to investigate the mechanism of adenomyosis in this context.

Methods: Formalin-fixed paraffin-embedded archival tissues were used in the current retrospective study. A total of 76 patient samples and 3 groups were used. Group 1: adenomyotic tissue (n=28), Group 2: eutopic endometrial tissue (n=30), and Control Group (n=18). In these groups, Sterol regulatory element binding protein 1 (SREBP1) molecule, fatty acid synthase (FASN), acetyl-CoA carboxylase (ACACA), ATP-citrate lyase (ACLY), HMG-CoA reductase (HMGCR), and HMG-CoA synthase (HMGCS) markers were evaluated by using RT-PCR method.

Results: Statistically significant differences (p<0.05) were found between the groups regarding expression levels of HMGCR, HMGCS, ACLY, ACACA, and SREBP1. HMGCR, HMGCS, ACLY, and SREBP1 gene expression levels between Group 1 and Group 2 and HMGCS, ACACA, ACLY, and SREBP1 gene expression levels between Group 1 and Control Group were determined as statistically different. A significant difference was detected only in HMGCR gene expression levels between Group 2 and the Control Group.

Conclusions: These results show that genes involved in lipid metabolism may be associated with the molecular pathogenesis of adenomyosis.

Keywords: adenomyosis; lipogenesis; gene expression; SREBP; RT-PCR

Introduction

Adenomyosis is defined as a benign uterine condition. Adenomyosis occurs as a result of the progression of endometrial tissues in the uterus towards the muscle layer of the uterus. It is characterized by the presence of endometrial glands and stroma in the myometrium, as well as widespread uterine hypertrophy [1]. The etiology of adenomyosis is unknown. Although many mechanisms have been suggested such as the invagination of the endometrium into the myometrium and tissue damage and repair mechanism, it has not been supported by a definite etiological finding with experimental data [1, 2].

Lipids, which have essential functions in biological life, are heterogeneous compounds that dissolve easily in organic solvents such as ether, chloroform, benzene, and acetone. However, they are extremely poorly soluble in water. Lipids are chemical compounds that can turn into fatty acids and complex alcohols or fatty acid esters by hydrolysis [3].

In studies carried out to understand the difference between cancer cells and healthy cells, differences in many
metabolic processes such as energy metabolism, angiogenesis, growth factors, lipid metabolism and changes in signaling pathways have been determined [4]. Proliferation increase in cells that have undergone neoplastic transformation requires acceleration of lipid synthesis since they will be used as nutrients because they are rich in membrane production and energy. A change occurs in terms of lipid metabolism in cells that begin to differentiate [5]. Studies show that acceleration of lipid synthesis is necessary for tumor growth and cancer cells need fatty acids in many ways [6, 7]. Despite this, there are many points waiting to be clarified in terms of lipid metabolism in tumor-like tissues where benign conditions such as adenomyosis are maintained.

Recent research has revealed that several genes are expressed in patients with adenomyosis differently in ectopic and eutopic endometrial tissue. These genes, which differ in their expression levels, are involved in regulating cellular proliferation, tissue morphology, apoptosis, and angiogenesis processes [8, 9]. In addition, it has been determined that there are metabolic differences in the myometrium of women with adenomyosis. It has been reported that these differences cause changes in inflammation, oxidative stress, and energy metabolism and play a role in the progression of adenomyosis [10].

Biochemical, molecular, and genetic studies in adenomyosis are relatively new and few. In the current study, we focused on the lipid metabolism of the adenomyotic tissues and the possible roles of related genes in the molecular pathology of adenomyosis. Thus, we investigated the expression of lipid metabolism related genes such as the Sterol regulatory element binding protein 1 molecule (SREBP1), acetyl-CoA carboxylase (ACACA), ATP citrate lyase (ACLY), fatty acid synthase (FASN), HMG-CoA reductase (HMGCR) and HMG-CoA synthase (HMGCS), some for the first time, to provide insight the pathogenesis of the disease.

Materials and methods

Ethics approval

The project named “Relation of SREBP Molecule and Lipogenesis in Adenomyosis” was approved by the Ethics Committee of Mersin University (2020/450).

