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Araştırma Makalesi /Research Article

Pterjiyum Vakalarında Aktin İlişkili Protein 2/3 Kompleks Alt Birimi 3 (ARPC3) Gen Ekspresyonunun Değerlendirilmesi

Evaluation of Actin Related Protein 2/3 Complex Subunit 3 (ARPC3) Gene Expression in Cases Pterygium

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Öz

Amaç: Pterjiyum, konjonktival invazyon, fibro-vaskülarizasyon ve kolajenin elastik dejenerasyonu ile karakterize olan anormal bir konjonktival yara iyileşme sürecidir. Pterjiyumun patogenezinde hücresel proliferasyon, anti-apoptoz, inflamasyon ve anjiyogenez yer almaktadır. Pterjiyumun hücresel proliferasyon ve anti-apoptoz içermesi, araştırmacıların pterjiyumu dejeneratif bir hastalıktan ziyade bir tümör analoğu olarak düşünmesine neden olmaktadır. ARPC3, ARP2/3 kompleksinin alt birimlerinden biridir. Bu genin aktivasyonu proliferasyon, invazyon ve migrasyon süreçlerini destekler. Bu çalışmanın amacı, pterjiyum vakalarında ARPC3 gen ekspresyonlarını araştırmaktır.
Gereç ve Yöntem: Pterjiyum örnekleri ve sağlıklı konjonktival dokular (n=27), ameliyat geçiren tek bir hastanın tek gözünden elde edilmiştir. ARPC3 mRNA seviyelerinin pterjiyum ve sağlıklı konjonktival dokulardaki ekspresyon profili, qPCR kullanılarak incelenmiştir. ARPC3'ün RQ değeri, evrensel genin ekspresyon seviyesine göre ayarlanarak Livak yöntemi ile hesaplanmış ve kalibratör için RQ değeri 1 olarak alınmıştır.
Bulgular: ARPC3'ün mRNA ekspresyonu, kontrol dokularına kıyasla pterjiyum dokularında artmıştır (kat değişimi 1.54, p=0.091). Ancak, bu artış anlamlı değildir.
Sonuç: Bildiğimiz kadarıyla, bu, pterjiyumun etiyopatogenezini anlamada ARPC3 geninin rolünü değerlendiren ilk makaledir. Çalışmamızın sonuçları, pterjiyumun etiyopatogenezini anlamaya katkıda bulunmaktadır.
Anahtar Kelimeler: ARPC3, ekspresyon, pterjiyum, qPCR

Abstract

Objective: Pterygium is an aberrant conjunctival wound healing process, which is characterized by conjunctival invasion, fibro-vascularization, and elastic degeneration of collagen. Cellular proliferation, anti-apoptosis, inflammation, and angiogenesis are involved in the pathogenesis of pterygium. The fact that pterygium contains cellular proliferation and anti-apoptosis makes researchers think that pterygium is a tumor analog rather than a degenerative disease. ARPC3 is one of the subunits of the ARP2/3 complex. Activation of this gene promotes proliferation, invasion and migration processes. The purpose of this study was to investigate ARPC3 gene expressions in cases with pterygium.
Material and Method: Pterygium samples and healthy conjunctival tissues (n=27) were obtained from a single eye of a single patient underwent surgery. The expression profile in pterygium and healthy conjunctival tissues of ARPC3 mRNA levels were examined using qPCR. The RQ of ARPC3 was calculated by the Livak method, with adjustment to expression level of housekeeping gene and RQ value for the calibrator was equal to 1.
Results: mRNA expression of ARPC3 was increased in pterygium tissues compared to control tissues (fold change 1.54, *p*=0.091). However, this increase was not significant.
Conclusions: To our knowledge, this is the first manuscript to evaluate the role of ARPC3 gene in understanding etiopathogenesis of pterygium. The results of our study contribute to understanding the etiopathogenesis of pterygium**.**
Keywords: ARPC3, expression, pterygium, qPCR

