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miRNA-129-3P Expression in Synovial Fluid of Patients with Osteoarthritis

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ABSTRACT

Objective: The aim of this study was to investigate the miRNA-129-3p expressions in the synovial fluid of patients with a primary knee osteoarthritis (OA), and thereby contribution to the elucidating underlying molecular mechanisms in OA pathophysiology.

Methods: Patient group included 31 individuals with an advanced knee OA. A total of 13 patients with anterior cruciate ligament rupture who had no cartilage damage, were chosen as a control group. Synovial fluid samples were collected during the total knee arthroplasty and an arthroscopic reconstruction. After the centrifugation, samples were stored in a -80 °C cooler. The miRNA-129-3p expressions were examined by reverse transcriptase-polymerase chain reaction using the RNU44 molecule as a reference. The 2-ΔΔCt method was used to calculate the expression of molecules. Statistical comparisons were undertaken by the Student's t-test. Pearson's chi-square test was used to determine the differences in the ratios or relationships between the categorical variables. Statistical significance was set at $p<0.05$ for all the cases.

Results: In this study, the miRNA-129-3p, which we thought to be associated with the ciliogenesis, IL-17, and osteoprotegerin, was found 1.54 times higher in the synovial fluid of the patients compared to a control group ($p<0.01$).

Conclusion: It is thought that the miRNA-129-3p may play a role in the OA pathophysiology. Extensive studies are required to use the miRNA-129-3p as a biomarker, or a treatment target for OA.

Keywords: Osteoarthritis, miRNA-129-3p, ciliogenesis, synovial fluid

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INTRODUCTION

It is widely unknown how the path of mechanical transformation in osteoarthritis (OA) changes, and how chondrocytes perceive and respond to the compression. A recent study suggested that the primary cilia of chondrocytes played a role in detecting and transmitting the mechanical stimulation such as (1): McGlashan et al. (2) reported that in normal cartilage cells, the number and length of the cilia were the lowest in the superficial zone and increased as they moved away from the articular surface. This was the first study showing that the primary cilia are present in chondrocytes during the OA progression, and that the total percentage of cilia cells in degenerative cartilage increased with the OA severity (2). Cao et al. (3) reported that the ciliary genesis of miRNA-129-3p was regulated by the actin dynamics and CP_{110}, a ciliary gene blocker. The removal of the CP_{110} promoted the growth of a ciliary axoneme. The authors suggested that the miRNA-129-3p increased the formation of cilia by reducing the CP_{110} (3).

Tsai et al. (4) showed that the miRNA-129-3p was the most repressed miRNA with its IL-17 binding potential after the stimulation of the osteoblasts by osteopontin in the arthritis model. In the same study, the miRNA-129-3p in osteoblasts was reported to bind directly to the 3′-UTR region of the *human IL-17* gene, suppressing IL-17 translation and eliminating the monocyte migration (4). In another study by Liu et al. (5), found that synovial IL-17 levels were significantly higher in the OA patients compared to the control group, and showed a negative correlation with the OA severity. IL-17 has also been reported as a pain sensitizer in the rodent models of arthritis (6).

The studies demonstrate that the miRNA-129-3p may play a role in the cartilage degeneration and inflammatory cascade of the OA and provides insight into a potential miRNA-based treatment strategies for delaying the hyperalgesia and the regulation of ciliary functions by IL-17 mediated monocyte migration. In our study, we aimed to investigate the differences in the expression of a synovial fluid in the patients with an advanced OA, considering the role of miRNA-129-3p in IL-17-osteopontin relationship and ciliogenesis. To the best of our knowledge, the literature does not contain any microarray studies on OA conducted with these expectations.

METHODS

This research experiment was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to the study. The necessary approval was obtained from the Faculty Ethics Committee of Istanbul University-Cerrahpaşa (decision no: 83045809-604.01.02, date: 05.09.2017).

