Biologics: Targets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

siRNAs, tRNAs, and rRNAs in Osteoarthritis: Biological Functions and Therapeutic Opportunities

Mina Wang[1,*], Yidan Dai[1,*], Xiaobo Ge[1,*], Libin Zheng[1,2], Miaoxin Zhai[3], Wenshan Li[1,2], Guannan Liu[1], Xiaoyu Cheng[1], Jiangyan Wei[1], Xin Yang[1], Lu Liu[1], Huilin Liu[1], Jingqing Sun[1], Bin Li[1], Fang Yuan[1]

[1]Department of Acupuncture and Moxibustion, Beijing Hospital of Traditional Chinese Medicine, Beijing, People's Republic of China; [2]Graduate School, Beijing University of Chinese Medicine, Beijing, People's Republic of China; [3]Yinghai Hospital in Daxing District, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Fang Yuan; Bin Li, Email yuanfang@bjzhongyi.com; libin@bjzhongyi.com

Abstract: Osteoarthritis (OA) is a prevalent chronic disease, characterized by progressive joint degeneration and primarily affects older adults. OA leads to reduced functional abilities, a lower quality of life, and an increased mortality rate. Currently, effective treatment options for OA are lacking. Non-coding RNAs (ncRNAs) are functional RNAs transcribed from DNA but not translated into proteins. Among ncRNAs, small interfering RNAs (siRNAs), transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs) have become significant in the field, which is intricately linked to the progression of OA and perform significant regulatory functions in transcription, post-transcription, and post-translation, making them potential biological targets for the prevention, diagnosis, and treatment of OA. This review summarizes the general functions of siRNAs, tRNAs, and rRNAs and their application in OA. The primary focus has been on regulating cartilage degradation. Other participations include regulating synovium, protecting anterior cruciate ligament cells, and diagnosis. No clinical trials were found as challenges such as effective delivery systems, immune responses, long-term effects, and interactions between therapies need to be demonstrated first.

Keywords: osteoarthritis, non-coding RNAs, small interfering RNAs, transfer RNAs, ribosomal RNAs

Introduction

Osteoarthritis, commonly abbreviated as OA, is a highly prevalent chronic disorder characterized by the gradual and progressive deterioration of articular joints, predominantly affecting the elderly demographic. Risk factors of OA include aging, obesity, female gender, knee injuries and high-impact sports, among which increasing age and obesity are considered the most significant contributors.[1] OA leads to reduced functional abilities, lower quality of life, and concomitant increased mortality.[2] Over 7.6% of the global population (596 million individuals) are affected, and the average annual costs range from US $700 to US $15,600 per person.[3] The primary symptoms of OA include pain, stiffness, and restricted mobility, which are caused by articular cartilage, synovitis, subchondral bone thickening, osteophyte formation and infrapatellar fat pad (IPFP) inflammation.[4] The earliest pathological changes in OA are typically observed on the surface of articular cartilage, where fibrillation occurs in localized regions subjected to maximal load. In response to matrix loss, there is a marked proliferation of chondrocytes, the sole cell type present within cartilage. Some chondrocytes undergo a phenotypic transformation into hypertrophic chondrocytes, resembling those found in the hypertrophic zones of growth plates. As OA progresses, significant degradation and loss of the extracellular matrix occur due to the sustained production of proteases induced by pro-inflammatory cytokines, such as IL-1, IL-6, and IL-8. These cytokines facilitate an autocrine and paracrine signalling loop that compels chondrocytes to generate additional cytokines and proteases.[5] Consequently, as significant damage to the matrix occurs, areas devoid of

Received: 24 February 2025
Accepted: 17 August 2025
Published: 3 September 2025

cells become apparent due to chondrocyte apoptosis.[6] The changes in bone associated with OA include subchondral sclerosis, which arises from increased collagen production, as well as the formation of osteophytes and bone cysts at more advanced stages. Osteophytes are characterized as outgrowths of bone and cartilage that occur in the joint area. The direction of osteophyte growth is influenced by the size of the joint and local cartilage narrowing, with exceptions noted for the lateral tibia and medial patella.[7] Biomechanical factors play a significant role in supporting the development of osteophytes. Furthermore, most patients exhibiting symptoms of OA demonstrate synovial inflammation and hypertrophy.[8] The IPFP is the largest adipose tissue structure within the knee joint, comprising adipocytes, immune cells, and blood vessels. Additionally, the IPFP is situated near both cartilage and bone surfaces, which may help mitigate loading impacts and absorb forces transmitted through the knee joint. This positioning suggests a potential protective role for IPFP in maintaining joint health. Research has demonstrated that the IPFP secretes various cytokines and adipokines that contribute to pro-inflammatory and pro-catabolic processes in cartilage, thereby promoting the progression of OA.[9] Recent studies indicate that the infrapatellar fat pad in OA transforms into a stiffer adipose tissue, likely as a result of fibrosis. This alteration may contribute to changes in joint mechanics and promote inflammation.[10]

Currently, conventional treatments provide only palliative relief from symptomatic discomfort but do not impede the progression of OA.[11] These conventional treatments mainly involve the use of analgesics and anti-inflammatory medications, such as nonsteroidal anti-inflammatory drugs, are beneficial for relieving pain and improving joint function.[12] However, none of these medications can effectively slow or halt the progression of OA, and long-term treatment is often required. Moreover, excessive use of these drugs may result in diminished therapeutic efficacy and the occurrence of significant adverse effects.[13] The lack of effective treatment options for OA indicates an incomplete understanding of the underlying pathology. Over the last two decades, numerous studies have explored the complex gene expression regulatory networks involved in OA. Recent evidence has highlighted the multifaceted regulatory roles played by ncRNAs in maintaining cartilage homeostasis, as well as their active involvement in the pathological progression of OA.[14]

