



Small extracellular vesicles in combination with sleep-related circRNA3503: A targeted therapeutic agent with injectable thermosensitive hydrogel to prevent osteoarthritis

Shi-Cong Tao^{a,*}, Ji-Yan Huang^c, Yuan Gao^d, Zi-Xiang Li^e, Zhan-Ying Wei^f, Helen Dawes^g, Shang-Chun Guo^{a,b,**}

^a Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai, 200233, China

^b Institute of Microsurgery on Extremities, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai, 200233, China

^c Department of Stomatology, Shanghai Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, 185 Pu'an Road, Shanghai, 200021, China

^d School of Medicine, Shanghai Jiao Tong University, 227 South Chongqing Road, Shanghai, 200025, China

^e Medical College of Soochow University, Soochow University, Changzhou, 215123, Jiangsu, China

^f Shanghai Clinical Research Centre of Bone Diseases, Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, 200233, China

^g Faculty of Health and Life Science, Oxford Brookes University, Headington Road, Oxford, OX3 0BP, UK

ARTICLE INFO

Keywords:

Osteoarthritis
Extracellular vesicles
Exosomes
Circular RNAs
PDLLA-PEG-PDLLA

ABSTRACT

Osteoarthritis (OA), characterized by chondrocyte apoptosis and disturbance of the balance between catabolism and anabolism of the extracellular matrix (ECM), is the most common age-related degenerative joint disease worldwide. As sleep has been found to be beneficial for cartilage repair, and circular RNAs (circRNAs) have been demonstrated to be involved in the pathogenesis of OA, we performed RNA sequencing (RNA-seq), and found circRNA3503 was significantly increased after melatonin (MT)-induced cell sleep. Upregulation of circRNA3503 expression completely rescued the effects of interleukin-1 β (IL-1 β), which was used to simulate OA, on apoptosis, ECM degradation- and synthesis-related genes. Mechanistically, circRNA3503 acted as a sponge of hsa-miR-181c-3p and hsa-let-7b-3p. Moreover, as we previously showed that small extracellular vesicles (sEVs) derived from synovium mesenchymal stem cells (SMSCs) can not only successfully deliver nucleic acids to chondrocytes, but also effectively promote chondrocyte proliferation and migration, we assessed the feasibility of sEVs in combination with sleep-related circRNA3503 as an OA therapy. We successfully produced and isolated circRNA3503-loaded sEVs (circRNA3503-OE-sEVs) from SMSCs. Then, poly(D,L-lactide)-b-poly(ethylene glycol)-b-poly(D,L-lactide) (PDLLA-PEG-PDLLA, PLEL) triblock copolymer gels were used as carriers of sEVs. Through *in vivo* and *in vitro* experiments, PLEL@circRNA3503-OE-sEVs were shown to be a highly-effective therapeutic strategy to prevent OA progression. Through multiple pathways, circRNA3503-OE-sEVs alleviated inflammation-induced apoptosis and the imbalance between ECM synthesis and ECM degradation by acting as a sponge of hsa-miR-181c-3p and hsa-let-7b-3p. In addition, circRNA3503-OE-sEVs promoted chondrocyte renewal to alleviate the progressive loss of chondrocytes. Our results highlight the potential of PLEL@circRNA3503-OE-sEVs for preventing OA progression.

1. Introduction

Cartilage damage is a key feature of osteoarthritis (OA), a widely-prevalent degenerative and debilitating synovial joint disease. The

composition of the synovial fluid, which lubricates and nourishes joint cartilage, is controlled by fibroblast-like synoviocytes in the synovium [1], which are likely to be the same cell type as synovium mesenchymal stem cells (SMSCs) [2]. Homeostasis of the articular cartilage is maintained via a delicate balance among a series of processes [3]. As the only

Peer review under responsibility of KeAi Communications Co., Ltd.

** Corresponding author. Institute of Microsurgery on Extremities, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai, 200233, China.

* Corresponding author.

E-mail addresses: jerrytao1990@outlook.com, tsc13579@sjtu.edu.cn (S.-C. Tao), achuni@126.com (S.-C. Guo).

<https://doi.org/10.1016/j.bioactmat.2021.04.031>

Received 12 February 2021; Received in revised form 14 April 2021; Accepted 19 April 2021

2452-199X/© 2021 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC

BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations

OA	osteoarthritis	PI	propidium iodide
ECM	extracellular matrix	FCM	flow cytometry
Col-II	type II collagen	SMSC-sEVs	sEVs derived from SMSCs
sEVs	small extracellular vesicles	Wnt5a/b-dKO-sEVs	sEVs derived from SMSCs with dual Wnt5a and Wnt5b knockout
circRNAs	circular RNAs	circRNA3503-OE-sEVs	sEVs derived from SMSCs with circRNA3503 overexpression
SMSCs	synovium mesenchymal stem cells	dKO-OE-sEVs	sEVs derived from circRNA3503-overexpressing SMSCs with dual Wnt5a and Wnt5b knockout
MT	melatonin	DLS	dynamic light scattering
RNA-seq	RNA sequencing	TEM	transmission electron microscope
rRNA	ribosomal RNA	SPF	specific pathogen-free
PAGE	polyacrylamide gel electrophoresis	TNF- α	tumour necrosis factor- α
RT-PCR	reverse transcription-polymerase chain reaction	IL-1 β	interleukin-1 β
cDNA	complementary DNA	PGC-1 α	PPARGC1A
WB	western blotting	Col-X:	type X collagen
ER	endoplasmic reticulum	ceRNAs	competing endogenous RNAs
DPBS	Dulbecco's phosphate-buffered saline	MMPs	matrix metalloproteinases
PFA	paraformaldehyde		

cell type that forms articular cartilage, chondrocytes maintain a dynamic equilibrium between the synthesis and degradation of ECM components, including type II collagen (Col-II) and aggrecan, under normal conditions [4]. However, in an osteoarthritic state, this matrix equilibrium is disrupted to favour ECM degradation, leading to the progressive loss of cartilage, proliferation of chondrocytes in ECM-depleted regions of cartilage, and eventually chondrocyte apoptosis [5]. Both chondrocytes and the ECM are implicated in osteoarthritic cartilage injury. Thus, there is an urgent need to explore a new and effective therapeutic strategy with promising targets to maintain the balance in cartilage, including the balances in the synthesis/degradation of ECM components and in the death/survival of chondrocytes.

Currently, although the detailed pathogenesis of OA has not yet been elucidated and articular cartilage is thought to have limited regeneration potential [6], a number of promising intra-articular treatments, including small-molecule and biologic therapies, devices and gene therapies, are currently in clinical development [7]. Although induced articular cartilage regeneration may be possible in OA [8], preventative or therapeutic treatment approaches for OA and related articular cartilage injuries remain challenging, because of the difficulty of finding suitable carriers to reach the chondrocytes.

Although treatment approaches for OA have encountered difficulties due to the inaccessibility of chondrocytes, recent findings have demonstrated that small extracellular vesicles (sEVs), also called exosomes, have great promise as intercellular messengers and drug carriers for the therapeutic delivery of genetic material and drugs [9]. As described in our previous study, sEVs derived from SMSCs have the potential to reach chondrocytes within cartilage to maintain homeostasis of the ECM in OA [10]. Since EVs can penetrate cartilage [11], sEVs derived from SMSCs may be a promising carrier for nucleotide drugs to penetrate and target cartilage in OA.

According to our previous studies, sEVs can act as good carriers of nucleic acids [12–14], and miR-140 carried by sEVs has shown the potential to delay the progression of OA [10]. However, the use of miRNAs has some drawbacks, such as the insufficient stability and rapid clearance of miRNAs [14]; thus, the identification of a more stable type of nucleic acid, with the potential to improve therapeutic efficacy, would represent an improvement over currently-used therapeutic agents. Recent studies have shown that their high degree of environmental resistance and stability [15] are important properties that may make circular RNAs (circRNAs) ideal therapeutic agents [16]. CircRNAs are a class of covalently closed single-stranded circularized RNA molecules without free 5' or 3' ends, making them more stable than their linear counterparts [17]. In contrast to linear RNAs, circRNAs are highly stable

in vivo [18], and like linear RNAs, can be sorted into sEVs [19].

Interest in the functions of circRNAs and their roles in regulating cartilage homeostasis in OA has recently grown [20]. CircRNAs do not encode proteins but have recently emerged as important regulators of apoptosis in chondrocytes, thereby highlighting their potential role in chondrocyte injury leading to OA onset and progression [21]. Several differentially-expressed circRNAs that can promote or inhibit apoptosis by regulating the expression of miRNAs or their downstream proteins have been identified in chondrocytes [22]. Mechanistically, circRNAs can be degraded by siRNAs and may act as “miRNA sponges” that regulate the expression of target genes [23]. However, the mechanisms by which circRNAs regulate chondrocytes and cartilage in OA remain largely unidentified and require further investigation.

Melatonin (MT), a ubiquitous molecule formed in the pineal gland at night [24], is widely known to have a role in the regulation of sleep circuits and the circadian rhythm [25]. Mounting evidence suggests that MT, which is involved in both sleep-promoting and phase-shifting activities [26], can lull cells to sleep [27,28]. Notably, some studies have shown that MT is related to OA [29], but the exact mechanisms remain to be explored.

Synthetic poly(D,L-lactide)-b-poly(ethylene glycol)-b-poly(D,L-lactide) (PDLLA-PEG-PDLLA; PLEL) triblock copolymer gels have attracted attention in research into drug delivery systems and translational medicine, due to features such as their injectable, reversible, and thermosensitive abilities [30]. In particular, their ability to self-assemble into core-shell-like micelles at room temperature, and to transform into a physically-crosslinked non-flowing gel structure under physiological conditions, is considered to be a very good characteristic for nanoparticle/nano-drug delivery [30,31]. A growing number of studies suggest that triblock gels have preminent biosecurity for intra-articular injection [30,32,33]. However, to date PLEL has not been reported as a carrier of the cell-derived nanovesicles-sEVs-for intra-articular injection to prevent OA progression.

In this study, we aimed to combine sleep-related circRNAs with SMSC-derived sEVs, and to evaluate their potential for OA treatment. Specifically, we first used MT to induce chondrocyte sleep and identified differentially-expressed circRNAs with the potential to protect cartilage and prevent OA progression. After the identification of circRNA3503 as a candidate, sEVs carrying circRNA3503 (circRNA3503-OE-sEVs) were produced by circRNA3503 overexpression in SMSCs. PLEL was then used as a carrier of sEVs, and the effectiveness of PLEL@circRNA3503-OE-sEVs was evaluated using *in vitro* and *in vivo* models of OA. Our results will highlight the potential of PLEL@circRNA3503-OE-sEVs for preventing OA progression, and will provide some hints for the

application of sEV-based regenerative medicine in the future.

2. Materials and methods

All methods can be found in the accompanying Supplemental Information file.

3. Results

3.1. Identification and validation of the sleep-related circRNA3503

To generate circRNA profiles and identify sleep-related circRNAs, we employed RNA-seq of rRNA-depleted total RNA from chondrocytes cultured with or without MT (0.1 mM). We used a volcano plot (Fig. 1A) to visualize the candidate circRNAs. We found that the most promising candidate was circRNA3503 (Fig. 1A), which is spliced from the ZNF430 gene (Fig. 1B) on chr19:21033457-21034184+ and composed of exon 4 (whole exon 4 of the ZNF430 pre-mRNA) and e3 (a portion of exon 3 of the ZNF430 pre-mRNA), giving a final length of 224 nt (Fig. 1C).

After identifying circRNA3503 as a promising circRNA differentially expressed upon MT treatment, we used CircPrimer [34] for annotation (Table 1), graphic sketching (Fig. 1C), and the design of both divergent primers and linear primers (also known as convergent primers) (Table 2). The divergent primers, whose PCR product contains the sequence of the back-splicing junction region, were specially designed primers for circRNAs [34]. To validate circRNA3503 expression in chondrocytes, RT-PCR was performed with divergent and linear primers. CircRNA3503 could be detected with both the linear and divergent primers by using polyacrylamide gel electrophoresis (PAGE) after polymerase chain reaction (PCR) (Fig. 1D).

Next, to establish the effect of MT on circRNA3503, we performed RT-PCR and PAGE, and confirmed that MT upregulated the expression of circRNA3503 but not that of ZNF430 (the linear transcript of the host gene) (Fig. 1E). Then, we used RNase R resistance, a very important feature unique to circRNAs which is used as a key point to identify circRNAs in many studies [20], to verify that circRNA3503 was indeed circular. While circRNA3503 was resistant to RNase R, the corresponding linear transcript, ZNF430, was degraded by RNase R (Fig. 1F).

