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ORIGINAL ARTICLE

THE EXPRESSION LEVELS OF microRNAs ASSOCIATED WITH T AND B CELL DIFFERENTIATION/STIMULATION IN ANKYLOSING SPONDYLITIS

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ABSTRACT

Spondyloarthropathies (SpAs), are a group of chronic inflammatory diseases with a number of genetic, physiopathological, clinical and radiological features. Ankylosing spondylitis (AS) is the most common type of spondylo-arthropathies, and >90.0% of patients with ankylosing spondylitis are human leukocyte antigen-B27 (*HLA-B27*)-positive. In recent years, non-*HLA* genetic factors have been reported to have an effect on ankylosing spondylitis. MicroRNAs (miRNAs), are endogenous non coding RNA molecules containing 18-23 nucleotides that play a role in the post-transcriptional regulation of gene expression. In this study, we aimed to determine the expression levels of miRNAs associated with T- and B-cell differentiation/ stimulation in peripheral blood mononuclear cells and their relationship with the etiology of the AS in patients and healthy controls. In a molecular study, peripheral blood mononuclear cell isolation, and total RNA isolation were performed first. In the second step, cDNA synthesis and quantitative real-time PCR (qPCR) expression analysis were completed. Ultimately, in the patient and control group, the expression levels of miR-142-5p and miR-143 were found to be significantly different (p <0.05). According to current knowledge, miR-142-5p and miR-143 expressions were found to be important for those diseases that share similar etiology with AS. We suggest that miR-142-5p and miR-143 may play a role in the pathogenesis, especially miR-142-5p may be a potential biomarker and a target molecule for the treatment.

Keywords: Ankylosing spondylitis (AS); Expression level of microRNA (miRNA); miR-142-5p, miR-143.

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INTRODUCTION

Spondyloarthropathies (SpAs) are a group of chronic inflammatory diseases with a number of genetic, physiopathological, clinical and radiological features. However, their etiology is not completely understood [1]. The term 'seronegative spondyloarthropathy' is used to describe different disease groups such as ankylosing spondylitis (AS), Reiter's syndrome (reactive arthritis), psoriatic arthritis and enteropathic arthritis [2]. Common findings of these diseases are inflammatory arthritis and lumbar pain and their association with enthesitis and human leukocyte antigen-B27 (*HLA-B27*) HLA class 1 antigen.

Ankylosing spondylitis is the most common type of SpA, and >90.0% of patients with AS are *HLA-B27*-positive [3]. The *HLA-B27* is responsible for the presentation of microbial peptides to CD8-positive cytotoxic T lymphocytes and the resulting activation of the acquired immune system. Although >90.0% of patients with AS are *HLA-B27*-positive, antigen positivity alone cannot be considered as a cause of the disease, and a history of infection causing antigen positivity is found in only 50.0% of these patients [4]. In addition, it has been hypothesized that CD8-positive cytotoxic T lymphocytes are activated by the molecular similarity of pathogen microorganisms, due also to the patient's own peptide structure. Another hypothesis is that the misfolded *HLA-B27* molecule accumulates in the endoplasmic reticulum (ER) and increases the intra-ER stress and causes an unfolded protein response by activating intracellular signaling pathways [5].

In recent years, non *HLA* genetic factors have been reported to have an effect on AS. The risk of disease development is 5-16 times higher in *HLA-B27*-positive first-degree relatives of patients with AS than in *HLA-B27*-positive individuals in the population. In identical twins, AS coexistence is 50.0-60.0%, whereas in fraternal twins, it is approximately

24.0%. Genome-wide association studies (GWAS) have reported that non *MHC* genes have an effect on disease pathogenesis; the presence of interleukin-23 (IL-23) signaling pathways, aminopeptidases, peptide presentation and molecules that stimulate the innate immune system are such examples [6]. Recent studies have shown that *ARTS1* and *IL-23R* gene variants causing amino acid changes are effective in this particular patient group [7]. *NDUFS4* and *MAPK7* gene products have an effect on the pathogenesis of AS and may be the target of indomethacin in the treatment [8].