In the past, researchers have disregarded the importance of ncRNAs, labelling them as "junk RNA". However, with advancements in molecular biology technology, a growing body of research has revealed the crucial roles played by ncRNAs in epigenetic regulation, controlling gene and protein expression, and influencing the initiation and progression of various pathophysiological states within biological processes.[15]

ncRNAs are a class of functional RNA molecules that are transcribed from DNA yet do not undergo the process of translation into proteins, distinguishing them from other types of RNA that serve as templates for protein synthesis. They are categorised into transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small RNAs, long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), microRNAs (miRNAs) and small interfering RNAs (siRNAs). ncRNAs play a role in various pathological processes associated with osteoarthritis, including cell proliferation, migration, chondrogenesis, induction of chondrocyte differentiation, extracellular matrix formation, inflammation and apoptosis.[16] miRNAs and lncRNAs are two important types of small RNAs, and together with circRNAs, they have been extensively studied for their maintenance of cartilage homeostasis, regulation in skeletal homeostasis and inflammatory response.[17] For instance, Exosomes derived from human bone marrow mesenchymal stem cells (MSCs) overexpressing miR-320c have been shown to downregulate matrix metalloproteinase 13 (MMP13) expression while upregulating the expression of SOX9 and COL2A1 during chondrogenic differentiation of human bone marrow MSCs.[18] Zhou et al[19] proposed that exosomal miR-126-3p derived from synovial fibroblasts exhibits anti-inflammatory properties on articular cartilage and effectively inhibits the expression of inflammatory cytokines, including IL-1β, IL-6, and TNF-α, in chondrocytes. Yan et al[20] found that LncRNA H19 functions as a competing endogenous RNA against miR-29b-3p, thereby upregulating FoxO3 expression in chondrocytes and promoting sustained cartilage repair. Moreover, Mao et al[21] reported that the upregulation of exosomal circRNA_0001236 promoted chondrogenesis in human mesenchymal stem cells (hMSCs) by enhancing the expression of Col2a1 and Sox9, while simultaneously suppressing MMP13 expression. Although siRNAs, tRNAs, and rRNAs participate in regulating chondrocyte inflammation, proliferation, apoptosis, autophagy, and extracellular matrix (ECM) metabolism in OA pathogenesis, they have not been well studied. Therefore, we aim to provide a comprehensive framework for understanding the biological role and therapeutic potential of siRNAs, tRNAs and rRNAs in OA.

Types and Functions of ncRNAs

siRNAs

siRNAs are a class of non-coding double-stranded RNAs molecules, typically ranging from 19 to 25 nucleotide base pairs in length. Notably, siRNA molecules are not naturally present in cells under physiological conditions. They are generally synthetic in nature or originate from double-stranded RNA that is artificially introduced into cells. Characterized by their diminutive size, usually less than 10 nanometres, and a molecular weight approximating 15 kDa, siRNAs feature a 2-nucleotide-3′ overhang akin to endogenous miRNAs.[22] Generated from precursor molecules such as long double-stranded RNAs (dsRNAs) and small hairpin RNAs under the catalytic activity of the enzyme dicer (Figure 1A), siRNAs mediate gene silencing by impeding the translation process of specific target genes, a phenomenon known as RNA interference (RNAi).[23] Therefore, siRNAs are used as a critical therapeutic tool in many diseases, such as genetic diseases, metabolic diseases and cancer.[24]

The primary function of siRNAs is to regulate gene expression through a process known as RNAi. Upon introduction into a cell, siRNA molecules specifically target and bind to complementary messenger RNA (mRNA) sequences. This binding event triggers the degradation or inhibition of the targeted mRNA, thereby preventing its protein translation. By effectively silencing the expression of specific genes, siRNAs play a crucial role in various biological processes, including developmental regulation, cellular homeostasis, and defence against viral infections.[25,26]

siRNAs share functional similarities with miRNAs, albeit with a notable distinction: miRNAs typically modulate the expression of numerous genes through imperfect base pairing, whereas siRNAs exhibit a more precise binding affinity to a singular gene at a defined locus.[27]