The study has two groups: patient, and control group. All the participants presented to our orthopedics clinic in 2017 and 2018. Patient group included 31 individuals with an advanced primary knee OA. They were classified into stage 3-4 OA according to the Kellgren-Lawrence classification (5). Thirteen patients with an anterior cruciate ligament (ACL) rupture were chosen as a control group. During the arthroscopic reconstruction, ACL rupture cases evaluated as stage 0, both according to Kellgren-Lawrence and Outerbridge classifications were included in the control group. The gender distribution of the participants is 17 men [8 patients (47.1%) and 9 controls (52.9%) p=0.018] and 27 women [23 patients (85.2%) and 4 controls (14.8%) p=0.033]. Synovial fluid used as a material. Patients with the malignancies, systemic infection, poor general condition, cognitive dysfunction, or psychosis, under 18 years of age, pregnant women, puerperal and breastfeeding women were excluded.

The synovial fluid of the patient and control groups were collected on a voluntary basis within the framework of the ethical rules. Volunteers in both the groups were clearly informed about the purpose and content of the study before the participation. A full physical examination was performed in all the patients. The direct radiographs and the magnetic resonance imaging of the groups were examined. Routine blood tests were evaluated before the operation.

A total of 1.5-3 mL of synovial fluid was taken from the participants. The materials were centrifuged at 3,000 g for 5 mins; then, the supernatant was transferred to the different Eppendorf tubes and stored in a -80 °C cooler until the day of the experiment. Total RNA isolation was performed with an EXTRACTME miRNA kit. The isolated RNAs were evaluated by a nano spectrophotometer prior to the complementary DNA (cDNA) synthesis. A TRANSCRIPTME RNA kit was used for cDNA synthesis from the template RNA. In the last step, miRNA was evaluated by the real-time polymerase chain reaction (PCR) method.

Statistical Analysis

In the mean ± standard deviation (SD) comparisons, the Student's t-test was used by providing the necessary conditions such as: normal distribution and covariance. Pearson's chi-square test was conducted to determine the differences in the ratios or relationships between the categorical variables. Statistical significance was set at $p<0.05$ for all the cases.

RESULTS

In this study, miRNA-129-3p expression in the synovial fluid of the patients was found to be 1.54 times higher than the control group ($p<0.01$) (Table 1) (Figure 1). Age was significantly different in the patient group compared to the control group (mean ± SD patient age: 62.77±5.42, control age: 33.15±8.91, $p<0.001$) (Table 1). Body mass index (BMI) was significantly higher in the patient group compared to the control group (mean ± SD patient BMI: 32.34±6.58, control BMI: 25.86±2.45, $p<0.001$).

DISCUSSION

In this study, the miRNA-129-3p, which we thought to be associated with the ciliogenesis, IL-17, and osteoprotegerin (OPN), was found

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Table 1. Mean ± standard deviation, median, and p-values of the data obtained from the patient and control groups

	Control Median ± SD	Patient Median ± SD	-
	Control Median (min-max)	**Patient Median (min-max)**	**p**
Age	33.15±8.91	62.77±5.42	0.001
	35 (18-47)	63 (53-72)	-
Length	168.62±5.72	163.97±7.07	0.06
	168 (160-178)	165 (150-180)	-
Weight	73.62±8.26	86.29±14.25	0.002
	78 (55-85)	85 (65-135)	-
BMI	25.86±2.45	32.34±6.58	0.001
	26.1 (21.5-29.4)	31.2 (23.9-52.7)	-
ΔCT	-3.44±0.64	-4.06±0.77	0.01
	-3.35 (-4.98 to -2.57)	-4.24 (-5.74 to -2.07)	-

