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Expression of MiR-140 and MiR-199 in Synovia and its Correlation with the Progression of Knee Osteoarthritis

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The aim of this study was to explore the expression of miR-140 and miR-199 in synovia of patients with knee osteoarthritis (KOA) and its correlation with the progression of this disease. We used the Kellgren and Lawrence grading (KLG) system.

Material/Methods: There were 110 patients with early (KLG <2), middle (KLG=2) and late (KLG >2) stage KOA and 60 healthy individuals (control) included in this study.

Results: The relative expression levels of miR-140 (1.07±0.091) and miR-199 (1.03±0.110) in synovia of the control group were higher than those of KOA groups (0.511±0.130, 0.298±0.168) and the difference exhibited statistical significance ($P<0.01$). Expression of miR-140 in the middle and the late stage KOA groups (0.322±0.118 and 0.110±0.088 respectively) were 58.80% and 81.29% lower, respectively, compared to the early stage KOA group (0.588±0.172), which was significant ($P<0.05$). Expression of miR-199 in the middle and the late stage KOA groups (0.210±0.124 and 0.056±0.068 respectively) were 39.41% ($P<0.05$) and 83.72% ($P<0.01$) respectively lower than that in the early KOA group (0.344±0.147). The severity of OA was significantly negatively correlated with the expressions of miR-140 and miR-199 (r=–0.859, $P<0.05$; r=–0.724, $P<0.001$ respectively). Matrix metalloproteinase (MMP)-3 levels of the early stage, middle stage and late stage KOA groups were 1.320±0.118, 1.488±0.210, and 1.955±0.023 respectively; and IL-1β mRNA was 1.401±0.204, 1.522±0.210, and 1.889±0.217 respectively, which were obviously higher than those in the control group (1.020±0.085), ($P<0.05$).

Conclusions: Expression levels of miR-140 and miR-199 in synovia might act as an early diagnostic marker for KOA. These expression levels might also act as indicators of OA progression to some extent.

MeSH Keywords: Osteoarthritis, Hip • Osteoarthritis, Knee • Osteoarthritis, Spine

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Background

Knee osteoarthritis (KOA) is a kind of chronic arthropathy and relevant clinical manifestations include knee joint pain, swelling, joint malformation, and activity limitation together with pathological changes leading to cartilage degeneration, synovial lesions, and hyperplasia which mostly occur in middle aged and elderly adults [1–3]. Due to rapid growth in the aging population of China, the incidence of KOA is gradually increasing and due to its high morbidity rate, the quality of life of patients is reduced, and a heavy economic burden is exerted on patients' families and society [4]. The occurrence and progression of KOA is caused by a multitude of factors with complex pathogenesis [5]. Remodeling of the cartilage and subchoracular bone tissue is believed to be associated with intra-articular inflammation and chronic degenerative changes in the cartilage tissue. Disorders linked to gene regulation are considered to be a vital factor in causing such structural and functional changes in cartilage [2,5].

MicroRNAs (miRNAs) are endogenous, short, non-coding RNAs consisting of 21 to 25 bases that complementarily pair with a target gene to affect its stability and function [6]. Studies have shown that miRNA might enable the regulation of important physiological and pathological processes of the body, including maintenance of stability and homeostasis of degenerative changes in articular chondrocytes [7]. MiRNA-140 (miR-140) participates in the formation of articular cartilage and is therefore capable of causing degenerative changes in articular cartilage leading to the generation of osteoarthritis [8]. The expression of miR-140 is downregulated in the articular cartilage of OA patients and chronic OA mouse models, while it is highly expressed in normal cartilage. OA-like changes such as articular cartilage fibrosis and other degenerative changes occur in mice when miR-140 is inhibited, whereas overexpression of miR-140 helps in delaying the development of arthritis [7]. Interleukin-1β (IL-1β) is an important inflammatory factor that affects the development of OA. Studies have demonstrated that the miR-140 expression in cartilage tissue is regulated by IL-1β [9]. It is reported that miR-199 regulates cartilage transformation by regulating IL-1β expression, which promotes the normal function of chondrocytes [10]. Matrix metalloproteinase 3 (MMP-3) is an MMP capable of causing degradation of the articular cartilage matrix [11]. Studies have shown that miR-140 expression in osteoarthritic cartilage gradually decreases with increasing cartilage degeneration, while MMP-3 expression gradually increases [11]. *In vitro* experiments have verified that miRNAs may negatively regulate MMP-3 expression and inhibit decomposition and metabolism of cartilage utilizing various cellular pathways and cytokines.

