Therapeutic effect and mechanisms of intra-articular injections of microRNA-140-5p on early-stage osteoarthritis in rats

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Abstract

Background MicroRNAs (miRs) have received extensive attention in osteoarthritis (OA) pathogenesis in recent years, and our previous study have confirmed that single intra-articular injection (IAJ) of miR-140-5p alleviates early-stage OA (EOA) progression in rats. This study aims to further investigate the effects of single IAJ of miR-140-5p on different stage OA and multiple IAJs of miR-140-5p on EOA, as well as the potential mechanisms. **Methods** Firstly, OA model was surgically induced in rats, 9 rats were treated with IAJ of Cy5-miR-140-5p at 1 week after surgery, and fluorescence distribution was measured. Then, 72 rats were treated with single IAJ of miR-140-5p at different time after surgery or multiple IAJs of miR-140-5p at 1 week after surgery, and OA progression were evaluated macroscopically and histologically. Finally, bioinformatics analyses were performed and the potential targets and molecular mechanisms of miR-140-5p were predicted. **Results** Strong fluorescence was observed in the chondrocytes and joint where Cy5-miR-140-5p was injected. Behavioural scores, chondrocyte numbers and cartilage thickness in cartilage were higher, while pathological scores were lower in the miR-140-5p group than in the control group. Specifically, the earlier a single IAJ of miR-140-5p, the better the therapeutic effect, and multiple IAJs exhibited better therapeutic effect than single IAJ on EOA. Bioinformatics analyses predicted 84 potential target genes of rno-miR-140-5p and revealed that these genes enrich in various biological processes and pathways. **Conclusions** IAJs of miR-140-5p effectively alleviate EOA progression by modulating various biological processes and pathways, and may be a promising therapeutics for EOA.

Background

Osteoarthritis (OA) is a major cause of disability and a leading source of societal cost in older people, and is becoming more prevalent than in previous decades as the number of ageing adults increases [1–3]. Although the specific mechanisms leading to OA have not been fully elucidated, OA is characterized by various epigenetic changes, which refer to alterations in gene promoter that lead to a reduced expression of a specific protein [4, 5]. Unlike genetic inheritance, epigenetic changes can be reversed and easily controlled, and its mechanisms consist mainly of DNA methylation, histone modifications and regulatory microRNAs (miRs) [6].

miRs regulate their target genes (mRNAs) at the post-transcriptional level and have received extensive attention in OA pathogenesis in recent years [7–9]. Our previous studies reported that miR-140-5p levels were significantly reduced in OA chondrocytes and synovial fluid, and single intra-articular injection (IAJ) of exogenous miR-140-5p agomir effectively alleviates early-stage OA (EOA) progression in rats [10]. However, the effects of single IAJ of miR-140-5p on different stage OA and multiple IAJs of miR-140-5p on EOA, as well as the related molecular mechanisms were not addressed.

Therefore, this study aims to validate whether the intra-articularly injected miR-140-5p could enter the cartilage and be taken up by chondrocytes first, then to investigate the effect of single IAJ of miR-140-5p on different stage OA and multiple IAJs of miR-140-5p on EOA, and finally to explore the possible mechanistic network of miR-140-5p by bioinformatics methods.

Methods

All animal procedures were performed according to the Guidelines for Animal Experimentation of Sichuan University, and with the approval of the Institutional Ethics Committee of West China Hospital, Sichuan University.

Rats and OA model

Twelve-week-old male and female (1:1) Sprague-Dawley rats were purchased from the Chengdu Dossy Experimental Animals Co.,Ltd, and housed at the Laboratory Animal Centre of Sichuan University under standard diurnal light/dark conditions, fed a standard commercial diet and allowed access to tap water ad libitum. All animals received humane care, and all procedures were carried out according to the Guide for the Care and Use of Laboratory Animals. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg; Tocris, Bristol, UK), and euthanized by injection of excessive pentobarbital sodium. OA was induced in the right hind knee by anterior cruciate ligament transection and destabilization of the medial meniscus (ACLT + DMM) as described elsewhere [11]. All surgical procedures were performed under sterile conditions, and the rats were allowed to move, eat and drink freely after surgery.

