Journal of Orthopaedic Science xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Orthopaedic Science

journal homepage: http://www.elsevier.com/locate/jos

Original Article

Comprehensive analysis of microRNA expression in lumbar facet joint capsules and synovium of patients with osteoarthritis: Comparison between early-stage and late-stage osteoarthritis samples from a single individual

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ARTICLE INFO

Article history:
Received 3 August 2022
Received in revised form
16 November 2022
Accepted 18 January 2023
Available online xxx

Keywords:
Facet joint
Osteoarthritis
MicroRNA
Next generation sequencing

ABSTRACT

Background: MicroRNA is attracting attention as a therapeutic target for osteoarthritis. We focused on joint capsules and synovium in lumbar facet joint osteoarthritis. The purpose of this study was to identify microRNAs that are upregulated in lumbar facet joint capsules and synovium with osteoarthritis.
Methods: We included patients who underwent spinal fusion for degenerative lumbar spine diseases. We selected patients who had both early-stage and late-stage facet joint osteoarthritis in a single individual. We extracted joint capsule and synovium samples from these patients and isolated microRNAs. During the screening phase, we compared early-stage and late-stage osteoarthritis samples from the same individual. We identified microRNAs with >2-fold change in expression in 75% or more of patients with late-stage osteoarthritis using next generation sequencing. During the technical validation phase, the same samples were used for real-time polymerase chain reaction. We identified microRNAs with >2-fold change in expression in 62.5% or more of patients with late-stage osteoarthritis.
Results: Of 40 patients who underwent spinal fusion, we selected eight patients with both early-stage and late-stage facet joint osteoarthritis. During the screening phase, we identified eight upregulated microRNAs out of 2274 microRNAs in late-stage OA. In late-stage OA, two microRNAs (miR-133a-5p and miR-144-3p) were upregulated in seven patients and six microRNAs (miR-133a-3p, miR-133b, miR-206, miR-20a-5p, miR-301a-3p, and miR-32-5p) were upregulated in six patients. During the technical validation phase, we found significant upregulation of miR-144-3p expression in late-stage osteoarthritis compared with early-stage osteoarthritis. Expression of the other microRNAs was not significantly different according to the paired-t test. However, miR-133a-3p, miR-133b, and miR-206 were upregulated >2-fold in 62.5% or more of patients with late-stage osteoarthritis.
Conclusions: Some of the microRNAs identified in this study might be involved in joint capsule degeneration or synovitis.

1. Introduction

The bilateral facet joints and intervertebral discs form three-joint complexes that connect adjacent vertebrae and stabilize the spine [1,2]. The facet joints are synovial joints with cartilage overlying subchondral bone, a synovial membrane, and a joint capsule [3,4]. Facet joints can develop osteoarthritis (OA), and lumbar facet joint OA can cause low back pain, which is known as facet pain or facet syndrome [1,3].

Recently, endogenous small non-coding ribonucleic acids (RNAs), which include microRNAs, have been considered as potential biomarkers for various diseases and therapeutic targets [5]. Approximately 20–25 nucleotides long non-coding RNAs are called microRNAs [5]. They are involved in various disease conditions via regulation of post-transcription gene expression. Identification of

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https://doi.org/10.1016/j.jos.2023.01.008

Please cite this article as: K. Ishida, S. Tanishima, A. Tanida *et al.*, Comprehensive analysis of microRNA expression in lumbar facet joint capsules and synovium of patients with osteoarthritis: Comparison between early-stage and late-stage osteoarthritis samples from a single individual, Journal of Orthopaedic Science, https://doi.org/10.1016/j.jos.2023.01.008

microRNAs as biomarkers for OA and discovery of drugs that can suppress OA progression can lead to advances in treatment [6]. Methods such as microarray or next generation sequencing (NGS) are available for comprehensive microRNA analysis [7]. Various studies have investigated microRNA expression in the synovium, synovial fluid, or cartilage in human OA [8,9].

To identify biomarkers of OA, we focused on microRNAs expression in the joint capsule and synovium. When we performed spinal fusion for lumbar degenerative diseases, it was possible to obtain tissues from multiple facet joints in an individual. These patients often had late-stage facet joint OA. Furthermore, some individuals have both late-stage and early-stage facet joint OA. Comparing multiple facet joint specimens collected from a single individual is a great advantage because microRNAs are considered to have wide inter-individual differences. There have been no reports on microRNA expression in human facet joint capsules or synovium. The purpose of this study was to identify microRNAs that are upregulated in lumbar facet joint capsules and synovium with OA.