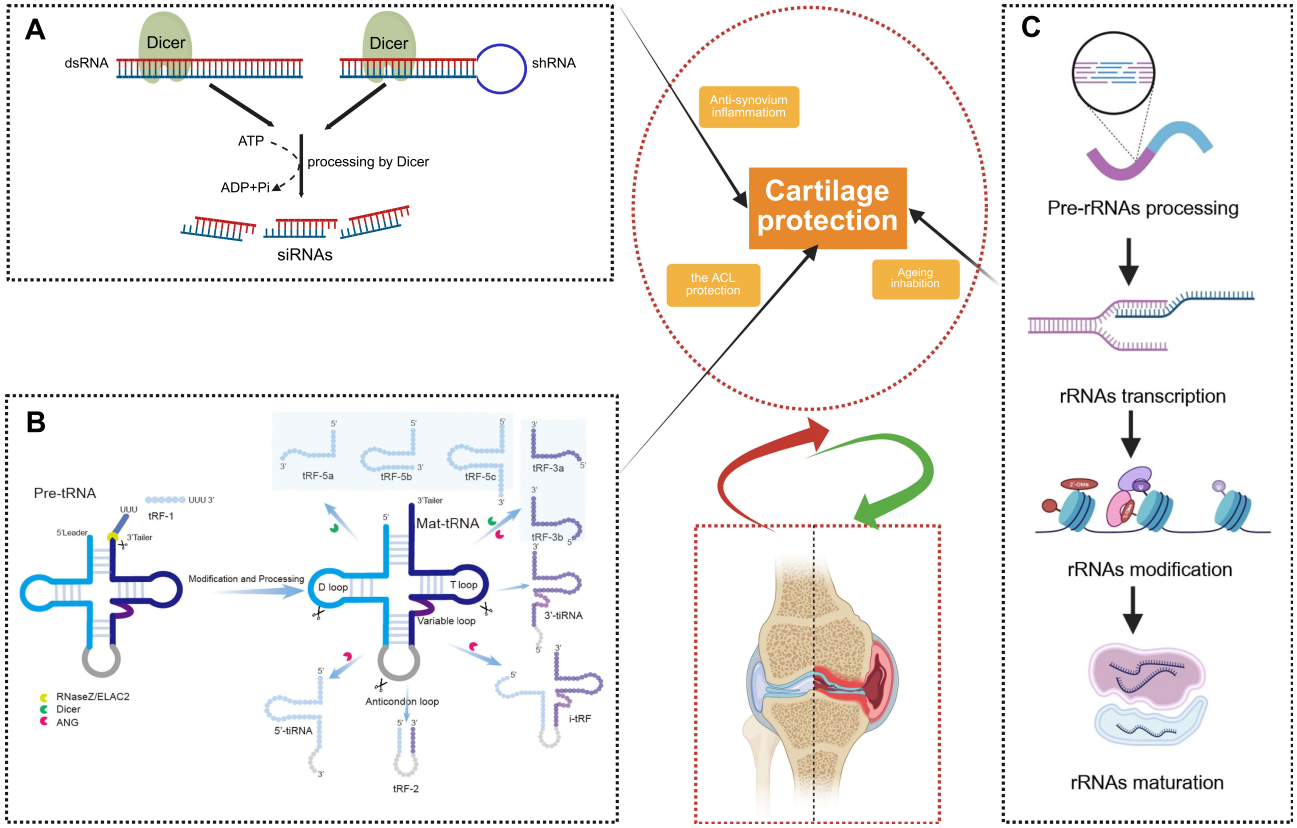

Figure 1 Biogenesis of three non-coding RNAs and their roles in regulation OA. (**A**) Biogenesis of siRNAs. Dicer processes dsRNA and shRNA to generate siRNAs in an ATP-dependent manner, yielding ADP and inorganic phosphate. (**B**) Biogenesis of tRNAs. pre-tRNA undergoes modification and processing by enzymes (eg, RNase Z/ELAC2, Dicer, ANG) to produce distinct tRFs. (**C**) Biogenesis of rRNAs. pre-rRNA transcription, modification, and maturation are essential for ribosomal function. siRNAs, tRNAs, and rRNAs collectively modulate OA pathogenesis through cartilage protection, synovial inflammation suppression, ACL preservation and anti-aging effects.
Abbreviations: ADP, adenosine diphosphate; ANG, angiopoietin; ATP, adenosine triphosphate; dsRNAs, double-stranded RNAs; OA, osteoarthritis; rRNA, ribosomal RNA; shRNAs, short hairpin RNA; shRNA, short hairpin RNA; siRNAs, small interfering RNAs; tRF, tRNA-derived fragment; tiRNAs, tRNA-derived stress-induced RNAs.

https://doi.org/10.2147/BTT.S521180

Compared to traditional pharmaceutical agents like small molecules and antibodies, siRNAs present distinct advantages, including abundant disease targets, elevated success rates in development, shortened developmental timelines, sustained and potent efficacy, and the versatile characteristics inherent in platform-based modalities.[28,29] In recent times, the therapeutic application of siRNA has exhibited promising prospects in advancing a multitude of drug candidates during preclinical and clinical investigative endeavors.[30]

tRNAs

tRNAs are RNA molecules typically comprised of 73–90 nucleotides, featuring distinct structural elements including a D-loop, T loop, variable loop, and the anticodon loop (Figure 1B).[31] Among cellular RNAs, tRNAs are notably abundant, constituting approximately 12% of the total RNA in many cell types.[32] tRNAs are essential for protein synthesis, as they enable the decoding of mRNA codons and ensure the delivery of corresponding amino acids to ribosomes, thereby supporting the elongation of polypeptide chains.[31] In addition to their canonical function in translation, tRNAs are involved in a variety of other cellular activities, including the regulation of neuronal homeostasis through the action of certain decoders,[33] the modulation of pro-metastatic gene expression,[34] and the mobilization of adaptive translation by nucleotide changes.[35]

tRNA-derived small RNAs (tsRNAs) are RNA fragments of specific lengths generated by certain ribonucleases, including dicer and angiogenin (ANG). These tsRNAs are generated as a result of the specific and targeted clipping of tRNAs within designated cells and tissues, occurring under particular and defined circumstances.[36] With the ability to bind to proteins or mRNAs, tsRNAs have a wide range of applications in various biological processes. They play crucial roles in RNA epigenetic, translational regulation, and silencing.[37,38] Moreover, tsRNAs regulate cell proliferation and apoptosis[37] by interfering with cell cycle process.

tRNA-derived fragments (tRFs) and tRNA-derived stress-induced RNAs (tiRNAs) are two main types of tsRNA. Both may originate from the splitting of specialized, mature tRNAs or their respective precursors.

