Long non-coding RNA PMS2L2 is down-regulated in osteoarthritis and inhibits chondrocyte proliferation by up-regulating miR-34a

Fei Yang\textsuperscript{a}, Min Zhao\textsuperscript{b}, Qinghua Sang\textsuperscript{b}, Changhong Yan\textsuperscript{b} and Zhenjun Wang\textsuperscript{c}

\textsuperscript{a}Department of Orthopedics, Yanqing District Hospital, Beijing (Yanqing Hospital Peking University Third Hospital), Beijing, PR China; \textsuperscript{b}Department of General Surgery, Yanqing District Hospital, Beijing (Yanqing Hospital Peking University Third Hospital), Beijing, PR China; \textsuperscript{c}Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing, PR China

\textbf{ABSTRACT}

Long non-coding RNA (IncRNA) PMS2L2 has been reported to participate in endotoxin-induced inflammatory responses. As these types of responses can promote osteoarthritis (OA), it was of interest to ascertain if PMS2L2 may be involved in OA. To explore any potential participation of PMS2L2 in OA, synovial fluid was extracted from both OA patients and healthy controls (n = 62 each) and PMS2L2 expression of each sample determined by RT-qPCR. In addition, as miR-34a has a potential binding site on PMS2L2, hypothetical interactions between PMS2L2 and miR-34a in chondrocytes were analyzed by performing over-expression experiments. Furthermore, the role of PMS2L2 and miR-34a in the regulation of chondrocyte proliferation was analyzed using CCK-8 and BrdU assays. The results showed that PMS2L2 expression in OA patient synovial fluid was lower compared to that in control group fluid, and the extent of this reduction was related to disease stage. In \textit{in vitro} studies, it was seen that endotoxin treatment of chondrocytes led to decreased PMS2L2 expression. It was found that PMS2L2 over-expression caused increased miR-34a expression in OA patient chondrocytes but not in cells from healthy controls. In contrast, miR-34a over-expression in either cell population did not affect PMS2L2 expression. Lastly, over-expression of both PMS2L2 and miR-34a led to inhibited chondrocyte proliferation. Of note, a combined over-expression of PMS2L2 and miR-34a resulted in stronger effects on proliferation compared to that from either single over-expression. Based on the findings that PMS2L2 is down-regulated during ongoing states of OA, and that changes in PMS2L2 expression can lead to increases in chondrocyte expression of miR-34a – resulting in inhibition of chondrocyte proliferation in OA. From these findings, one may conclude that finding means to regulate PMS2L2 could be a promising new target in the development of regimens for the treatment of OA.

\textbf{Introduction}

Osteoarthritis (OA), a long-term chronic disease, is the most common type of arthritis caused by inflammation and breakdown of protective cartilage (Glyn-Jones et al. 2013). The progression of OA eventually leads to loss of cartilage in the joints, i.e., degenerative arthritis (Zhang and Jordan 2010; Vina and Kwoh 2018). OA affects millions of people every year and it is estimated that > 10% of males and 13% of females older than 65 years old will develop OA (Loeser 2017; Rahmati et al. 2017). Besides aging, other factors such as obesity, joint over-use, and joint injury also contribute to the development of OA (Kulkarni et al. 2016). At present, there is no cure for OA; current treatments mainly focus on the relief of symptoms, such as chronic pain (Sinusas 2012). Thus, therapeutic approaches are needed to improve the recovery of OA.

Studies on the mechanisms underlying the progression and pathogenesis of OA have revealed the participation of a considerable number of molecular factors (Charlier et al. 2016; Saito and Tanaka 2017; Kim et al. 2018). These factors promote or inhibit OA progression in multiple ways, including the regulation of apoptosis/proliferation of chondrocytes which are the only mature cells found in healthy cartilage (Charlier et al. 2016). It would seem then that regulating expression of these factors may contribute to mitigation of OA.