2. Materials and methods

2.1. Study design and outline

This study was a non-randomized observational study. We outlined this study in Fig. 1. During the screening phase, we analyzed 2274 microRNAs using NGS. During the technical validation phase, we analyzed microRNAs shown to be upregulated in late-stage OA during the screening phase using real-time polymerase chain reaction (PCR).

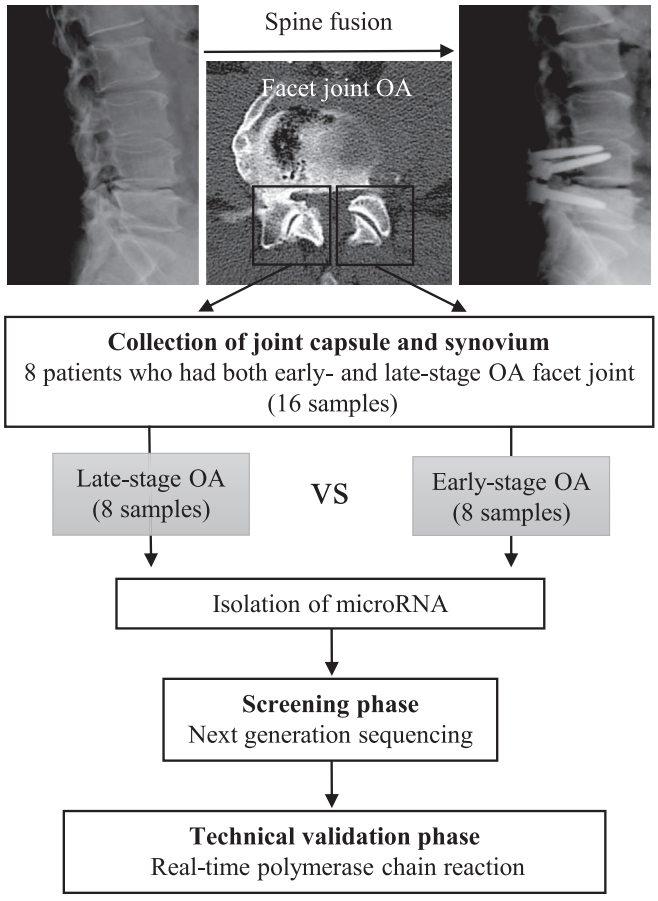

Fig. 1. Study outline. OA, osteoarthritis.

2.2. Participants and inclusion criteria

We included Japanese patients aged 20–80 years who underwent posterior spinal fusion surgery (posterolateral fusion or transforaminal lumbar interbody fusion) at the L3–4, L4–5, or L5–S1 disc levels for degenerative lumbar spine diseases like spinal stenosis, degenerative spondylolisthesis, degenerative scoliosis, isthmic spondylolisthesis, and disc herniation. All surgeries were performed at the senior authors' hospital from May 2018 to December 2019. We excluded patients who had a history of lumbar surgery, lumbar infection, autoimmune disease, severe renal failure, dialysis, or cancer under treatment. Furthermore, patients with an uncertain understanding of our study because of diseases such as dementia or mental illness were excluded.

2.3. Selection of patients

The first author (an orthopedic surgeon) and second author (a spine surgeon) classified facet joint OA severity based on computed tomography images according to the Weishaupt classification [10] as grade 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). Grade 0–1 was defined as early-stage OA and grade 2–3 was defined as late-stage OA. Inter-rater reliability of OA severity between both authors was moderate (kappa value, 0.51), and the OA severity was adopted from the first author's evaluation. We selected patients who had both early-stage and late-stage facet joint OA for analysis. A total of 16 samples were obtained from eight patients. We excluded samples with insufficient amounts of joint capsule from the analysis.