Collection of tissue samples

In the current study, 90 archival tissues were used. The tissues were collected in three groups as follows: Group 1; adenomyotic tissues (ectopic endometrium, n=35) and Group 2; normally located endometrial tissues (eutopic endometrium, n=35) of adenomyosis patients. Endometrial tissues from women without adenomyosis were used as the Control Group (n=20). Tissue samples were collected surgically from 35 adenomyosis patients. The patients were selected carefully after clinical and histopathological examination (Figure 1). Tissue collection was carried out between June 2017 and December 2021 in the Department of Pathology and Department of Obstetrics and Gynecology, Hospital of Mersin University, Turkey. The women enrolled in the study groups were of reproductive age, in the proliferative period, and diagnosed with adenomyosis. Adenomyotic tissues including the endometrial gland and stroma were used. Individuals without adenomyosis were used for tissues in the Control Group. Group 1 and Group 2 were 37–54 years old (mean: 45.58±4.26). The individuals in the Control Group were 38–47 years old (mean: 42.44±3.12). Moreover, approximately 90 % of the patients in Group 1 and Group 2 were multiparous.

The Mersin University Ethical Review Board gave their approval to this work.

Reagents

The innuPREP FFPE total RNA kit (Analytikjena PN: 845-KS-2050050) was used to isolate RNA from FFPE tissues. cDNA was synthesized from the obtained RNAs by RT-PCR. High-Capacity cDNA Reverse Transcription Kit (Thermo Cat. No: 4368814) was used for cDNA synthesis. The gene expression procedure was carried out using TaqMan® Gene Expression Master Mix (Appliedbiosystems PN: 4371135).

RNA extraction, cDNA synthesis and RT-qPCR expression method

After the RNA extraction step that used innuPREP FFPE total RNA kit, the cDNA synthesis step was applied. High-Capacity cDNA Reverse Transcription Kit protocol was performed for the cDNA synthesis from the RNAs isolated from FFPE tissue samples. The Thermal Cycler protocol for converting RNA to cDNA is as follows: 25°C for 10 min, 37°C for

Figure 1: Benign endometrial glands surrounded by endometrial stroma, with leiomyomatous smooth muscle bordering the endometrial stromal component (H&E × 40).
120 min, 85 °C for 5 s, and 4 °C for 1 min. After this stage, the gene expression method was performed. TaqMan® Gene Expression Master Mix, cDNAs, and primers (forward and reverse) designed for the genes we investigated were used in the gene expression method. The method performed with Roche LightCycler 480 II includes the steps as follows: 50 °C for 2 min (incubation phase), 95 °C for 10 min (activation phase), amplification step (95 °C for 15 s then 60 °C for 60 s, 40 cycles), 40 °C for 30 s (cooling phase). The ACTB (beta-actin) is a housekeeping gene and was used as the control gene in the current study. 2^ΔΔCt values were determined after experimental stages and employed in statistical analysis.

Statistical analysis

The IBM SPSS Statistics tool was used for statistical analysis. The mean and standard deviation were used to express the data. In the comparison of the expression of genes for the groups in the study, for normal and non-normal data distributions the ANOVA test for the normal distribution and the Kruskal–Wallis tests were utilized. Statistical significance was considered as a p-value of <0.05.

Results

After examining samples regarding RNA quantity and quality, a total of 76 patient samples were used in the study. The number of the samples in the groups was as follows: Group 1 (n=28); adenomyotic tissues, Group 2 (n=30); normally located endometrial tissues of adenomyosis patients, and Control Group (n=18); endometrial tissues from women without adenomyosis.

Except the FASN gene, statistically significant differences (p<0.05) in the expression of all genes were observed in comparison of the groups (Table 1).

**SREBP1** gene expression levels were significantly different between Group 1–Group 2 (p=0.029) and Group 1–Control Group (p=0.024) indicating a statistically significant decrease in **SREBP1** expression in adenomyotic tissues (Group 1) compared to eutopic endometrial tissues (Group 2) and endometrial tissues of women without adenomyosis.