Both miR-140 and miR-199 play a crucial part in the process of chondrocyte degenerative changes, but its mode of action and its role in the pattern of KOA progression are rarely reported. This study explored the relationship between miR-140 and miR-199 expression levels and the occurrence and development of KOA, with particular reference to their mode of action, by studying expression levels of miR-140 and miR-199 in synovia in order to provide new ideas for diagnosing and treating KOA patients.

Material and Methods

Patients

There were 110 KOA patients and 60 healthy individuals (controls), who were admitted to our hospital between January 2017 and May 2018, and who were selected as the participants of this study. All patients were diagnosed using KOA diagnostic criteria recommended by the American Rheumatism Association [12]. These KOA patients were classified according to the Kellgren and Lawrence grading (KLG) system into the early (KLG <2), middle (KLG=2), and late (KLG >2) stage KOA groups [13]. Exclusion criteria: patients with infectious arthritis, diabetes, and autoimmune diseases. The Ethics Committee of the First Affiliated Hospital of Xi'an Medical College (General Medical College of Xi'an Medical College) has approved the study (approval dated 2015.08.19), and all study participants provided written informed consent before participating in the study.

Materials

DEPC, toluidine blue stain, isopropyl alcohol, and chloroform were obtained from Guangzhou Xindi Laboratory Equipment Co., Ltd, China; miR-140 and miR-199 primers were obtained from Ambien, Austin, TX, USA; reverse transcription kit, real-time quantitative polymerase chain reaction (PCR) kit and Trizol kit were obtained from Invitrogen Corporation Carlsbad, CA, USA; real-time quantitative PCR detector equipped with the PRISM 7500 real-time PCR System were from ABI Corporation, Foster, CA, USA).

For collection and preservation of synovia, the control group and the treatment group were routinely disinfected in the supine position and 2% lidocaine was applied for localized anesthesia. Approximately 2 to 5 mL of synovial fluid was drawn from the knee articular joint in the middle of the medial patella and placed in serum tubes. Following centrifugation at 4500 rpm for 20 minutes, the supernatant was placed in a centrifuge tube and stored at –70°C.

Fluorescent quantitative PCR

U6snRNA was used as an internal reference to detect the expression of miRNAs, as well as MMP-3 and IL-1β mRNA expression.

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Total RNA was extracted by the Trizol method and the target gene was reverse transcribed into cDNA as per the reverse transcription kit instructions; the target gene was further amplified by transcription as per the real-time quantitative PCR kit instructions. The reaction conditions were pre-denaturation at 95°C for 2 minutes, at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 30 seconds for a total of 50 cycles. Real-time fluorescence quantitative PCR results were expressed as Ct values and the expression of RNA was calculated as $2-\Delta\Delta Ct$.

$\Delta\Delta Ct=\Delta Ct-(\Delta Ct)_{control}$ ΔCt was calculated by subtracting Ct value of internal reference gene from the Ct value of target gene in the same specimen $(\Delta Ct)_{control}=\Delta Ct$ of the control group.

Statistical analysis

SPSS 13.0 software (IBM, Armonk, NY, USA) was applied to conduct statistical analysis. GraphPad Prism 5.0 software was used for plotting. The χ^2 test was applied to analyze qualitative data; Analysis of variance or *t*-test was applied to analyze normally distributed quantitative data and the SNK-Q test was applied to analyze the differences between group means. Correlation analysis was carried out as per the Spearman correlation method. All *P* values are based on a 2-tailed probability at 0.05 and 0.01 significance levels.