Non-coding RNA (ncRNA) such as long ncRNA (IncRNA) and microRNA (miRNA) can potentially directly mediate OA progression by regulating inflammation and cell behavior (Pearson et al. 2016; Kolhe et al. 2017). For example, it has been reported that miRNA directly target downstream protein-coding genes at both the transcriptional and the translational level to affect synthesis of several OA-related proteins (Pearson et al. 2016; Kolhe et al. 2017). Those same authors also showed that lncRNA can interact with proteins, DNA, and/or miRNA to indirectly affect protein production during OA (Pearson et al. 2016; Kolhe et al. 2017). Nevertheless, to date, precise functions for ncRNA and a complete understanding of the gene regulatory network involved in OA remain unclear.

Previous studies have shown that lncRNA PMS2L2 and miR-34a play similar roles in the chondrocyte proliferation and...
apoptosis (Yan et al. 2016; Li et al. 2019), indicating a potential for interactions between them. This could be important because proliferation of chondrocytes is a key event in the pathogenesis of OA (Hwang and Kim 2015). However, no previous studies have reported interactions between PMS2L2 and miR-34a in any pathological or physiological processes. Accordingly, the present study was carried out to explore the potential for interactions between PMS2L2 and miR-34a in OA.

Material and methods

Study population

This study was approved by the Ethics Committee of Yanqing District Hospital, Beijing. OA patients (n = 62; 20 males, 42 females, age range 52–69 [mean 60.1 ± 5.8] years) and healthy controls (HC; n = 62; 20 males, 42 females, age range 52–69 [mean 60.2 ± 5.7] years) were recruited at Yanqing District Hospital in the period from August 2017 to August 2019. All patients signed informed consent forms prior to entry into the study. All HC received a systemic physiologic exam and their normal physiologic functions were confirmed. All OA patients were diagnosed for the first time; all OA patients were staged based on disease severity. Based on those findings, the 62 OA patients were further sub-categorized: 18 Stage 2 cases (mild), 20 Stage 3 cases (moderate), and 24 Stage 4 cases (severe). The OA patients included 34 that were knee-affected and 28 that were hip-affected. Patients with other clinical disorders and any who had already initiated non-OA-related treatment therapies (to control immune response over-activities) before this study were excluded.

RNA preparation

Cultured chondrocyte lines (10^6 total) from LPS, test cytokines, or transfections) as well as synovial fluid samples (100 µl/subject) from OA patients and healthy controls underwent RNA isolation using Trizol reagent (Invitrogen). To isolate extracellular RNA, synovial fluid samples were first centrifuged (12,000 × g, 4°C, 10 min). The resulting cell-free supernatant was then used for RNA extraction. Total RNA were digested with DNase I (Invitrogen) at 37°C for 2 h to remove genomic DNA. RNA integrity was confirmed using 6% urea-PAGE gels; RNA purity and concentration were determined spectrophotometrically based on OD 260/280 ratios. RNA samples with a ratio > 2.0 were considered as pure.

RT-qPCR

RNA (from 10^6 cells or 100 µl synovial fluid sample) isolates were subjected to reverse transcription using a Quantitect Reverse Transcription Kit (Qiagen, Shanghai, China). With the resulting cDNA samples as template, PMS2L2 expression was determined by performing qPCR using Quantitect SYBR Green PCR Kit (Qiagen) with 18S rRNA as the internal control. To determine mature miR-34a expression levels, an All-in-One™ miRNA qRT-PCR Detection Kit (Genecopoeia, Rockville, MD) was used to perform poly (A) addition, miRNA reverse transcription, and then qPCR. In this case, U6 was used as the internal control. Each PCR was performed with three replicates. The 2^(-ΔΔCt) method was used to normalize expression levels of PMS2L2 and miR-34a to corresponding internal controls. AGC (target gene Ct – internal control Ct) was calculated for all samples. The sample with the greatest ΔCt value was set to the value of ‘1’; all other samples were normalized to this sample to calculate relative gene expression levels.