IAJ of miR-140-5p

The miR-140-5p agomir and miR agomir negative control (scrambled 22nt nucleotides, miR-Scr) were synthesized by Guangzhou RiboBio Co., Ltd (China), and the working solutions were prepared immediately before injection. One week after surgery (EOA), 9 rats were injected with Cy5-labelled miR-140-5p agomir (5 nmol / 100 μ l) in the right hind knee and with Normal Saline (NS) in the left hind knee under ultrasonic guidance through a medial patellar approach using an insulin syringe with a 29G needle (BD, USA), and then, the rats were allowed unrestricted weight-bearing and motion. Twenty-four, 48 and 72 hours later, 3 rats were randomly selected and fluorescence was measured using a SpectrumCT in vivo imaging system (PerkinElmer, USA). Then, the bilateral hind knee tissues including femoral condyle, tibial plateau, meniscus and synovium were obtained, washed, coated with OCT and quickly frozen, and 6- μ m serial sections were prepared and naturally dried away from light. Fluorescent images of the sections were acquired by a fluorescent microscope (Zeiss, Germany).

For subsequent experiments, 72 EOA rats were established and randomly divided into control (n = 18), SIAJ-1W / MIAJ-1 (n = 18), SIAJ-4W (n = 12), SIAJ-8W (n = 6), MIAJ-2 (n = 12) and MIAJ-3 (n = 6) groups. All rats in the control group received no treatment, while equal amount of miR-140-5p agomir (5 nmol / 100 μ l) were injected into the right hind knee as shown in Fig. 2A. Six rats in each group were randomly selected at 4, 8 and 12 weeks after surgery (T1, T2 and T3, respectively) for further assessment as described below, and 6 normal rats were designated as normal controls.

Behavioural and cartilage degeneration assessment

Behavioural changes were assessed by measuring the number of rears performed by the rats as described previously [10]. Then, the rats were euthanized and the gross morphologic changes in their femoral condyle were photographed and scored using the following 0–4 scale: 0 = surface appears normal; 1 = minimal fibrillation or slight yellowish discoloration of the surface; 2 = erosion extending to the superficial or middle layers; 3 = erosion extending to the deep layer; and 4 = erosion extending to the subchondral bone [10]. Finally, the femoral condyles were routinely fixed with 4% paraformaldehyde at 4 °C for 48 h and then decalcified with 10% EDTA at pH 7.4 for 4–6 weeks. After dehydration, all tissues were embedded in paraffin and sagittally sectioned at 6-µm thickness. The sections were dewaxed and hydrated before being stained with haematoxylin & eosin (HE) and toluidine blue. The chondrocyte numbers in an equal area and the cartilage thickness were measured as reported previously [12], and the severity of the cartilage lesions was graded using the modified Mankin score. All sections were assessed independently by three blinded assessors (SHB, YTM and CY), and the final results were determined via a consensus.

Bioinformatics analysis of the possible mechanism of rno-miR-140-5p

The mature sequence of rno-miR-140-5p was obtained in NCBI and UCSC database, and its putative targets were determined using the miRDB, miRmap (the cut-off values was set to 80), PicTar, TargetScan and DIANA microT-CDS (the threshold was set to 0.8) databases. To make the predicted target genes more convincing, only the target genes predicted by at least three databases were selected for further analysis. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to perform enrichment analyses for the target genes of rno-miR-140-5p, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, and $p < 0.05$ was considered statistically significant. Finally, the interactive relationships among the proteins encoded by the target genes were mapped using the Search Tool for the Retrieval of Interacting Genes (STRING) database, and the interactions with a combined score > 0.7 (high confidence) were considered significant and visualized.

Statistical analysis

Quantitative data were presented as the mean and 95% confidence intervals (95%CI) and assessed for normality of distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The t-test or Mann-Whitney U test was performed to identify the differences between two groups, while one-way analysis of variance (ANOVA) with Tukey's post hoc analysis or Kruskal-Wallis H test with Student-Newman-Keuls (SNK) post hoc analysis was conducted for multiple group comparisons. All statistical analyses were performed using SPSS software (IBM, USA), and $P < 0.05$ were considered statistically significant.