2.4. Tissue collection and storage

All surgeries were performed under general anesthesia in the prone position with open surgical techniques. The posterior joint capsules, including the synovium of the facet joint to be fused, were pinched with forceps, and incised sharply with a scalpel. The tissues were received in the operating room, separated into three or more pieces with scissors using magnifying glasses (2.0 ×), and placed into 2.0-mL tubes. The excised joint capsule was removed from attached tissues such as muscle, fat, bone, calcifications, or ossifications. They were extracted into only fibrous tissue and synovium. We performed these procedures aseptically. The tubes containing samples were snap frozen in liquid nitrogen, and stored in a −80 °C deep freezer. To confirm that tissues were properly harvested, the tissue obtained from some of patients was fixed in 10% paraformaldehyde, stained with hematoxylin and eosin, and observed under a microscope (Olympus Co., Ltd., Tokyo, Japan). Microscopic images were obtained using an Olympus DP22 microscopic digital camera (Olympus Co., Ltd.) with an Olympus U-TV 0.5 XC-3 camera adaptor (Olympus Co., Ltd.) with a digital system. The images were captured using Olympus cellSens standard software, version 2.1 (Olympus Co., Ltd.). We verified that the joint capsule samples included only fibrous tissue and synovium based on histological examination (Fig. 2A and B). Fig. 2A and B were obtained from the same patient. Both tissue samples were composed of fibrous connective tissue and synovium. Fig. 2A was early-stage OA sample and showed proliferation of intervening collagenous synovial stroma and mild tissue thickening. Fig. 2B was late-stage OA and showed remarkable proliferation of intervening collagenous synovial stroma and marked tissue thickening.

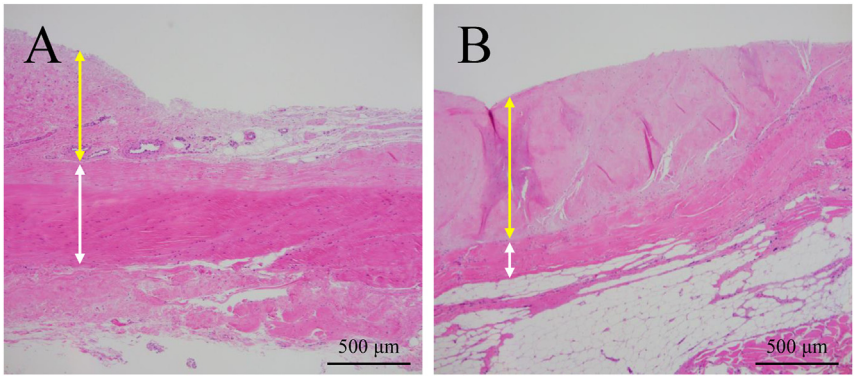

Fig. 2. Histology of the lumbar facet joint capsule and synovium based on hematoxylin and eosin staining (×40). (A) Early-stage osteoarthritis, Weishaupt classification grade 1. (B) Late-stage osteoarthritis, Weishaupt classification grade 2. Double-headed yellow arrows indicate layers of synovium and stroma. White arrows indicate a layer of fibrous connective tissue.

2.5. Screening phase

We provided the tissue samples to DNA Chip Research Inc. (Tokyo, Japan) for NGS. Total RNA was isolated from each frozen fresh facet joint capsule sample using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions, followed by purification using the RNeasy Mini Kit (Qiagen, Inc., Hilden, Germany). Purified microRNAs were ligated with 5′ and 3′ adaptors at both ends. Ligated microRNAs were further purified and reverse transcribed into complementary deoxyribonucleic acid (cDNA). After cDNA cleanup, library amplification was performed. A pre-sequencing quality control step was performed using the High Sensitivity DNA Assay and the 2100 Bioanalyzer Expert (Agilent Technologies, Inc., Santa Clara, CA, USA). The libraries were pooled in equal volumes and sequenced on an Illumina NextSeq 500 platform (Illumina, Inc., San Diego, CA, USA) with 75 bp single-end. Raw sequences were normalized with the trimmed mean of M-values method. Clean reads were aligned against the reference human genome hg19 (http://www.genome.ucsc.edu/index.html) using Strand NGS software, version 3.4 (Strand Life Sciences, Inc., Bangalore, India). Sequences of mature microRNAs were obtained from miRbase 20.0 (http://www.mirbase.org/). We identified microRNAs with >2-fold change in 75% or more of patients as either high or low expression.

2.6. Technical validation phase

Real-time PCR was performed using a TaqMan® microRNA assay (Thermo Fisher Scientific, Inc.). cDNA for each microRNA of interest was synthesized from an input of 5 μL of total RNA using TaqMan® microRNA reverse transcription reagents and specific reverse transcription primers. Reverse transcription was performed under the following thermal conditions: 30 min at 16 °C, followed by 30 min at 42 °C and 5 min at 85 °C, and maintained at 4 °C. Real-time PCR with TaqMan® probes was performed on the ViiA™ 7 real-time PCR System (Thermo Fisher Scientific Inc.) under the following thermal conditions: 10 min at 95 °C followed by 40 cycles of 9 °C for 15 s and 60 °C for 1 min. All assays were performed in triplicate. Cycle threshold (Ct) values were determined using QuantStudio real-time PCR software (Thermo Fisher Scientific, Inc.). Triplicate Ct values were averaged and normalized to the geometric mean of U6B small nuclear RNA Ct values, which was used as an endogenous control [11]. The normalized expression was calculated as $2^{-\Delta\Delta Ct}$. We identified microRNAs with >2-fold change in 62.5% or more of patients as high or low expression using Microsoft Excel 2019 software (Microsoft, Redmond, WA, USA). For statistical analysis, we compared the expression of each microRNA during early-stage versus late-stage OA using the paired *t*-test in EZR version 1.53 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). P values less than 0.05 were considered statistically significant.