In 1958, Zamecnik and Hoagland conducted research that led to the identification of tRNAs with the ability to translocate 14C-labeled amino acids to newly synthesized proteins.[39] Within the nucleus, the precursor tRNAs (pre-tRNAs) are first transcribed by RNA polymerase III and subsequently undergo modifications, including 5′ and 3′ end processing by ribonuclease P (RNase P) and RNase Z, and further modifications during tRNA maturation.[40] The specific cleavage of tRNAs at designated positions mediated by distinct ribonucleases gives rise to a spectrum of tRFs, which encompasses tRF-1, tRF-2, tRF-3, tRF-5, as well as i-tRF.[38]

The presence of seed sequences in tRFs that correspond to crosslink-centered areas of target mRNAs is noteworthy.[41] A considerable number of research studies have robustly indicated that tRFs fulfill a role akin to that of miRNAs, ultimately resulting in the silencing of mRNA.[42] The tRFs-mRNA hybrid molecules have the capacity to interact with Argonaute (AGO) complexes in a fashion akin to miRNAs. They utilize a specific 5 “seed sequence” comprising 7–8 nucleotide bases to facilitate the recruitment of target mRNAs into the AGO/RNA-induced silencing complex (RISC).[43] This process serves to regulate post-transcriptional gene expression. Advances in high-throughput sequencing technology have facilitated numerous studies, revealing a crucial role for tRFs in the pathogenesis of various diseases. tRF-3022b, for instance, may affect colorectal cancer tumour proliferation and M2 macrophage polarization by binding to macrophage migration inhibitory factor (MIF) and galectin 1 (LGALS1).[44]

tiRNAs are induced in response to cellular stress. When cells encounter various stressors such as oxidative stress, heat shock, or nutrient deprivation, they undergo rapid changes in gene expression to adapt and survive. These tiRNAs are typically short RNA fragments, ranging from 18 to 22 nucleotides in length, and are derived from tRNA molecules.[45] They are generated through specific cleavage of tRNAs, often occurring at or near the anticodon loop region. The precise mechanisms underlying the generation of tiRNAs during stress conditions are still being investigated, but they likely involve stress-responsive enzymes or ribonucleases.[46]

tiRNAs are associated with stress response and adaptation in cellular processes. They can regulate gene expression by interacting with other RNA molecules, such as mRNAs, miRNAs, or lncRNAs.[47] Additionally, tiRNAs may influence protein synthesis, translation efficiency, and cellular signalling pathways, thereby contributing to the cellular stress response.[48]

rRNAs

rRNAs, the primary component of the most abundant cellular molecule, the ribosome, comprising about 80% of total cellular RNA, derive from a precursor transcript known as pre-rRNA, which stems from multicopy genes housed within the nucleolus (Figure 1C).[49,50] In eukaryotes, the synchronized function of all three nuclear RNA polymerases, specifically, Pol I, II, and III, holds a pivotal significance in the biosynthesis of ribosomes.[50] In rRNA structures, the dominant conformational state is A-form RNA, which is prevalent in both double-helical and single-stranded regions.[51] A significant quantity of nucleolar RNAs is prone to the formation of nucleic acid structures referred to R loops.[52] These structures function as modulators of genome dynamics and functionality, thereby exerting an influence on transcriptional regulation.

rRNA synthesis, processing, and modifications are essential for ribosome biogenesis in humans and play a pivotal role in protein synthesis and cellular function across all organisms.[53,54] The transcription of rRNA genes serves as a critical regulatory step in the ribosome biogenesis pathway. This process is essential for cellular adaptation, stress response, development, proliferation, and meeting the energy demands of cells.[55] The crucial role that abundant and meticulously regulated rRNA production plays within a functional nucleolus in sustaining cell viability underscores the necessity for the synchronized action of essential enzymes, related factors, and epigenetic modifiers.[56] The dysfunction of rRNAs may lead to various disorders, such as Parkinson's disease (PD),[57] breast cancer,[58,59] and obesity.[60]

The Role of ncRNAs in OA

siRNA in OA

Through the suppression or enhancement of protein expression, siRNA treatment may be designed to either upregulate or downregulate a specific gene and function as OA disease-modifying medications (Figure 2). The primary cell types addressed in OA are chondrocytes and synoviocytes. Aberrant chondrocyte and synoviocyte metabolism represent an adaptive response to alterations in the inflammatory microenvironment and may contribute significantly to cartilage degeneration and the progression of OA. Under conditions of environmental stress, these cells tend to adjust their metabolic profiles by transitioning between different metabolic pathways, for instance, shifting from oxidative phosphorylation to glycolysis. Such metabolic shifts are associated with mitochondrial dysfunction, increased anaerobic