Results

Intra-articularly injected miR-140-5p enters cartilage and be taken up by chondrocytes

The surgical wound healed well and no complications were observed throughout the observational period in all rats. As shown in Figs. 1A and 1B, strong fluorescence was observed in and around the right knee where Cy5-miR-140-5p was injected, while no fluorescence in the left knee where NS was injected, as well as in the other parts of the body. Meanwhile, strong fluorescence was observed in the cytoplasm of almost all chondrocytes in the superficial and middle layers of the medial femoral and tibial cartilage, while little and no obvious fluorescence in deep layer of cartilage and subchondral region, respectively (Figs. 1C and 1D). Furthermore, strong fluorescence was also observed in the meniscus and synovium (Figs. 1E and 1F).

Single IAJ of miR-140-5p exhibits better therapeutic effect on early-stage OA

Rearing behaviour is an indicator of joint pain and spontaneous activity levels. As shown in Fig. 2B, the number of rears was higher in the SIAJ groups than in the control group at T1 to T3, and the differences between the SIAJ-1W and the control groups at T2 and T3 and the difference between the SIAJ-1W and the SIAJ-8W groups were statistically significant. Gross images of the medial femoral condyle are shown in Fig. 2C. The cartilage surface was smooth in normal rats, while cartilage degeneration characterized by fibrillation, pitting, ulceration, osteophyte formation and even subchondral bone exposure was observed at T1 to T3, and the rats in the SIAJ groups displayed less extensive cartilage lesions than those in the control group. Quantitatively, pathological lesion scores were significantly lower in the SIAJ-1W group than in the control group at T1 to T3, and the score in the SIAJ-1W group was also significantly lower than that in the SIAJ-8W group at T3 (Fig. 2D).

The typical images of HE and toluidine blue staining are shown in Figs. 3A and 3B. Compared with the control group, the chondrocyte numbers and cartilage thickness in the SIAJ-1W group were significantly higher at T2 and T3 (Figs. 3C and 3D), while the Mankin score was significantly lower in the SIAJ-1W group at T1 to T3 and in the SIAJ-4W group at T2 (Fig. 3E). Moreover, the chondrocyte numbers and cartilage thickness were significantly higher, while the Mankin score was significantly lower in the SIAJ-1W group than in the SIAJ-8W group at T3; meanwhile, the cartilage thickness at T3 was significantly higher, while the Mankin score at T2 was significantly lower in the SIAJ-1W group than in the SIAJ-4W group.

Multiple IAJs of miR-140-5p exhibit better therapeutic effect than single IAJ on early-stage OA

As shown in Fig. 2E, the number of rears was higher in the MIAJ groups than in the control group at T1 to T3, and the differences at T2 and T3 were statistically significant. Gross images of the medial femoral condyle are shown in Fig. 2C, the rats in MIAJ groups displayed less extensive cartilage lesions compared with the control group, and pathological lesion scores were significantly lower in the MIAJ groups than in the control group at T1 to T3, and the MIAJ-3 showed the best results (Fig. 2F). Compared with the

control group, the chondrocyte numbers and cartilage thickness in the MIAJ groups were significantly higher at T2 and T3 (Figs. 3F and 3G), while the Mankin score was significantly lower at T1 to T3 (Fig. 3H). Moreover, the Mankin score was significantly lower in the MIAJ-3 group than in the MIAJ-1 group at T3.

Bioinformatics analyses of the potential mechanisms by which rno-miR-140-5p regulates OA progression

The gene ID of rno-miR-140-5p was MIMAT0000573, its mature sequence was CAGUGGUUUUACCCUAUGGUAG, which is highly conserved in various species and consistent with that of hsa-miR-140-5p. The number of predicted target genes of rno-miR-140-5p in the miRDB, miRmap, PicTar, TargetScan, and DIANA microT-CDS databases was 166, 472, 223, 284 and 85, respectively (4A). A total of 84 target genes were predicted by at least three databases and were used for further analyses (Table 1).