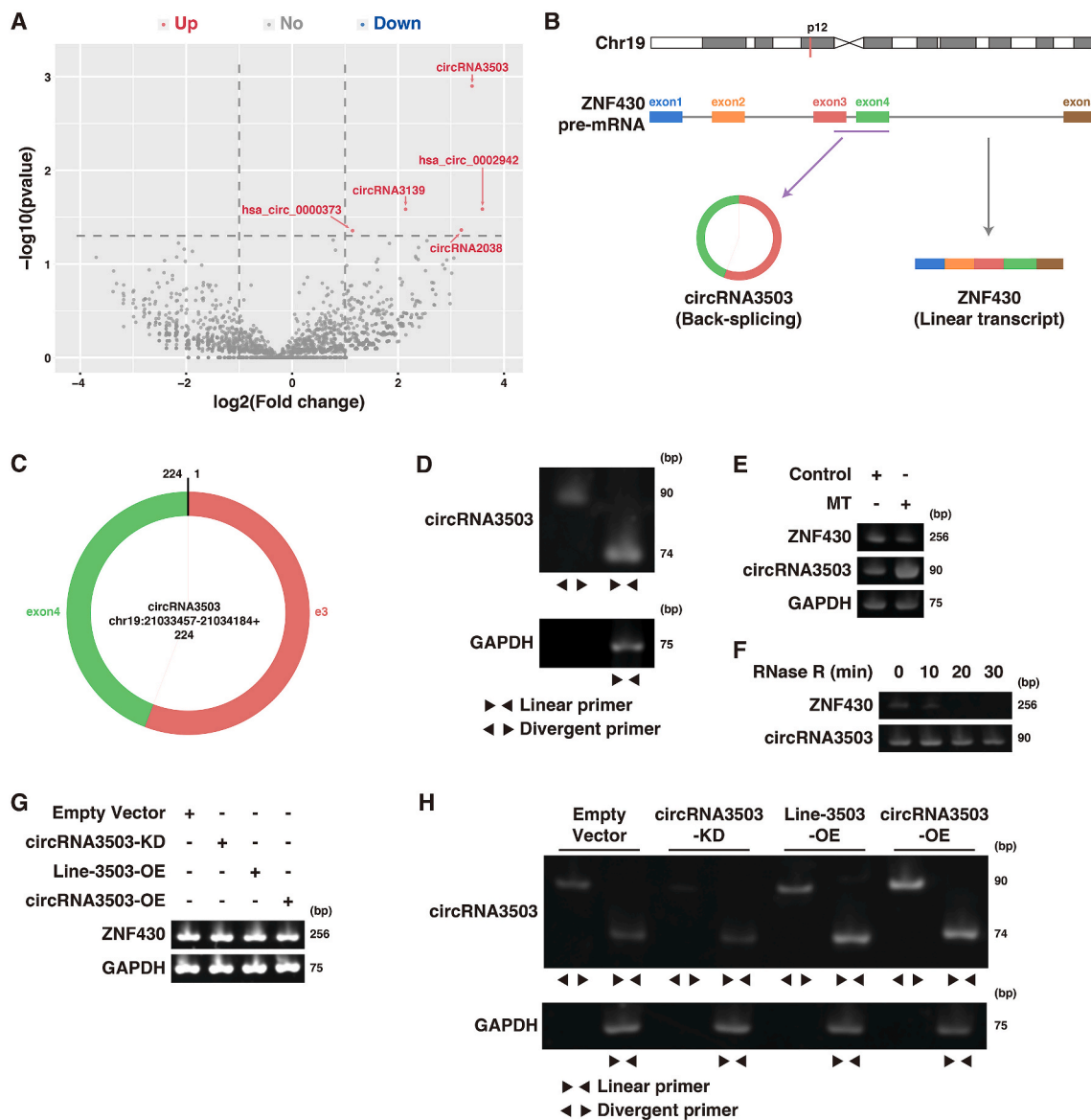


Fig. 1. Identification and validation of sleep-related circRNA3503. (A) Volcano plot visualisation of different circRNA expression patterns. (B) Schematic diagram of the relationship between the ZNF430 and circRNA3503 sequences. (C) Schematic diagram of the structure of circRNA3503. (D, E, F, G, H) PAGE assays to detect the expression levels under different conditions. Experiments were repeated at least three times, and representative results are shown.

Table 1

Nucleotide sequence of circRNA3503.

>circRNA3503 (chr19:21033457-21034184+) (GRCh38.90) 224 nt
GCCATTGACATTTAGGGATGTGGCCATAGAATTTCTCTGGAGGATGGCA ATGCCTGGACACTGCTCAGCAGGATTTGTATAGAAAAGTGATGTTAGAGA ACTACAGAAACCTGGTCTTCTTGGCAGGTATTGCTGTTTCTAAGCCAGACCT GATCACCTGTCTAGACGAAGAAAAGAGCCCTGGAATATGAAGAGACATGC GATGGTAGATCAACCCCA
G

The first ("G") and last ("G") letters constitute the back-splice junction.

Table 2

Primers specific for circRNAs and mRNAs used in this study.

Target	Forward Primer	Reverse Primer
circRNA3503 (divergent primers)	TGA AGA GAC ATG CGA TGG TAG AT	CAT TGC CAC TCC TCC AGA GA
circRNA3503 (linear primers)	CCT GGT CTT CTT GGC AGG TA	AGG GCT CTT TTC CTT GCT CT
ZNF430	CTG GAA GCC TAG AAA TGG AGA AC	TTA GAA ACA GCA ATA CCT GCC A
GAPDH	CTC TGC TCC TCC TGT TCG AC	ATG GTG TCT GAG CGA TGT GG

These results not only further suggested that circRNA3503 was indeed a circRNA but also that the level of the circular version rather than that of the linear version of the gene was significantly increased by MT. Therefore, we refer to circRNA3503 as sleep-related circRNA3503 in this study.

After identifying and confirming circRNA3503, to regulate its

expression for subsequent in-depth experiments, we transfected three plasmids: circRNA3503-KD (a short hairpin RNA to knock down circRNA3503 expression), circRNA3503-OE (a plasmid for the overexpression of circRNA3503-OE), and Line-3503-OE (a linear circRNA3503 expression vector without Alu elements). First, we confirmed that these plasmids had no significant effect on ZNF430 expression (Fig. 1G). CircRNA3503-KD transfection significantly inhibited the expression of circRNA3503 (Fig. 1H), and circRNA3503-OE significantly upregulated the expression of circRNA3503, regardless of the use of the linear or divergent primers for detection (Fig. 1H). After transfection of Line-3503-OE, the amount of product obtained with the linear primers was significantly increased, but the amount of product obtained with the divergent primers did not show such a significant change when compared with that obtained with transfection of circRNA3503-OE (Fig. 1H). Thus, the plasmids needed to regulate the levels of circRNA3503 for subsequent experiments were validated. In the following experiments, divergent primers were used as the default primers for the measurement of circRNA3503 levels.

3.2. Observation of circRNA3503 in an OA cell model

One of the best recognized and most widely-used methods of OA cell modelling is treatment with inflammatory cytokines (TNF- α and IL-1 β) [35]. Both TNF- α and IL-1 β increased the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) and simultaneously decreased the expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9) (Fig. 2A). Interestingly, we also noted that circRNA3503 expression was inhibited by either TNF- α or IL-1 β (Fig. 2B). Taken together, these results showed that circRNA3503 is related to inflammation and OA.

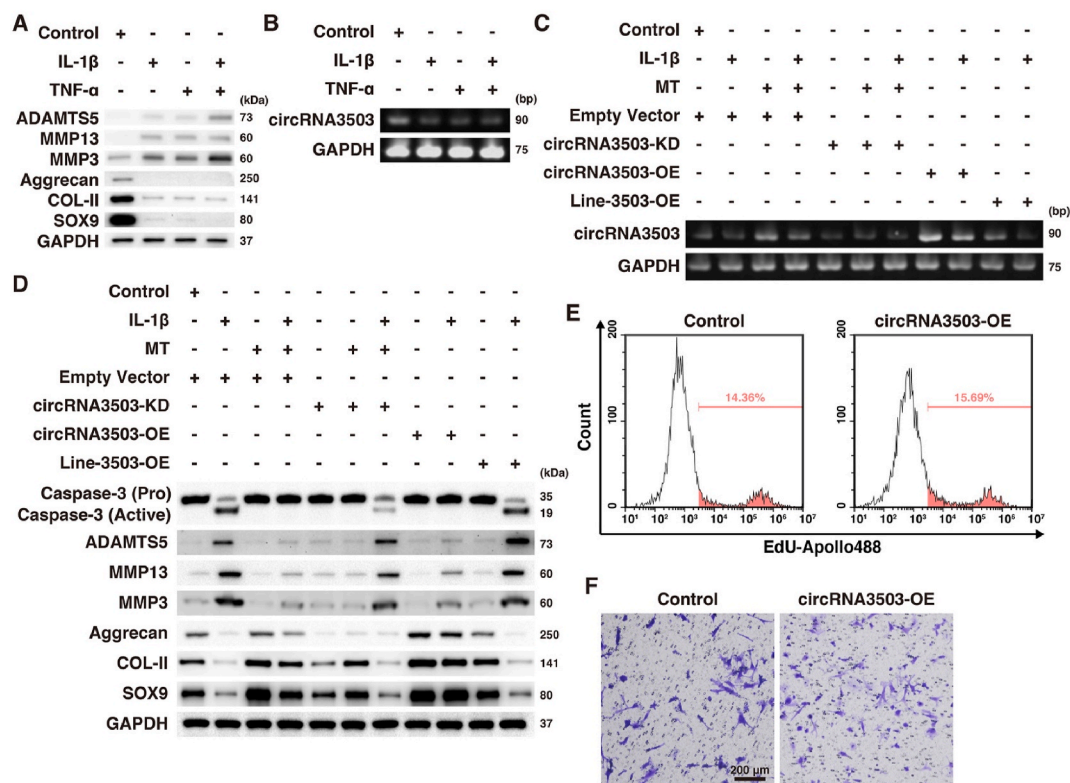


Fig. 2. Effect of circRNA3503 in an OA cell model. (A) The expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) and genes related to ECM synthesis (Aggrecan, COL-II, and SOX9) was measured by WB. (B, C) The expression level of circRNA3503 under different conditions was measured by PAGE after RT-PCR. (D) The activation of Caspase-3, expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) and expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9) under different conditions were measured by WB. (E) The proliferative abilities of chondrocytes were measured by EdU assays using FCM. (F) The migratory abilities of chondrocytes were measured by Transwell assays (Scale bar: 200 μ m). Experiments were repeated at least three times, and representative results are shown.

Although we showed that OA induction inhibited circRNA3503 expression, there have been no studies on the function of circRNA3503 in the progression of OA. Therefore, we designed a series of follow-up experiments to determine the exact role of circRNA3503 in OA and thus whether it can be used as a therapeutic target. After altering the expression of circRNA3503 (using circRNA3503-KD to knock down circRNA3503 expression, circRNA3503-OE to overexpress circRNA3503, or Line-3503-OE to assess a linear form of circRNA3503) in chondrocytes and/or stimulating chondrocytes with IL-1 β or MT, the results of our experiments showed the following phenomena (Fig. 2C and D):

- (1) IL-1 β downregulated the expression of circRNA3503 (consistent with an earlier experiment), elevated caspase-3 activation, increased the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3), and decreased the expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9).
- (2) MT upregulated the expression of circRNA3503, slightly inhibited the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3), slightly increased the expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9), and had no significant effect on caspase-3 activation.
- (3) MT completely rescued the effects of IL-1 β on caspase-3, genes related to ECM degradation, and genes related to ECM synthesis. Thus, MT functions in maintaining cartilage homeostasis.
- (4) No significant effects of circRNA3503-KD on caspase-3 or genes related to ECM degradation were observed, but circRNA3503-KD had a moderate inhibitory effect on the expression of genes related to ECM synthesis; thus, the downregulation of circRNA3503 expression was not the immediate cause of caspase-3 activation, the increases in the expression of the genes related to ECM degradation, or the decreases in the expression of the genes related to ECM synthesis.
- (5) circRNA3503-KD blocked the rescue effects of MT; thus, although circRNA3503 downregulation was not the immediate cause of OA progression, an adequate expression level of circRNA3503 may be the key to cartilage homeostasis.
- (6) CircRNA3503-OE significantly upregulated the expression of circRNA3503 and completely rescued the effects of IL-1 β on caspase-3, genes related to ECM degradation, and genes related to ECM synthesis. Thus, these results implied that circRNA3503 is the key to cartilage homeostasis, even though circRNA3503 downregulation may not directly cause damage to the physiological function of cartilage.
- (7) Line-3503-OE transfection did not upregulate the expression of circRNA3503 or rescue the effects of IL-1 β . Thus, only circRNA3503, not its linear counterpart, can functionally maintain cartilage homeostasis.