CCK-8 assay

Proliferation of chondrocytes (with and without over-expressed materials) was analyzed using a CCK-8 assay kit (Dojindo, Kumamoto, Japan). In brief, chondrocytes were seeded into wells of 96-well plates (4000 cells/0.1 ml medium/well) and then

Transient cell transfections

To perform over-expression experiments, a backbone vector expressing PMS2L2 was constructed using a pcDNA3.1 vector (Invitrogen, Carlsbad, CA). Mimic miR-34a, miR-34a inhibitor, and negative control (NC) miRNA were also purchased from Invitrogen. For each transfection, vector (1 µg) or miRNA (50 nM) was transfected into chondrocytes using lipofectamine 2000 (Invitrogen). Transfection with empty vector or NC miRNA was also performed (NC group). Cells without transfection were cultured under normal conditions and used as the control (C) cells. In another set, cells were co-transfected with PMS2L vector and either miR-34a or miR-34a inhibitor (50 nM). Well content was replaced with clean medium at 24 h after transfection. qRT-PCR analyses of random cultures from each set were then used to confirm cell transfection efficiency (Livak and Schmittgen 2001).

Synovial fluid preparation

Synovial fluid was extracted from affected sites of the OA patients and corresponding sites in the healthy controls. In brief, following administration of local anesthesia (2% lidocaine, topical), ~ 2 ml of synovial fluid was collected from the joint cavity into a 10-ml syringe using standard harvesting protocols. All isolated synovial fluid samples were immediately subjected to RNA extraction (see below) for analyses of PMS2L2 and miR-34a expression levels.

Chondrocyte lines and in vitro studies

For in vitro studies, chondrocytes originated from healthy (402-05A) and an OA patient (402OA-05A) were purchased from Sigma (St. Louis, MO). Chondrocyte growth medium (PromoCell, Heidelberg, Germany) was used for cultivation. All cells were grown in 60-mm flasks in an incubator maintained at 37°C with a 5% CO2 atmosphere and 95% relative humidity. Cells used in all experiments were harvested at ~ 85% confluency before being re-seeded into 96-well plates for subsequent 48 h treatment with various doses of lipopolysaccharide (LPS, Type 026:B6 from Escherichia coli, Sigma) or select cytokines (i.e., interleukin [IL]-2 or IL-7; Sigma) dissolved in culture medium. Control wells received vehicle only. Three replicate wells (3000 cells/0.1 ml medium/well) were used for each group.

Material and methods

Study population

This study was approved by the Ethics Committee of Yanqing District Hospital, Beijing. OA patients (n = 62; 20 males, 42 females, age range 52–69 [mean 60.1 ± 5.8] years) and healthy controls (HC; n = 62; 20 males, 42 females, age range 52–69 [mean 60.2 ± 5.7] years) were recruited at Yanqing District Hospital in the period from August 2017 to August 2019. All patients signed informed consent forms prior to entry into the study. All HC received a systemic physiologic exam and their normal physiologic functions were confirmed. All OA patients were diagnosed for the first time; all OA patients were staged based on disease severity. Based on those findings, the 62 OA patients were further sub-categorized: 18 Stage 2 cases (mild), 20 Stage 3 cases (moderate), and 24 Stage 4 cases (severe). The OA patients included 34 that were knee-affected and 28 that were hip-affected. Patients with other clinical disorders and any who had already initiated non-OA-related treatment therapies (to control immune response over-activities) before this study were excluded.
cultured at 37°C. CCK-8 solution from the kit was added to wells to reach a 10% final concentration (v/v) every 24 h for a total of 96 h. After a final 1 h of incubation, the OD450 values in the wells were measured using a BioTek microplate reader (Winooski, VT). Data were expressed in terms of mean OD ± SD.

**BrdU assay**

In separate plates, chondrocytes (with and without over-expressed materials) were seeded into wells of 96-well plates (4000 cells/0.1 ml medium/well) and cultivated at 37°C for 48 h. At that point, BrdU stock solution from a kit (BrdU Cell Proliferation Assay kit, Sigma, Shanghai) was added to each well to a final concentration of 10 mg/ml. The cells were incubated a further 2 h before 0.5 ml of kit-provided horseradish peroxidase-conjugated anti-BrdU-antibody was added to each well at the manufacturer-recommended dilution. After incubation of the plate at 37°C for 20 min, wells were gently washed with PBS, the kit-provided peroxidase substrate was added to each well, and the plate incubated a further 1 h. The OD450 value from each well was then measured in the BioTek microplate reader. Data were expressed in terms of mean OD ± SD.

