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ORIGINAL ARTICLE

Efficacy of ABCA1 Transporter Proteins in Patients with Endometrial Cancer: an *In Vitro* Study

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ABSTRACT

Objectives: Endometrial carcinoma (EC) is a typical gynecological malignant tumor that occurs more frequently every year. Obesity is a significant contributor to the development of EC and its prognosis. Lipid metabolism and malignant tumors have a long history of association. Elevated cholesterol levels are made possible by adenosine triphosphate-binding cassette protein A1 (ABCA1) deficiency, which eventually promotes cancer cell survival. The aim of this study was to examine at the ABCA1 gene expression levels in EC patients. The relationship between ABCA1 and the occurrence, progression, and prognosis of EC is discussed in this article as a potential mechanism.

Materials and Methods: The samples of 45 endometrial adenocarcinoma patients were retrospectively included in this study and they were further divided into Grade 1 (15), Grade 2 (15), Grade 3 (15) tumors, control group. Twenty-nine endometrial tissues without a confirmed diagnosis of endometrial cancer made up the control group. ABCA1 gene expression was examined using real-time polymerase chain reaction.

Results: According to the results, the gene expressions of the patient group were higher than the control group When each Grade was compared with the control group, statistically significant results were obtained. After analyzing the data, it was found that the patient group was generally higher than the control group ($p < 0.05$) and there were differences in the grades of the patient group ($p < 0.05$). When the ABCA1 expressions of the grade groups and control groups were compared separately, a difference was found between Grade 1, Grade 2 and Grade 3 and the control group ($p= 0.0001$).

Conclusion: According to the findings of our study, a key component in the growth of EC tumors is the increase in cholesterol production caused by a reduction in ABCA1.

Keywords: ABCA1, endometrial cancer, cholesterol, ATP binding cassette protein, gene expression

INTRODUCTION

The most frequent gynecological tumor in developed nations is endometrial cancer (EC), a tumor that develops in the endometrium, and its frequency is rising.[1]

The main risk factor is having access to estrogens, both endogenous and exogenous, which are connected to conditions such as diabetes, obesity, early menarche age, nulliparity, late-onset menopause, and older age (55 years).[2,3] EC has been largely divided into two categories over the past 30 years on the basis of histological features, hormone receptor expression, and grade.[4] The most typical subtype of EC has a fair prognosis and is low-grade, endometrioid, diploid, and hormone receptor-positive, referred to as type I. Non-endometrioid, high-grade, aneuploid, TP53-mutated, and hormone receptor-negative tumors with a poor prognosis and a higher likelihood of metastasis are referred to as type II endometrial malignancies.[4]

According to certain studies, alterations in cancer cell metabolism, particularly aberrant cholesterol metabolism, play a significant role in their increased ability to proliferate and invade.[5]

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Adenosine triphosphate (ATP)-binding cassette (ABC) transporters are a family of lipid transporters that are confined to the plasma membrane.[6] ABC transporter A1 (ABCA1) is the first ABC protein to be discovered.[7] The liver, gut, brain, and macrophages all exhibit significant levels of the transmembrane protein ABCA1. Its main job is to facilitate the transfer of unbound phospholipids and free cholesterol from cells to apolipoprotein I to produce nascent high-density lipoprotein (nHDL) particles.[8] When ATP is hydrolyzed for energy, free cholesterol and intracellular phospholipids are ejected from cells and joined with non- or low-fat apo A-I to produce HDL, which subsequently kickstarts the process of reverse cholesterol transport.[6]

ABCA1 and Apo A1 interact through the Janus kinase/signal transducer activator of the transcription 3 signaling pathway. Tyrosine kinase/transcription factor 3 has anti-inflammatory effects and reduces chronic inflammation; both can be activated. This decreases the generation of pro-inflammatory cytokines, which cause increased cholesterol production from cells and inhibits ABCA1 degradation to prevent macrophage activation.[9] Dysregulation of cholesterol homeostasis, or decreased lipid export and increased cholesterol production in cancer cells, is a significant contributor to tumor formation.[10]

In this study, it was aimed to examine ABCA1 gene expression levels in patients with EC.