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INTRODUCTION

Pterygium is a degenerative ocular surface disease characterized by conjunctival invasion, fibro-vascularization, and elastic degeneration of collagen[1]. Pterygium frequently shows a triangular wing shape, starting from the nasal region expanding toward the corneal, but it can also arise from the temporal area. In some patients pterygium tissue may grow until the pupil[2,3]. It can be bipolar if it affects both temporal and nasal regions of the conjunctiva or unipolar if it affects only the temporal or nasal region[3]. The pterygium tissue consists of parts: head (apex), neck, and body. The head is an avascular region at the apex while the neck, which connects the body and head, has finely branched neovessels. The body is at the bulbar conjunctiva, where straight and radial vessels are available[3,4]. Symptoms of red eye, lacrimation, irritation, foreign body sensation, and burning may accompany the growth of pterygium tissue onto the cornea[1]. Epidemiological data show that sunlight exposure is a trigger for pterygium pathogenesis[5]. Cellular proliferation, anti-apoptosis, loss of heterozygosity, microsatellite instability, inflammation, inheritance, viral infections, oxidative stress, epithelial-mesenchymal transition, extracellular matrix remodeling, and angiogenesis are involved in the pathogenesis of the disease[6]. Several studies have demonstrated that postoperative inflammation and surgical trauma were inducers of the recurrence of pterygium. Anti-inflammation, fibrovascular growth-inhibiting, and anti-metabolism agents have been used for the treatment of pterygium recurrence.

However these drugs cause some side effects[7]. In pterygium, preneoplastic manifestations are also observed. For example; the activation of extracellular signal-regulated kinases-mitogen-activated protein kinase (ERK-MAPK) and nuclear factor-kβ (NF-kβ) signaling pathways, whose abnormal activations are observed in tumorigenic processes, have been shown to be effective in the development of pterygium[8]. In addition, tumor-like features such as high and invasive growth in the pterygium, dysplasia symptoms in surgically resected pterygium, proliferation, and migration are observed[8,9]. Clarifying the pathogenesis of pterygium is important for its treatment and prevention, as pterygium is a disease with contradictory features and multifaceted pathology[8,10].

Cellular movement is essential for many normal biological processes such as activation of the immune system, tissue repair and regeneration, whereas abnormally activated cell migration is implicated in many diseases[11]. The actin-related protein 2/3(ARP2/3) complex arranges actin-related processes such as membrane trafficking, endocytosis, cell migration, cell division, phagocytosis, and infection[12]. Actin-Related Protein 2/3 Complex Subunit 3 (ARPC3) is a member of the ARP2/3 complex, which consists of seven proteins, and is involved in the regulation of actin dynamics[13]. The role of the ARP2/3 complex in cell migration has been the subject of many studies. The complex uses filopodia and lamellipodia, finger-like projections composed of polymerized actin so cells can migrate. Filopodia are typically involved in activities such as migration and wound healing[13].

Actin-rich protrusions can also be formed by cancer cells, allowing them to degrade the extracellular matrix and attack the environment[11]. It is known that the ARP2/3 complex plays a fundamental role in the processes of invasion and migration in many types of cancer, and its subunits of the complex exhibit aberrant expression patterns in tumor cells[14,15]. ARP2/3 members are upregulated in hepatocellular carcinoma (HCC). Higher expression of ARP2/3 members has been shown to be significantly associated with poor prognosis in HCC patients. It has been reported that there is a relationship between Actin Related Protein 2 (ACTR2) and Wiskott-Aldrich syndrome protein family verprolin homologous protein 2 (WAVE2) expression and poor prognosis of the disease in colon, breast, and lung cancer. Overexpression of the ARPC2 gene has also been used as a marker to differentiate melanomas from benign and atypical nevi. Increased ACTR2 and Actin Related Protein 3 (ACTR3) expression in colon cancer cells has been associated with cancer stages[15]. Frentzas et al. reported that ARPC3 is expressed in all human cancer cells studied[16].

Pterygium also is an aberrant conjunctival wound-healing process[17]. Fibroblasts are migratory cells that play important roles in epithelial tissue formation and wound healing. Studies on fibroblasts and ARPC3 have identified significant defects in the directional migration of ARPC3 deficient or mutant fibroblasts, drawing attention to the central role of the ARP2/3 complex in cell migration[18,19]. Given the roles of

ARPC3 in the directional migration of fibroblasts and the migration and metastasis of cancer cells, it should be considered in the etiopathogenesis of pterygium. There is no study on whether ARPC3 has a role in the etiopathogenesis of pterygium. For these reasons, we aim to evaluate ARPC3 expressions in cases with pterygium.