**MTT assay**

In separate plates, chondrocytes (with and without over-expressed materials) were seeded into 96-well plates (4000 cells/0.1 ml medium/well) and cultivated at 37°C for 48 h. At that point, MTT solution (20 μl of 5 mg/ml stock; Sigma) was added to each well. After a further incubation at 37°C for 4 h, 150 μl DMSO (Sigma) was added to each well to disrupt the cells and allow formazan that had formed to be released for measure. OD490 values in each well were measured in the microplate reader. Data were expressed in terms of mean OD ± SD.

**Statistical analysis**

Three replicates were included in each experiment. Data are presented as means ± SD values. Results from the OA and HC groups were compared using an unpaired t-test. Results obtained with multiple groups were compared using one-way analysis of variance (ANOVA) test followed by a post-hoc Tukey's test. A p-value < .05 was considered as significant. All data were analyzed using SPSS software (v. 16.0, SPSS, Chicago, IL).

**Results**

**Altered expression of PMS2L2 and miR-34a in OA patients was affected by disease stage**

The expression of PMS2L2 in synovial fluid samples from OA patients (n = 62) and healthy controls (HC, n = 62) were determined using RT-qPCR. The results show that in comparison to the HC, OA subjects exhibited significantly lower synovial fluid expression levels of PMS2L2 (Figure 1(A), p < 0.01). Differences
in synovial fluid expression levels of PMS2L2 in OA patients as a function of disease stages were also detected, with expression levels decreasing as disease stage worsened (Figure 1(B), \( p < 0.05 \)). The expression of miR-34a was also determined by RT-qPCR, and the results showed that miR-34a was accumulated to low amount in OA group compared to that in the control group (Figure 1(C), \( p < 0.01 \)). Moreover, decreased accumulation of miR-34a was observed with the increased disease stage (Figure 1(D), \( p < 0.05 \)).

LPS treatment resulted in the down-regulation of PMS2L2 in two types of chondrocytes

Chondrocytes derived from OA patients and HC were cultivated in medium containing 0, 1, 2, 5, and 10 mg/ml LPS for 48 h, followed by measuring of expression levels of PMS2L2 through RT-qPCR. The results showed that LPS treatment led to decreased PMS2L2 expression in both the HC and OA chondrocyte cell lines. In OA chondrocytes, 10 mg LPS/ml resulted in a 63% reduction in expression of PMS2L2 compared to that in the 0 mg LPS/ml group (Figure 2(A)). In contrast, this same LPS dose resulted in a 95% reduction in the HC chondrocytes (Figure 2(B)). Thus it would seem that with regard to LPS effects on PMS2L2 expression, HC chondrocytes were more ‘sensitive’ than OA chondrocytes. It is worth noting that no LPS-induced morphological changes were observed in either of the two types of chondrocytes. In addition, the effects of IL-2 and IL-7 (each known to contribute to OA [Malemud 2010]) on expression of PMS2L2 in the cells were also evaluated. These analyses indicated that neither IL-2 nor IL-7 (each provided at 5, 10, 15, and 20 ng/ml in place of LPS) affected PMS2L2 expression in either line (data not shown).

Over-expression of PMS2L2 resulted in up-regulation of miR-34a in OA chondrocytes

OA chondrocytes were transfected with PMS2L2 expression vector or miR-34a mimic. All successful transfections were confirmed by RT-qPCR (Figure 3(A)). The analyses here found that over-expression of PMS2L2 resulted in a significant up-regulation of miR-34a in the OA chondrocytes (Figure 3(B)). In contrast, over-expression of miR-34a did not affect expression of PMS2L2 in these same cells (Figure 3(B)). Similarly, HC chondrocytes were also over-expressed for PMS2L2 or miR-34a (Figure 3(C)); the results showed that changes in PMS2L2 and miR-34a levels did not affect the expression of one another (Figure 3(D)).