As our previous study revealed [10], increased chondrocyte proliferation and migration may be very important for protecting cartilage during OA progression, so we performed EdU and Transwell assays. However, no significant effect of circRNA3503-OE on chondrocyte proliferation or migration was observed (Fig. 2E and F).

3.3. Downstream mechanism of circRNA3503-mediated cartilage homeostasis

Via bioinformatic analysis, we predicted two candidate downstream mechanisms of circRNA3503: (1) circRNA3503 promotes the expression of PPARC1A (PGC-1 α) by acting as a sponge for miR-181c-3p and (2) circRNA3503 promotes the expression of SOX9 by acting as a sponge for hsa-let-7a-3p, hsa-let-7b-3p, hsa-let-7f-1-3p, or hsa-miR-98-3p (Fig. 3A). Thus, we first measured the expression levels of these candidate miRNAs

in chondrocytes, the results of which showed that the expression levels of hsa-miR-181c-3p and hsa-let-7b-3p were significantly higher than those of the other candidates (hsa-let-7a-3p, hsa-let-7f-1-3p, and hsa-miR-98-3p), whether in healthy chondrocytes or in OA chondrocytes (Fig. 3B and Fig. S1) (Table 3). Furthermore, hsa-miR-181c-3p was significantly upregulated in OA chondrocytes compared to healthy chondrocytes. Hence, we focused subsequent experiments on both hsa-miR-181c-3p and hsa-let-7b-3p.

To investigate and confirm the functions of hsa-miR-181c-3p and hsa-let-7b-3p, we employed miRNA mimics (miR-181c-3p-mimic and let-7b-3p-mimic, respectively) and miRNA inhibitors (miR-181c-3p-inhibitor and let-7b-3p-inhibitor, respectively). Furthermore, because many studies have suggested that ER stress plays a key role in OA progression [29] and based on the findings of our previous research [36], we believed the PERK/eIF2 α /Bcl-2 axis to be one of the most promising candidate signalling pathways responsible for the caspase-3 activation and increased expression of genes related to ECM degradation caused by inflammatory cytokines. Thus, we also employed GSK2656157 (which inhibits stress-induced PERK phosphorylation and decreases downstream p-eIF2 α expression [37], thus inhibiting ER stress-induced apoptosis [36]) and Sal003 (a small molecule that promotes eIF2 α phosphorylation [38]).

The results showed that let-7b-3p-mimic significantly inhibited the expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9), that let-7b-3p-inhibitor and circRNA3503-OE promoted the expression of genes related to ECM synthesis (Aggrecan, COL-II, and SOX9), and that circRNA3503-OE rescued the effect of let-7b-3p-mimic (Fig. 3C). Hence, these results verified the first potential mechanism of circRNA3503: circRNA3503 promotes the expression of SOX9 by acting as a sponge of hsa-let-7b-3p.

Next, our further exploration focused on circRNA3503 and its function in ER stress. The results of previous research [39,40] suggested that p-eIF2 α inhibits I κ B and that inhibition of I κ B promotes the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) via the activation of NF- κ B. Interestingly, PGC-1 α has been reported to inhibit NF- κ B by promoting I κ B activity [41,42]. In addition, p-PERK and p-eIF2 α promote apoptosis by inhibiting Bcl-2 [36]. Nevertheless, enhanced PGC-1 α expression has been reported to increase the expression of Bcl-2 [43], which might rescue ER stress-induced apoptosis. Therefore, we speculated that circRNA3503-induced PGC-1 α could rescue the promotional effects of IL-1 β on caspase-3 activation and the expression of genes related to ECM degradation.

The following experimental results supported our hypothesis (Fig. 3D):

- (1) IL-1 β promoted the phosphorylation of eIF2 α , inhibited the expression of Bcl-2 and activated caspase-3 but inhibited I κ B and promoted the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3).
- (2) miR-181c-3p-mimic enhanced the effects of IL-1 β .
- (3) miR-181c-3p-inhibitor promoted the expression of PGC-1 α and rescued the effects of IL-1 β , which activated caspase-3 and increased the expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3).
- (4) GSK2656157 rescued the effects of IL-1 β by inhibiting the phosphorylation of eIF2 α .
- (5) Like miR-181c-3p-inhibitor, circRNA3503-OE rescued the effects of IL-1 β on caspase-3 activation and increased expression of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3). These results also support our hypothesis that the induction of PGC-1 α by the circRNA3503/miR-181c-3p ceRNA mechanism rescues the effects of IL-1 β on caspase-3 activation and increased expression of genes related to ECM degradation.

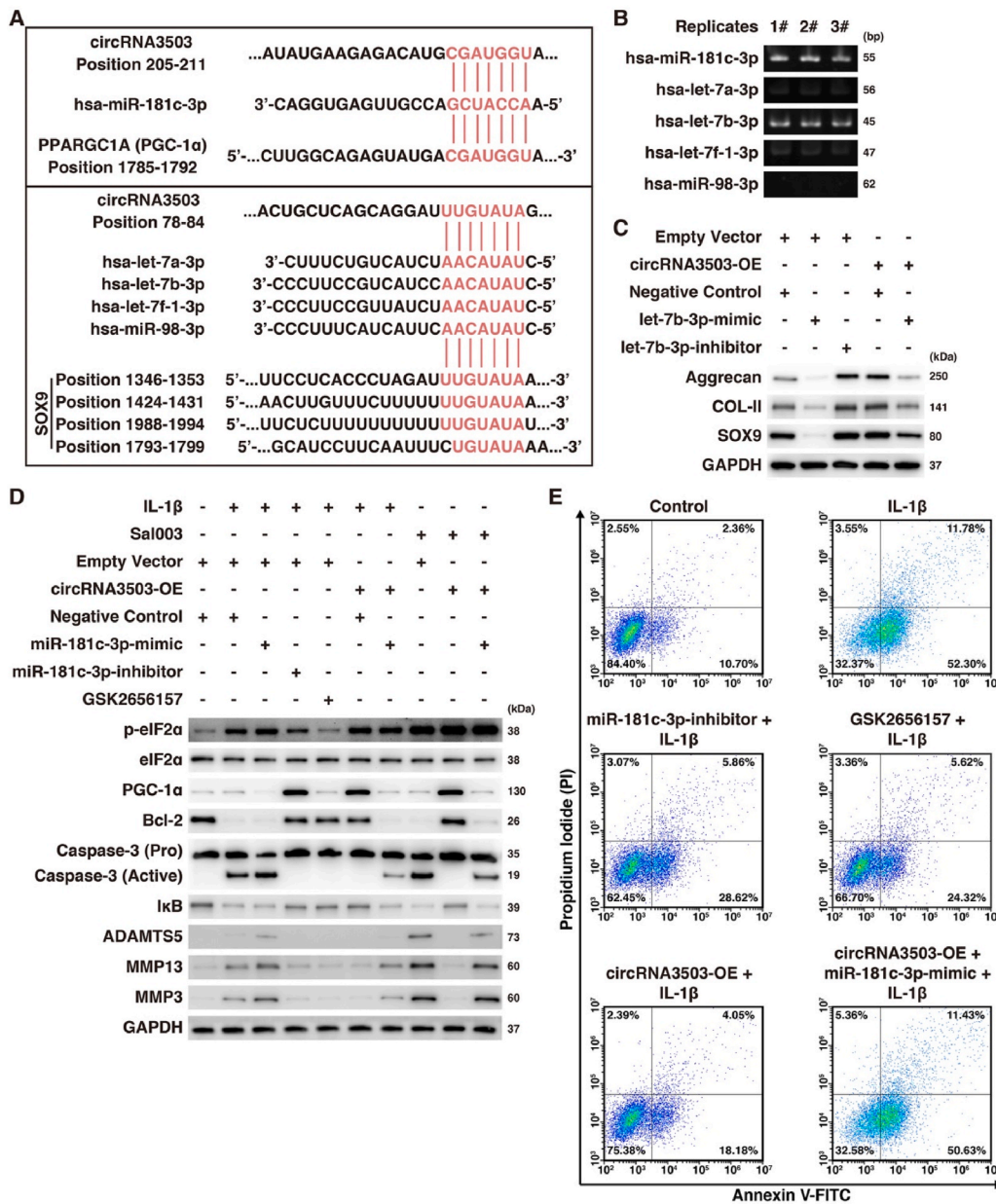


Fig. 3. Downstream mechanism of circRNA3503-mediated cartilage homeostasis. (A) Downstream mechanism prediction by bioinformatic analysis. (B) The expression levels of downstream miRNA candidates measured by PAGE after RT-PCR. (C) The expression of genes related to ECM synthesis (aggrecan, COL-II, and SOX9) measured by WB. (D) The phosphorylation of eIF2 α , activation of Caspase-3, and expression levels of PGC-1 α , Bcl-2, I κ B, and genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) measured by WB. (E) Apoptosis detected by FCM analysis of Annexin V/PI staining. Experiments were repeated at least three times, and representative results are shown.

Table 3
Specific primers for miRNAs used in this study.

Target	Hairpin Reverse Transcription Primer	Forward Primer	Reverse Primer
hsa-let-7a-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GAA AGA CA	GGG CTA TAC AAT CTA CTG TC	ACT GGT GTC GTG GAG T
hsa-let-7b-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GGG AAG GC	GCG GCT ATA CAA CCT AC	AGT CGG CAA TTC AGT T
hsa-let-7f-1-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GGG AAG GC	GGC GGC TAT ACA ATC TAT	GAG TCG GCA ATT CAG T
hsa-miR-98-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GGG AAA G	GCG AGG CTA TAC AAC TTA CTA C	CAA CTG GTG TCG TGG A
hsa-miR-181c-3p	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG GTC CAC TC	AAC CAT CGA CCG TTG A	AAC TGG TGT CGT GGA GTC

- (6) miR-181c-3p-mimic blocked the function of circRNA3503-OE, a result that also supports our hypothesis regarding the circRNA3503/miR-181c-3p ceRNA mechanism.
- (7) Sal003, which caused the phosphorylation of eIF2 α , produced results similar to those observed after IL-1 β -induced ER stress, which further supports the hypothesis that ER stress plays an important role in cartilage destruction in OA progression.

Furthermore, to confirm the effect on apoptosis, we performed flow cytometry with Annexin V/PI staining. The results were consistent with

the caspase-3 activation measured by WB (Fig. 3E).

3.4. Building a synergistic OA therapy: SMSC-sEVs and sleep-related circRNA3503

Through the above experiments, we found that sleep-related circRNA3503 has therapeutic potential in OA, and then explored the downstream molecular mechanism of circRNA3503. Considering the features of sEVs derived from SMSCs (SMSC-sEVs) described in our previous study [10], we hypothesized that SMSC-sEVs and sleep-related