MicroRNAs (miRNAs) are endogenous non coding RNA molecules containing 18-23 nucleotides that play a role in the pos-transcriptional regulation of gene expression. MicroRNAs play roles in cell proliferation, growth and differentiation, apoptosis, intracellular metabolic processes and the pathogenesis of some human diseases [9]. Studies have also reported their role in the etiology of AS. Although some studies have pointed out that miRNA-130a may have an effect on the etiology of AS, others have shown that miR-16, miR-221 and let-7i expressions are increased in this patient group [10,11]. Herein, we compared the expression levels of miRNAs in the peripheral blood mononuclear cells in patient and control groups associated with T- and B-cell activation and differentiation, that has pivotal role at the etiopathogenesis of AS.

MATERIALS AND METHODS

Study Design. This study was planned as a case-control study, and approval was obtained from Marmara University Faculty of Medicine Ethics Committee, İstanbul, Turkey. A total of 50 patients with AS who were admitted to Marmara University Pendik Training and Research Hospital Physical Therapy and Rehabilitation (PTR) Outpatient, İstanbul, Turkey, clinics between January and July 2017, who had acute bilateral sacroiliitis detected at sacroiliac magnetic resonance imaging (MRI) and met the Assessment of SpondyloArthritis International Society (ASAS) 2009 criteria for axial SpAs were included in the patient group. The control group was comprised of 50 healthy volunteers who met the criteria mentioned below.

Inclusion Criteria (for the patient group). Age 18-45 years; detection of acute bilateral sacroiliitis using sacroiliac MRI; meeting the ASAS 2009 criteria for axial SpAs.

Exclusion Criteria (for patient and control groups). Patients with advanced stage AS (stage 4 sacroiliitis according to the modified New York criteria); having previously received and/or currently receiving anti-tumor necrosis factor (TNF), treatment; being pregnant or lactating; having autoimmune or any other chronic inflammatory disease; being diagnosed with cancer; having psychiatric disorders that may affect cognitive functions.

Age and sex, disease duration, erythrocyte sedimentation rate (ESR), *HLA-B27* results of the patient group were recorded using the patient evaluation form. Direct radiographic methods, such as antero-posterior pelvis and lum-bosacral and cervical vertebrae were used in the radiological evaluation of patients, and T1, T2 and STIR sequences in MRI in combination with direct X-ray, were used in the evaluation of the sacroiliac joints.

Disease activity in the patient group was evaluated using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scale [12]. The functional capacity of patients was calculated using the Bath Ankylosing Spondylitis Functional Index (BASFI) scale [13]. In this study, the measurement parameters were evaluated using the Bath Ankylosing Spondylitis Metrology Index (BAS MI), scale [14].

MicroRNA Analysis. Isolation of Mononuclear Cells from Peripheral Blood. Peripheral blood mononuclear cells (PBMC) were isolated with the density gradient separation method using the Ficoll-Hypaque solution. Samples were studied on the day of blood collection without delay and then centrifuged at 400 ×g for 20 min. at room temperature, and the ‘buffy coat’ region containing mononuclear lymphocyte cells was separated from the lower phase containing erythrocytes and granulocytes and the upper phase containing platelets.

Total RNA Isolation. Total RNA was isolated from PBMC cells using the miRNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions.

cDNA Synthesis. From the isolated total RNA, cDNA was synthesized with reverse transcription using the miScript II RT Kit (Qiagen GmbH) according to the manufacturer’s instructions. Total RNA (125 ng) has been used for cDNA analysis according to the miScript protocol. The miScript HiSpec (Qiagen GmbH) buffer used in the synthesis ensured that only miRNAs and small nucleolar RNAs were specifically converted into cDNA.