Over-expression of PMS2L2 and miR-34a promoted OA chondrocyte proliferation

The effects of over-expression of PMS2L2 and miR-34a on OA chondrocyte proliferation were analyzed using CCK-8 (Figure 4(A)) and BrdU (Figure 4(B)) assays. In both assays, over-expression of PMS2L2 or miR-34a inhibited OA chondrocyte proliferation. In contrast, neither PMS2L2 nor miR-34a over-expression affected HC chondrocyte proliferation (Supplementary Figure 1(A,B)). In addition, in the OA populations, the combined over-expression of both PMS2L2 and miR-34a resulted in an even stronger effect than attained with either singly over-expressed.

To ascertain if these changes might be related to alterations in chondrocyte viability, MTT assays were performed. The results showed that over-expression of PMS2L2 and/or miR-34a reduced OA chondrocyte viability (Figure 4(C)), but did not affect HC chondrocyte viability (Supplementary Figure 1(C)). Moreover, it was found that a co-presence of the miR-34a inhibitor totally reversed the inhibitory effects of PMS2L2 on OA chondrocyte proliferation in and viability in each assay system (Figure 4(A–C)).

Discussion

This study explored the potential for interactions between PMS2L2 and miR-34a in chondrocytes and how these, in turn, might be related to the pathology of OA. The main results of the current study revealed first that PMS2L2 expression was down-regulated in synovial fluids of patients with OA (and this was related to diseases stage/severity) and that ex vivo, changes in PMS2L2 levels could impact on the expression of miR-34a in OA chondrocytes (but apparently not in cells from healthy controls).

The role of PMS2L2 in human diseases has only been investigated in endometrial adenocarcinoma (EA) (Zhang et al. 2019). It was observed that PMS2L2 was down-regulated in EA and

Figure 2. Effects of LPS treatment on PMS2L2 expression in chondrocyte cell lines. (A) OA and (B) HC chondrocytes were cultivated in medium supplemented with 0, 1, 2, 5, or 10 mg LPS/ml for 48 h, and then RT-qPCR was used to measure PMS2L2 expression. Experiments were performed in three biological replicates. Mean±SD values are shown. * \( p < 0.05 \), ** \( p < 0.01 \).
Figure 3. PMS2L2 over-expression effects on miR-34a expression by chondrocyte cell lines. (A) OA chondrocytes were over-expressed for PMS2L2 or miR-34a; confirmation with RT-qPCR. (B) Role of PMS2L2 and miR-34a in regulating expression of one another explored using RT-qPCR. (C) HC chondrocytes were over-expressed for PMS2L2 or miR-34a; confirmation with RT-qPCR. (D) Role of PMS2L2 and miR-34a in regulating expression of one another explored using RT-qPCR. C: control cells without transfection. NC: negative control cells transfected with empty pcDNA3.1 vector or NC miRNA. PMS2L2: cells with PMS2L2 over-expression. miR-34a: cells with miR-34a over-expression. *p < 0.05.

Figure 4. Effects of PMS2L2 and miR-34a over-expression on chondrocyte cell proliferation and viability. Only outcomes with OA cells shown. Effects of PMS2L2 and miR-34a over-expression on proliferation were analyzed using (A) CCK-8 and (B) BrdU assays. (C) Cell viability was analyzed via MTT assay. Experiments were performed in three biological replicates. Mean ± SD values are shown. C: control cells without transfection. NC miRNA: negative control cells transfected with NC miRNA. pcDNA3.1: negative control cells transfected with empty pcDNA3.1 vector. PMS2L2: cells with PMS2L2 over-expression. miR-34a: cells with miR-34a over-expression. PMS2L2 + miR-34a: cells co-over-expressed with PMS2L2 and miR-34a. PMS2L2 + miR-34a inhibitor: cells co-transfected with PMS2L2 expression vector and miR-34a mimic. *p < 0.05.
PMS2L2 could negatively regulate EA cell viability. Zhang et al. (2019) showed that down-regulation of PMS2L2 could contribute to the development of chemoresistance in EA cells against carboplatin. A recent study reported that a presence of PMS2L2 in ATDC5 chondrocytes could protect these cells from expected effects from exogenous LPS (Li et al. 2019). Those authors speculated this protective effect was likely attributable to the ability of PMS2L2 to bind to miR-203. Therefore, it is plausible to assume that PMS2L2 might impact on other miRNA and chondrocyte functions during the course of normal and/or disease states.