MATERIALS AND METHODS

Collection of pterygium specimens

Pterygium samples and healthy conjunctival tissues (n=27) were obtained from a single eye of a single patient underwent surgery at Gaziosmanpasa University Hospital and all tissues were analyzed in the pathology laboratory. Our study was confirmed by the local Ethics Committee of Gaziosmanpasa University, Türkiye (Number: 19-KAEK-024), conducted in line with the principles of the Declaration of Helsinki. All patients obtained full written informed consent for this research.

ARPC3 gene expression analyses

Total RNA isolation was performed employing the RNA isolation kit (ThermoFisher Scientific, USA), according to the producer's protocol. The quantity and quality of isolated RNA was spectrophotometrically analyzed employing Qubit™ RNA High Sensitivity Assay Kit (Invitrogen, USA) and all tissues were electrophoresed on 1% agarose gel to assess total RNA integrity. Reverse transcription–polymerase chain reaction (RT-PCR) was applied using the master Mix (GeneAll, South Korea) in a 20 µL reaction volume, according to the producer's instructions. The cDNA concentrations were detected with the Qubit dsDNA Assay Kit (Invitrogen, USA). The quantity of cDNA required for PCR was calculated separately for each sample. The expression level of the target gene was analyzed by qPCR in the Applied Biosystem StepOnePlus (Singapore) with beta actin has been used as the housekeeping gene. The qPCR mixture contained 3µL cDNA product, 10 µL 2X SYBR Green Master Mix (Applied Biosystems Life Technologies, USA), 1 µL forward and reverse primers with 0,4 µL ROX reference dye and RNase-free water (Invitrogen, USA) in a 20 µL reaction volume. The qPCR reactions were carried out with non-template a negative control. The expression level of the ARPC3 gene was determined by the $2^{-\Delta\Delta Ct}$ value[20].

Statistical analysis

The t-test was used to compare the level of ARPC3 gene expression between primary pterygium and healthy tissues. The mRNA expression level of the ARPC3 gene was calculated by the $2^{-\Delta\Delta Ct}$ method, adjusting according to the beta actin expression level, and the relative expression value (RQ) of the calibrator was equal to 1.20[20]. Calculations were based on the SPSS software version 16.0 (SPSS, Inc., Chicago, IL) program. Outcomes of $p<0.05$ were regarded as statistically significant. The fold change was determined according to the range of 0.9 – 1.1. Accordingly, if it was less than 0.9, gene expression was considered to decrease, and if it was greater than 1.1, gene expression was considered to increase[20].

RESULTS

The patients in the study were 9 female and 18 male, with an age range of 43–78 years and a mean age±SD, 58±8.43. To evaluate expression levels of ARPC3 were analyzed by qPCR in pterygium and healthy conjunctiva tissues.
According to the results of qPCR analysis, when the expression level of the ARPC3 gene was compared with normal conjunctival tissues, it was determined that ARPC3 gene expression increased in pterygium tissues. However, this increase was not statistically significant (fold change 1.54, $p=0.091$). The fold change is shown in Figure 1. ARPC3 expression level is shown in Table 1.

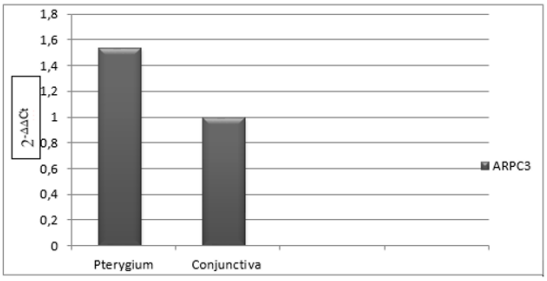

Figure1. This bar graph shows fold change values of ARPC3 gene in pterygium and conjunctiva tissues. The RQ value of ARPC3 was measured by qPCR, calculated using the $2^{-\Delta\Delta Ct}$ method, with adjustment to the expression level of beta actin.