Results

Basic information on the participants

The control group consisted of 60 healthy individuals. The treatment group of 110 KOA patients included 38, 35, and 37 cases in the early, middle, and late stage KOA groups, respectively. No obvious difference ($P>0.05$) existed in sex, age, and body mass index (BMI) between the control group and each of the early, middle, and late KOA groups.

Expression of miR-140 and miR-199 in synovia of KOA and the control groups

The real-time quantitative PCR results (Figure 1) show that the expression level of miR-140 in synovia of KOA groups (1.07±0.091) was obviously lower ($P<0.01$) than that of the control group (0.511±0.130), and the expression level of miR-199 in synovia of KOA groups (0.298±0.168) was obviously lower ($P<0.01$) than that of the control group (1.03±0.110).

Expression of miR-140 and miR-199 in synovia of KOA groups

Expression of miR-140 in the middle and late stage KOA groups (0.322±0.118 and 0.110±0.088 respectively) were 58.80% and

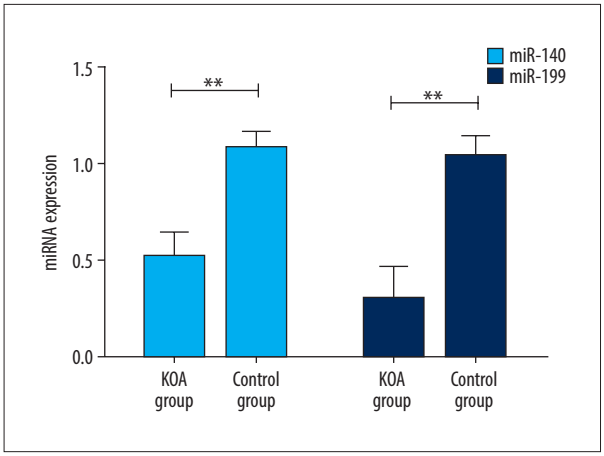

Figure 1. Comparative expression of miR-140 and miR-199 in synovia of KOA and control groups. Real time quantitative PCR results displayed that the expression level of miR-140 in synovia of KOA groups (1.07±0.091) was obviously lower ($P<0.01$) than that of the control group (0.511±0.130) and the expression level of miR-199 in synovia of KOA groups (0.298±0.168) was obviously lower ($P<0.01$) than that of the control group (1.03±0.110). PCR – polymerase chain reaction; miR-140 – micro-RNA 140; KOA – knee osteoarthritis, miR-199 – micro-RNA 199.

81.29%, respectively, lower than that in the early stage KOA group (0.588±0.172), which exhibited statistical significance ($P<0.05$). Expression of miR-199 in the middle and late stage KOA groups were (0.210±0.124 and 0.056±0.068 respectively) 39.41% ($P<0.05$) and 83.72% ($P<0.01$) respectively, lower than that in the early stage KOA group (0.344±0.147) and exhibited statistical significance at ($P<0.05$) and ($P<0.01$) respectively (Figures 2, 3)

Analysis of the correlation between miR-140 and miR-199 expression levels and progression of KOA

The expression level of miR-140 in synovia had obvious and negative correlation with the progression of KOA ($r=-0.859$; $P<0.001$); the expression level of miR-199 in synovia had obvious and negative correlation with the progression of KOA ($r=-0.724$, $P<0.001$) and the KOA disease course and the (Figures 4, 5).