BMI: body mass index, min: minimum, max: maximum, SD: standard deviation

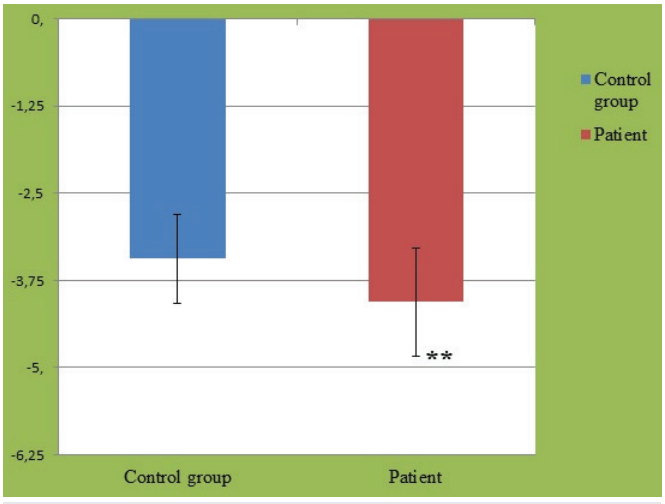

Figure 1. Comparison of the ΔCT values of the advanced osteoarthritis and control groups**

1.54 times higher in the synovial fluid of the patients compared to a control group (p<0.01).

In a study by McGlashan et al. (2), primary cilia were found in chondrocytes during the OA progression, and the total percentage of cilia cells in a degenerative cartilage increased with the OA severity (1). Cao et al. (3) suggested that the increased expression of miRNA-129-3p increased cilia cells by regulating the CP_{110} and actin dynamics. The number and length of a primary cilia in the degenerative joint chondrocytes increased in OA (2). The miRNA-129-3p is defined micro-RNA that increases in the ciliogenesis over CP_{110} inhibition (3). Therefore, the high expression of miRNA-129-3p in the synovial fluid in patients with an advanced OA may also be related to the ciliogenesis.

OPN and IL-17 are known to be involved in the pathogenesis of OA (5,7). Tsai et al. (4) showed that the human *IL-17* gene was one of the target genes of the miRNA-129-3p. They reported that the miRNA-129-3p expression was decreased through the Syk-PI3K-Akt pathway, and increased IL-17 because of the osteoblast stimulation by an osteopontin (4). Thus, it could be stated that the OPN and IL-17 are associated with the miRNA-129-3p.

In a study performed by Liu et al. (5) with 226 OA patients and 106 control subjects, the IL-17 levels were found to be negatively correlated with the OA severity. In another study, Snelling et al. (8) found that in 152 patients with an advanced OA, the IL-17 levels were elevated only in the 14 patients, while IL-17 could not be detected in 138 patients. The reduced levels of IL-17 in the OA patients in these studies may be associated with an increase in the miRNA-129-3p in an advanced OA, and its consequent binding to the *IL-17* gene and reduced IL-17 translation.

Dong et al. (7) found that the osteopontin levels decreased in patients with an advanced OA. Matsui et al. (9) showed that the osteopontin deficiency exacerbated both aging-related and instability-induced OA. Decreased levels of osteopontin in the OA may be associated with the increased miRNA-129-3p levels. These studies indirectly support our thesis.

It is known that increase in the mechanical load due to obesity and aging take place in the etiology of OA (10). In our study, BMI was found to be significantly higher in the advanced OA group compared to the control group (32.34±6.58 and 25.86±2.45, respectively; p<0.001). It is known that the risk of OA increases by 60% in a people with the BMI of ≥30 (10). The increase in miRNA-129-3p expression is prominent in OA patients with a BMI of >30, suggesting that this expression is associated with the obesity. In our study, age was also significantly different in the advanced OA group compared to the control group (62.77±5.42 and 33.15±8.91, respectively; p<0.001). Age may also play an important role in the increase of the miRNA-129-3p.

Study Limitations

Limitations of our research were the limited number of participants in the patient and control groups, as well as the lack of homogeneity among the groups, the ability to test miRNA-129-3p expression only in the synovial fluid (could also be tested in a peripheral blood), and the lack of examination of the other mediators associated with OA pathogenesis, such as OPN, IL-17.