Table 1
The target genes of rno-miR-140-5p predicted by at least three database

ADCY6	AFTPH	AKIRIN2	ANO6	BCL2L1	BCL2L2	BCL9
CAPN1	CDH11	CELF1	CORO2A	CREB3L1	CSK	CSNK1G3
CTCF	CUL3	DPYSL2	EGR2	ELAVL2	ENO4	ENTPD5
EPB41L2	FAM175B	FAM214A	FBN1	FGF9	FOXP2	GALNT16
GIT1	GPR85	HDAC4	HNRNPH3	HS2ST1	IGFBP5	IPO7
JAG1	KBTBD2	LAMC1	LHFPL2	LRP4	MAP3K12	MBNL3
MGAT1	MMD	MYCBP2	NCSTN	NFAT5	NFE2L2	NLK
NUMBL	OSBPL6	PDGFRA	PITX2	PPTC7	PRDM1	PTTG1IP
R3HDM1	RFX7	RNF19A	SASH3	SEPT2	SH3GL2	SIAH1
SLC30A5	SLC38A2	SMOX	SNX2	SNX27	SPRY4	ST5
STRADB	TGFBR1	TJP1	TMEM260	TPCN1	TTK	TTYH3
VEGFA	WDR3	WNT1	WNT9A	ZBTB10	ZFP800	ZHX1

GO enrichment analysis revealed that the predicted target genes mainly locate in the cytoplasm, nucleus, membrane, etc.; participate in the molecular functions of protein binding, protein homodimerization activity, metal ion binding, etc.; and involve in various biological processes, such as regulation of transcription, cell proliferation and protein phosphorylation (Fig. 4B). KEGG pathway analysis found that the predicted target genes were significantly enriched in 10 pathways (Fig. 4C and Table 2), among which the PI3K-Akt, Notch, Wnt and MAPK pathways are well known to be associated with OA pathogenesis

(Figure S1-S4). The STRING database was used to predict the functional protein association networks of the target genes, and 27 pairs of protein-protein interactions were identified (Fig. 4D).

Table 2

Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis for predicted target genes of rno-miR-140-5p (P < 0.05)

ID	Term	P	Genes
rno05200	Pathways in cancer	0.001	WNT1, FGF9, TGFBR1, VEGFA, ADCY6, PDGFRA, BCL2L1, WNT9A, LAMC1
rno05166	HTLV-I infection	0.003	WNT1, EGR2, TGFBR1, ADCY6, PDGFRA, BCL2L1, WNT9A
rno04916	Melanogenesis	0.012	WNT1, ADCY6, CREB3L1, WNT9A
rno04151	PI3K-Akt signaling pathway	0.024	FGF9, VEGFA, PDGFRA, CREB3L1, BCL2L1, LAMC1
rno04330	Notch signaling pathway	0.027	NCSTN, JAG1, NUMBL
rno04310	Wnt signaling pathway	0.031	WNT1, NLK, SIAH1, WNT9A
rno04010	MAPK signaling pathway	0.036	FGF9, TGFBR1, NLK, PDGFRA, MAP3K12
rno05212	Pancreatic cancer	0.039	TGFBR1, VEGFA, BCL2L1
rno04144	Endocytosis	0.042	GIT1, TGFBR1, PDGFRA, SNX2, SH3GL2
rno04520	Adherens junction	0.049	TJP1, TGFBR1, NLK

Discussion

The cartilage lesions mainly affect the superficial layer in EOA, and are gradually worsened and deepened with the progress of OA, suggesting that it is of great clinical significance to begin intervention in the early-stage of OA. In this study, we firstly verified that the intra-articularly injected miR-140-5p could rapidly enter the cartilage and reach the chondrocyte cytoplasm; then, we found that the earlier a single IAJ of miR-140-5p, the better the therapeutic effect, and multiple IAJs of miR-140-5p exhibit better therapeutic effect than single IAJ on EOA; finally, the bioinformatics analysis predicted the potential targets of miR-140-5p and the possible mechanisms by which miR-140-5p participates in OA pathogenesis. These results suggest that IAJs of miR-140-5p is an effective and promising strategy for the treatment of EOA, and also provide support for further exploration of miR-based OA therapeutics.

Intra-articular treatments have emerged in recent years and may offer safe therapeutics that have few extra-articular adverse effects [13–16], and we firstly proved in this study that the intra-articularly injected

miR-140-5p could rapidly enter the cartilage and reach the chondrocyte cytoplasm, while no extra-articular uptake, and exhibit a protective role in OA. Based on these results, we further discovered that the potential target genes of rno-miR-140-5p were enriched in various biological processes and mainly located within 10 pathways, implying that the potential mechanisms by which rno-miR-140 attenuates OA progression may be the regulation of these pathways.