Expression of MMP-3 and IL-1β in synovia of KOA groups

Expression of MMP-3 in KOA early, middle and late groups (1.320±0.118, 1.488±0.210 and 1.955±0.023 respectively) increased by 29.91%, 44.19%, and 89.44% respectively in comparison with that in the control group (1.032±0.066), which exhibited statistical significance ($P<0.05$). The mRNA of IL-1β in early, middle, and late KOA groups (1.401±0.204, 1.522±0.210 and 1.889±0.217 respectively) increased by 37.35%, 49.22%,

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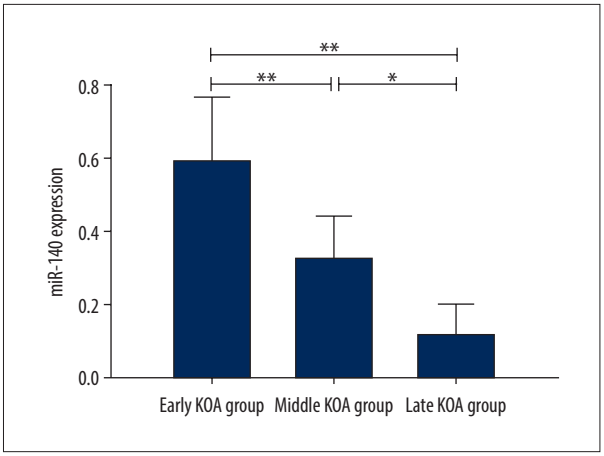

Figure 2. Comparative expression of miR-140 in synovia of KOA groups. The mRNA expressions of miR-140 in the middle and late stage KOA groups (0.322±0.118 and 0.110±0.088 respectively) were 58.80% and 81.29% respectively lower than that in the early stage KOA group (0.588±0.172), which exhibited statistical significance (P<0.05). miR-140 – micro-RNA 140; KOA – knee osteoarthritis; mRNA – messenger RNA.

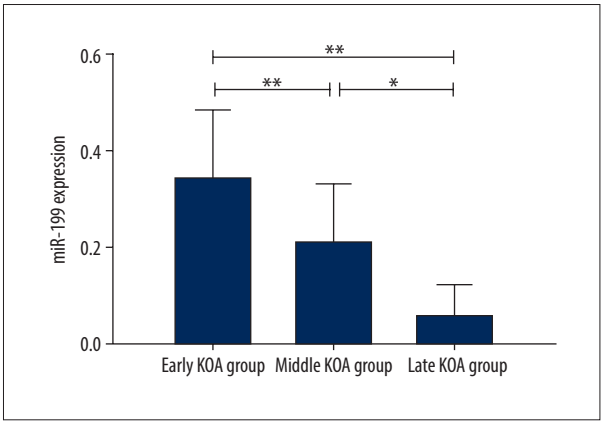

Figure 3. Comparative expression of miR-199 in synovia of KOA groups. Expression of miR-199 in the middle and late stage KOA groups (0.210±0.124 and 0.056±0.068 respectively) were 39.41% (P<0.05) and 83.72% (P<0.01) respectively lower than that in the early stage KOA group (0.344±0.147) (P<0.01). * P<0.05, ** P<0.01. miR-199 – micro-RNA 199; KOA – knee osteoarthritis.

and 85.20%, respectively, in comparison with those in the control group (1.020±0.085) which exhibited statistical significance (P<0.05) (Figures 6, 7).

Discussion

KOA is a kind of chronic arthropathy, and relevant manifestations include degenerative changes in cartilage and hyperosteogeny. Risk factors include age, weight, poor lifestyle,

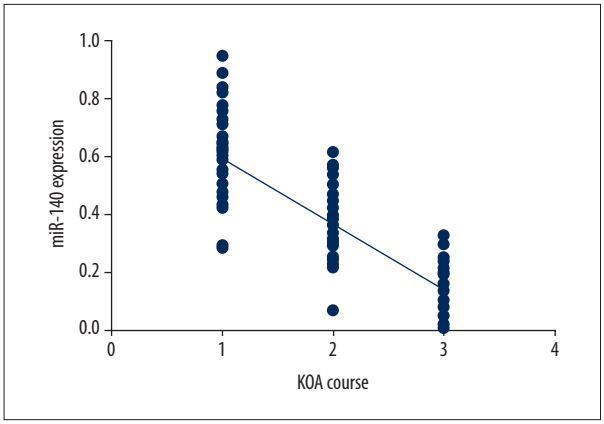

Figure 4. Correlation analysis of miR-140 expression during different KOA stages. Spearman correlation analysis displayed that the expression level of miR-140 in synovia had negative correlation with the progression of KOA (r=–0.859, P<0.001). miR-140 – micro-RNA 140; KOA – knee osteoarthritis.