MATERIALS AND METHODS

Four groups of endometrial tissues were used in the study: patient groups, Group 1 including patients with EC Grade 1 (n= 15), Group 2 including patients with EC Grade 2 (n= 15), Group 3 including patients with EC Grade 3 (n= 15), and control group including patients with endometrial tissues without EC diagnosis (n= 29). The control group was classified into two: Control 1, which included 14 proliferative samples, and Control 2, which included 15 secretory phase healthy samples. During this study, formali-fixed paraffin embedded (FFPE) tissue samples retrospectively analyzed which were stored in Mersin University Faculty of Medicine, Department of Obstetrics and Gynecology and Pathology Department between 2010-2020. The Mersin University Ethics Committee accepted this study as a component of the "The Role of De Novo Lipogenesis and Cholesterol Synthesis Enzymes in Endometrial Cancer Development" project (2020/418, date: 10.06.2020).

The samples to be included in the study were graded as Grade 1, 2, and 3 according to their differentiation, belonging to patients diagnosed with endometrioid adenocarcinoma, which is the most common type of EC, without any prognostic factor. The International Federation of Gynecology and Obstetrics grading system for carcinomas of the uterine corpus is exclusively designated for endometrioid carcinomas and is based on architectural features as follows.[11]

Grade 1, 5% or less non-squamous solid growth pattern, Grade 2, 6-50% non-squamous solid growth pattern, Grade 3, > 50% non-squamous solid growth pattern.

Samples were taken from healthy individuals who were not diagnosed with EC. Control group samples were collected and stored in the same way as the patient group samples. The proper locations on the preparations were indicated after samples with little to no necrosis and no detection-tracking artifact were evaluated under a microscope (Figure 1). The right regions were then extracted from microtome sections of FFPE tissue blocks using the marked regions as a guide. From the paraffin block, 4-mm diameter by 10 µm thick microtome sections were removed and placed on clean slides. After that, 8-10 pieces of leaf tissue were removed from the slides.

All primers are composed of TaqMan Gene Expression Assays (ABI/Thermo, ThermoFisher Scientific).

Sample separation from the paraffin block was performed according to the protocol of the innu PREP FFPE Total RNA Kit from Analytikjena (PN: 845-KS-2050050/Jena-Germany). After the necessary procedures were done, total RNA was filtered into the tube for cDNA extraction. High-capacity cDNA Reverse Transcription Kits from Appliedbiosystems (PN: 4375222 Carlsbad, CA, USA) were used to convert the RNAs obtained into complementary DNA. TaqMan Gene Expression Master Mix from Appliedbiosystems (PN: 4371135/Carlsbad, CA, USA) was used for the gene expression method. The Uracil-DNA glycosylase enzyme is activated by maintaining the mixture at 50 C° for 2 minutes throughout the initial stage of the real-time PCR (RT-PCR) procedure. For 10 minutes, AmpliTaq Gold was maintained at 95 C° to stimulate the ultra pure enzyme.