The present study showed that PMS2L2 expression was lower in the synovial fluids of OA patients compared to in HC subject fluids. This suggested that PMS2L2 could be important in controlling the proliferation of chondrocytes during OA, i.e., its absence might allow for chondrocyte overgrowth – a characteristic of OA (Goldring 2000). On the other hand, the study here also found that over-expression of PMS2L2 selectively inhibited OA chondrocyte proliferation and not that HC chondrocytes. An underlying mechanism to explain this outcome is not clear yet. It is possible that a function of PMS2L2 in controlling chondrocyte overgrowth or excess proliferation might depend on other changes (genotypic and/or phenotypic) induced in these cells during states of ongoing OA.

Along those lines, it is known that inflammation is crucial for the development and subsequent progression of OA (Huang et al. 2016). In the present study, LPS treatment of the chondrocyte cultures resulted in down-regulation of PMS2L2 in both the OA and HC cultures. Interestingly, the inducible decrease in expression levels of PMS2L2 was less obvious in OA chondrocytes than in HC counterpart lines. A possible explanation for this is that the expression of PMS2L2 was already down-regulated in OA chondrocytes (see above in regard to synovial fluids) such that any LPS-inducible further down-regulation would be difficult to discern. In contrast, in the HC chondrocytes, LPS treatment mimics conditions associated with OA (i.e., local hyper-inflammation), and dramatic decreases in PMS2L2 expression could become more evident when compared to expression levels in untreated HC cells.

The present studies also found that PMS2L2 could impact on the expression of one type of miRNA, i.e., over-expression of PMS2L2 resulted in significant up-regulation of miR-34a formation in OA chondrocytes (but not in HC chondrocytes). Those influencing effects would be in line with results from earlier studies that PMS2L2 and miR-34a play roles in chondrocyte proliferation and apoptosis (Li et al. 2019; Yan et al. 2016), and that there seemed to be potential interactions between the two during these processes. In a recent study, miR-34a was reported to inhibit proliferation of chondrocytes in OA patients (Yan et al. 2016). Those authors proposed that this effect was mediated by an inactivation of the SIRT1/p53 signaling in the cells. In the present study, an inhibitory effect of miR-34a on OA chondrocyte proliferation (but not in the control cells) was also noted, indicating that any role for miR-34a in regulating chondrocyte proliferation during OA might depend on OA-related factors themselves (and thus not present in the HC cultures).

To date, upstream regulators of miR-34a in the pathological processes associated with OA have not been elucidated. As PMS2L2 could up-regulate miR-34a in the OA chondrocytes, it is possible PMS2L2 could serve as an activator of miR-34A during inhibition of OA chondrocyte proliferation. Interestingly, PMS2L2 did not induce any miR-34a accumulation in the HC cells. One could speculate that as with the potential factors regulating miR-34a impacts on chondrocyte proliferation, there could be OA-related factors that allow for the PMS2L2 to impart an miR-inducing effect in the cells of OA patients. Future studies will start to focus on the identification of these factors.

Conclusions

The results of the present study indicated that PMS2L2 was likely to have an important role in the pathology of OA. For example, the expression levels of PMS2L2 were repeatedly found to be reduced in synovial fluids of OA patients. Moreover, increasing the expression levels of PMS2L2 in cultures of chondrocytes obtained from OA patients and control subjects revealed that this IncRNA could differentially impact on proliferation by the two cell types. It was also observed that PMS2L2 could selectively affect the expression of miR-34a in the two cell lines, as well as their ability to proliferate in response to miR-34a. We speculated that there might be OA-specific factors that affect the ability of PMS2L2 to regulate chondrocytes during OA and that many of these factors might also affect miR-34a and its capacity to control chondrocyte functionality. Further study of both PMS2L2 and miR-34a, along with their inter-activities, in chondrocytes during OA is still needed.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Yanqing Hospital at Peking University Third Hospital.