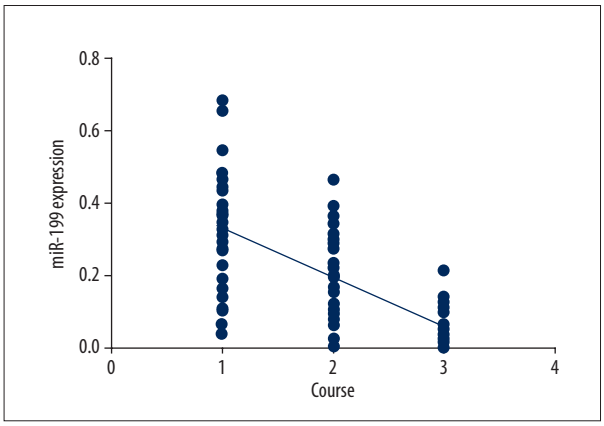

Figure 5. Correlation analysis of miR-199 expression and different KOA stages. Spearman correlation analysis displayed that the expression level of miR-199 in synovia had negative correlation with the course of KOA (r=–0.724, P<0.001). miR-199 – micro-RNA 199; KOA – knee osteoarthritis.

immunity, and genetic causes [14,15]. Previous studies have indicated a close relationship between the occurrence and progression of KOA and miRNA, as miRNA may promote regeneration of chondrocytes by regulating the expression of certain genes which participate in important physiological processes such as promoting the development of articular cartilage and protecting normal articular cartilage from destruction by OA [6,16]. Studies indicate that miR-140 and miR-199 might play a crucial part in the occurrence and progression of OA and that their expression levels in synovia may be relevant to the occurrence and progression of OA, and even further, that miR-140 and miR-199 might regulate KOA generation by acting on IL-1β and MMP-3 [10,17,18]. IL-1β is an important

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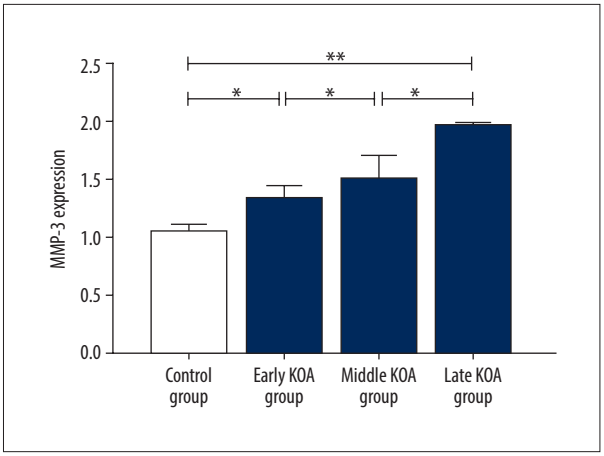

Figure 6. Expression of MMP-3 in synovia of KOA groups. Real-time quantitative PCR results displayed that expression of MMP-3 mRNA in early, middle, and late KOA groups was obviously increased by 29.91%, 44.19%, and 89.44% respectively in comparison with that in the control group. * $P<0.05$, ** $P<0.01$. MMP – matrix metalloproteinase; KOA – knee osteoarthritis; PCR – polymerase chain reaction; mRNA – messenger RNA.