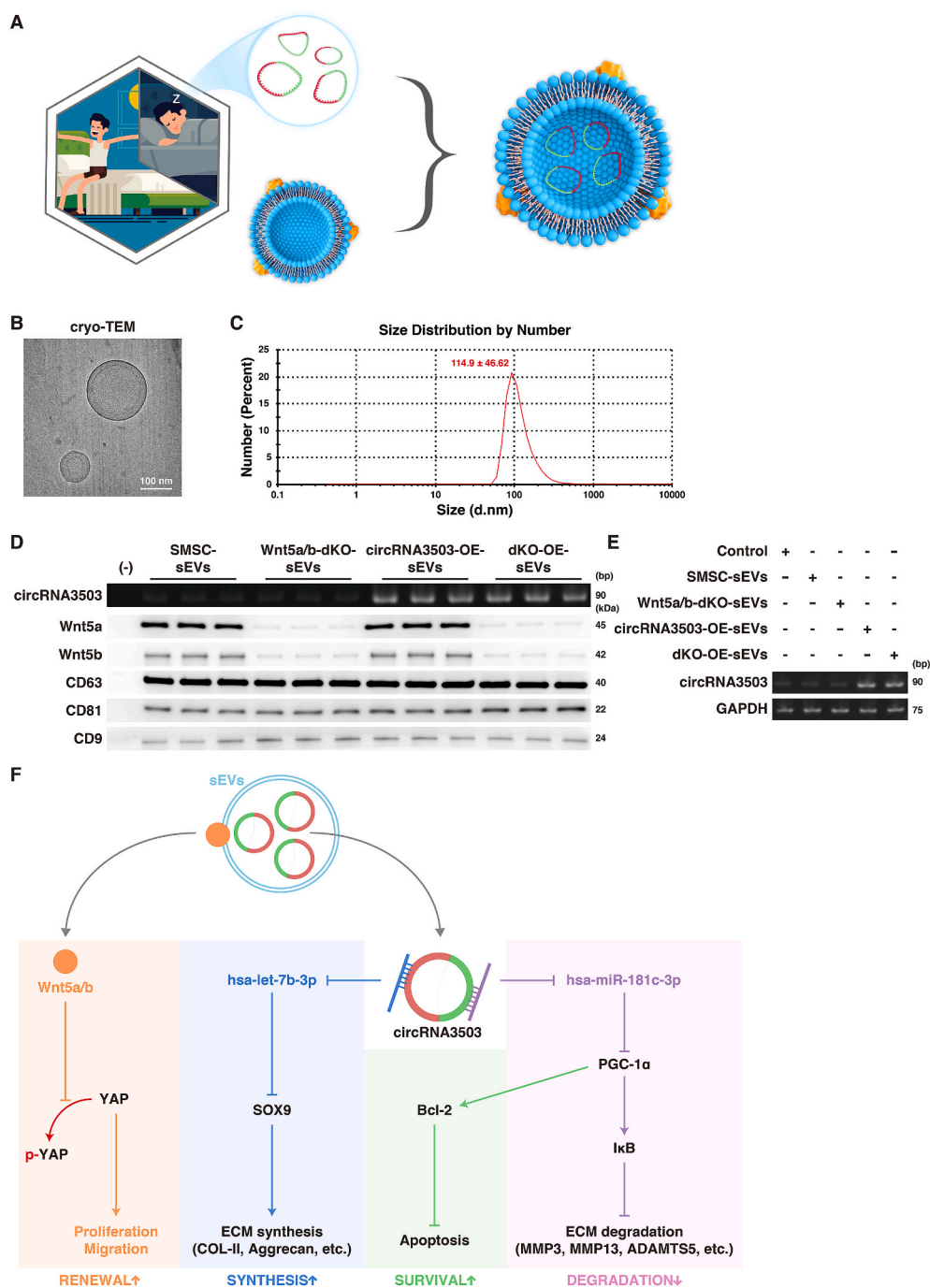


Fig. 4. Building a synergistic OA therapy: SMSC-sEVs and sleep-related circRNA3503. (A) Schematic diagram of the approach for combining SMSC-sEVs and sleep-related circRNA3503 used in this study. (B) Morphology of sEVs determined by cryo-TEM (scale bar: 100 nm). (C) Size distribution of sEVs determined by DLS. (D) The circRNA3503 content of the indicated sEVs measured by PAGE after RT-PCR, and the protein levels of Wnt5a, Wnt5b, CD63, CD81 and CD9 in the indicated sEVs measured by WB. (E) circRNA3503 content of the indicated sEVs measured by PAGE after RT-PCR. (F) Schematic diagram of the mechanism of circRNA3503-OE-sEVs. Experiments were repeated at least three times, and representative results are shown.

circRNA3503 would act synergistically and could serve as a better treatment for OA than either treatment alone (Fig. 4A).

First, after isolating sEVs, we aimed to identify and characterize the SMSC-sEVs by transmission electron microscopy (TEM), scanning electron microscopy (SEM), cryo-TEM, dynamic light scattering (DLS), and western blotting (WB). Morphological analysis by cryo-TEM showed electron-dense spherical nanovesicles with a clear lipid bilayer/membrane (Fig. 4B), which is consistent with previous studies [44,45]. TEM (with negative stain) and SEM images showed that the nanovesicles were cup- or sphere-shaped (Fig. S2), which is also consistent with previous studies [44–47]. Furthermore, DLS was used to determine the size distribution of sEVs and showed that the particle size distribution was 114.9 ± 46.62 (Fig. 4C), which is consistent with the size observed by TEM, SEM, and cryo-TEM. Specific surface markers of sEVs (CD63,

CD81, and CD9) were detected by WB, which indeed showed the expression of these markers on the SMSC-sEVs (Fig. 4D). The above results suggest that the nanovesicles that we isolated were indeed sEVs that showed features consistent with those revealed by previous studies [14,48–52].

Based on the above experimental results combined with the findings of our previous study, which showed that Wnt5a and Wnt5b are the key molecules in SMSC-sEVs that enhance the proliferation and migration of chondrocytes [10], we aimed to assess the contributions of Wnt5a/Wnt5b and circRNA3503. Thus, we prepared the following four types of sEVs:

- (1) SMSC-sEVs: sEVs derived from SMSCs;

- (2) Wnt5a/b-dKO-sEVs: sEVs derived from SMSCs with dual Wnt5a and Wnt5b knockout;
- (3) circRNA3503-OE-sEVs: sEVs derived from SMSCs overexpressing circRNA3503;
- (4) dKO-OE-sEVs: sEVs derived from SMSCs overexpressing circRNA3503 with dual Wnt5a and Wnt5b knockout.

To validate the sEVs, the Wnt5a and Wnt5b contents of all four types of sEVs were confirmed by WB, while the circRNA3503 content was confirmed by PAGE after PCR (Fig. 4D). We further confirmed the circRNA3503 expression in chondrocytes after treatments by these four sEVs (Fig. 4E).

The schematic diagram in Fig. 4F summarizes these findings and illustrates the mechanism of circRNA3503-OE-sEVs in preventing OA progression. Wnt5a/b can enhance the regeneration/renewal ability of chondrocytes by increasing proliferation and migration via inhibition of the phosphorylation of Yes-associated protein (YAP). In addition, circRNA3503 can enhance ECM synthesis in cartilage and inhibit ER stress-induced chondrocyte apoptosis and ECM degradation in cartilage.

3.5. Validation of the effects of circRNA3503-OE-sEVs in vitro

The effects of the four types of sEVs were next evaluated through a series of experiments.

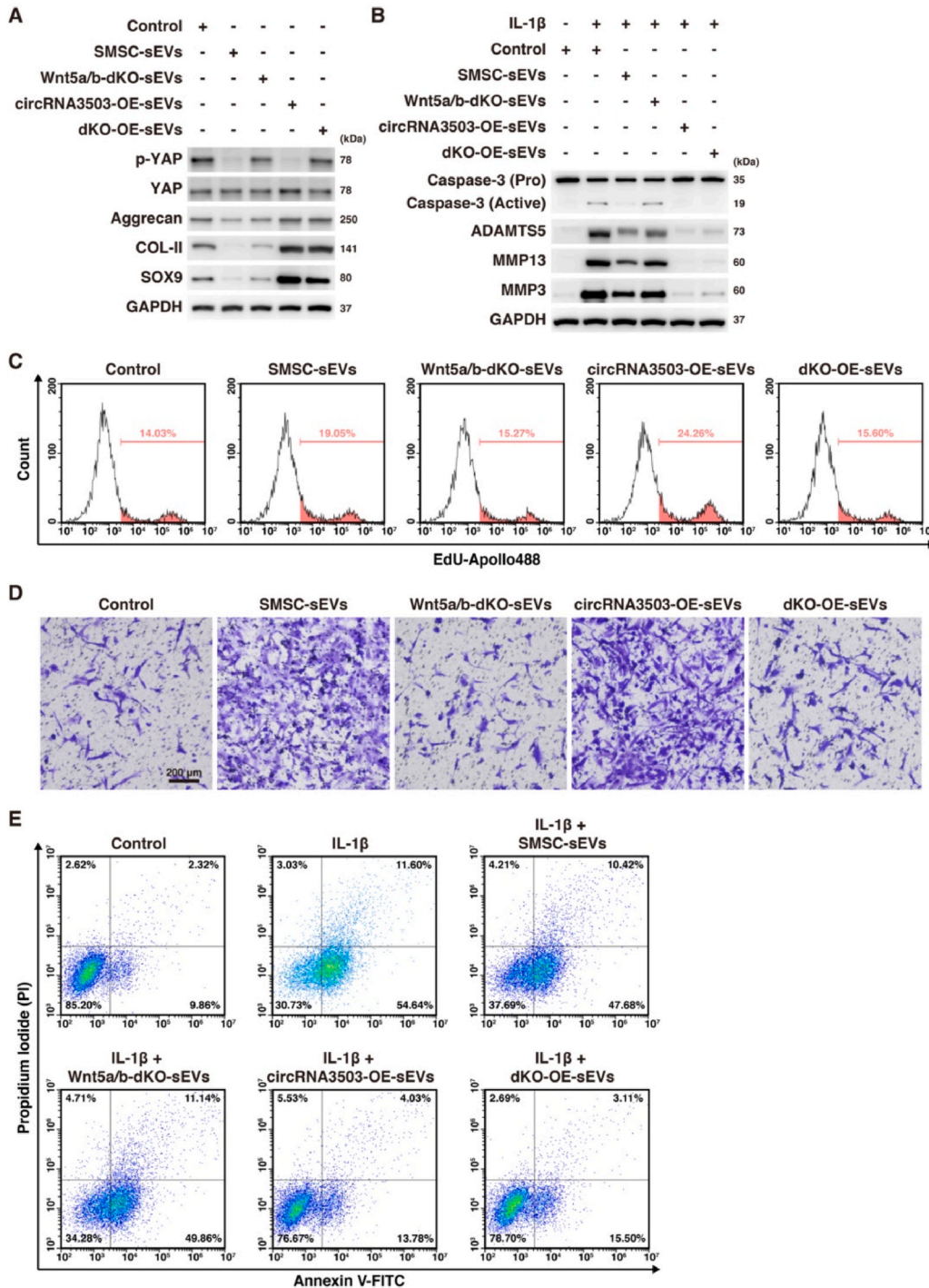


Fig. 5. Validation of the function of circRNA3503-OE-sEVs *in vitro*. (A) The phosphorylation level of YAP and expression levels of genes related to ECM synthesis (COL-II, Aggrecan, and SOX9) were measured by WB. (B) The activation of Caspase-3 and expression levels of genes related to ECM degradation (ADAMTS5, MMP13, and MMP3) were measured by WB. (C) The proliferative abilities of chondrocytes after treatment with different types of sEVs were measured by EdU assays. (D) The migratory abilities of chondrocytes after treatment with different types of sEVs were measured by Transwell assays. (E) Apoptosis was detected by FCM analysis of Annexin V/PI staining. Experiments were repeated at least three times, and representative results are shown.

Similar to the findings of our previous study [10], Wnt5a/b carried by sEVs inhibited the phosphorylation of YAP in chondrocytes (Fig. 5A), and non-phosphorylated YAP (activated YAP) was shown in a previous study to promote chondrocyte proliferation and migration, but a side effect (the inhibition of ECM synthesis) eliminated their ability to treat OA [10]. Without Wnt5a/b (Wnt5a/b-dKO-sEVs or dKO-OE-sEVs), sEVs lost the ability to inhibit YAP phosphorylation (Fig. 5A). CircRNA3503 overexpression overcame the side effect of SMSC-sEVs, and circRNA3503-OE-sEVs even significantly enhanced ECM synthesis (Fig. 5A).

Next, we tested the ability of sEVs to enhance resistance to ER stress. SMSC-sEVs inhibited caspase-3 activation and the expression of ECM degradation-related genes but could not fully rescue IL-1 β -induced apoptosis or enhanced expression of ECM degradation-related genes (Fig. 5B). This phenomenon might be caused by the presence of activated YAP, which has been reported to have anti-inflammatory and anti-apoptotic functions [53,54]. The effects of Wnt5a/b-dKO-sEVs were less pronounced (Fig. 5B). Both circRNA3503-OE-sEVs and dKO-OE-sEVs fully rescued the IL-1 β -induced apoptosis and enhanced expression of ECM degradation-related genes caused by OA modelling (Fig. 5B); thus, circRNA3503 is effective in preventing OA progression.

Next, to further confirm the effects of circRNA3503-OE-sEVs, EdU assays were used to evaluate changes in proliferative ability (Fig. 5C), Transwell assays were used to evaluate changes in migratory ability (Fig. 5D), and Annexin V/PI assays were used to evaluate the ability of sEVs to inhibit apoptosis (Fig. 5E). These results further confirmed that circRNA3503 was the major contributor to the anti-apoptotic function of the circRNA3503-OE-sEVs, while the function of these sEVs in promoting renewal was accounted for by Wnt5a/b. Therefore, these results show that the combination of SMSC-sEVs and sleep-related circRNA3503 is a potential treatment to prevent OA progression.

3.6. Therapeutic effect of PLEL@circRNA3503-OE-sEVs

In the Fourier transform infrared (FTIR) spectrum (Fig. S3A), C=O stretching vibration (ν C=O) appears at 1638.46 cm^{-1} and 1742.77 cm^{-1} , which was attributed to the ester carbonyl bond. C–O–C stretching vibration (ν C–O–C) appears at 1094.97 cm^{-1} , which was attributed to repetitive $-\text{OCH}_2\text{CH}_2$ units in PEG. O–H stretching vibration (ν O–H) appears at 3333.11 cm^{-1} , which was attributed to terminal hydroxyl groups ($-\text{OH}$) of the PLEL copolymers. These results confirmed that the purchased commercial PLEL triblock copolymers were synthesized successfully [30,55].