Table 1. mRNA expression level of ARPC3

	ARPC3 (mean ± SD)	*p* value
Pterygium tissue (n= 27)	1.54±0.33	*p*=0.091
Conjunctiva tissue (n=27)	1	

SD: Standard Deviation

DISCUSSION

Pterygium is a degenerative ocular surface disease characterized by conjunctival invasion, fibrovascularization, and elastic degeneration of collagen[1]. Cellular proliferation, anti-apoptosis, inflammation, epithelial-mesenchymal transition, oxidative stress, extracellular matrix remodeling, and angiogenesis are involved in the pathogenesis of the disease[6]. Abnormal cell cycle kinetics can also cause cellular proliferation and escape from apoptosis. Therefore, the abnormal cell cycle plays a critical role in the formation and growth of pterygium.[6] The observation of cell proliferation, cell migration, and local angiogenesis in pterygium formation indicates uncontrolled cell proliferation as a mechanism[21]. The fact that the pathogenesis of pterygium contains many features similar to cancer such as cellular proliferation, antiapoptosis, invasion, migration, and recurrence suggests that pterygium is a tumor analog rather than a degenerative disease[21-23]. The fact that the MAPK signaling pathway, which is observed in almost all cancers, is also active in pterygium, the association between p53 mutation and the pathogenesis of pterygium in many studies, the relationship between K-Ras proto-oncogene mutations and disease severity, loss of heterozygosity, and high expression of Ki-67, the protein associated with proliferation in patients with pterygium, support the view that pterygium is pre-malignant[21, 23,24].

Cell migration, defined as the movement of cells to a specific point, plays a critical role in tissue formation, repair, and regeneration. Abnormally activated cell migration is involved in many diseases, especially cancer[25]. In addition, transformed basal epithelial cell migration is thought to be one of the key processes in pterygium formation[26]. The ARP2/3 complex is known to perform a role in a lot of basic processes, including endocytosis, adhesion, cell division, and cell migration. The mechanism of metastasis of tumor cells is mainly based on microfilaments, actin-associated proteins, and pseudopods.

High expression of the ARP2/3 complex promotes pseudopodia formation and causes its increase. Increasing pseudopodia increases cancer migration and metastasis.[27]

In studies, it has been determined that ARP2/3 subunits are highly expressed in many cancer types such as colorectal, breast, prostate, bladder, and stomach cancers[14,28,29,30,31]. Despite the crucial role of the ARP2/3 complex in a wide variety of cellular processes, studies on the mechanisms and functions of some proteins in the complex, including ARPC3, are relatively scarce[27].

According to a study, ARPC3 expression was increased in bladder cancers, kidney cancers, brain and central nervous system cancers, head and neck cancers, esophageal cancers, and liver cancers, but decreased in leukemia and lung cancer[15]. In a study on colon cancer, it was reported that increased expression of ACTR2 and ACTR3 was associated with the malignancy stage of the tumor[32]. In a study performed on pancreatic cancer cell lines and normal pancreatic samples, Actin Related Protein 2/3 Complex Subunit 2 (ARPC2) and Actin Related Protein 2/3 Complex Subunit 1B (ARPC1B) were expressed at low levels, while ARPC3 and Actin Related Protein 2/3 Complex Subunit 4 (ARPC4) were found to be expressed at high levels. As a result of the silencing of ARP2/3 complex and its subunits, a decrease in cell migration was observed[33]. In the study of Huang et al., it was reported that ARPC2, ACTR3, and Actin Related Protein 2/3 Complex Subunit 5 (ARPC5) are overexpressed in HCC patients and this may be associated with poor prognosis. Therefore, they reported that ARPC2, ACTR3, and ARPC5 could be used as a biomarker and promising molecular targets for the treatment of HCC in the future[15]. In a study by Song et al. on patients with HCC, it was reported that overexpression of ARPC3 is directly proportional to disease prognosis and that ARPC3 is a risk factor for HCC. In addition, it has been reported that ARPC3 may also cause failures in immunotherapy treatment.[34] In our study, the relationship between pterygium, a disease with premalignant features, and ARPC3 gene expression levels was investigated for the first time. The increase observed in pterygium tissues was not statistically significant.

Finally, this study has two main limitations. First, the number of pterygium tissues was relatively small in our study. Second, the pterygium tissue

consists of parts: head (apex), neck, and body[35]. While the head region is avascular, intense neovascularization is observed in the neck and trunk, and different expression levels are observed[3,4]. For these reasons, examining the expression of the ARPC3 gene in different parts of the pterygium tissue will be significant in elucidating the etiopathogenesis of the disease. Understanding the etiopathogenesis of the disease will help find further treatment strategies to prevent pterygium and recurrence.

Authors' Contributions

All authors contributed to the design and design of the study to review, read, and approve the final version of the manuscript. Material preparation and analysis were done by Kubra SAHIN, Nihan BOZKURT, Sadegul SAVKIN. The first version of the manuscript was written by Kubra SAHIN.

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