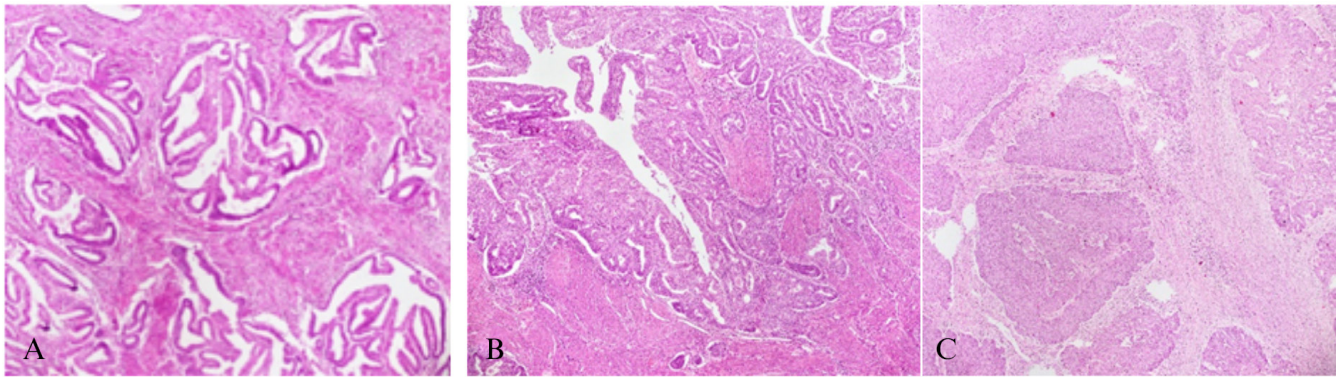

Figure 1. Determination of target tissues by marking in archive tissue preparations. A) Grade 1 (H&E X 100), B) Figo Grade 2 (H&E X 40), C) Figo Grade 3 (H&E X 40)

Amplification was carried out in 2 stages; Step 1) The transition of DNA from double-stranded to single-stranded structure (denaturation) for 15 seconds at 95 C°, Step 2) Beta-actin was used as an endogenous control in the quantitative analysis of RT-PCR to normalize the distinguishing expression of tissues. Calculated values of Delta Ct ($^{\Delta Ct}$) and $2^{-\Delta\Delta Ct}$ were employed in the statistical analysis.[12]

Statistical analysis

The IBM SPSS 22.0 Statistics program was used for statistical analysis. The Shapiro-Wilk test was used for the normality test. Because the variables did not show normal distribution in the comparisons between the groups, the Mann-Whitney U test was used for the comparisons of two groups, and the Kruskal-Wallis H test was used for the comparisons of more than two groups. When a difference was found as a result of Kruskal-Wallis H test, Conover's Post Hoc Test was used. Significant *p* values were defined as those 0.05.

RESULTS

This study involved 29 healthy participants and 45 patients with EC. All samples were used anonymously. It was revealed that there was a statistically significant difference between the means of ABCA1 gene expression in the patient and control groups when the means of the two groups were compared (p= 0.001) (Figure 2).

There was a significant difference in terms of ABCA1 gene expressions between the groups determined according to the stages of the patients ($p < 0.05$) (Figure 3).

Post Hoc test results showed a difference between the control and Grade 1 (p= 0.0001), Grade 2 (p= 0.0001) and Grade 3 (p= 0.0001), respectively. There was no difference between grades ($p > 0.05$). Although there was no statistically significant difference between the grades, when we compared the mean values, ABCA1 gene expression showed the highest increase in Grade 1. There was a slight decrease in Grade 3 and a serious decrease in expression in Grade 2 (Table 1).

No statistically significant difference was determined when the expressions in the secretory and proliferative phase samples were compared among themselves in the control group ($p > 0.05$).

DISCUSSION

The absence of a progesterone antagonistic effect with extended estrogen activity is linked to the pathophysiology of EC, which leads to sustained endometrial growth, atypical hyperplasia, and ultimately carcinogenesis. Obesity, which is among the factors that cause an increase in endogenous estrogen, constitutes 26-47% of all cases, and the risk of EC is 2-10%. Lipid metabolism in patients is directly related to tumor cell proliferation. Therefore, it is important to examine lipid metabolism in patients with EC for whom obesity is an important risk factor.[13]

All vertebrates require cholesterol, which is obtained from cells through de novo synthesis and uptake of lipoproteins from the blood using lipoprotein receptors. But more is not always better.[14] Although every cell in the body is capable of producing cholesterol, the majority of them do not have effective metabolic pathways and must instead expel the