The PI3K-Akt, Notch, Wnt and MAPK signaling pathways are well known to be involved in OA pathogenesis, and regulate a variety of biological processes, including inflammation, cell growth, survival and metabolism [17–19]. For examples, activation of the PI3K-AKT pathway in chondrocytes accelerates cartilage degradation, while inhibition of the PI3K-AKT pathway attenuates cartilage degradation and inflammatory responses [20, 21]. Notch pathway plays a critical role in cell fate via regulating differentiation and apoptosis [22], and is activated in OA [23]. Meanwhile, these pathways are involved in a wide variety of cellular processes, and there is also close interactions between them. For examples, Guo et al. reported that Notch2 negatively regulates cell invasion by inhibiting the PI3K-AKT signaling pathway in gastric cancer [24], and Villegas et al. found that PI3K-AKT cooperates with oncogenic Notch by inducing nitric oxide-dependent inflammation [25]. However, few studies have reported the interactions between these pathways in chondrocytes and OA pathogenesis.

Although the central role of cartilage degeneration in OA has been recognized, OA is a total joint disease, while not just a dysfunction of cartilage[12]. Although we found in this study that the intra-articularly injected miR-140-5p has no extra-articular distribution, it is substantially uptaken by the intra-articular non-cartilage tissues such as synovium and meniscus. Peng et al. reported that synovial fibroblasts (SFs) also respond to miR-140-5p which could inhibits the proliferation and migration of SFs and promotes apoptosis of SFs in autoimmune arthritis mice [26]. Li et al. reported that miR-140 plays an important role in fracture healing [27], and Genemaras et al. found that miR-140-5p can be detected in meniscal cells [28]. However, few studies reported the specific effect of miR-140-5p on osteoblasts, osteoclasts or meniscus cells. Therefore, there is still a need to further investigate the effects of miR-140-5p on intra-articular cells and tissues other than chondrocytes and cartilage.

Previous studies have confirmed that drugs or genes injected directly into the joint cavity will be cleared rapidly due to the action of lymphatic vessels and synovial blood vessels, and non-targeted aggregation further reduces the bioavailability, thus multiple, high-dose injections which might make the administration more cumbersome and increase the risk of infection are often required [29]. In order to enhance the cartilage-targeting delivery efficiency and minimize dosage requirements, construction of carriers with good safety, precise cartilage-targeting and high delivery efficiency is essential. A number of delivery vectors for miRs have been reported, such as adenoviruses, collagen scaffolds and hydrogels [30, 31], and biochemical modifications that can selectively bind to cartilage or play specific biological functions may further improve the vectors. For example, Li et al. reported that N-Cadherin peptidomimetic modified self-assembling polypeptide nanomaterials enhances the chondrogenic differentiation of mesenchymal stem cells (MSCs) [32].

The cartilage matrix is dense and highly negatively charged (mainly from aggrecans), and is a major obstacle to the entry of drugs or genes into cartilage. Previous studies have reported that non-specific electrostatic interaction between cationic carrier and negatively charged extra-cellular matrix (ECM) is beneficial to the rapid penetration of drugs or genes into cartilage, and the high negative charge state of the ECM will greatly increase the residence time of the cationic carrier, thereby transforming the cartilage from the barrier into a reservoir [33]. Therefore, if a functionalized vector was modified by appropriate cationic groups or molecules, it can not only serve as a carrier for cartilage-targeting and efficiently delivery of the negatively charged miRs, but also synergistically enhance the repair of cartilage lesions, possessing good application prospects [34].

Conclusions

Taken together, our results suggest that IAJs of miR-140-5p effectively alleviate EOA progression in rats, and IAJs of miRs may be a promising OA therapeutics although there are still many issues that need to be further addressed.

List Of Abbreviations

miRs: MicroRNAs; OA: Osteoarthritis; EOA: Early-stage osteoarthritis; IAJ: Intra-articular injection; NS: Normal Saline; HE: haematoxylin & eosin; DAVID: Database for Annotation, Visualization and Integrated Discovery; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; STRING: Search Tool for the Retrieval of Interacting Genes; MSCs: Mesenchymal stem cells; ECM: Extra-cellular matrix.