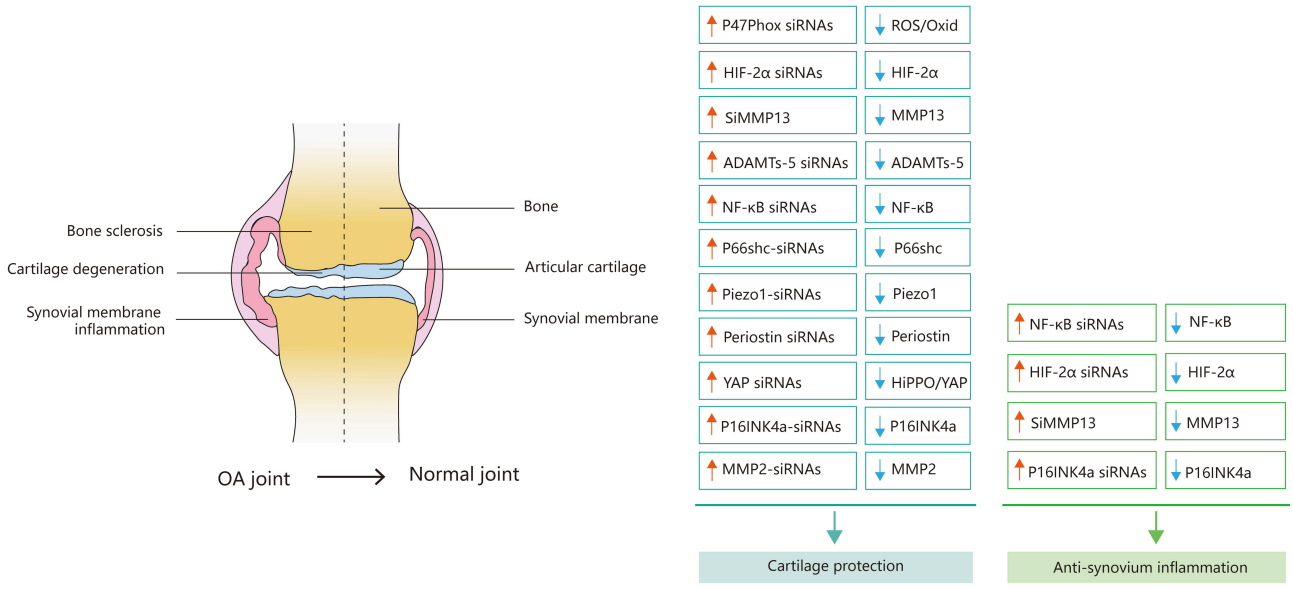

Figure 2 Molecular mechanisms underlying therapeutic siRNA modulation of key pathways for chondroprotection and synovitis amelioration in osteoarthritis joint restoration.

Abbreviations: ADAMTs, a disintegrin and metalloproteinase with thrombospondin-like motifs; HIF-2α, Hypoxia induced factor-2α; MMP, matrix metalloproteinase; NF-κB, nuclear factor-kappa B; OA, osteoarthritis; meniscectomy; p16INK4a, cyclin-Dependent Kinase Inhibitor p16; ROS, reactive oxygen species; siRNAs, small interfering RNAs.

https://doi.org/10.2147/BTT.S521180

glycolysis, as well as dysregulation of lipid and amino acid metabolism. This metabolic flexibility is primarily governed by the AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) signaling pathways.[61]

Chondrocytes are the sole cellular components of articular cartilage, and their functional integrity is crucial for maintaining cartilage homeostasis. Chondrocyte necrosis, autophagy and apoptosis are vital feature of OA. Chondrocyte necrosis is generally less common in OA, as it is typically an acute process that occurs only when tissues are exposed to severe mechanical injury or highly toxic substances.[62] Autophagy, a cellular degradation system that regulates energy metabolism, plays a critical role in the progression of osteoarthritis. In the early stages of OA, autophagy is upregulated in chondrocytes, serving as an adaptive mechanism to protect these cells from various environmental stressors. However, as OA progresses, chondrocytic autophagy declines gradually, resulting in the accumulation of damaged organelles and macromolecules within the cells, which ultimately promotes chondrocyte apoptosis.[63] Chondrocyte apoptosis, a non-classical form of chondrocyte death, occurs under abnormal chondrocyte conditions as a mechanism to eliminate cellular remnants without inducing inflammation, such as in cases of hyperthermia. Chondroptosis contributes to the degradation of the cartilage matrix and a reduction in tissue integrity.[64]

siRNAs can regulate cartilage ECM, including ECM inflammation and metabolic homeostasis. Table 1 lists several cells and key pathways that were targeted using siRNAs.

Prevention of Cartilage Degradation

ECM degradation and the inflammatory response are significant factors in the underlying mechanisms that contribute to the development of OA.[88] It is predominantly comprised of Collagen II and proteoglycan, whereas chondrocytes, as individual cellular constituents, primarily sustain cartilage homeostasis through the regulation of catabolic and anabolic metabolic processes.[89] In OA, the gene-silencing capability of custom-designed siRNAs targeting specific pathways implicated in disease pathogenesis has opened new avenues for therapeutic intervention. The potential strategy of knocking down or silencing various pathogenic pathways, including matrix metalloproteinases (MMPs), nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), and the JAK/signal transducer and activator of transcription (JAK/STAT) pathway, has been explored.

Matrix-degrading enzymes, such as MMPs and ADAMTs, exert a vital role in cartilage destruction. TNF-α, IL-1β and IL-6 enhance gene expression, leading to the degradation of the ECM and an inflammatory response.[90] According to contemporary research, MMPs (1, 3, and 13) and ADAMTs (4 and 5) predominantly destroy Aggrecan, whereas MMPs, especially MMP13, degrade Collagen II.[91–93] Thus, by reducing MMPs expression, Aggrecan and Collagen II can be effectively accumulated, ECM degradation can be delayed and inflammatory mediators can be reduced. MMP13 stands as a pivotal proteolytic enzyme in the process of cartilage degradation observed in OA. Sean K Bedingfield et al[65] expanded the application of polymeric siRNA nanopolyplexes (NPs) to develop a targeted delivery system capable of selectively recognizing early OA cartilage lesions. This was achieved by incorporating a collagen type II-specific monoclonal antibody (mAbCII, E4-D4 clone). The selected mAbCII clone specifically binds to an epitope located within the cyanogen bromide-derived peptide fragment 10 of collagen type II. The investigation revealed that a localized injection of NPs tailored with an antibody against type II collagen and embedded with MMP13 siRNAs, which target the degradation of type II collagen, led to a significant decrease in MMP13 expression. Moreover, it sustained the integrity of cartilage and the joint structure in mouse models of post-traumatic osteoarthritis (PTOA).[65] Additionally, the same team[67] implemented a therapeutic intervention incorporating microplates composed of nanoparticles and loaded with specific siRNAs targeting MMP13 (siMMP13-μPLs) within a mouse model of PTOA subjected to mechanical loading, these nanoparticles were injected in the joint of PTOA mice. The objective of this treatment was to effectively knock down the expression of the MMP13 gene and consequently decrease the production of MMP13 protein within articular tissues, throughout the entire duration of the 28-day study period. Silencing of MMP13 reduced the deterioration of articular cartilage, synovial proliferation and pro-inflammatory gene expression, which supports the possibility of long term therapy of siMMP13-μPL for PTOA. MMP-2 primarily contributes to joint destruction in OA by degrading the ECM, promoting inflammation, and facilitating cell migration.[94] In OA joints, inflammatory cytokines such as IL-1β and TNF-α enhance the expression of MMP-2. Subsequently, MMP-2 can activate chemokines and growth factors, including TGF-β, thereby forming a positive feedback loop that exacerbates inflammation and extracellular matrix degradation.