For a thermosensitive triblock copolymer like PLEL, when no lower than a critical concentration, three basic physical phases (sol, gel, and precipitation) appear during heating. Sol–gel–precipitation phase transition was measured using a test-tube inversion method. The lower critical gelation temperature (LCGT) was defined as the temperature of the sol–gel transition point, while the upper critical gelation temperature (UCGT) was defined as the temperature of the gel–precipitation transition point [30,55]. In Fig. S3B, although the incorporation of sEVs into PLEL did slightly narrow the gel window (temperature range between LCGT and UCGT), either PLEL or PLEL@sEVs formed a gel below 37 °C when the concentration was no less than 13% (wt%).

Furthermore, a dynamic rheological assay was employed for quantitative monitoring of the sol–gel transition (Fig. S3C). In rheology, a sol–gel transition is defined as the point where the gap between storage modulus (G') and loss modulus (G'') starts to widen. The results were consistent with the test-tube inversion experiment. Although PLEL@sEVs had a slightly narrower gel window than PLEL, they all appeared in the sol phase at room temperature and converted into the gel phase at body temperature (Fig. S3C). Additionally, the dynamic rheological data also showed that incorporation of sEVs into PLEL had negligible influence on the mechanical strength and strain capacity (Fig. S3C).

Both PLEL and PLEL@sEVs had very good bio-degradability (Fig. S3D). Different groups of the PLEL gel complex not only showed

good sol–gel phase transition when heated up to body temperature (Fig. S4A), but also exhibited a very good *in-situ* rapid gelation capability upon direct injection into 37 °C water, an *in vitro* simulated articular cavity injection (Fig. S4B). Moreover, the PLEL@sEVs showed good slow-release performance (Fig. 6A), which conferred a better long-term effect on PLEL@sEVs, including delivery of circRNA3503, compared to direct injection of sEVs (Fig. S5).

Transwell chambers and low adhesive plates were used to establish a co-culture system to observe the chondrogenic potential and cartilage matrix deposition capacity of chondrocytes treated with different combinations of PLEL gel followed by IL-1 β stimulation (Fig. 6B).

When incubated with IL-1 β , the cartilage matrix deposition capacity of chondrocytes significantly decreased (Fig. 6C). OA + PLEL@Saline, PLEL@SMSC-sEVs, and PLEL@Wnt5a/b-dKO-sEVs showed no very significant improvement (Fig. 6C). However, the situation was significantly improved with the PLEL@circRNA3503-OE-sEVs or PLEL@dKO-OE-sEVs, and, between these two, PLEL@circRNA3503-OE-sEVs showed the most significant effect on maintaining chondrogenic potential, promoting the cartilage matrix deposition capacity of chondrocytes, and rescuing cells from the destructive effect of IL-1 β (Fig. 6C).

Finally, we evaluated the function of sEV-loaded PLEL hydrogel in preventing OA progression *in vivo* (Fig. 7). The OA group showed severe cartilage damage, indicating that our model of OA was successfully established. In the OA + PLEL@Saline group, it did not have any effect on the features of OA. In the OA + PLEL@SMSC-sEVs group, the cartilage defect was covered with some regenerated tissue, but this regenerated tissue had a disorganized arrangement and did not stain normally, distinguishing it from normal cartilage. In the OA + PLEL@Wnt5a/b-dKO-sEVs group, no ability to induce the production of even disorganized regenerated tissue was observed. In the OA + PLEL@circRNA3503-OE-sEVs group, PLEL@circRNA3503-OE-sEVs substantially prevented OA disease progression. However, in the PLEL@dKO-OE-sEVs group, the cartilage-protective function of the sEVs was significantly weaker than that of the PLEL@circRNA3503-OE-sEVs. Immunohistochemical (IHC) assays showed that only PLEL@sEVs carrying circRNA3503 (PLEL@circRNA3503-OE-sEVs and PLEL@dKO-OE-sEVs) had a significant capability to protect cartilage ECM, and PLEL@circRNA3503-OE-sEVs had the best protective effects compared with other interventions (Fig. S6).

In summary, with our new therapeutic agent (summarized in Fig. 8), circRNA3503-OE-sEVs, Wnt5a/b from the SMSC-sEVs enhanced the regenerative/renewal abilities of chondrocytes to neutralize cartilage defects caused by OA progression, and circRNA3503 (1) enhanced ECM synthesis in cartilage to abrogate the decreased ECM synthesis caused by OA progression, (2) suppressed chondrocyte apoptosis caused by OA-induced ER stress, and (3) suppressed ECM degradation in cartilage caused by OA-induced ER stress. In addition, the injectable thermosensitive hydrogel PLEL is a very suitable sEV sustained-release delivery system for intraarticular injection. The combination of these effects renders the administration of PLEL@circRNA3503-OE-sEVs a potential therapeutic approach to prevent OA disease progression.

4. Discussion

CircRNAs have attracted increasing attention in the OA research field as therapeutic targets, molecules for early diagnosis, and therapeutic drugs. In this study, we focused on the epigenetic regulatory effects of sleep-related circRNA3503 in chondrocytes and uncovered the chondroprotective properties of both sleep-related circRNA3503 and circRNA3503-containing sEVs (circRNA3503-OE-sEVs). CircRNA3503-OE-sEVs could interact with chondrocytes and had a significant effect on the ECM, revealing a potential novel therapeutic approach for OA.

Sleep is a period of restoration during which physiological changes are believed to promote a microenvironment suitable for cells to proliferate, migrate and differentiate. Cell division and protein synthesis have been demonstrated to reach their highest levels during sleeping

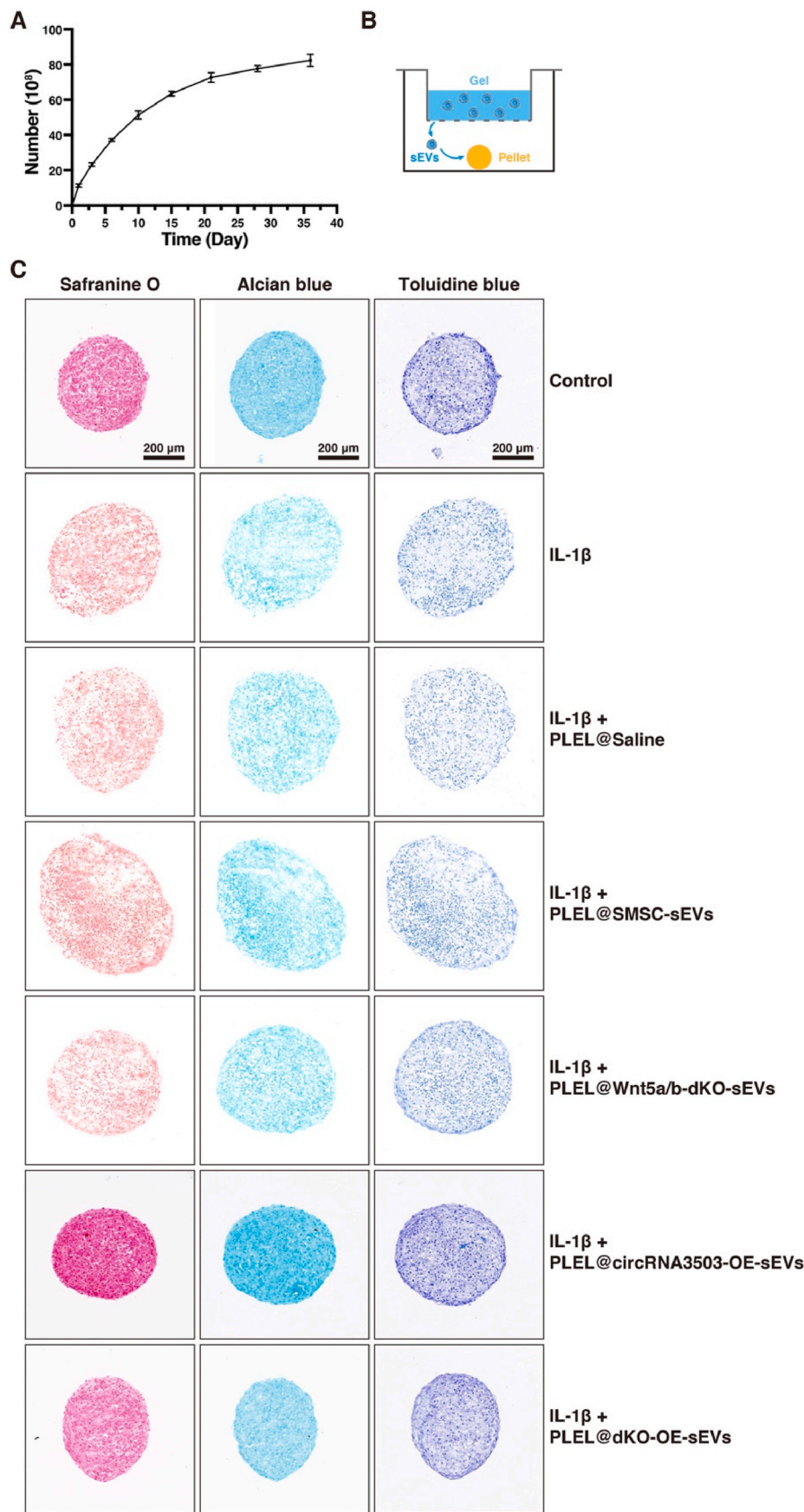


Fig. 6. Validation of the therapeutic effect of PLEL@circRNA3503-OE-sEVs *in vitro*. (A) *In vitro* total sEVs released from PLEL@sEVs hydrogels incubated in DPBS. (B) Schematic diagram of the co-cultural system for gel (upper) and chondrocyte pellets (lower). (C) As indicated at day 21, the pellets with different treatments were stained with Safranin O, Alcian blue and Toluidine blue. Experiments were repeated at least three times, and representative results are shown. Error bars show standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