**Table 1:** Comparison of the study groups for expression of lipid metabolism related genes.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Group 1 (n=28) mean±SD</th>
<th>Group 2 (n=30) mean±SD</th>
<th>Control (n=18) mean±SD</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGCR</td>
<td>0.901±0.389</td>
<td>1.348±0.622</td>
<td>0.853±0.487</td>
<td>0.004b</td>
</tr>
<tr>
<td>HMGCS</td>
<td>1.020±1.074</td>
<td>1.556±0.787</td>
<td>1.867±0.962</td>
<td>0.0001b</td>
</tr>
<tr>
<td>ALCY</td>
<td>0.941±0.418</td>
<td>1.391±0.563</td>
<td>1.604±0.861</td>
<td>0.001b</td>
</tr>
<tr>
<td>ACACA</td>
<td>1.307±0.593</td>
<td>1.251±0.586</td>
<td>0.955±0.670</td>
<td>0.016b</td>
</tr>
<tr>
<td>FASN</td>
<td>1.226±0.579</td>
<td>1.375±0.463</td>
<td>1.295±0.657</td>
<td>0.352</td>
</tr>
<tr>
<td>SREBP1</td>
<td>0.918±0.542</td>
<td>1.391±0.786</td>
<td>2.273±4.117</td>
<td>0.009b</td>
</tr>
</tbody>
</table>

*p-Values show the results of the ANOVA test for the normal distribution and the Kruskal–Wallis test for the non-normal distribution of data. SD, standard deviation, *p*<0.05, statistically significant.

Discussion

Adenomyosis occurs in the reproductive period of life in women and is diagnosed usually in multiparous patients. Although adenomyosis is a prevalent condition, its etiology and pathophysiology are still uncertain [11]. According to some earlier investigations, the expression of some genes has been found different in adenomyosis [12]. However, molecular processes regarding lipid metabolism in the development of adenomyosis remain unclear. This study could guide further studies on lipid metabolism in adenomyosis.

**FASN** catalyzes the processes that result in the production of 16-carbon palmitate from malonyl-CoA and acetyl-CoA substrates. It is one of the most studied lipid metabolism enzymes in tumors. Increased fatty acid production as a result of an increase in **FASN** has been detected in many malignancies and has been linked to a bad prognosis in many samples [13]. Since **FASN** has not been studied in adenomyosis patients before, the current study is the first
report between FASN and adenomyosis. The results of the study show that there is no difference in FASN expression between the experimental groups.

Transcription levels of enzymes related to fatty acid synthesis and transcription factors involved in regulating these enzymes are essential for lipid metabolism. One of these transcription factors is SREBP [14]. There are two SREBP genes in mammalian genomes: SREBP1 and SREBP2 [15]. While the genes involved in fatty acid biosynthesis are activated by SREBP1, SREBP2 is the transcription factor of the genes of cholesterol metabolism [16, 17]. Alter in SREBP expression has been detected in a variety of cancer types including glioblastoma, breast cancer, and prostate cancer. Emerging data reveals that the relationship between

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Figure 2: Intergroup comparison of $2^{-\Delta\Delta Ct}$ values of SREBP1 [Group 1 (IQR: 0.769; Q1: 0.469; Q3: 1.242), Group 2 (IQR: 1.150; Q1: 0.867; Q3: 1.881) and Control Group (IQR: 1.279; Q1: 1.032; Q3: 1.795)] (p<0.05).

Figure 3: Intergroup comparison of $2^{-\Delta\Delta Ct}$ values of ACACA [Group 1 (IQR: 1.162; Q1: 0.974; Q3: 1.808), Group 2 (IQR: 1.146; Q1: 0.866; Q3: 1.531) and Control Group (IQR: 0.823; Q1: 0.531; Q3: 0.987)] (p<0.05).

Figure 4: Intergroup comparison of $2^{-\Delta\Delta Ct}$ values of ACLY [Group 1 (IQR: 0.913; Q1: 0.599; Q3: 1.212), Group 2 (IQR: 1.287; Q1: 0.991; Q3: 1.770) and Control Group (IQR: 1.586; Q1: 0.932; Q3: 2.024)] (p<0.05).
oncogenic signaling and tumor metabolism may be mediated by the SREBP1 molecule, a crucial regulator of lipid metabolism [18]. SREBP1 is a possible molecular target for cancer treatment due to the relevance of increased lipogenesis for uncontrolled cell proliferation. Considering a benign tumor-like disease such as adenomyosis, no study can be associated with SREBP yet. The findings of our study suggest for the first time that the SREBP molecule may be effective in adenomyosis. The findings indicate a statistically significant decrease in SREBP1 expression in adenomyotic tissue (Group 1) compared to eutopic endometrial tissue (Group 2) and the Control Group. This decrease reveals the presence of lipid metabolism changes through the SREBP molecule in the development of adenomyosis. Even though there is no statistically significant difference between eutopic and control tissue, the difference in expression in ectopic adenomyosis tissue supports the idea that the SREBP molecule may be effective in the development of adenomyosis.