CONCLUSION

OA is a degenerative joint disease with a chronic inflammation and increasing global prevalence. There are symptomatic and surgical treatment options for this condition. Symptomatic treatment does not affect the progression of the disease, but the surgical treatments also have certain disadvantages. The molecular mechanism of the disease has not yet been fully elucidated. Recent studies showed that the miRNAs played an important role in the pathogenesis of the disease. Although the miRNA expression differences in the OA patients have been shown in studies, there is still a limited information about the varying levels of circulating miRNA. The miRNAs have been found to reduce inflammation,

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and OA progression or have anabolic function in the cartilage. It is considered that the injectable form of these miRNAs may be developed for the local treatment of OA in the joints. Thus, it is anticipated that the miRNA-based therapy may provide another approach to the treatment process without the potentially harmful side effects. In our study, the increased expression of miRNA-129-3p in an advanced OA patients compared to the control group indicates that the miRNA-129-3p may be involved in the pathogenesis of the OA. However, further prospective studies are needed for miRNA-129-3p to be used as a biomarker in the OA or as a therapeutic target.

Ethics Committee Approval: The necessary approval was obtained from the Faculty Ethics Committee of Istanbul University-Cerrahpaşa (decision no: 83045809-604.01.02, date: 05.09.2017).

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REFERENCES

1. Wann AK, Zuo N, Haycraft CJ, Jensen CG, Poole CA, McGlashan SR, et al. Primary cilia mediate mechanotransduction through control of ATP-induced Ca2+ signaling in compressed chondrocytes. FASEB J 2012; 26: 1663-71.
2. McGlashan SR, Knight MM, Chowdhury TT, Joshi P, Jensen CG, Kennedy S, et al. Mechanical loading modulates chondrocyte primary cilia incidence and length. Cell Biol Int 2010; 34: 441-6.
3. Cao J, Shen Y, Zhu L, Xu Y, Zhou Y, Wu Z, et al. miR-129-3p controls cilia assembly by regulating CP110 and actin dynamics. Nat Cell Biol 2012; 14: 697-706.
4. Tsai CH, Liu SC, Wang YH, Su CM, Huang CC, Hsu CJ, et al. Osteopontin inhibition of miR-129-3p enhances IL-17 expression and monocyte migration in rheumatoid arthritis. Biochim Biophys Acta Gen Subj 2017; 1861: 15-22.
5. Liu Y, Peng H, Meng Z, Wei M. Correlation of IL-17 Level in Synovia and Severity of Knee Osteoarthritis. Med Sci Monit 2015; 21: 1732-6.
6. Richter F, Natura G, Ebbinghaus M, von Banchet GS, Hensellek S, König C, et al. Interleukin-17 sensitizes joint nociceptors to mechanical stimuli and contributes to arthritic pain through neuronal interleukin-17 receptors in rodents. Arthritis Rheum 2012; 64: 4125-34.
7. Dong X, Zheng Y, Liu HY. [The clinical significance of serum and joint fluid osteopontin, and thrombin-cleaved osteopontin levels in osteoarthritis]. Zhonghua Nei Ke Za Zhi 2013; 52: 1023-7.
8. Snelling SJ, Bas S, Puskas GJ, Dakin SG, Suva D, Finckh A, et al. Presence of IL-17 in synovial fluid identifies a potential inflammatory osteoarthritic phenotype. PLoS One 2017; 12: e0175109.
9. Matsui Y, Iwasaki N, Kon S, Takahashi D, Morimoto J, Matsui Y, et al. Accelerated development of aging-associated and instability-induced osteoarthritis in osteopontin-deficient mice. Arthritis Rheum 2009; 60: 2362-71.
10. O'Conor CJ, Leddy HA, Benefield HC, Liedtke WB, Guilak F. TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. Proc Natl Acad Sci U S A 2014; 111: 1316-21.