Quantitative qPCR Phase. To measure the expression levels of miRNA, the pathway-focused miScript miRNA PCR ARRAY kit (Qiagen GmbH) was used according to the manufacturer’s instructions in duplicates for 100 samples (50 patient and 50 control samples). The Rotor Gene® device (Qiagen GmbH) and 100-well disk-shaped ready-to-use systems were used for the expression analysis of miRNAs associated with T- and B-cell activation and differentiation. *SNORD61*, *SNORD95*, *SNORD 96A*, *SNORD68*, *SNORD72* and *RNU6B* were used as internal controls to normalize data obtained during the relative quantitation phase in the ΔΔ cycle threshold [$2^{(-\Delta\Delta Ct)}$] method. Data were analyzed with a suitable analysis software using the relative quantitation method based on the CT principle of the commercial kit (http://pcrdataanalysis. sabiosciences.com/mirna.)

Statistical Analysis. The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, UT, USA) program was used for statistical analysis. In addition to descriptive statistical methods (mean, standard deviation, median, frequency, ratio and minimum and maximum), Student's *t*-test was used to compare quantitative data showing normal distribution between the two groups, and Mann-Whitney U test was used to compare quantitative data not showing normal distribution between the two groups. Pearson χ^2 test was used to compare qualitative data. Pearson correlation analysis and Spearman correlation analysis were used to investigate the correlation normally and non normally distributed variables, respectively. A p value of <0.05 was considered to be statistically significant.

RESULTS

In our study, of all participants, 58.0% (n = 58) were females and 42.0% (n = 42) were males, and the mean age was 37.24 ± 9.42 (range: 18-60) years (Table 1). There was no statistically significant difference between the ages and sex distribution of participants in the patient and control groups (p >0.05). Ages of participants in the patient group ranged from 18 to 59 years, with a mean age of 37.76 ± 9.34 years. Overall, 58.0% (n = 29) of the patients were females and 42.0% (n = 21) were males.

Table 2 presents data of 84 different miRNAs associated with T- and B-cell activation and differentiation studied in duplicates in 50 patients and 50 healthy volunteers using the signaling pathway targeted miScript miRNA PCR Array kit (Qiagen GmbH) (Table 2). Results of data analysis conducted with a suitable analysis software using the relative quantitation method based on the Ct principle of the commercial kit are presented in Table 3. Differences in the expression between the groups were analyzed by converting the Ct values obtained in the study using the $2^{(-\Delta\Delta Ct)}$ formula. In the miRNAs analyzed, the expression of *miR-143* and *miR-142-5p* was significantly higher in the patient group than in the control group (p <0.05) (Table 3).

Table 1. Distribution of descriptive characteristics.

Descriptive Characteristics (n=100)		
Age (years)	median (min-max)	37 (18-60)
	mean±SD	37.24±9.42
Gender n (%)	females	58 (58.0)
	males	42 (42.0)

Table 2. miRNAs analyzed in the signaling pathway targeted miScript miRNA PCR array study.