2.7. Prediction of target genes

To predict microRNAs target genes, we used the databases: TargetScan (http://www.targetscan.org/); PITA (https://genie.weizmann.ac.il/pubs/mir07/index.html); miRanda (http://www.microrna.org). The genes that appeared in two or more these databases were considered as potential targets.

3. Results

3.1. Patient characteristics

We enrolled 40 patients (mean age ± SD, 68.2 ± 9.0 years; range, 33–80 years; 23 males and 17 females) during the study period. The location and OA grade of the lumbar facet joint capsules collected are shown in Fig. 3. Of these patients, 33 patients had a sufficient amount of joint capsule samples from each facet joint. Six patients (Patients 5, 7, 8, 16, 37, and 38 in Fig. 3) had a small amount of joint capsule from one facet joint obtained during surgery, but the samples from the other joints were sufficient. We excluded one patient because of insufficient amount of joint capsule obtained from each joint (Patient 13). In eight of 40 enrolled patients (mean age, 63.3 years; range 33–79 years; six males and two females), some lumbar facet joint capsules had early-stage OA and some had late-stage OA. We included the 16 joint capsule samples from these eight patients for analysis. The clinical characteristics of these eight patients are shown in Table 1.

3.2. Screening phase

The average number of total sequencing reads was approximately 14.1 million, and microRNA reads was approximately 7.1 million (Fig. 4). A large proportion of all sequencing reads were microRNA reads. We performed NGS on 16 samples from eight patients to identify microRNA expression in late-stage OA versus early-stage OA in lumbar facet joint capsules. Coincidentally, all samples with early-stage OA were from the left facet joint and all samples with late-stage OA were from the right. We sequenced 2274 microRNAs and identified eight microRNAs. The fold change in microRNA expression in facet joint capsules with late-stage OA

	Lumbar spine levels						
Sample No.	L3–4		L4–5		L5–S1		
1			L	L			
2			L	L			
3			L	L			
4	L	L	L	L			
5	L	L	—	E			Patient 1
6			L	L			
7					L	—	
8			L	—			
9			E	E			
10					L	L	
11	L	L	L	L			
12			L	E			Patient 2
13			—	—			
14			L	L			
15			L	E			Patient 3
16	L	E	—	L			Patient 4
17			L	L			
18	L	L	L	L			
19			L	L			
20	L	L	L	L			
21			L	L			
22	L	L	L	L			
23			L	L			
24			L	L			
25			L	L			
26					L	L	
27			L	L			
28			L	L			
29	L	L	L	L			
30	L	L					
31			L	L			
32			L	L			
33			L	E			Patient 5
34					L	L	
35			L	E			Patient 6
36			L	L	E	E	Patient 7
37	—	L	L	L			
38	L	L	L	—			
39	L	E					Patient 8
40			L	L			

Fig. 3. Location and osteoarthritis grade of the lumbar facet joint capsule samples. L, late-stage osteoarthritis; E, early-stage osteoarthritis. Minus means small or insufficient amount of tissue samples.

Table 1
Clinical characteristics of eight patients analyzed with next generation sequencing.

Patient	Age (years)	Sex	Diagnosis	Surgical procedure	Level and side of facet joint	
					Early-stage OA	Late-stage OA
					Weishaupt grade 0–1	Weishaupt grade 2–3
1	71	Male	LSS	PLF + TLIF	L3–4, left	L4–5, right
2	69	Male	LSS, DLS	TLIF	L4–5, left	L4–5, right
3	61	Male	LSS	PLF	L4–5, left	L4–5, right
4	64	Male	LSS, DLS	TLIF	L3–4, left	L3–4, right
5	79	Female	LSS	PLF	L4–5, left	L4–5, right
6	33	Male	LDH	TLIF	L4–5, left	L4–5, right
7	57	Male	L5 IS	PLF + TLIF	L5–S, left	L4–5, right
8	72	Female	LSS, L3 DS	TLIF	L3–4, left	L3–4, right

OA, osteoarthritis; LSS, lumbar spine stenosis; PLF, posterolateral fusion; TLIF, transforaminal lumbar interbody fusion; DLS, degenerative lumbar scoliosis; LDH, lumbar disc herniation; IS, isthmic spondylolisthesis; DS, degenerative spondylolisthesis.