Declarations

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Author Contributions

SHB and SB contributed to the study concept and design. SHB, YTM, CY, MRW, WLM, LMW, LSY and ZY contributed to the acquisition, analysis, and interpretation of the data. SHB drafted the article and SB critically revised the article for important intellectual content. All authors gave final approval of the version to be submitted for publication.

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Availability of data and materials

Nor applicable. The datasets used and/or analysed during the current study are not publicly available. Data are however available from the corresponding author on reasonable request.

Authors' information

Not applicable.

Ethics approval and consent to participate

This study was performed with the approval of the Ethical Committee for Medical and Health Research of West China Hospital, Sichuan University. Consent to participate was not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet. 2019;393(10182):1745-59.
2. Miyaji N, Nishida K, Tanaka T, Araki D, Kanzaki N, Hoshino Y, Kuroda R, Matsushita T. Inhibition of Knee Osteoarthritis Progression in Mice by Administering SRT2014, an Activator of Silent Information Regulator 2 Ortholog 1. Cartilage. 2020;1947603519900795.
3. Driban JB, Bannuru RR, Eaton CB, Spector TD, Hart DJ, McAlindon TE, Lu B, Lo GH, Arden NK. The incidence and characteristics of accelerated knee osteoarthritis among women: the Chingford cohort. BMC Musculoskelet Disord. 2020;21(1):60.
4. Wang W, Yu Y, Hao J, Wen Y, Han J, Hou W, Liu R, Zhao B, He A, Li P, et al. Genome-wide DNA methylation profiling of articular cartilage reveals significant epigenetic alterations in Kashin-Beck

- disease and osteoarthritis. *Osteoarthritis Cartilage*. 2017;25(12):2127-33.
5. Chen K, Zhu H, Zheng MQ, Dong QR. LncRNA MEG3 Inhibits the Degradation of the Extracellular Matrix of Chondrocytes in Osteoarthritis via Targeting miR-93/TGFBR2 Axis. *Cartilage*. 2019;1947603519855759.
 6. Nugent M. MicroRNAs: exploring new horizons in osteoarthritis. *Osteoarthritis Cartilage*. 2016;24(4):573-80.
 7. Hecht N, Johnstone B, Angele P, Walker T, Richter W. Mechanosensitive MiRs regulated by anabolic and catabolic loading of human cartilage. *Osteoarthritis Cartilage*. 2019;27(8):1208-18.
 8. Yang DW, Qian GB, Jiang MJ, Wang P, Wang KZ. Inhibition of microRNA-495 suppresses chondrocyte apoptosis through activation of the NF-kappaB signaling pathway by regulating CCL4 in osteoarthritis. *Gene Ther*. 2019;26(6):217-29.
 9. Sun Y, Kang S, Pei S, Sang C, Huang Y. MiR93-5p inhibits chondrocyte apoptosis in osteoarthritis by targeting lncRNA CASC2. *BMC Musculoskelet Disord*. 2020;21(1):26.
 10. Si HB, Zeng Y, Liu SY, Zhou ZK, Chen YN, Cheng JQ, Lu YR, Shen B. Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. *Osteoarthritis Cartilage*. 2017;25(10):1698-707.
 11. Thysen S, Luyten FP, Lories RJ. Targets, models and challenges in osteoarthritis research. *Dis Model Mech*. 2015;8(1):17-30.
 12. Si HB, Yang TM, Li L, Tian M, Zhou L, Li DP, Huang Q, Kang PD, Yang J, Zhou ZK, et al. miR-140 Attenuates the Progression of Early-Stage Osteoarthritis by Retarding Chondrocyte Senescence. *Mol Ther Nucleic Acids*. 2019;19:15-30.
 13. Pi Y, Zhang X, Shao Z, Zhao F, Hu X, Ao Y. Intra-articular delivery of anti-Hif-2alpha siRNA by chondrocyte-homing nanoparticles to prevent cartilage degeneration in arthritic mice. *Gene Ther*. 2015;22(6):439-48.
 14. Miller LE, Bhattacharyya S, Parrish WR, Fredericson M, Bisson B, Altman RD. Safety of Intra-Articular Hyaluronic Acid for Knee Osteoarthritis: Systematic Review and Meta-Analysis of Randomized Trials Involving More than 8,000 Patients. *Cartilage*. 2019;1947603519888783.
 15. Yoshioka K, Katayama M, Nishiyama T, Harada K, Takeshita S, Kawamata Y. Biocompatibility study of different hyaluronan products for intra-articular treatment of knee osteoarthritis. *BMC Musculoskelet Disord*. 2019;20(1):424.
 16. Tsukada S, Kurosaka K, Nishino M, Maeda T, Yonekawa Y, Hirasawa N. Intra-articular tranexamic acid as an adjunct to intravenous tranexamic acid for simultaneous bilateral total knee arthroplasty: a randomized double-blind, placebo-controlled trial. *BMC Musculoskelet Disord*. 2019;20(1):464.
 17. Xie L, Xie H, Chen C, Tao Z, Zhang C, Cai L. Inhibiting the PI3K/AKT/NF-kappaB signal pathway with nobiletin for attenuating the development of osteoarthritis: in vitro and in vivo studies. *Food Funct*. 2019;10(4):2161-75.
 18. Chen H, Shi B, Feng X, Kong W, Chen W, Geng L, Chen J, Liu R, Li X, Chen W, et al. Leptin and Neutrophil-Activating Peptide 2 Promote Mesenchymal Stem Cell Senescence Through Activation of