Table 1 Roles of siRNAs in OA

siRNAs	Targeted Tissue	Therapeutic Effects	Targeted Pathway	Carrier	Animal Model	Administration Route	Results	Ref
mAbCII-siNP /siMMP13	Cartilage	Anti-inflammation	MMP13, type II collagen	Functionalized NPs antibody	Male C57 mice, Post-traumatic OA	Intra-articular injections	Reduced angiogenesis, immune response and proteolysis; protected cartilage integrity and overall joint structure.	[65]
ERMs@siM13	Cartilage	Regeneration; Anti-inflammation	MMP13	Micelle	Male C57 mice, Post-traumatic OA	Intra-articular injections	Restored the balance of cartilage matrix metabolism; diagnosed early-stage PTOA.	[66]
siMMP13-μPL	Cartilage; Synovium	Regeneration; Anti-inflammation	MMP13	1.17 μg nucleic acid/mg PLGA	C57BL/6 mice, Post-traumatic OA	Intra-articular injections	Decreased cartilage degeneration, synovial hyperplasia and pro-inflammatory gene expression.	[67]
Periostin siRNA	Cartilage	Regeneration	Periostin, NF-κB	p5RHH peptidic NP	C57BL/6J mice, DMM model	Intra-articular injections	Suppressed IL-1β–induced MMP-13 and ADAMT-4 expression in chondrocytes; mitigated the severity of joint degeneration.	[68]
p5RHH–NF-κB siRNA NP	Cartilage; Synovium	Regeneration; Anti-inflammation	NF-κB	p5RHH peptidic NP	Male C57BL/6 mice; Noninvasive Mechanical Injury Model	Intra-articular injections	Reduced chondrocyte apoptosis and reactive synovitis	[69]
CAP-Exo /siMMP13	Cartilage	Regeneration; Anti-inflammation	MMP13	CAP, CAP-Exo	ACLT model	Intra-articular injections	Reduced the MMP13 level, increased collagen COL2A1 and proteoglycan in cartilage; attenuated cartilage degeneration.	[70]
siHIF-2α-loaded MIL-101-NH2 @CCM complex	Cartilage	Regeneration; Anti-inflammation	HIF-2α	MIL-101-NH2 nanoparticles (MC NPs)	C57BL/6 mice, DMM model	Intra-articular injections	Downregulated the expression of catabolic markers and upregulated the expression of cartilage-specifc markers in chondrocytes.	[71]
p66shc-siRNA-loaded nanoparticles	Cartilage	Regeneration; Anti-inflammation	ROS-associated proteins p-p66shc expression level	Encapsulate PLGA NPs	MIA-induced OA rats	Intra-articular injections	Reduced mitochondrial dysfunction-induced cartilage damage; alleviated pain and inflammation.	[72]
NF-kBP65-SPECIFIC siRNA928-948	Cartilage	Anti-inflammation	NF-κB	siPORTTM Lipid (Ambion)	Articular chondrocytes (from SD male rats)	Transfection	Decreased COX-2, NOS-2, MMP-9 and TNF-α in chondrocytes.	[73]
Ad-siRNA NF-κBp65	Cartilage; Synovium	Anti-inflammation	NF-κBp65	Adenoviral vector	SD male rats, pMMx model	Intra-articular injections	Mitigated synovial inflammation by inhibition of f IL-1β, TNF-α and cartilage degradation.	[74]

(*Continued*)

https://doi.org/10.2147/BTT.S521180

https://doi.org/10.2147/BTT.S521180

Table 1 (Continued).