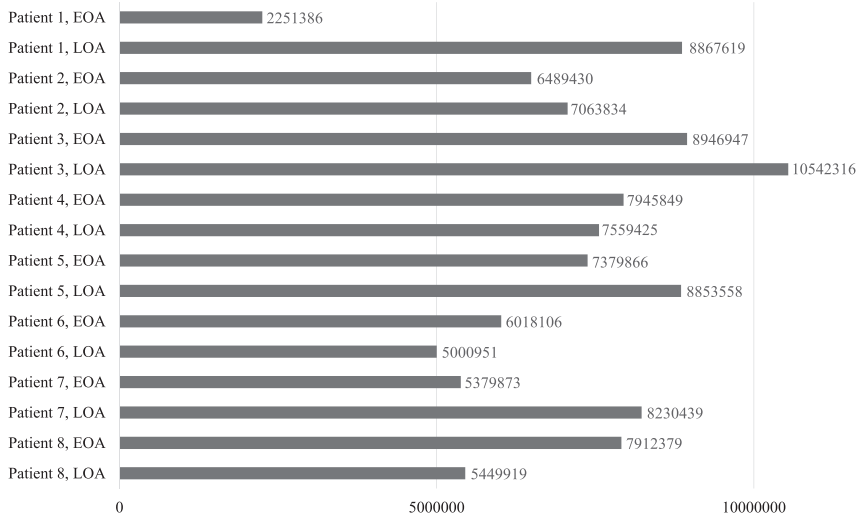

Fig. 4. Sequencing reads of microRNA expression in the joint capsules of each lumbar facet joint. EOA, early-stage osteoarthritis; LOA, late-stage osteoarthritis.

versus early-stage OA in the same individual is shown in Table 2. In late-stage OA, two microRNAs (miR-133a-5p and miR-144-3p) were significantly upregulated in seven patients. Six microRNAs (miR-133a-3p, miR-133b, miR-206, miR-20a-5p, miR-301a-3p, and miR-32-5p) were significantly upregulated in six patients. There were no downregulated microRNAs (fold change >2).

3.3. Technical validation phase

We analyzed the eight microRNAs identified during the screening phase using NGS for technical validation with real-time PCR. We used the same 16 samples obtained from the same eight patients used in NGS for real-time PCR. The comparison of microRNA expression in facet joint capsules between early-stage and late-stage OA based on real-time PCR is shown in Fig. 5. Expression of miR-144-3p was significantly upregulated in late-stage OA compared with early-stage OA ($p = 0.005$). Expression of the other microRNAs by OA stage was not significantly different. Fold change in microRNA expression in facet joint capsules with late-stage OA relative to early-stage OA in an individual based on real-time PCR in shown in Table 3. We identified three upregulated microRNAs (miR-133a-3p, miR-133b, and miR-206) with >2-fold change in 62.5% or more of patients with late-stage OA compared with early-stage OA. Upregulation of the other microRNAs was not observed.

Table 2
Fold change in microRNA expression of facet joint capsules with late-stage versus early-stage osteoarthritis in the same individual based on next generation sequencing.

	Fold change (late-stage OA versus early-stage OA)							
	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8
miR-133a-3p	5.07	0.91	0.03	21.74	22.88	32.97	7.80	2.01
miR-133a-5p	6.46	2.54	0.04	23.55	27.78	100.49	12.48	2.01
miR-133b	4.84	0.87	0.03	24.16	23.47	40.78	6.72	2.35
miR-144-3p	3.22	9.16	1.72	4.88	2.08	2.86	2.77	2.03
miR-206	4.52	2.22	0.04	15.51	30.87	57.85	4.27	1.74
miR-20a-5p	3.27	2.13	1.36	2.97	2.26	2.50	2.19	0.76
miR-301a-3p	2.39	3.24	1.48	3.91	2.32	2.25	3.98	0.52
miR-32-5p	3.23	2.37	1.43	2.32	2.27	2.72	2.31	0.74

OA, osteoarthritis; Pt, patient.