T-Cell Differentiation CD4−/CD8− T-cell associated:	*let-7d-5p, let-7e-5p, miR-126-3p, miR-128, miR-146b-5p, miR-15a-5p, miR-17-5p, miR-17-3p, miR-181c-5p, miR-191-5p, miR-19a-3p, miR-199a-5p, miR-20a-5p, miR-20b-5p, miR-221-3p, miR-222-3p, miR-223-3p, miR-28-5p, miR-29c-3p, miR-30e-5p, miR-342-3p, miR-423-5p, miR-93-5p, miR-98-5p*
CD4+/CD8+ T-cell associated:	*let-7b-5p, miR-181a-5p, miR-181b-5p, miR-181d, miR-19b-3p.*
CD4+ Naive T-cell associated:	*miR-132-3p, miR-146a-5p, miR-182-5p, miR-184, miR-25-3p, miR-326, miR-92a-3p*
CD8+ Naive T-cell associated:	*let-7a-5p, let-7c, let-7f-5p, let-7g-5p, miR-130b-3p, miR-139-5p, miR-142-3p, miR-150-5p, miR-155-5p, miR-15a-3p, miR-16-5p, miR-26a-5p, miR-26b-5p, miR-29b-3p, miR-30b-5p, miR-30c-5p, miR-30d-5p*
CD8+ Effector cell associated:	*miR-147a, miR-148a-3p, miR-18a-5p, miR-27a-3p, miR-27b-3p*
CD8+ Memory cell associated:	*let-7i-5p, miR-106b-5p, miR-142-5p, miR-15b-5p, miR-17-5p, miR-21-5p, miR-23a-3p, miR-23b-3p, miR-24-3p, miR-29a-3p, miR-31-5p*
B-Cell Differentiation Naive B-cell associated:	*let-7a-5p, let-7b-5p, let-7d-5p, let-7g-5p, let-7i-5p, miR-101-3p, miR-132-3p, miR-142-3p, miR-142-5p, miR-150-5p, miR-181c-5p, miR-195-5p, miR-204-5p, miR-214-3p, miR-221-3p, miR-222-3p, miR-223-3p, miR-29b-3p, miR-30e-5p, miR-331-3p, miR-92a-3p*
Germinal centre associated:	*miR-106b-5p, miR-130b-3p, miR-132-3p, miR-148a-3p, miR-15a-5p, miR-15b-5p, miR-16-5p, miR-17-5p, miR-17-3p, miR-181a-5p, miR-181b-5p, miR-191-5p, miR-19a-3p, miR-19b-3p, miR-210, miR-23b-3p, miR-25-3p, miR-28-5p, miR-30d-5p, miR-93-5p, miR-98-5p*
Memory cell associated:	*miR-100-5p, miR-125b-5p, miR-145-5p, miR-146a-5p, miR-155-5p, miR-21-5p, miR-23a-3p, miR-24-3p, miR-26a-5p, miR-26b-5p, miR-27a-3p, miR-27b-3p, miR-29a-3p, miR-29c-3p, miR-30b-5p, miR-30c-5p, miR-34a-5p*
Expressed in the Treg cells:	*miR-100-5p, miR-125b-5p, miR-146a-5p, miR-181c-5p, miR-20b-5p, miR-21-5p, miR-31-5p, miR-335-5p, miR-365a-3p, miR-99a-5p*
T-cell activation associated:	*let-7e-5p, let-7g-5p, let-7i-5p, miR-143, miR-140-3p, miR-142-5p, miR-146a-5p, miR-146b-5p, miR-155-5p, miR-15a-5p, miR-15a-3p, miR-181a-5p, miR-181c-5p, miR-195-5p, miR-20b-5p, miR-214-3p, miR-223-3p, miR-23a-3p, miR-23b-3p, miR-25-3p, miR-26a-5p, miR-26b-5p, miR-27a-3p, miR-27b-3p, miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-30a-5p, miR-30b-5p, miR-30e-5p, miR-342-3p, miR-368, miR-574-3p, miR-98-5p*

In the patient group, *HLA-B27* allele was positive in 54.0% (*n* = 27) (Table 4). The ESR levels of patients ranged from 2 to 59 mm/hour, with a mean level of 21.36 ± 15.51 (Table 4). Symptom duration ranged from 1 to 25 years, with a mean duration of 6.60 ± 5.08 years and median duration of 5 years (Table 4).

The BASDAI measurements ranged from 0 to 8, with a mean of 2.75 ± 2.03; BASFI measurements ranged from 5 to 12, with a mean of 5.36 ± 1.19 and BASMI measurements ranged from 0 to 9.8, with a mean of 1.55 ± 1.93 (Table 4). No significant correlation was found between any parameters and *miR-142-5p* and *miR-143* measurements (p >0.05) (Table 5).

In the patient group, *miR-142-5p* and *miR-143* measurements were not significantly different between the sexes (p >0.05). There was no significant difference in *miR-142-5p* and *miR-143* measurements according to the presence of extraspinal findings (p >0.05). There was no significant difference in *miR-142-5p* and *miR-143* measurements according to *HLA-B27* allele status (p >0.05) (Table 5).