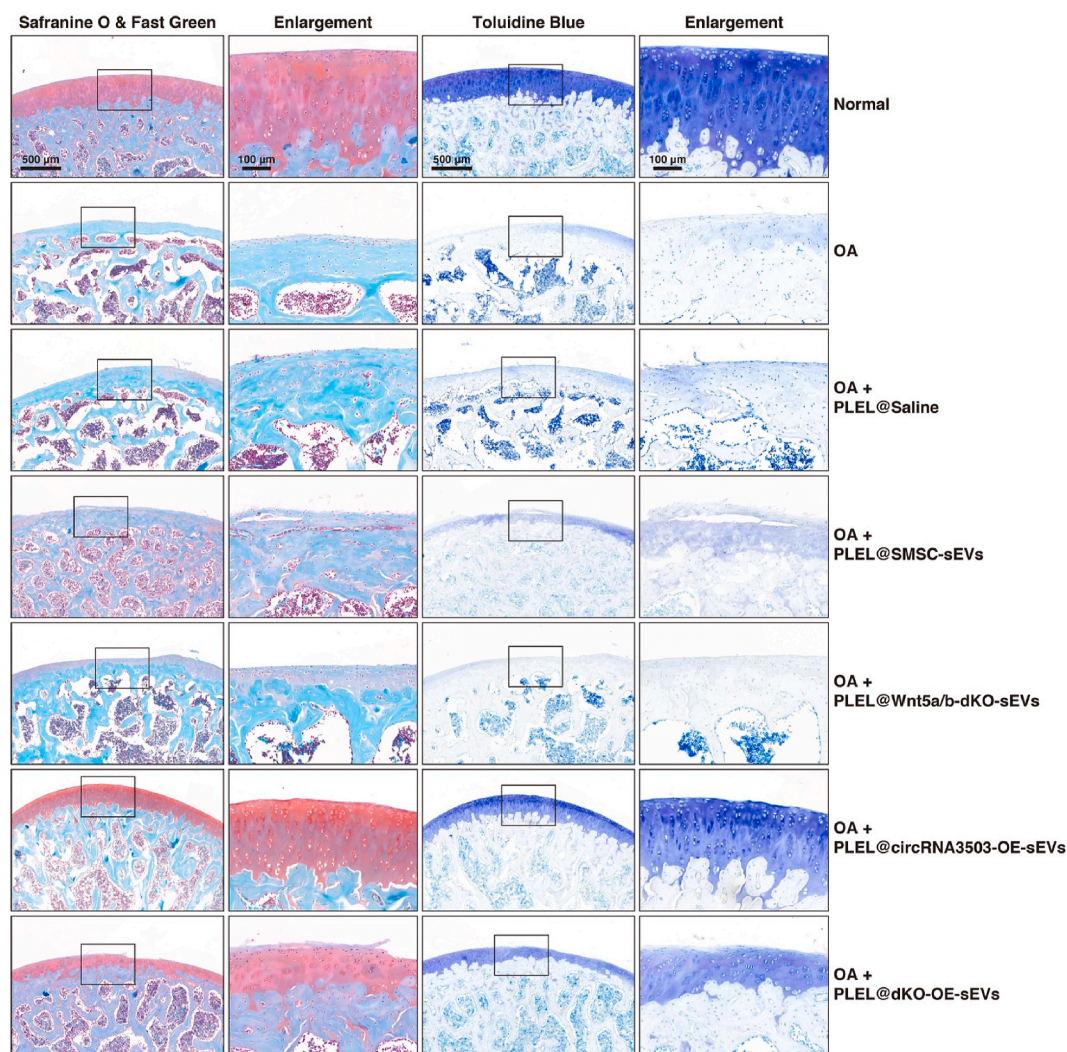


Fig. 7. Validation of the function of PLEL@circRNA3503-OE-sEVs *in vivo*. Histologic analysis with Safranin O & Fast Green and Toluidine Blue staining for different groups ($n = 10$ for each group; Scale bar: 500 μm for low magnification, and 100 μm for enlargement). Experiments were repeated at least three times, and representative results are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hours and diminish throughout the day. These effects can be mediated by MT, which is secreted in response to neural optic signals, which act in harmony to regulate many biological functions during sleep. Similarly, the rate of damaged tissue healing is greater during sleep than while awake [56]. The healing process is faster during sleep, which is required to maintain health. Some studies have demonstrated that sleep restriction can induce pathological alterations in condylar cartilage [57] and synovial membrane destruction in OA [58]. Moreover, chronic sleep deprivation is a validated method used to establish cartilage injury in the temporomandibular joint [59]. MT administration can alter the circadian clock and promote sleep [60], and growing evidence has indicated that MT treatment can promote the chondrogenic differentiation of human mesenchymal stem cells (MSCs) [61]; upregulate the expression of chondrogenic markers, including Col-II, SOX9, and aggrecan, at both the mRNA and protein levels; downregulate the expression of hypertrophic markers, such as type X collagen (Col-X); enhance cartilage ECM synthesis [62]; and inhibit matrix metalloproteinase (MMP) activity [63]. Therefore, MT treatment can significantly reduce cartilage damage [64]. Herein, we utilized MT treatment to screen sleep-related circRNAs and constructed sEVs carrying a sleep-related circRNA (circRNA3503) to promote the restoration of damaged chondrocytes for cartilage repair and regeneration in OA.

Recent studies have shown that some circRNAs may be involved in

the initiation and progression of OA, and circRNAs have been found to be differentially expressed *in vitro* in chondrocytes isolated from patients with OA compared to those isolated from control individuals [65]. Here, our research results demonstrated that circRNA3503 promotes chondrocyte survival by inhibiting apoptosis, modulating cartilage ECM synthesis, and alleviating ECM degradation by acting as a miRNA sponge for hsa-miR-181c-3p and hsa-let-7b-3p to maintain cartilage homeostasis and prevent OA progression.

Although OA is a disorder of the whole joint, the progressive destruction of cartilage, including destruction caused by chondrocyte apoptosis and ECM degradation, is a hallmark of OA and indicates that the interaction between chondrocytes and the ECM leads to unbalanced catabolic and anabolic responses and a vicious cycle of changes that results in cartilage destruction [3]. The ECM is synthesized by chondrocytes, which maintain cartilage homeostasis by maintaining equilibrium between cartilage anabolism and catabolism.

Accumulating evidence suggests that chondrocyte apoptosis plays a key role in cartilage destruction and matrix depletion in OA [29]. Chondrocytes are solely responsible for the maintenance of ECM integrity and synthesis of ECM components, and any biological event that undermines their wellbeing or alters their phenotypic stability can therefore compromise cartilage homeostasis and trigger the onset of OA [66]. Here, we demonstrated that circRNA3503 treatment both

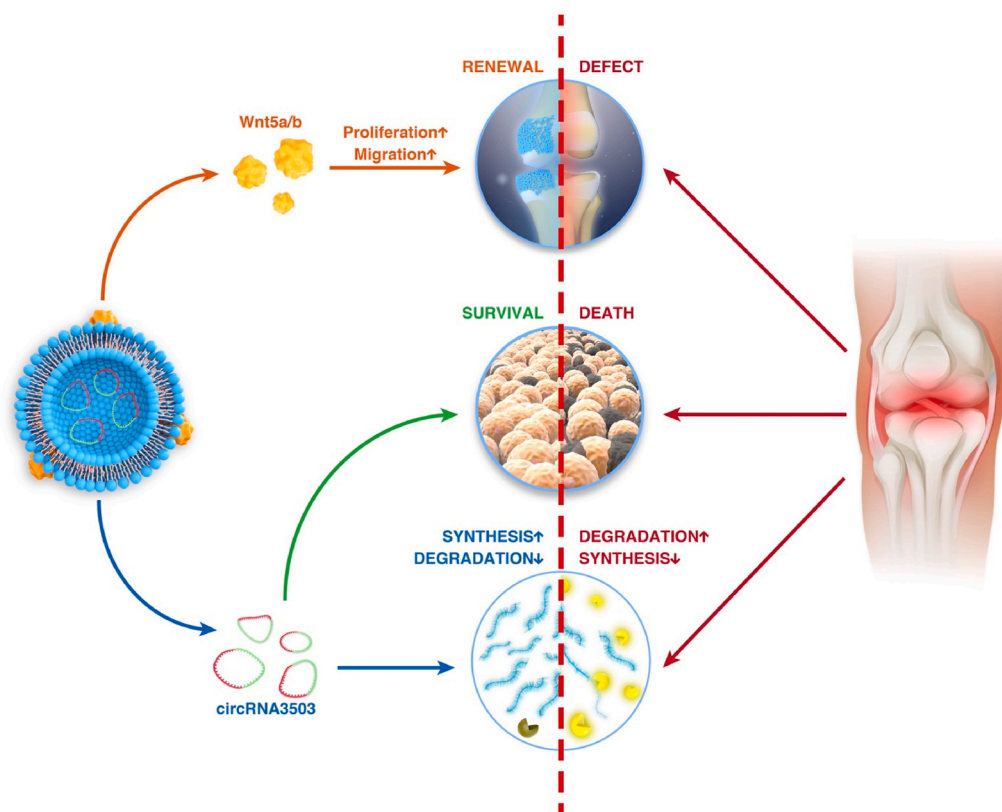


Fig. 8. Schematic diagram summarising the mechanism by which circRNA3503-OE-sEVs prevent OA progression. Wnt5a/b on circRNA3503-OE-sEVs enhances the ability of chondrocytes to regenerate/renew, which neutralises cartilage defects caused by OA progression. circRNA3503 inside circRNA3503-OE-sEVs can enhance cartilage ECM synthesis, which neutralises the decreased ECM synthesis caused by OA progression. circRNA3503 can also suppress the cartilage ECM degradation and chondrocyte apoptosis caused by OA-induced ER stress.

inhibited chondrocyte death and maintained chondrocyte survival, regulated SOX9 expression by functioning as a ceRNA and participated in the process of chondrocyte ECM synthesis. In this study, after circRNA3503 intervention, in chondrocytes damaged by IL-1 β exposure, balance between ECM catabolism and anabolism was restored, maintaining phenotypic stability. These changes in chondrocyte metabolism rescued changes to the composition of the surrounding ECM. The rescue of cartilage ECM homeostasis prevented cartilage deterioration. Furthermore, intervention with circRNA3503-OE-sEVs inhibited chondrocyte apoptosis and promoted cartilage regeneration.

Cartilage is predominantly composed of ECM, which has a low cell density, and changes in the ECM play an important role in the progression of OA [67]. The main ECM proteins in cartilage are Col-II and aggrecan, which are synthesized by chondrocytes and in turn maintain the phenotypic stability of chondrocytes. The local microenvironment, specifically the ECM, is well known to play a major role in the regulation of cell behaviour and function [68]. During the pathological progression of OA, increased catabolism in the ECM causes the disruption of cartilage homeostasis [66]. Upon circRNA3503 overexpression, synthesis of the ECM was increased, while degradation of the ECM was decreased, through regulation of hsa-miR-181c-3p and hsa-let-7b-3p. Therefore, the shift towards catabolism over anabolism caused by OA was rescued. Furthermore, excessive production of the matrix-degrading enzymes MMP-13 and ADAMTS-5, which ultimately result in cartilage degradation [69], was rescued. However, circRNA3503 intervention was not shown to promote the proliferation or migration of chondrocytes in this study.

The synovium, a key component of synovial joints, produces synovial fluid to provide lubrication and nutrients for cartilage, which greatly contributes to maintaining cartilage homeostasis [70]. EVs are abundant in the synovial fluid, which is thought to be a specialized form of ECM [71]. In recent years, spontaneous chondrogenesis has been observed, and this ability to undergo cartilage repair is thought to be derived from residual stem cells in the joint, especially those residing in the synovium

“stem cell pool”. In our previous study, we reported that SMSC-sEVs promoted the proliferation and migration of chondrocytes via Wnt5a/b signalling [10]. Thus, in this study, we chose to combine SMSC-sEVs and sleep-related circRNA3503 and investigate whether their effects would be synergistic. Our observations demonstrated that circRNA3503-OE-sEVs could maintain cartilage stability through the synergistic effects of these components (increased proliferation, reduced apoptosis, and enhanced cartilage homeostasis by both promoting ECM synthesis and inhibiting ECM degradation).

Macrophages, which play a very important role in the progression of OA [72,73], participate in various stages of inflammation such as initiation, progression, and resolution [72–75], and activated macrophages can be classified into classically-activated (M1, proinflammatory) and alternatively-activated (M2, anti-inflammatory) phenotypes [72–74]. Interestingly, more and more studies have implied that sEVs derived from mesenchymal stem cells can regulate the macrophage phenotype (higher percentage of anti-inflammatory M2 macrophages and lower percentage of proinflammatory M1 macrophages) [76–78]. Currently, there is no clear theory about the in-depth mechanisms. Recent studies have shown that Wnt5a regulates the macrophage phenotype (inducing M2 polarization) [79,80], and this regulatory effect may be mediated by YAP activation [81]. Further, this phenotype regulatory function could be one of the factors which made the therapeutic effects of PLEL@circRNA3503-OE-sEVs relatively better than those of PLEL@dKO-OE-sEVs. However, the in-depth mechanism of sEV-induced macrophage phenotype regulation still needs to be further studied in future.

PDLLA-PEG-PDLLA triblock copolymer gels have attracted attention in the fields of research into regenerative medicine and oncology as carriers of nanoparticles/nano-drugs, and the advantage of triblock gels in intra-articular injection has attracted more and more attention [30–33,82,83]. In this current study, we demonstrate for the first time that PLEL showed good performance in slow release sEVs, and PLEL@circRNA3503-OE-sEVs showed a very good potential to protect

cartilage and delay the progression of OA. We anticipate that our exploration of PLEL@circRNA3503-OE-sEVs will not only provide a specific therapeutic method for OA but also inspire further research.

Ethics statement

For clinical samples (including synovium and cartilage) collected in our department, written informed consent was obtained from each volunteer or patient, and ethical approval was granted by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital at the Shanghai Jiao Tong University School of Medicine. The welfare of the experimental animals was prioritized, and all animal experiments were approved by the Animal Care Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital at the Shanghai Jiao Tong University School of Medicine and followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All procedures were conducted in accordance with the Declaration of Helsinki and standard guidelines.

Funding

The present study was supported by the National Natural Science Foundation of China (grant numbers 81301589, 81802226, 81871834 and 82072530), the Shanghai Pujiang Program (grant number 2019PJJD038), Shanghai "Rising Stars of Medical Talent" Youth Development Program (Youth Medical Talents – Specialist Program), and the Shanghai Jiao Tong University K.C. Wong Medical Fellowship Fund.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Shi-Cong Tao acquired the data, designed the experiments and wrote a draft of the manuscript; Zi-Xiang Li and Shi Zhan prepared the experimental materials and assisted with the *in vitro* and *in vivo* assays; Ji-Yan Huang and Yuan Gao interpreted and analysed the results; Helen Dawes gave basic suggestions and helped to complete the manuscript; Shang-Chun Guo performed the *in vitro* and *in vivo* assays, and supervised this study. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

CRediT authorship contribution statement

Shi-Cong Tao: Data curation, Methodology, Writing – review & editing, Funding acquisition. **Ji-Yan Huang:** Formal analysis, Visualization. **Yuan Gao:** Visualization. **Zi-Xiang Li:** Resources, Investigation, Shi Zhan: Resources, Investigation. **Zhan-Ying Wei:** Conceptualization. **Helen Dawes:** Investigation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

We thank the Shanghai Institutes for Biological Sciences, Fudan University, Tongji University, Shanghai University of Traditional Chinese Medicine and the Animal Experimental Centre of Shanghai Sixth People's Hospital for expert assistance. We also wish to acknowledge current and previous members of our laboratories. In addition, we thank

LC-Bio, BioTNT, and RiboBio for providing technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioactmat.2021.04.031>.