- the Phosphatidylinositol 3-Kinase/Akt Pathway in Patients With Systemic Lupus Erythematosus. *Arthritis Rheumatol.* 2015;67(9):2383-93.
19. Huang X, Zhong L, van Helvoort E, Lafeber F, Mastbergen S, Hendriks J, Post JN, Karperien M. The Expressions of Dickkopf-Related Protein 1 and Frizzled-Related Protein Are Negatively Correlated to Local Inflammation and Osteoarthritis Severity. *Cartilage.* 2019;1947603519841676.
 20. Fu D, Shang X, Ni Z, Shi G. Shikonin inhibits inflammation and chondrocyte apoptosis by regulation of the PI3K/Akt signaling pathway in a rat model of osteoarthritis. *Exp Ther Med.* 2016;12(4):2735-40.
 21. Xue JF, Shi ZM, Zou J, Li XL. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. *Biomed Pharmacother.* 2017;89:1252-61.
 22. Saito T, Tanaka S. Molecular mechanisms underlying osteoarthritis development: Notch and NF-kappaB. *Arthritis Res Ther.* 2017;19(1):94.
 23. Hosaka Y, Saito T, Sugita S, Hikata T, Kobayashi H, Fukai A, Taniguchi Y, Hirata M, Akiyama H, Chung UI, et al. Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. *Proc Natl Acad Sci U S A.* 2013;110(5):1875-80.
 24. Guo LY, Li YM, Qiao L, Liu T, Du YY, Zhang JQ, He WT, Zhao YX, He DQ. Notch2 regulates matrix metalloproteinase 9 via PI3K/AKT signaling in human gastric carcinoma cell MKN-45. *World J Gastroenterol.* 2012;18(48):7262-70.
 25. Villegas SN, Gombos R, Garcia-Lopez L, Gutierrez-Perez I, Garcia-Castillo J, Vallejo DM, Da Ros VG, Ballesta-Illan E, Mihaly J, Dominguez M. PI3K/Akt Cooperates with Oncogenic Notch by Inducing Nitric Oxide-Dependent Inflammation. *Cell Rep.* 2018;22(10):2541-9.
 26. Peng JS, Chen SY, Wu CL, Chong HE, Ding YC, Shiau AL, Wang CR. Amelioration of experimental autoimmune Arthritis through targeting of synovial fibroblasts by intraarticular delivery of microRNAs 140-3p and 140-5p. *Arthritis Rheumatol.* 2016;68(2):370-81.
 27. Li KC, Chang YH, Yeh CL, Hu YC. Healing of osteoporotic bone defects by baculovirus-engineered bone marrow-derived MSCs expressing MicroRNA sponges. *Biomaterials.* 2016;74:155-66.
 28. Genemaras AA, Ennis H, Kaplan L, Huang CY. Inflammatory cytokines induce specific time- and concentration-dependent MicroRNA release by chondrocytes, synoviocytes, and meniscus cells. *J Orthop Res.* 2016;34(5):779-90.
 29. Bottini M, Bhattacharya K, Fadeel B, Magrini A, Bottini N, Rosato N. Nanodrugs to target articular cartilage: an emerging platform for osteoarthritis therapy. *Nanomedicine.* 2016;12(2):255-68.
 30. Maudens P, Meyer S, Seemayer CA, Jordan O, Allemann E. Self-assembled thermoresponsive nanostructures of hyaluronic acid conjugates for osteoarthritis therapy. *Nanoscale.* 2018;10(4):1845-54.
 31. Bottini M, Magrini A, Fadeel B, Rosato N. Tackling chondrocyte hypertrophy with multifunctional nanoparticles. *Gene Ther.* 2016;23(7):560-4.