Table 3. Comparison of expression levels of miRNA in the patient and control groups.

miRNA	**AVG Ct**		$2^{[-Avg(\Delta(Ct)]}$		***p* Value**	**Ratio Patient/Control**
	Control	Patient	Control	Patient		
miR-143	26.00	25.04	0.136139	0.203892	0.025721	1.4977
miR-142-5p	26.80	24.68	0.078435	0.26217	0.000003	3.3425

Table 4. Distribution of disease characteristics.

Patient Group (*n*=50)		***n* (%)**
HLA-B27	negative positive	23 (46.0) 27 (54.0)
ESR	median (min-max) mean±SD	17 (2-59) 21.36±15.51
Symptom duration (years)	median (min-max) mean±SD	5 (1-25) 6.60±5.08
BASDAI	median (min-max) mean±SD	2.5 (0-8) 2.75±2.03
BASFI	median (min-max) mean±SD	5 (5-12) 5.36±1.19
BASMI	median (min-max) mean±SD	1 (0-9.8) 1.55±1.93
miR-142-5p	median (min-max) mean±SD	24.1 (21.4-30.5) 24.68±2.06
miR-143	median (min-max) mean±SD	24.2 (21.6-35.8) 25.10±3.11
Control Group (*n*=50)		*n* (%)
miR-142-5p	median (min-max) mean±SD	26.4 (22.45-34.7) 26.88±2.88
miR-143	median (min-max) mean±SD	25.4 (21.28-33.7) 26.02±2.71

HLA-B27: human leukocyte antigen-B27; ESR: erythrocyte sedimentation rate; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index.

Table 5. Correlation between age and disease characteristics and *miR-142-5p* and *miR-143* measurements.

Patient Group (*n*=50)	***miR-142-5p***		***miR-143***	
	r^a	p Value	r^b	p Value
Age (years)	0.047	0.746	0.126	0.384
ESR	–0.070	0.641	–0.081	0.589
Symptom duration (years)	–0.184	0.200	–0.172	0.233
BASDAI	–0.268	0.060	–0.266	0.062
BASFI	–0.026	0.861	–0.114	0.447
BASMI	0.062	0.671	0.229	0.110

ESR: erythrocyte sedimention rate; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index;
BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index.

[a] Pearson correlation coefficient.
[b] Spearman's correlation coefficient.

DISCUSSION

Recently, several studies have investigated the role of miRNAs in the pathogenesis of AS. Because the disease is considered to be associated with chronic inflammation and immunity, these studies have focused on miRNAs that have an effect on these mechanisms. In AS, CD4+ and CD8+ T-cells may have an effect in the immunological process and a contribution to the inflammation process, which have been discussed in studies conducted on peripheral blood and joint tissues of this patient group [12-16].

Concerning AS and miRNA, studies were mostly focused on T-cell differentiation and activation processes. The T-cell differentiation and activation in diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SS), inflammatory bowel disease (IBD) and psoriatic arthritis (PsA), which share a similar etiology and are associated with autoimmune and chronic inflammatory processes, were discussed in the latest literature. Functional studies with the aim of determining the defective signaling pathways were conducted. Thus, *miR-143* and *miR-142-5p*, which are associated with T- and B-cell differentiation and activation, were examined and found to be significantly more expressed in the patient group than in the control group (p <0.05), consistent with previous studies.

In the present study, miR-142-5p expression in the PBMCs was significantly higher in the patient group compared to the control group (p <0.001), with the expression level being 3.3-times more in the patient group. The study of Beltz [15] has shown that interferon expression, which is important in innate and acquired immune responses, is regulated by regulatory and transcription factors under strict control of miR-142. In their study, Sun *et al.* [16] reported that *miR-142* plays a regulatory role in T-cell differentiation, development, cytokine release and TLR4 stimulation associated with *IL-6* expression. *In vitro* cell culture studies have also reported that *miR-142* is effective in TLR4-related *IL-6* production and the resulting immunological process [16]. Beltz [15] and Sun *et al.* [16] have shown that *miR-142* affects T-cell differentiation and function and cytokine production processes, which seems to support the significant increase in the expression level of *miR-142-5p* and T-cell defect suspected in the aetiology of AS.