References

- [1] G. Nygaard, G.S. Firestein, Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes, *Nature reviews, Rheumatology* 16 (6) (2020) 316–333.
- [2] F. Li, Y. Tang, B. Song, M. Yu, Q. Li, C. Zhang, J. Hou, R. Yang, Nomenclature clarification: synovial fibroblasts and synovial mesenchymal stem cells, *Stem Cell Res. Ther.* 10 (1) (2019) 260.
- [3] M.L. Tiku, H.E. Sabaawy, Cartilage regeneration for treatment of osteoarthritis: a paradigm for nonsurgical intervention, *Therapeut. Adv. Musculoskeletal Dis.* 7 (3) (2015) 76–87.
- [4] F. Iannone, G. Lapadula, The pathophysiology of osteoarthritis, *Aging Clin. Exp. Res.* 15 (5) (2003) 364–372.
- [5] L.J. Sandell, T. Aigner, Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis, *Arthritis Res.* 3 (2) (2001) 107–113.
- [6] D.J. Huey, J.C. Hu, K.A. Athanasiou, Unlike bone, cartilage regeneration remains elusive, *Science (New York, N.Y.)* 338 (6109) (2012) 917–921.
- [7] I.A. Jones, R. Togashi, M.L. Wilson, N. Heckmann, C.T. Vangsness Jr., Intra-articular treatment options for knee osteoarthritis, *Nat. Rev. Rheumatol.* 15 (2) (2019) 77–90.
- [8] K. Wiegant, P.M. van Roermund, F. Intema, S. Cofana, F. Eckstein, S. C. Mastbergen, F.P. Lafeber, Sustained clinical and structural benefit after joint distraction in the treatment of severe knee osteoarthritis, *Osteoarthritis Cartilage* 21 (11) (2013) 1660–1667.
- [9] J.P.K. Armstrong, M.M. Stevens, Strategic design of extracellular vesicle drug delivery systems, *Adv. Drug Deliv. Rev.* 130 (2018) 12–16.
- [10] S.C. Tao, T. Yuan, Y.L. Zhang, W.J. Yin, S.C. Guo, C.Q. Zhang, Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model, *Theranostics* 7 (1) (2017) 180–195.
- [11] S.E. Headland, H.R. Jones, L.V. Norling, A. Kim, P.R. Souza, E. Corsiero, C.D. Gil, A. Nerviani, F. Dell'Accio, C. Pitzalis, S.M. Oliani, L.Y. Jan, M. Perretti, Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis, *Sci. Transl. Med.* 7 (315) (2015) 315ra190.
- [12] M. Li, Q.F. Ke, S.C. Tao, S.C. Guo, B.Y. Rui, Y.P. Guo, Fabrication of hydroxyapatite/chitosan composite hydrogels loaded with exosomes derived from miR-126-3p overexpressed synovial mesenchymal stem cells for diabetic chronic wound healing, *J. Mater. Chem. B* 4 (42) (2016) 6830–6841.
- [13] S.C. Tao, B.Y. Rui, Q.Y. Wang, D. Zhou, Y. Zhang, S.C. Guo, Extracellular vesicle-mimetic nanovesicles transport LncRNA-H19 as competing endogenous RNA for the treatment of diabetic wounds, *Drug Deliv.* 25 (1) (2018) 241–255.
- [14] S.C. Tao, S.C. Guo, C.Q. Zhang, Modularized extracellular vesicles: the dawn of prospective personalized and precision medicine, *Adv. Sci. (Weinheim, Baden-Wuerttemberg, Germany)* 5 (2) (2018) 1700449.
- [15] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, J. Kjems, Natural RNA circles function as efficient microRNA sponges, *Nature* 495 (7441) (2013) 384–388.
- [16] Y. Wang, J. Liu, J. Ma, T. Sun, Q. Zhou, W. Wang, G. Wang, P. Wu, H. Wang, L. Jiang, W. Yuan, Z. Sun, L. Ming, Exosomal circRNAs: biogenesis, effect and application in human diseases, *Mol. Canc.* 18 (1) (2019) 116.
- [17] K.W. Hon, N.S. Ab-Mutalib, N.M.A. Abdullah, R. Jamal, N. Abu, Extracellular Vesicle-derived circular RNAs confers chemoresistance in Colorectal cancer, *Sci. Rep.* 9 (1) (2019) 16497.
- [18] W.R. Jeck, J.A. Sorrentino, K. Wang, M.K. Slevin, C.E. Burd, J. Liu, W.F. Marzluft, N.E. Sharpless, Circular RNAs are abundant, conserved, and associated with ALU repeats, *RNA (New York, N.Y.)* 19 (2) (2013) 141–157.
- [19] Y. Li, Q. Zheng, C. Bao, S. Li, W. Guo, J. Zhao, D. Chen, J. Gu, X. He, S. Huang, Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis, *Cell Res.* 25 (8) (2015) 981–984.
- [20] S. Shen, Y. Wu, J. Chen, Z. Xie, K. Huang, G. Wang, Y. Yang, W. Ni, Z. Chen, P. Shi, Y. Ma, S. Fan, CircSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene, *Ann. Rheum. Dis.* 78 (6) (2019) 826–836.
- [21] S. Jiang, Y. Liu, B. Xu, Y. Zhang, M. Yang, Noncoding RNAs: new regulatory code in chondrocyte apoptosis and autophagy, *Wiley Interdiscipl. Rev. RNA* 11 (4) (2020) e1584.
- [22] Z.B. Zhou, G.X. Huang, Q. Fu, B. Han, J.J. Lu, A.M. Chen, L. Zhu, circRNA, 33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p, *Mol. Ther. : J. Am. Soc. Gene Ther.* 27 (3) (2019) 531–541.
- [23] L. Szabo, J. Salzman, Detecting circular RNAs: bioinformatic and experimental challenges, *Nat. Rev. Genet.* 17 (11) (2016) 679–692.
- [24] K.J. Schippers, S.A. Nichols, Deep, dark secrets of melatonin in animal evolution, *Cell* 159 (1) (2014) 9–10.
- [25] B. Stauch, L.C. Johansson, J.D. McCorvy, N. Patel, G.W. Han, X.P. Huang, C. Gati, A. Batyuk, S.T. Slocum, A. Ishchenko, W. Brehm, T.A. White, N. Michaelian, C. Madsen, L. Zhu, T.D. Grant, J.M. Grandner, A. Shiriaeva, R.H.J. Olsen, A. R. Tribo, S. Yous, R.C. Stevens, U. Weierstall, V. Katritch, B.L. Roth, W. Liu,