siRNAs	Targeted Tissue	Therapeutic Effects	Targeted Pathway	Carrier	Animal Model	Administration Route	Results	Ref
NO-Hb @siRNA@PLGA-PEG (NHsPP)	Cartilage	Anti-inflammation	Pro-inflammatory cytokines, macrophage	Photothermal-triggered NO nanogenerators	OA model mice	In situ injections	Inhibited the inflammatory response and prevented cartilage erosion	[75]
p16INK4a-siRNA	Cartilage; Synovium	Anti-inflammation	p16INK4a	PLGA NPs	Male C57BL/6 mice, pMMx model	Intra-articular injections	Alleviated pain behavior; reduced cartilage damage; downregulated proinflammatory cytokines in synovium.	[76]
p16INK4a-specific siRNAs	Cartilage	Regeneration	p16INK4a	None	Cartilage samples from patients undergoing total knee replacement	Transfection	Decreased the response of chondrocytes to catabolic cytokines and increased the response to anabolic growth factor.	[77]
ADAMTs-5 siRNAs	Cartilage	Regeneration	ADAMTs-5	Lentivirus-mediated	Male SD rats, ACLT +PMMxmodel	Intra-articular injection	Downregulated the expression of ADAMTs-5 protein and prevented the degradation of cartilage.	[78]
HIF-2α-siRNA	Cartilage; Synovium	Regeneration; Anti-inflammation	HIF-2α	Chondrocyte homing peptide/PEI NP	Male Chinese Kun Ming mice; ACLT, MCLT model	Intra-articular injections	Maintained cartilage integrity, alleviated synovium inflammation, and inhibited the expression of catabolic proteins.	[79]
MMP-2 siRNA	Cartilage	Regeneration; Anti-inflammation	MMP-2	Positively charged nanoparticles (AcPEI-NPs)	Human chondrocyte cells line C20A4	Transfection	Prevented matrix degradation, protected chondrocytes against degeneration and supported ECM homeostasis in articular cartilage.	[80]
p47phox si_NPs	Cartilage	Anti-oxidative stress; Regeneration	ROS/Oxidative Stress	PLGA NPs	MIA-induced OA rats	Intra-articular injections	Decreased ROS production, attenuated oxidative stress, reduced chondrocyte cell death and cartilage degradation.	[81]
YAP siRNA	Cartilage	Regeneration	Hippo/YAP	NA	Mouse, ACLT model	Intra-articular injections	Inhibited catabolic genes expression and chondrocytes apoptosis; reduced aberrant bone formation; prevented cartilage degradation	[82]
MMP13 siRNA	Cartilage	Regeneration; Anti-inflammation	MMP13	NA	C57BL/6J mice, DMM model	Intra-articular injections	Reduce cartilage degeneration and decreased OARSI score	[83]

p5RHH-NF-κB p65 siRNA	Cartilage	Regeneration	NF-κB	Self-assembling peptidic NP platform	Human umbilical endothelial cells, chondrocytes and cartilage explants	Transfection	Reduced chondrocyte apoptosis, preserved chondrocyte viability and maintained cartilage homeostasis.	[84]
MMP13 or ADAMTs5 siRNAs	Cartilage	Regeneration	MMP13 or ADAMTs5	None	C57BL/6 mice, DMM model	Intra-articular injections	Inhibited cartilage degradation and protected the type II collagen network.	[85]
Piezo1 siRNAs	BMSCs; Cartilage	Regeneration	Piezo1	Lentivirus-mediated	Male SD rats DMM model	Intra-articular injection	Promoted the differentiation of BMSCs into chondrocytes and inhibited the progression of OA	[86]
COL1A1 siRNAs	Cartilage	Regeneration	COL1A1	NA	Human and rabbit articular chondrocytes	Transfection	Decreased the type I collagen mRNA and protein; improved the differentiated chondrocyte phenotype.	[87]

Abbreviations: ACLT, anterior cruciate ligament transection/tear; ADAMTs, a disintegrin and metalloproteinase with thrombospondin-like motifs; BMSCs, bone marrow mesenchymal stem cell; CAP, cartilage affinity peptide; COL2A1, collagen type II alpha 1; COX-2, cyclooxygenase-2; DMM, destabilization of medial meniscus; HIF 2α, Hypoxia induced factor 2α; IL-1β, interleukin 1β; MCLT, medial collateral ligament transection; MIA, monosodium iodoacetate; MMP, matrix metalloproteinase; NF-κB, nuclear factor-kappa B; NOS-2, nitric oxide synthase 2; NP, Nanoparticle; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; PLGA, poly(D,L-lactide-coglycolide) acid; pMMx, partial medial meniscectomy; PTOA, post-traumatic osteoarthritis; ROS, reactive oxygen species; siRNAs, small interfering RNAs; TNF-α, tumour necrosis factor alpha; YAP, Yes-associated protein.

https://doi.org/10.2147/BTT.S521180

Moreover, MMP-2 may promote synovial cell invasion and angiogenesis, further contributing to synovitis progression and joint space narrowing.[95] Raffaele Conte et al.[80] Found that MMP-2 silencing mediated by siRNA-loaded positively charged nanoparticles (AcPEI-NPs) effectively counteracts chondrocyte de-differentiation, prevents matrix degradation, protects chondrocytes from degeneration, and supports the maintenance of ECM homeostasis in articular cartilage.

NF-κB signalling pathway exerts a crucial regulatory function in inflammatory signalling and thus constitutes an essential therapeutic target,[96] which results in the expression of TNF-α, IL-1β, IL-6, nitric oxide (NO), MMPs and the ADAMTs. Yan et al[69] showed that siRNAs targeting NF-κB injected in the joint can improve joint stability, reduced synovitis and inhibited cartilage degeneration in PTOA mouse model. Lian et al[73] used the NF-κBp65-specific siRNAs inhibited the expression of NF-κBp65 and activation of NF-κB, reducing significantly the expression of cyclooxygenase-2 (COX-2), nitric oxide synthase 2 (NOS-2) and MMP-9 induced by IL-1β and TNF-α in cultured rat chondrocytes. Chen et al[74] employed specifically targeted NF-κBp65 siRNAs to effectively inhibit the activation of NF-κB and the expression of NF-κBp65 within the cartilage and synovium of the knee joint in rats. Notably, this approach resulted in a substantial downregulation of the expression levels of COX-2, NOS-2, TNF-α, and IL-1β. Furthermore, it mitigated synovial inflammation and minimized the progression of cartilage deterioration during the initial, crucial stages of OA.