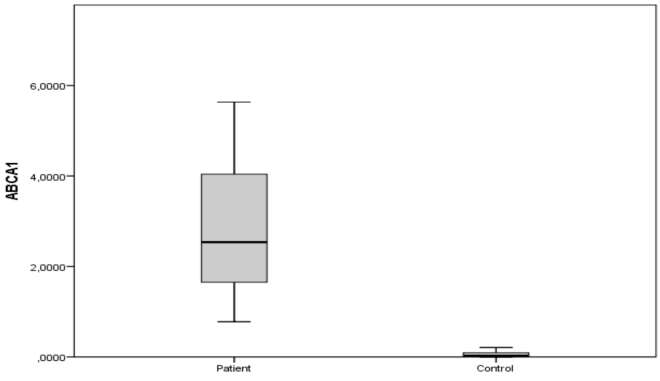

Figure 2. Comparison of *ABCA1* gene expression
ABCA1: ABC transporter A1

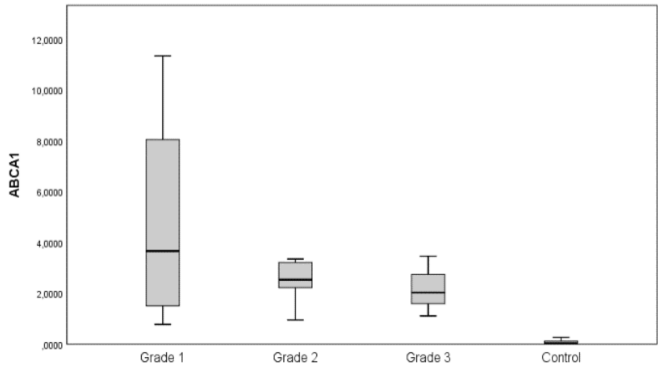

Figure 3. Comparison of ABCA1 gene expression in groups
ABCA1: ABC transporter A1

Table 1. Comparison of patient, baseline characteristics of case Grade1, Grade2, Grade3, and the controls

Gene	Controls (n= 29) mean ± SD	Patients (n= 45) mean ± SD			*p* value
		3.797 ± 3.710			0.0001*
		Grade 1 (n= 15) mean ± SD	Grade 2 (n= 15) mean ± SD	Grade 3 (n= 15) mean ± SD	
ABCA1	0.113 ± 0.207	4.699 ± 3.522	4.262 ± 5.111	2.432 ± 1.275	0.0001*

Mean, * shows a statistically significant difference between Grade 1, Grade 2, and Grade 3 and the control group (p= 0.0001), SD: Standard deviation

substance from cells through a variety of transporters. The ability of one of them, ABCA1, to join with apo A-I on the cell surface to create new HDL and use ATP energy to encourage the outflow of free cholesterol and phospholipids from cells is one of its key functions. This in turn starts the process of reverse cholesterol transport, which transports cholesterol from peripheral tissues back to the liver.[15,16] The ability of cells to move has been shown to be influenced by the cholesterol regulation in the plasma membrane.[17] Furthermore, it has been proposed that the adherence and migrating of cancer cells are significantly influenced by lipid rafts rich in cholesterol.[18,19] The expression of ABCA1 surface protein can be controlled by the activity of phosphoinositide 3 kinase (PI3K), which dramatically raises the risk of cancer cells migrating into the bloodstream and forming metastases.[20] According to our study results, ABCA1 expression was increased in the patient group compared with that in the control group ($p < 0.05$). In our team's previous study, PI3K increased in the patient group compared with the control group.[21]

According to previous studies, cancer-specific ABCA1 methylation and protein expression loss directly caused higher intracellular cholesterol levels in cancer cells, creating a microenvironment conducive to the spread of the disease.[22]

When the patient grade groups were compared with the control group, an increase was observed in each group compared with the control group ($p= 0.001$). When the grades were compared with each other, there was no statistically significant difference; however, when the mean values were examined, an increase was observed in Grade 1 and Grade 2 groups and a significant decrease in Grade 3. This shows that disease progression is inversely related to ABCA1 expression. The decrease in ABCA1 expression in Grade 3 differentiation of the disease indicates that cell proliferation may be increased and expression may be correlated with disease progression. In addition, our previous study showed that in accordance with the working mechanism of PI3K and ABCA1, PI3K, like ABCA1, increases as expected in the early stages of cancer and decreases as the tumor progresses.[21]