3.4. Prediction of target genes

No gene were predicted as miR-133a-3p potential targets using the databases: TargetScan; PITA; miRanda, and 100 genes were predicted as miR-133b, 39 genes as miR144-3p, and 265 genes as miR-206 potential targets. The hypergeometric method was used to calculate the p values, cut-off: 0.05. Five genes (ANP32E, acidic nuclear phosphoprotein 32 family member E; CAP1, cyclase associated actin cytoskeleton regulatory protein 1; FLI1, Fli-1 proto-oncogene; NEUROD1, neuronal differentiation 1; RARB, retinoic acid receptor beta) appeared as targets for two or more microRNAs (Table 4).

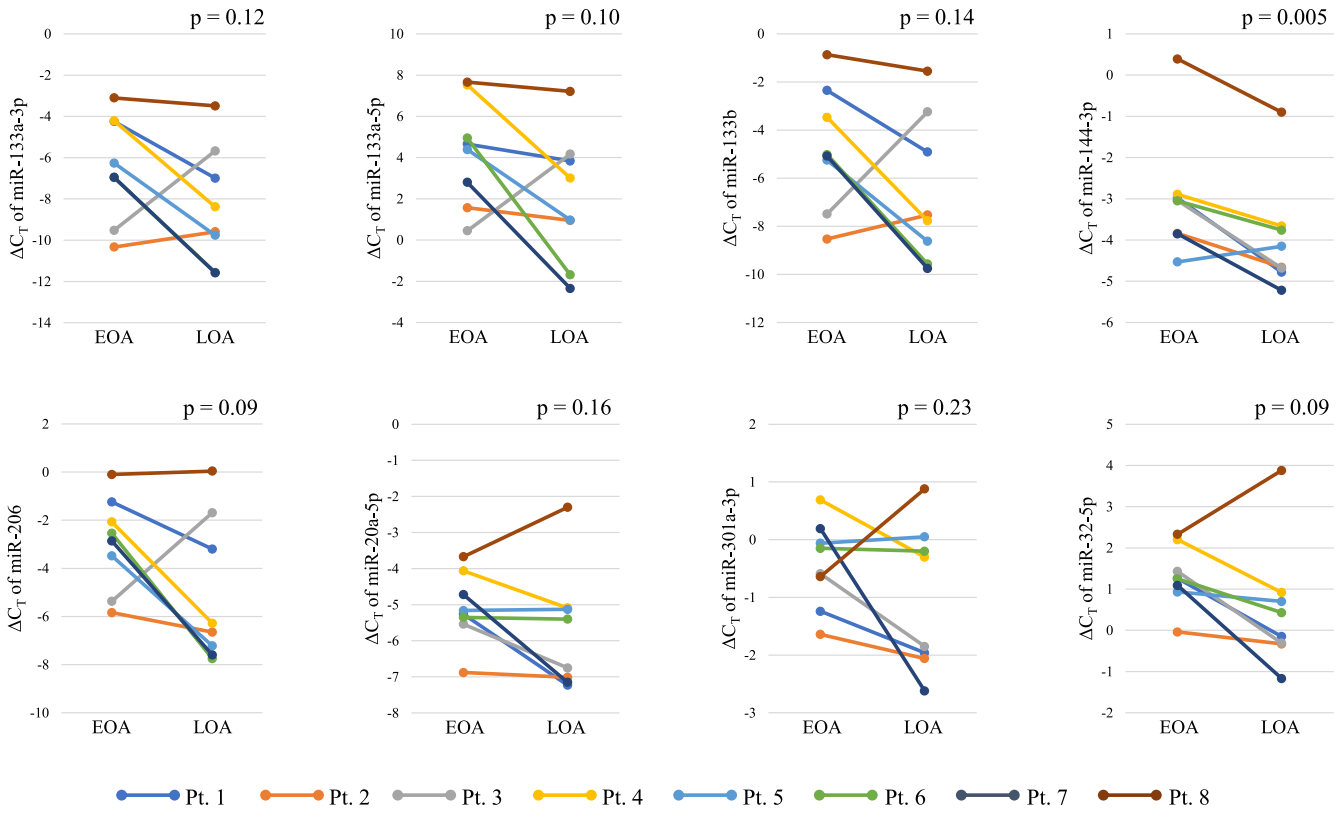

Fig. 5. Comparison of microRNA expression in facet joint capsules between early-stage and late-stage osteoarthritis based on real-time polymerase chain reaction. EOA, early-stage osteoarthritis; LOA, late-stage osteoarthritis. Pt, patient.

Table 3
Fold change in microRNA expression of facet joint capsules with late-stage versus early-stage osteoarthritis in the same individual based on real-time polymerase chain reaction.