The rate-limiting enzyme in the fatty acid synthesis pathway is acetyl-CoA carboxylase (ACC). It has two isoforms called ACC1 and ACC2, which have various physiological roles in different cell types. Each is encoded by different genes. The lipogenic enzyme ACC1 catalyzes the carboxylation of acetyl-CoA to malonyl-CoA and is encoded by the ACACA gene. Malonyl-CoA produced from ACC1 is used in fatty acid synthesis [19]. The cytosolic enzyme ATP-citrate lyase (ACLY) transforms mitochondrial citrate into acetyl CoA, a precursor for the production of both fatty acids and mevalonates. It has been reported that the expression of ACLY in proliferative cells changes and suppresses the proliferation of certain tumor cell types [20]. No previous studies have been conducted on the role of ACACA and ACLY in adenomyosis. In the study, when the adenomyotic tissue and the Control Group were evaluated, it was determined that there was a significant increase in the expression of ACACA and a significant decrease in the expression of ACLY. Considering that these enzymes catalyze sequential steps in fatty acid synthesis, the possibility that different molecular processes may play a role in this step of fatty acid synthesis should be considered. In addition, it stated before that the expression of FASN which enzyme exists in further steps of fatty acid synthesis was also evaluated in this study. After analyzing the results of ACACA, ACLY, and FASN, it is
thought that further research is required to understand how the fatty acid production pathway affects women with adenomyosis.

HMG-CoA reductase is a rate-limiting enzyme for cholesterol synthesis and is the target of cholesterol-lowering statin drugs [21]. This enzyme is inhibited in mammalian cells by cholesterol, which is produced through the intracellular degradation of low-density lipoprotein. It is stated that cholesterol biosynthesis plays a role in the development of tumor tissue and statin drugs inhibit cell proliferation by inhibiting HMG-CoA reductase [22]. The HMG-CoA synthase enzyme condenses acetyl-CoA with acetoacetyl CoA to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), which is the substrate of HMG-CoA reductase. HMG-CoA is an intermediate metabolite involved in both cholesterol synthesis and ketogenesis [23]. In a previous research, it was reported that the incidence of adenomyosis decreased in mice whose cholesterol levels were lowered using a hypocholesterolemic agent [24]. The current study demonstrated a significant decrease in both HMGCR and HMGCS in ectopic adenomyosis tissue compared to eutopic endometrial tissue. This shows the possibility that cholesterol metabolism is suppressed in the development of adenomyosis. In addition, both eutopic and ectopic tissues of adenomyosis patients were compared with the Control Group, it was determined that there were expression differences in the HMGCS and HMGCR genes. The differences in the expressions of these genes suggest that these enzymes and cholesterol metabolism may be effective in the molecular mechanism of adenomyosis. There is no previous study on the role of the cholesterol synthesis pathway in adenomyosis patients. Therefore, this study is the first report on this subject. Additionally, the data of the current research will be a guide for future research on molecular studies in adenomyosis. Furthermore, the molecular pathways investigated in this study may serve as a possible therapeutic/diagnostic purpose for individuals with adenomyosis. Research on the molecular basis of adenomyosis will help to find better ways to diagnose and treat the condition of patients.

Conclusions

In conclusion, this study suggests that lipid metabolism related genes are altered in the development and progression of adenomyosis. Future molecular studies to clarify the pathways and mechanisms involved will provide a better understanding of the pathogenesis of the disease. In this way, potential therapeutic targets may also be elucidated.

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Research ethics: This study was approved by the Ethics Review Board of Mersin University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Not applicable.


Competing interests: The authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

References