Talebi *et al.* [17] investigated the expression level of *mir-142*, that has a role in T-cell differentiation, and its target transcripts in patients with multiple sclerosis (MS), which is accompanied by autoimmunity related neuroin-flammation, and in mice with autoimmune encephalomyelitis as animal model of MS. They examined the expression level of *mir-142* in the brain tissue of six deceased MS patients and six deceased non-MS individuals and found significantly higher expression of *miR-142* in the MS group, and similar findings in the spinal cord tissues of mice with autoimmune encephalomyelitis. The amount of CD3-positive T-cells in the tissue significantly increased compared to the control group. Naive CD4+ T-cells isolated from mice splenocytes transfected with *miR-142-5p* and *miR-142-3p* imitators were investigated to identify whether they would differentiate into Th1, Th17 or Treg cell groups. In the presence of *miR-142-5p*, the cells differentiated into the Th1 cell phenotype. For *miR-142-5p*, *TGFBR2* and *SOCS1* were found to be the target genes both in splenocyte cells and in luciferase-dependent genetic studies. *SOCS1* is a molecule from the *SOCS* protein family, acts as a negative regulator of the cytokine signal. Several cytokines require *JAK-STAT* molecules to exert their effect. *SOCS* proteins block *JAK* proteins or cytokine receptors *via* cytokines such as *IL-2* and *IFN-γ*. *SOCS1* regulates T-cell differentiation and plays a key role in T-cell dependent immunopathologies. *STAT1* and *STAT5* also have an effect on Th1 differentiation, and *SOCS1* inhibits STAT1 and blocks *IFN-γ*-mediated *STAT1* activation. *SOCS1* also has an effect on Treg cells and performs its function by altering *Foxp3* expression, suppressing the inflammatory cytokine production of Treg cells. Normally, Treg cells do not secrete inflammatory cytokines, but *IFN-γ* and *IL-17* in the absence of *SOCS1* to cause *STAT1* and *STAT3* hyperactivity. In *SOCS1* knockout mice experiments, CD4+ naive T-cells have been shown to differentiate into Th1 lineage and develop autoimmune inflammatory diseases over time. In light of this information, *SOCS1,* the target of *mir-142-5p*, seems to be the main regulatory molecule in Treg cells and Th1 differentiation [17]. In the present study, the expression level of *mir-142-5p* in PBMCs was significantly higher in patients with AS, which has a similar aetiology with MS; thus, the expression level of *mir-142-5p* could create the inflammatory process in the sacroiliac joint by targeting similar genes.

Duijvis *et al.* [18] performed a miRNA and mRNA genome-wide expression analysis in *CD45RB* transfer colitis mice, an IBD model, and found a significant increase in the expression level of 11 miRNAs, including *mir-142-5p*, compared with that in the colon tissue of healthy mice. They targeted five miRNAs with the highest level in the colon tissue with appropriate anti-miRs and showed that anti-miR-142-5p-treated mice started to gain weight and improved clinical course with histologically decreased colonic inflammation. They performed mRNA expression analysis in the colon tissue before and after treatment and predicted that mRNAs with changing expression levels after treatment are targets for *miR-142-5p*. When genes whose expression levels changed after treatment were compared with those that were targets of *miR-142-5p* with in-silico analyses, *MAL* (*TIRAP*) and glial-derived neurotrophic factor *(GDNF)* were

considered direct target genes. *MAL (TIRAP)* acts as an intermediary molecule between the *TLR4* and *Myd88* molecules and triggers inflammation in the colon *via NF-κB* activation. *Cyp2c55*, showing the highest increase in expression level after *anti-miR-142-5p* treatment, is a molecule that converts arachidonic acid into epoxyeicosatrienoic acid having a powerful anti-inflammatory effect. In the study by Duijvis *et al.* [18], 250 different genes, whose expression levels were found to be altered by *anti-miR-142-5p* treatment, were analyzed to identify the responsible signalling pathway, and stated that *IL10RA* could be an upstream regulator. *IL10RA* is a receptor of *IL10* and its receptor-ligand binding produces an anti-inflammatory effect; it is inhibited in inflamed colon tissues and is activated by *anti-miR-142-5p* treatment. *IL10RA* is the target gene in *anti-miR-142-5p* treatment for supressing inflammation [18]. Based on these results by Duijvis *et al.* [18], it can be argued that in AS, sharing a similar aetiology, *miR-142-5p* molecule, with a significantly increased expression level was in the present study, might cause inflammation in the sacroiliac joint via *IL10RA* mechanism.