	Fold change in $2^{-\Delta\Delta Ct}$ (late-stage OA versus early-stage OA)							
	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8
miR-133a-3p	6.73	0.60	0.07	18.00	11.31	24.08	24.76	1.31
miR-133a-5p	1.77	1.54	0.08	22.94	10.70	99.73	35.51	1.37
miR-133b	5.90	0.50	0.05	19.70	10.34	23.43	25.46	1.60
miR-144-3p	3.48	1.77	3.12	1.71	0.77	1.64	2.58	2.45
miR-206	3.89	1.75	0.08	18.51	13.36	37.01	26.72	0.91
miR-20a-5p	3.92	1.09	2.31	2.04	0.98	1.04	5.39	0.39
miR-301a-3p	1.65	1.34	2.39	1.99	0.93	1.04	7.01	0.35
miR-32-5p	2.66	1.22	3.34	2.43	1.17	1.77	4.79	0.34

Ct, cycle threshold value; OA, osteoarthritis; Pt, patient.

Table 4
Predicted target genes of microRNAs.

Target gene	microRNA
ANP32, acidic nuclear phosphoprotein 32 family member E	miR-144-3p miR-206
CAP1, cyclase associated actin cytoskeleton regulatory protein 1	miR-133b miR-206
FLI1, Fli-1 proto-oncogene	miR-133b miR-206
NEUROD1, neuronal differentiation 1	miR144-3p mir-206
RARB, retinoic acid receptor beta	miR133b miR-206

4. Discussion

Comprehensive analyses of microRNA expression have been performed with microarrays or NGS [12]. Several reports have suggested that microarrays produced less reliable absolute quantitative expression measurements compared with sequencing [7,13]. In our study, we performed highly reliable microRNA profiling using screening microRNAs with NGS and technical validation with real-time PCR. This was the first study on microRNA expression in joint capsule and synovium of human lumbar facet joints with OA. We identified miR-144-3p upregulation in late-stage OA facet joint capsules. In addition, three microRNAs (miR-133a-3p, miR-133b, and miR-206) were upregulated with >2-fold change in 62.5% or more of patients with late-stage OA. It was unclear which of these four microRNAs identified in our study is the most strongly involved in the pathogenesis of OA because we did not conduct *in vivo* or *in vitro* studies. Whereas the samples in most studies comparing microRNA expression levels were obtained from multiple individuals, we compared between samples from a single individual in this study. Gene products such as microRNAs vary greatly between individuals, so that the one of the advantages of this study was elimination of errors due to inter-individual gene variation.

Many publications have investigated microRNA expression in OA [2,8,9,14,15]. Xie et al. reported 17 microRNAs inhibiting OA and 14 microRNAs promoting OA in their systematic review [2]. As one of the few publications focusing on the relationship between lumbar facet joint OA and microRNAs, Nakamura et al. reported

that miR-181a-5p and miR-4454 are upregulated in facet joint cartilage with OA [9]. Furthermore, treatment of rat facet joints with induced OA or mouse knee joints with intra-articular injections of locked nucleic acid miR-181a-5p antisense oligonucleotides have attenuated cartilage destruction and expression of catabolic, hypertrophic, apoptotic or cell death, and type II collagen breakdown markers [15]. It has been reported that miR-144-3p and miR-206 are promote chondrocyte apoptosis and are upregulated in OA cartilage [16,17]. In this study, both microRNAs were upregulated in late-stage OA facet joint capsules and synovium. Although we cannot deny the possibility that the tissues collected in our study contained worn cartilage components, it was unlikely that there was enough cartilage attached to the tissue samples to affect the difference in microRNA expression levels.

While there have been many published reports on the relationship between OA cartilage and microRNAs, we focused on the joint capsule and synovium in this study. Li et al. identified upregulation of seven microRNAs (miR-23a-3p, miR-24-3p, miR-27a-3p, miR-27b-3p, miR-29c-3p, miR-34a-5p, and miR-186-5p) in the synovial fluid of knee joints with late-stage compared to early-stage OA. While there have been several reports on microRNAs in the synovium or synovial fluid of patients with OA [8,14], there have been no reports on the lumbar facet joint capsule or synovium.