- V. Cherezov, Structural basis of ligand recognition at the human MT(1) melatonin receptor, *Nature* 569 (7755) (2019) 284–288.
- [26] P.C. Zee, P. Manthana, The brain's master circadian clock: implications and opportunities for therapy of sleep disorders, *Sleep Med. Rev.* 11 (1) (2007) 59–70.
- [27] J.S. Lee, D.J. Cua, Melatonin lulling Th17 cells to sleep, *Cell* 162 (6) (2015) 1212–1214.
- [28] M.F. Farez, I.D. Mascanfroni, S.P. Méndez-Huergo, A. Yeste, G. Murugaiyan, L. P. Garo, M.E. Balbuena Aguirre, B. Patel, M.C. Ysraelit, C. Zhu, V.K. Kuchroo, G. A. Rabinovich, F.J. Quintana, J. Correa, Melatonin contributes to the seasonality of multiple sclerosis relapses, *Cell* 162 (6) (2015) 1338–1352.
- [29] A. Hosseinzadeh, S.K. Kamrava, M.T. Joghataei, R. Darabi, A. Shakeri-Zadeh, M. Shahriari, R.J. Reiter, H. Ghaznavi, S. Mehrzadi, Apoptosis signaling pathways in osteoarthritis and possible protective role of melatonin, *J. Pineal Res.* 61 (4) (2016) 411–425.
- [30] Q. Tang, T. Lim, L.Y. Shen, G. Zheng, X.J. Wei, C.Q. Zhang, Z.Z. Zhu, Well-dispersed platelet lysate entrapped nanoparticles incorporate with injectable PDLA-PEG-PDLA triblock for preferable cartilage engineering application, *Biomaterials* 268 (2021) 120605.
- [31] X. Zhou, X. He, K. Shi, L. Yuan, Y. Yang, Q. Liu, Y. Ming, C. Yi, Z. Qian, Injectable thermosensitive hydrogel containing erlotinib-loaded hollow mesoporous silica nanoparticles as a localized drug delivery system for NSCLC therapy, *Adv. Sci. (Weinheim, Baden-Württemberg, Germany)* 7 (23) (2020) 2001442.
- [32] P. Liu, L. Gu, L. Ren, J. Chen, T. Li, X. Wang, J. Yang, C. Chen, L. Sun, Intra-articular injection of etoricoxib-loaded PLGA-PEG-PLGA triblock copolymeric nanoparticles attenuates osteoarthritis progression, *Am. J. Tourism Res.* 11 (11) (2019) 6775–6789.
- [33] A. Petit, M. Sandker, B. Müller, R. Meyboom, P. van Midwoud, P. Bruin, E. M. Redout, M. Versluis-Helder, C.H. van der Lest, S.J. Buwalda, L.G. de Leede, T. Vermonden, R.J. Kok, H. Weinans, W.E. Hennink, Release behavior and intra-articular biocompatibility of celecoxib-loaded acetyl-capped PCLA-PEG-PCLA thermogels, *Biomaterials* 35 (27) (2014) 7919–7928.
- [34] S. Zhong, J. Wang, Q. Zhang, H. Xu, J. Feng, CircPrimer, A software for annotating circRNAs and determining the specificity of circRNA primers, *BMC Bioinf.* 19 (1) (2018) 292.
- [35] X. Cheng, L. Zhang, K. Zhang, G. Zhang, Y. Hu, X. Sun, C. Zhao, H. Li, Y.M. Li, J. Zhao, Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200c and X linked inhibitor-of-apoptosis protein, *Ann. Rheum. Dis.* 77 (5) (2018) 770–779.
- [36] S.C. Tao, T. Yuan, B.Y. Rui, Z.Z. Zhu, S.C. Guo, C.Q. Zhang, Exosomes derived from human platelet-rich plasma prevent apoptosis induced by glucocorticoid-associated endoplasmic reticulum stress in rat osteonecrosis of the femoral head via the Akt/Bad/Bcl-2 signal pathway, *Theranostics* 7 (3) (2017) 733–750.
- [37] C. Atkins, Q. Liu, E. Minthorn, S.Y. Zhang, D.J. Figueroa, K. Moss, T.B. Stanley, B. Sanders, A. Goetz, N. Gaul, A.E. Choudhry, H. Alsaïd, B.M. Jucker, J.M. Axten, R. Kumar, Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity, *Canc. Res.* 73 (6) (2013) 1993–2002.
- [38] M. Costa-Mattioli, D. Gobert, E. Stern, K. Gamache, R. Colina, C. Cuello, W. Sossin, R. Kaufman, J. Pelletier, K. Rosenblum, K. Krnjević, J.C. Lacaille, K. Nader, N. Sonenberg, eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory, *Cell* 129 (1) (2007) 195–206.
- [39] M.C. Choi, J. Jo, J. Park, H.K. Kang, Y. Park, NF-κB signaling pathways in osteoarthritic cartilage destruction, *Cells* 8 (7) (2019).
- [40] E. Jimi, H. Fei, C. Nakatomi, NF-κB signaling regulates physiological and pathological chondrogenesis, *Int. J. Mol. Sci.* 20 (24) (2019).
- [41] T.W. Jung, S.H. Lee, H.C. Kim, J.S. Bang, A.M. Abd El-Aty, A. Hacımüftüoğlu, Y. K. Shin, J.H. Jeong, METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice, *Exp. Mol. Med.* 50 (9) (2018) 122.
- [42] X. Yang, S. Xu, Y. Qian, Q. Xiao, Resveratrol regulates microglia M1/M2 polarization via PGC-1α in conditions of neuroinflammatory injury, *Brain Behav. Immun.* 64 (2017) 162–172.
- [43] Y. Liu, J. Zhai, J. Chen, X. Wang, T. Wen, PGC-1α protects against oxidized low-density lipoprotein and luteinizing hormone-induced granulosa cells injury through ROS-p38 pathway, *Hum. Cell* 32 (3) (2019) 285–296.
- [44] Y. Yuana, R.I. Koning, M.E. Kuil, P.C. Rensen, A.J. Koster, R.M. Bertina, S. Osanto, Cryo-electron microscopy of extracellular vesicles in fresh plasma, *J. Extracell. Vesicles* 2 (2013).
- [45] S. Santasusagna, I. Moreno, A. Navarro, J.J. Castellano, F. Martínez, R. Hernández, C. Muñoz, M. Monzo, Proteomic analysis of liquid biopsy from tumor-draining vein indicates that high expression of exosomal ECMI is associated with relapse in stage I-III colon cancer, *Transl. Oncol.* 11 (3) (2018) 715–721.
- [46] F.N. Faruqi, J.T. Wang, L. Xu, L. McNickle, E.M. Chong, A. Walters, M. Gurney, A. Clayton, L.A. Smyth, R. Hider, J. Sosabowski, K.T. Al-Jamal, Membrane radiolabelling of exosomes for comparative biodistribution analysis in immunocompetent and immunodeficient mice - a novel and universal approach, *Theranostics* 9 (6) (2019) 1666–1682.
- [47] S.C. Guo, S.C. Tao, W.J. Yin, X. Qi, T. Yuan, C.Q. Zhang, Exosomes derived from platelet-rich plasma promote the re-epithelialization of chronic cutaneous wounds via activation of YAP in a diabetic rat model, *Theranostics* 7 (1) (2017) 81–96.
- [48] S.C. Tao, J.Y. Huang, Z.Y. Wei, Z.X. Li, S.C. Guo, EWSAT1 acts in concert with exosomes in osteosarcoma progression and tumor-induced angiogenesis: the "double stacking effect", *Adv. Biosyst.* 4 (9) (2020), e2000152.
- [49] S.C. Tao, S.C. Guo, Extracellular vesicles in bone: "dogrobbers" in the "eternal battle field", *Cell Commun. Signal.* 17 (1) (2019) 6.
- [50] S.C. Guo, S.C. Tao, H. Dawn, Microfluidics-based on-a-chip systems for isolating and analysing extracellular vesicles, *J. Extracell. Vesicles* 7 (1) (2018) 1508271.
- [51] S.C. Tao, S.C. Guo, Extracellular vesicles: potential participants in circadian rhythm synchronization, *Int. J. Biol. Sci.* 14 (12) (2018) 1610–1620.
- [52] S.C. Tao, S.C. Guo, C.Q. Zhang, Platelet-derived extracellular vesicles: an emerging therapeutic approach, *Int. J. Biol. Sci.* 13 (7) (2017) 828–834.
- [53] K.J. Kurppa, Y. Liu, C. To, T. Zhang, M. Fan, A. Vajdi, E.H. Knelson, Y. Xie, K. Lim, P. Cejas, A. Portell, P.H. Lizotte, S.B. Ficarro, S. Li, T. Chen, H.M. Haikala, H. Wang, M. Bahcall, Y. Gao, S. Shalhout, S. Boettcher, B.H. Shin, T. Thai, M.K. Wilkens, M. L. Tillgren, M. Mushajiang, M. Xu, J. Choi, A.A. Bertram, B.L. Ebert, R. Beroukhim, P. Bandopadhyay, M.M. Awad, P.C. Gokhale, P.T. Kirschmeier, J.A. Marto, F. D. Camargo, R. Haq, C.P. Paweletz, K.K. Wong, D.A. Barbie, H.W. Long, N.S. Gray, P.A. Jänne, Treatment-induced tumor dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway, *Canc. Cell* 37 (1) (2020) 104–122, e12.
- [54] Y. Song, J. Fu, M. Zhou, L. Xiao, X. Feng, H. Chen, W. Huang, Activated hippo/yes-associated protein pathway promotes cell proliferation and anti-apoptosis in endometrial stromal cells of endometriosis, *J. Clin. Endocrinol. Metab.* 101 (4) (2016) 1552–1561.
- [55] M. Qiu, D. Chen, C. Shen, J. Shen, H. Zhao, Y. He, Platelet-Rich plasma-loaded poly (d,l-lactide)-poly(ethylene glycol)-Poly(d,l-lactide) hydrogel dressing promotes full-thickness skin wound healing in a rodent model, *Int. J. Mol. Sci.* 17 (7) (2016).
- [56] H. Elkhenany, A. AlOkda, A. El-Badawy, N. El-Badri, Tissue regeneration: impact of sleep on stem cell regenerative capacity, *Life Sci.* 214 (2018) 51–61.
- [57] Y. Zhu, G. Wu, G. Zhu, C. Ma, H. Zhao, Chronic sleep restriction induces changes in the mandibular condylar cartilage of rats: roles of Akt, Bad and Caspase-3, *Int. J. Clin. Exp. Med.* 7 (9) (2014) 2585–2592.
- [58] G. Wu, L. Chen, G. Wei, Y. Li, G. Zhu, Z. Zhao, F. Huang, Effects of sleep deprivation on pain-related factors in the temporomandibular joint, *J. Surg. Res.* 192 (1) (2014) 103–111.
- [59] C. Liang, T. Yang, G. Wu, J. Li, W. Geng, Therapeutic effect of low-intensity pulsed ultrasound on temporomandibular joint injury induced by chronic sleep deprivation in rats, *Am. J. Tourism Res.* 11 (6) (2019) 3328–3340.
- [60] J.K. Wyatt, A. Ritz-De Cecco, C.A. Czeisler, D.J. Dijk, Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day, *Am. J. Physiol.* 277 (4 Pt 2) (1999) R1152–R1163.
- [61] Z. Wu, X. Qiu, B. Gao, C. Lian, Y. Peng, A. Liang, C. Xu, W. Gao, L. Zhang, P. Su, L. Rong, D. Huang, Melatonin-mediated miR-526b-3p and miR-590-5p upregulation promotes chondrogenic differentiation of human mesenchymal stem cells, *J. Pineal Res.* 65 (1) (2018), e12483.
- [62] W. Gao, M. Lin, A. Liang, L. Zhang, C. Chen, G. Liang, C. Xu, Y. Peng, C. Chen, D. Huang, P. Su, Melatonin enhances chondrogenic differentiation of human mesenchymal stem cells, *J. Pineal Res.* 56 (1) (2014) 62–70.
- [63] D.S. Rudra, U. Pal, N.C. Maiti, R.J. Reiter, S. Swarnakar, Melatonin inhibits matrix metalloproteinase-9 activity by binding to its active site, *J. Pineal Res.* 54 (4) (2013) 398–405.
- [64] H.D. Lim, Y.S. Kim, S.H. Ko, I.J. Yoon, S.G. Cho, Y.H. Chun, B.J. Choi, E.C. Kim, Cytoprotective and anti-inflammatory effects of melatonin in hydrogen peroxide-stimulated CHON-001 human chondrocyte cell line and rabbit model of osteoarthritis via the SIRT1 pathway, *J. Pineal Res.* 53 (3) (2012) 225–237.
- [65] Q. Liu, X. Zhang, X. Hu, L. Yuan, J. Cheng, Y. Jiang, Y. Ao, Emerging roles of circRNA related to the mechanical stress in human cartilage degradation of osteoarthritis, molecular therapy, *Nucleic Acids* 7 (2017) 223–230.
- [66] M. Rahmati, G. Nalesso, A. Mobasher, M. Mozafari, Aging and osteoarthritis: central role of the extracellular matrix, *Ageing Res. Rev.* 40 (2017) 20–30.
- [67] Y. Krishnan, A.J. Grodzinsky, Cartilage diseases, *Matrix Biol.* 71–72 (2018) 51–69.
- [68] S. Browne, M.G. Monaghan, E. Brauchle, D.C. Berrio, S. Chantepie, D. Papy-Garcia, K. Schenke-Layland, A. Pandit, Modulation of inflammation and angiogenesis and changes in ECM GAG-activity via dual delivery of nucleic acids, *Biomaterials* 69 (2015) 133–147.
- [69] H.J. Im, P. Muddasani, V. Natarajan, T.M. Schmid, J.A. Block, F. Davis, A.J. van Wijnen, R.F. Loeser, Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes, *J. Biol. Chem.* 282 (15) (2007) 11110–11121.
- [70] D.M. Lee, H.P. Kiener, S.K. Agarwal, E.H. Noss, G.F. Watts, O. Chisaka, M. Takeichi, M.B. Brenner, Cadherin-11 in synovial lining formation and pathology in arthritis, *Science (New York, N.Y.)* 315 (5814) (2007) 1006–1010.
- [71] A.M. Mustonen, P. Nieminen, A. Joukainen, A. Jaroma, T. Kääriäinen, H. Kröger, E. Lázaro-Ibáñez, P.R. Siljander, V. Kärjä, K. Härkönen, A. Koistinen, K. Rilla, First in vivo detection and characterization of hyaluronan-coated extracellular vesicles in human synovial fluid, *J. Orthop. Res. : Off. Publ. Orthopaedic Res. Soc.* 34 (11) (2016) 1960–1968.
- [72] A.S. Arabyat, H. Chen, J. Erndt-Marino, K. Burkhard, L. Scola, A. Fleck, L.Q. Wan, M.S. Hahn, Hyperosmolar Ionic Solutions Modulate Inflammatory Phenotype and sGAG Loss in a Cartilage Explant Model, *Cartilage*, 2020, 1947603520961167.
- [73] P. Diaz-Rodríguez, J. Erndt-Marino, H. Chen, J.F. Diaz-Quiroz, S. Samavedi, M. S. Hahn, A bioengineered in vitro osteoarthritis model with tunable inflammatory environments indicates context-dependent therapeutic potential of human mesenchymal stem cells, *Regen. Eng. Transl. Med.* 5 (3) (2019) 297–307.
- [74] P. Diaz-Rodríguez, H. Chen, J.D. Erndt-Marino, F. Liu, F. Totsingan, R.A. Gross, M. S. Hahn, Impact of select sphorolipid derivatives on macrophage polarization and viability, *ACS Appl. Bio Mater.* 2 (1) (2019) 601–612.
- [75] A.S. Arabyat, P. Diaz-Rodríguez, J.D. Erndt-Marino, F. Totsingan, S. Mekala, R. A. Gross, M.S. Hahn, Effect of poly(sphorolipid) functionalization on human mesenchymal stem cell osteogenesis and immunomodulation, *ACS Appl. Bio Mater.* 2 (1) (2019) 118–126.

- [76] G.R. Willis, A. Fernandez-Gonzalez, J. Anastas, S.H. Vitali, X. Liu, M. Ericsson, A. Kwong, S.A. Mitsialis, S. Kourembanas, Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation, *Am. J. Respir. Crit. Care Med.* 197 (1) (2018) 104–116.
- [77] T.J. Morrison, M.V. Jackson, E.K. Cunningham, A. Kissenpfennig, D.F. McAuley, C. M. O’Kane, A.D. Krasnodembskaya, Mesenchymal stromal cells modulate macrophages in clinically relevant lung injury models by extracellular vesicle mitochondrial transfer, *Am. J. Respir. Crit. Care Med.* 196 (10) (2017) 1275–1286.
- [78] S. Zhang, S.J. Chuah, R.C. Lai, J.H.P. Hui, S.K. Lim, W.S. Toh, MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity, *Biomaterials* 156 (2018) 16–27.
- [79] M. Asem, A.M. Young, C. Oyama, A. Claire De La Zerda, Y. Liu, J. Yang, T. S. Hilliard, J. Johnson, E.I. Harper, I. Guldner, S. Zhang, T. Page-Mayberry, W. J. Kaliney, M.S. Stack, Host Wnt5a potentiates microenvironmental regulation of ovarian cancer metastasis, *Canc. Res.* 80 (5) (2020) 1156–1170.
- [80] Q. Liu, C. Yang, S. Wang, D. Shi, C. Wei, J. Song, X. Lin, R. Dou, J. Bai, Z. Xiang, S. Huang, K. Liu, B. Xiong, Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression, *Cell Commun. Signal.* 18 (1) (2020) 51.
- [81] Y. Feng, Y. Liang, X. Zhu, M. Wang, Y. Gui, Q. Lu, M. Gu, X. Xue, X. Sun, W. He, J. Yang, R.L. Johnson, C. Dai, The signaling protein Wnt5a promotes TGF β 1-mediated macrophage polarization and kidney fibrosis by inducing the transcriptional regulators Yap/Taz, *J. Biol. Chem.* 293 (50) (2018) 19290–19302.
- [82] H.F. Darge, A.T. Andrgie, E.Y. Hanurrry, Y.S. Birhan, T.W. Mekonnen, H.Y. Chou, W.H. Hsu, J.Y. Lai, S.Y. Lin, H.C. Tsai, Localized controlled release of bevacizumab and doxorubicin by thermo-sensitive hydrogel for normalization of tumor vasculature and to enhance the efficacy of chemotherapy, *Int. J. Pharm.* 572 (2019) 118799.
- [83] J. Shao, C. Ruan, H. Xie, Z. Li, H. Wang, P.K. Chu, X.F. Yu, Black-phosphorus-incorporated hydrogel as a sprayable and biodegradable photothermal platform for postsurgical treatment of cancer, *Adv. Sci. (Weinheim, Baden-Wuerttemberg, Germany)* 5 (5) (2018) 1700848.