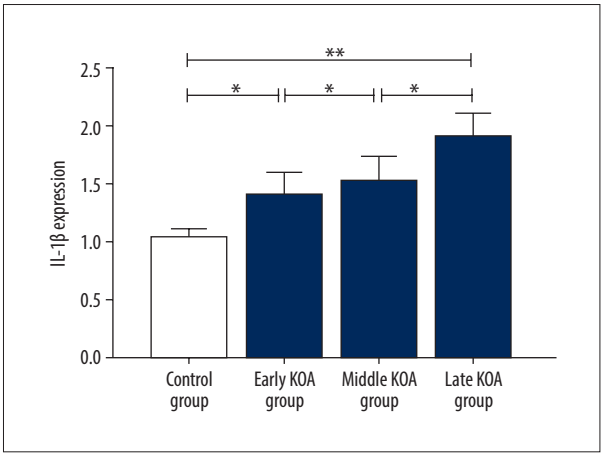

Figure 7. Expression of IL-1β in synovia of KOA groups. Fluorescent quantitative PCR results displayed that IL-1β mRNA expression in KOA groups was obviously increased by 37.35%, 49.22% and 85.20% in comparison with that in the control group. * $P<0.05$, ** $P<0.01$. IL-1β – interleukin-1β; KOA – knee osteoarthritis; PCR – polymerase chain reaction; mRNA – messenger RNA.

inflammatory factor that affects the occurrence and development of OA by regulating normal physiological functions of chondrocytes via activation of the signal transduction pathway *in vivo* [19]. Studies have demonstrated that a variety of miRNAs are associated with IL-1β expression [6]. During the progression of OA, chondrocytes secrete a large number of inflammatory factors such as MMP-3 and IL-1β which participate in the inflammatory reaction process in the articulations of joints [20]. As a therapeutic method, miRNA possesses the advantages of high efficacy, multiple target acquisition, and long duration of action, which is further enhanced by stability and non-toxicity to humans. Therefore, miRNA may be suitable for treating cartilage injury and degeneration tendencies in KOA patients [6,21,22].

The real-time quantitative PCR results demonstrated that the expression levels of miR-140 and miR-199 in synovia of KOA groups were obviously lower than those in the control group, and the expression level of miRNA in synovia gradually decreased with progression of the disease. The expression level of miRNA in synovia of KOA groups was negatively correlated with the progression of OA, suggesting that the low miR140 expression level in synovia may be used as a biochemical marker for the diagnosis of early KOA. As the disease progresses, miR-140 and miR-199 expression levels gradually decreased, indicating that miR-140 and miR-199 expression levels in synovia may be used as biochemical markers for evaluating the progression of KOA patients. Zhang et al. [23] found that a low-level of miR-140 expression is closely associated with the occurrence and development of KOA, which was consistent with our findings. Akhtar et al. [10] reported that the expression level of miR-199-3p was correlated to the severity of OA in rabbits, indicating that miR-199-3p might play a crucial part in the pathogenesis of OA by regulating cartilage and subchondral bone degeneration through the reprogramming of related signaling molecules and pathways.

This study examined the expression of MMP-3 and IL-1β mRNA in synovia of KOA groups. The results displayed that the levels of MMP-3 and IL-1β mRNA in synovia of KOA groups were higher than those in the control group, and that miRNA expression increased as the disease progressed. Current studies demonstrate that the degree of cartilage injury and the severity of disease in KOA patients were associated with the expression levels of proteases and certain inflammatory cytokines in synovia [24,25]. MiRNAs can modulate the expression of MMPs and cytokines, thereby maintaining the normal function of chondrocytes. Meanwhile, it also exhibits a biological effect on the occurrence and progression of osteoarthritis [26].

Conclusions

This study found a correlation between miR-140 and miR-199 expression levels and the progression of KOA. The expression levels of miR-140 and miR-199 had negative correlation with the progression of disease. This indicates that miR-140 and miR-199 might affect the expression levels of relevant metalloprotease and cytokines by regulating MMP-3 and IL-1β mRNA expression. This results in changes in the structure and function of osteoarticular chondrocytes, which further aggravates

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the pathogenesis of KOA. This was a clinical study and the specific mode of action involving the regulation of cytokines or metalloproteinases by miRNAs has not been fully researched. Therefore, further studies are needed to explore this issue. Overall, our study provides new latent therapeutic targets for treating KOA patients.

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Conflict of interests

None.

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