We demonstrated miR-144-3p upregulation in late-stage OA. Several reports have shown that miR-144-3p plays a role in promoting proliferation, migration aptitude, and collagen synthesis in cardiac fibrosis; it is upregulated in myocardial infarcts [18,19], and it has been reported that miR-144-3p is upregulated in fibroblasts of patients with idiopathic pulmonary fibrosis [20]. In addition, miR-144-3p suppresses transcription of interleukin-1β (IL-1β) by binding to the 3′ untranslated region of IL-1β mRNA [21]. Apelin, a major OA-related adipokine, suppresses the expression of miR-144-3p in human synovial fibroblasts and stimulates the synthesis of IL-1β [21]. The tissue samples collected in our study have significant fibrosis in the synovial stroma. The upregulation of miR-144-3p might reflect fibroblast proliferation. Considering that IL-1β inhibits miR-144-3p, inflammation should be suppressed in tissues with miR-144-3p upregulation, but we detected miR-144-3p upregulation in late-stage OA tissues. This result may have been due to the fact that the tissue samples collected in our study contained predominantly fibrosis rather than inflammation, suggesting that late-stage OA does not necessarily mean active inflammation.

In addition, we demonstrated upregulation of miR-133a-3p, miR-133b, and miR-206 in the fold change evaluation. Since miR-133 and miR-206 are muscle-specific microRNAs, they have been called myomiRs [22]. These microRNAs are necessary for cardiac or skeletal muscle development and function. In cardiac muscle, miR-133 represses fibroblast signatures and promotes cardiac reprogramming [23]. Although miR-133 levels are increased in fibroblasts with myocardial fibrosis, they are decreased in cardiomyocytes with cardiac hypertrophy or cardiac fibrosis [24]. In skeletal muscle, miR-206 potentially plays a role in hypertrophy, fiber type switching, and the pathophysiology of muscular dystrophy [25]. Similar to miR-144-3p mentioned above, upregulation of miR-133 and miR-206 might have been influenced by fibrosis in the joint capsule and synovium in our study.

Five genes (ANP32E, CAP1, FLI1, NEUROD1, and RARB) were predicted as potential targets of these four microRNAs. A review for genetics of OA have shown that over 100 polymorphic DNA variants have been associated with OA, and ANP32E have been listed for target gene [26]. CAP1 is a receptor for resistin, and involved in chemokine production in rheumatoid arthritis synovial tissue [27]. To consider the common denominator of synovial inflammation, CAP1 also might be involved in chemokine production in OA synovial tissue. Retinoic acid receptor alpha is expressed in synovial membrane in OA, and may play a role in the synovial inflammation [28]. Similarly, RARB may be involved in synovial inflammation. There were no reports showing that FLI1 and NEURD1 associated with OA.

This study had several limitations. Firstly, we had a small sample size. There were few patients with both early-stage and late-stage facet joint OA. Secondly, we did not perform comparative analysis between normal and OA. In case we included patients without OA, there would be large differences in patient backgrounds. Finally, analysis was based on only with >2-fold change between early-stage and late-stage OA. It would be worthwhile to investigate the microRNAs expression with ≤2-fold change. MiR-144-3p, miR-133a-3p, miR-133b, and miR-206 were upregulated in late-stage facet joint OA, suggesting that these microRNAs might be associated with synovitis, joint capsule degeneration, or OA. We are planning to conduct *in vitro* experiments using microRNA mimics or inhibitors to evaluate the role of the identified microRNAs and their target genes in the synovium or joint capsule.

In conclusion, we showed four microRNAs (miR-144-3p, miR-133a-3p, miR-133b, and miR-206) upregulation in lumbar facet joint capsules and synovium with late-stage OA. To eliminate errors due to individual gene variation, we compared differences in microRNA upregulation between early-stage and late-stage facet OA from a single individual. Therefore, some of these microRNAs are probably responsible for facet joint degeneration or synovitis.

Ethical statement

This study was approved by the institutional review board of the Tottori University Faculty of Medicine (approval number 17A143). This study was conducted in accordance with the Declaration of Helsinki. We informed patients about the study and obtained written consent for study participation.

Funding

This work was supported by the Grant-in-Aid for Scientific Research of Japan Society for the Promotion of Science, Grant Number JP16K10820.

Declaration of competing interest

None.

Acknowledgements

We would like to thank Akihiro Nakamura and Mohit Kapoor (Krembil Research Institute, Toronto, Canada) for their advice on study design. We would also like to thank Kaori Adachi (Research Initiative Center, Tottori University, Tottori, Japan) and Eiji Nanba (Research Strategy Division, Organization for Research initiative and Promotion, Tottori University, Tottori, Japan) for their advice on study design and help with real-time PCR analysis. We also thank Zenis Co., Ltd. (https://www.zenis.co.jp/) for English language editing.

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