According to a study on mitochondrial ABCA1, ABCA1 can keep cells functioning normally by keeping their cholesterol levels low, and it also has some inhibitory effects on the growth of cancer cells.[23]

According to a study, it is easier for tumors to progress when ABCA1 expression is downregulated. High amounts of Apo A1 can decrease EC formation, and HDL and Apo A1 both have the capacity to remove cholesterol from cells, which reduces lipid metabolism.[24]

Apo A1 levels should be used as a tumor marker for the early detection of EC because elevated levels can halt the progression of EC. High amounts of Apo A1 might slow down lipid metabolism, and HDL and Apo A1 may remove cholesterol from cells, lowering lipid metabolism.[13]

In another study, it was stated that ABCA1 deficiency provides high mitochondrial cholesterol and ultimately promotes cancer cell survival. Theoretically, this entails an increase in the retention of chemicals that encourage cell death in the mitochondria, perhaps as a result of a decrease in membrane fluidity and blockage of the mitochondrial permeability transition.[25,26] According to a different study that came to the opposite conclusion as this one, ABCA1 anti-tumor activity is dependent on efflux function and is mediated by lower mitochondrial cholesterol with a higher likelihood of the release of molecules that promote cell death, like cytochrome C, from mitochondria.[22]

Normal breast epithelium exhibits strong ABCA1 expression, and breast cancer exhibits lower ABCA1 expression, which appears to be related to a bad prognosis.[27] In particular, a study on breast cancer found that elevating protein expression of ABCA1 can maintain the body's cholesterol balance and inhibit the development of cancer cells by inhibiting the proliferative effects of high cholesterol levels on breast cancer cells in a mouse transgenic model.[28]

In addition, it was revealed that cholesterol efflux plays a significant part in the management of lung cancer. According to a study using microRNA 200b-3p, ABCA1 overexpression dramatically reduces lung cancer cell proliferation, migration, and penetrating lung adenocarcinoma samples, human cell lines A549 and H1299, by functioning as an oncogene.[29] In all prostate tumors, ABCA1 was downregulated, which was noticeable. There have been reports that cells with prostate cancer metastasis have elevated cholesterol levels.[5]

When our study and all these results are evaluated, it can be revealed that ABCA1 may play an important role in the development of EC.

CONCLUSION

Given the possibility of pharmacological regulation using new or existing drugs, future research should focus on understanding the effect of ABCA family transporter activity on the tumor microenvironment and EC cells. The potential of ABCA1, one of the major intracellular cholesterol efflux transporters, to transport cholesterol has enormous potential for use in treating cancer. According to our results, it was determined that it may be possible to develop new therapeutic approaches for the prevention and treatment of cancer by modifying ABCA1 gene expression.

Cancer cells have adapted to employ cholesterol efflux links to promote malignancy, whereas healthy cells reduce the possibility of cell harm from excess cholesterol. The results of our study support this finding, and it has been associated with decreased ABCA1 expression and poor prognosis in the later stages of the disease. Based on this result, we can say that cancer cell growth mechanisms can be interfered with by modifying the ABCA1 gene.

Ethics

Ethics Committee Approval: This research was approved by Mersin University Clinical Research Ethics Committee (approval number: 2020/418, date: 10.06.2020).

Informed Consent: Created anonymously.

Authorship Contributions

Surgical and Medical Practices: H.A., F.T., Concept: Ş.E.A., C.Y., N.C., Design: Ş.E.A., C.Y., N.C., H.A., F.T., S.E.E., Data Collection or Processing: Ş.E.A., F.T., Analysis or Interpretation: Ş.E.A., C.Y., S.E.E., Literature Search: Ş.E.A., Writing: Ş.E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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