32. Li R, Xu J, Wong DSH, Li J, Zhao P, Bian L. Self-assembled N-cadherin mimetic peptide hydrogels promote the chondrogenesis of mesenchymal stem cells through inhibition of canonical Wnt/beta-catenin signaling. *Biomaterials*. 2017;145:33-43.
33. Bajpayee AG, Grodzinsky AJ. Cartilage-targeting drug delivery: can electrostatic interactions help? *Nat Rev Rheumatol*. 2017;13(3):183-93.
34. Xiao X, Wang X, Wang Y, Yu T, Huang L, Chen L, Li J, Zhang C, Zhang Y. Multi-functional peptide-microRNA nanocomplex for targeted microRNA delivery and function imaging. *Chemistry*. 2018;24(9):2277-85.

Figures

Figure 1

Distribution of the intra-articularly injected miR-140-5p. (A) Representative in vivo fluorescence images after single intra-articular injection (IAJ) of Cy5-miR-140-5p agomir (the right hind knee) or normal saline (the left hind knee) (n = 3 in each group). (B) Representative fluorescence images of the medial femoral condyle (left upper), medial tibial plateau (left lower), meniscus (right upper) and synovium (right lower). (C-F) Representative fluorescence micrographs of sagittal frozen sections of the medial femoral condyle, medial tibial plateau, meniscus and synovium, respectively (100× scale bar, 200 μm; 400× scale bar, 50 μm).

Figure 2

General findings after intra-articular injections of miR-140-5p agomir in OA rats. (A) Flow chart of IAJ of miR-140-5p in rats. (B) Comparison of number of rears, as measured by behavioural testing and larger numbers of rears were indicative of less pain, between the SIAJ and the control groups (n = 6 in each

group). (C) Gross observation of the medial femoral condyle after single and multiple IAJs of miR-140-5p agomir (n = 6 in each group). (D) Comparison of cartilage pathological lesion scores between the SIAJ and the control groups (n = 6 in each group). (E, F) Comparisons of number of rears and cartilage pathological lesion scores between the MIAJ and the control groups, respectively (n = 6 in each group). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure 3

Histological staining and assessment after intra-articular injections of miR-140-5p agomir in early-stage OA rats (n = 6 in each group). (A, B) HE and toluidine blue staining of sagittal sections of the medial femoral condyle, respectively (left: 100× scale bar, 200 μm; right: 400× scale bar, 50 μm). (C) Comparison of chondrocyte numbers, as determined by HE staining, in 400×200-μm grids extending from the cartilage surface to the deep zone between the SIAJ and the control groups. (D) Comparison of medial femoral condyle cartilage thickness (from the cartilage surface to the deep zone), as determined by toluidine staining, between the SIAJ and the control groups. (E) Comparison of the modified Mankin scores, based on the HE and toluidine blue staining results and the score represented the most severe histologic changes, between the SIAJ and the control groups. (F-H) Comparisons of chondrocyte numbers, medial femoral condyle cartilage thickness and the modified Mankin scores between the MIAJ and the control groups. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure 4

Bioinformatics analyses of the potential mechanisms by which miR-140 attenuates OA. (A) The number of predicted target genes of rno-miR-140-5p in the miRDB, miRmap, PicTar, TargetScan, and DIANA microT-CDS databases. (B) Gene ontology (GO) enrichment analysis for the predicted target genes of rno-miR-140-5p. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for the predicted

target genes of rno-miR-140-5p ($P < 0.05$). (D) The interactive relationships among the proteins encoded by the target genes of rno-miR-140-5p.

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