As a postmenopausal osteoporosis disease model Teng *et al.* [19] analyzed a total miRNA expression analysis in bone marrow derived mesenchymal stem cells of mice with oophorectomy. In all *miRNAs*, the expression level of *miR-142-5p* increased the most compared with the control group. After *in silico* analysis, the expression of *VCAM-1*, was detected as target gene in osteoporosis development. *VCAM1* was decreased in HEK293T cells, as revealed by luciferase assay. *VCAM-1* has an important role in cell-cell recognition, inflammation, development of various organs and formation of immune responses and is also involved in the activation and migration of immune cells [19]. The increased expression of *miR-142-5p* and reduced expression of *VCAM-1* demonstrated in the osteoporosis model by Teng *et al.* [19] might be associated with the impaired cellular immune response and increased inflammatory process in patients with AS.

In our study, the expression of *miR-143* in PBMCs was significantly higher in the patient group compared to the control group (p <0.05), with the level being 1.5 times higher in the patient group. When Lin *et al.* [20] studied the expression level of *miR-143* at inflamed and non inflamed colon tissues of patients with Crohn's disease, there was a significantly increased expression in inflamed tissue compared to normal tissue. They showed that the expression of *ATG2B*, which is the target of *miR-143* playing a role in autophagy, was decreased in inflamed tissues. In case of increased expression of *miR-143* and decreased expression of *ATG2B* in inflamed colon tissue, auto-phagosomes and autolysosome formation were significantly decreased electron microscopically compared with those in normal tissues; this was reversed with the use of anti-*miR-143,* and the expression of *ATG2B* was increased. Increased expression of *miR-143* in cell culture suppressed expression of *IκBα* (indirectly activating the *NF-κB/REL* pathway) and increased mRNA expression of pro inflammatory cytokines. In light of all this information, it was demonstrated that *miR-143* suppresses autophagy by targeting *ATG2B* and increases the inflammatory process by affecting the *NF-κB* signalling pathway [20]. In our study, the expression level of *miR-143* was significantly increased in patients with AS, consistent with findings of Lin *et al.* [20].

Hong *et al.* [21] analyzed miRNA expression levels in fibroblast-like synovial cells of patients with RA and found that the expression of *miR-143* and *miR-145* significantly increased in the patient group compared with that in the osteoarthritis (OA) group. With microarray analysis using fibroblast-like synovial cells they found significant differences in the expression of 470 genes between the two groups. *IGFBP5* and *SEMA3A* were identified as the possible targets, and cell culture studies demonstrated that *IGFBP5* was the target of *miR-143*. Cell culture studies showed that *IGFBP5* inhibited the *TNFα* signal and indirectly suppressed the *NF-κB* signaling pathway. In summary, increased expression of *miR-143* in fibroblast-like synovial cells of patients with RA suppressed the expression of *IGFBP5* and activated the *NF-κB* signalling pathway *via* the activation of the *TNFα* signal [21]. Thus, *miR-143*, the expression level of which significantly increased in AS patients was considered to be the cause of the activation of pro inflammatory pathways through a similar mechanism.

To conclude, for further discussion of our hypothesis on whether specific miRNAs are potential biomarkers and target molecules in the treatment of AS. Obtained data should be validated by studies conducted using larger cohorts. The miRNA expression levels ight also be important at the affected target tissue level. Thus, the use of expression levels of *miR-142-5p* and *miR-143* might be effective as a non invasive potential tool for the diagnosis of AS and potential treatment targets in the future.

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