Ethical approval

All procedures were approved by the Ethics Committee of Yanqing District Hospital, Beijing (Yanqing Hospital, Peking University Third Hospital). Procedures operated in this research were completed in keeping with the standards set out in the Announcement of Helsinki and laboratory guidelines of research in China.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data availability statement

The data that support the findings of this study are available on request from corresponding author. Data are not publicly available due to containing information that could compromise the privacy of research participants.

References

Charlier E, Relic B, Deroyer C, Malaise O, Neville S, Collée J, Malaise M, de Seny D. 2016. Insights on molecular mechanisms of chondrocytes death in osteoarthritis. IJMS. 17(12):2146.
Glyn-Jones S, Palmer A, Agricola R, Price A, Vincent T, Weinans H, Carr A. 2015. Osteoarthritis. Lancet. 386(9991):376–387.
Goldring M. 2000. Role of the chondrocyte in osteoarthritis. Arthritis Rheumatism. 43(9):1916–1926.
Huang Z, Stabler T, Pei F, Kraus V. 2016. Both systemic and local LPS burden are associated with knee OA severity and inflammation. Osteoarth Cartil. 24(10):1769–1775.

Hwang H, Kim H. 2015. Chondrocyte apoptosis in the pathogenesis of osteoarthritis. Int J Mol Sci. 16(11):26035–26054.

Kim J, Yoo J, Kim H. 2018. Therapeutics in osteoarthritis based on an understanding of its molecular pathogenesis. IJMS. 19(3):674.

Kolhe R, Hunter M, Liu S, Jadeja R, Pundkar C, Mondal A, Mendhe B, Drewry M, Rojiani M, Liu Y, et al. 2017. Gender-specific differential expression of exosomal miRNA in synovial fluid of patients with osteoarthritis. Sci Rep. 7(1):2029.

Kulkarni K, Karssiens T, Kumar V, Pandit H. 2016. Obesity and osteoarthritis. Maturitas. 89:22–28.

Li X, Yu M, Chen L, Sun T, Wang H, Zhao L, Zhao Q. 2019. LncRNA PMS2L2 protects ATDC5 chondrocytes against LPS-induced inflammatory injury by sponging miR-203. Life Sci. 217:283–292.

Livak K, Schmittgen T. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-ΔΔCt) method. Methods. 25(4):402–408.

Loeser R. 2017. The role of aging in the development of osteoarthritis. Trans Am Clin Climatol Assoc. 128:44–54.

Malemud C. 2010. Anticytokine therapy for osteoarthritis: Evidence to date. Drugs Aging. 27(2):95–115.

Pearson M, Philp A, Heward J, Roux B, Walsh D, Davis E, Lindsay M, Jones S. 2016. Long intergenic noncoding RNAs mediate the human chondrocyte inflammatory response and are differentially expressed in osteoarthritis cartilage. Arthritis Rheumatol. 68(4):845–856.

Rahmati M, Nalesso G, Mobasher A, Mozafari M. 2017. Aging and osteoarthritis: Central role of the extracellular matrix. Ageing Res Rev. 40:20–30.

Saito T, Tanaka S. 2017. Molecular mechanisms underlying osteoarthritis development: Notch and NF-kB. Arthritis Res Ther. 19(1):94.

Sinusas K. 2012. Osteoarthritis: diagnosis and treatment. Am Fam Physician. 85(1):49–56.

Vina E, Kwoh C. 2018. Epidemiology of osteoarthritis: Literature update. Curr Opin Rheumatol. 30(2):160–167.

Yan S, Wang M, Zhao J, Zhang H, Zhou C, Jin L, Zhang Y, Qiu X, Ma B, Fan Q. 2016. MicroRNA-34a affects chondrocyte apoptosis and proliferation by targeting the SIRT1/p53 signaling pathway during the pathogenesis of osteoarthritis. Int J Mol Med. 38(1):201–209.

Zhang D, Sun X, Zhang Y. 2019. Down-regulation Of LncRNA PMS2L2 in endometrial adenocarcinoma upon carboplatin treatment. Cancer Manag Res. 11:8905–8910.

Zhang Y, Jordan J. 2010. Epidemiology of osteoarthritis. Clin Geriatr Med. 26(3):355–369.