Hypoxia-inducible factor 2α (HIF-2α), a pivotal regulatory molecule, serves as a catabolic mediator in cellular processes and its augmented expression level is significantly correlated with the occurrence and progression of OA.[97] An investigation conducted on animals demonstrated that the utilization of siRNAs NP complex to inhibit HIF-2α led to the alleviation of OA symptoms, the preservation of cartilage structure, and a decline in both cartilage degradation and synovitis.[79]

Yes-associated protein (YAP) is becoming increasingly recognized because of its role in OA. YAP was upregulated in human and mice osteoarthritis cartilage and chondrocytes.[82] Previous research indicates that YAP is involved in the regulation of chondrocyte differentiation in the course of bone repair and skeleton development, which is achieved by interacting with the SRY-box transcription factor 6 (SOX6) and collagen type X alpha 1 (COL10A1).[98] In addition, it has been demonstrated that siRNA-mediated YAP knockdown reduces the development of OA by decreasing bone formation and preventing the deterioration of cartilage in mice.[82]

Piezo proteins are mechanically activated ion channels that play an essential role in mediating mechanosensing functions across various cell types.[99] Piezo1 and Piezo2 are large transmembrane ion channels that are activated by mechanical stimulation. These proteins have been identified as critically important for mechanosensation in osteoblast lineage cells.[100] Recently, a study showed that chondrocyte expression of Piezo1 is not only physiologically essential for endochondral ossification, but also contributes to pathological processes associated with OA progression and osteophyte formation.[101] Li et al[86] found that Piezo1 siRNA silencing vector can promoted the differentiation of BMSCs into chondrocytes and inhibited the progression of OA.

Regulation of Synovium

Synovitis and synovial fibrosis within the joint are recognized as a risk factor for the development and progression of OA.[102,103] siRNAs were reported to alleviate synovium inflammation and synovial hyperplasia.[74,76] For example, Park et al[76] discovered that Cyclin-Dependent Kinase Inhibitor p16 (p16INK4a) expression in the synovium of OA patients increased. Based on this, they developed p16INK4a siRNAs-loaded PLGA nanoparticles (p16 si_NP) to reduce the expression of MMP13, TNF-α, IL-1β and IL-6 in mice fibroblast-like synoviocytes (FLSs) and chondrocytes. The utilization of RNAi nanomedicine to decrease the expression of p16INK4a in FLSs holds promise for facilitating the regeneration of osteoarthritic cartilage and alleviating associated pain. This finding implies that p16INK4a may represent a viable therapeutic target for the management of osteoarthritis, offering a potential new avenue for the development of innovative treatments.

Diagnostic Effects

siRNAs can also function as an effective approach for diagnosing OA and for tracking its progression during the early stages. In recent study, Zhou et al[66] constructed a micelle system functionalized with MMP13 enzyme-detachable, cyanine 5 (Cy5)-containing PEG, black hole quencher-3 (BHQ3), and cRGD ligands and loaded with siRNA silencing

MMP13 (siM13), namely ERMs@siM13. ERMs@siM13 can be cleaved by MMP13 in diseased cartilage tissues, leading to the detachment of the PEG shell and subsequent exposure of cRGD. The exposed ligand then facilitates micelle uptake by diseased chondrocytes through binding to αvβ3 integrins on the cell surface, thereby enhancing intracellular delivery of siM13 for targeted downregulation of MMP13. ERMs@siM13 have been developed to target over-expressed MMP13 in diseased cartilage, which can be diagnosed as well as intervene in early stages of PTOA.[66] Additionally, ERMs@siM13 are able to report in real time the extent of OA progression by responding to changes in MMP13 levels, reflecting the severity of PTOA.[66]

Specific Functions of siRNAs at Different Stage of OA

Osteoarthritis is typically categorized into early, middle, and late stages based on the Kellgren-Lawrence (KL) grading system,[104] with distinct pathophysiological characteristics observed at each stage. siRNA therapy demonstrates varying applicability and therapeutic effects across different stages of OA, with a primary focus on the early and middle stages. It aims to alleviate inflammation and delay disease progression through the regulation of specific gene expression.[105,106]

In the early stages of OA, cartilage damage is relatively mild, and the inflammatory response is more pronounced.[107] siRNA therapy primarily functions by targeting pro-inflammatory cytokines (such as TNF-α, IL-1β, and IL-6) and enzymes involved in cartilage degradation (eg, MMP-13 and ADAMTS-5).[74,78] Research has demonstrated that adenovirus vector-mediated delivery of NF-κB p65-specific siRNA can effectively inhibit the progression of experimental OA in its early phase.[74] Moreover, siRNA can also target and modulate the Wnt signaling pathway, which plays a crucial role in the onset and progression of OA.[108–110] By employing CRISPR/CasRX-mediated RNA knockdown to target β-catenin and Ihh signaling pathways, OA symptoms can be alleviated.[110] Additionally, siRNA can be utilized to suppress chondrocyte apoptosis—for example, by silencing microRNA-34a, which reduces chondrocyte apoptosis during the early stages of OA.[111]

As the disease progresses to the middle stage, cartilage damage becomes more severe, joint inflammation persists, and osteophyte formation begins.[112] At this stage, in addition to continuing to target inflammatory mediators and chondrodegradative enzymes, siRNA-based therapies can also focus on modulating extracellular ECM metabolism to promote cartilage repair. For example, siRNA nanoparticles targeting MMP13 have been shown to effectively inhibit the progression of post-traumatic OA.[113] Moreover, siRNA can be utilized to address mitochondrial dysfunction in chondrocytes. Specifically, p66shc siRNA nanoparticles have demonstrated the ability to improve mitochondrial function in OA-affected chondrocytes.[72] Additionally, the delivery of miR-27b-3p via extracellular vesicles has been found to alleviate OA symptoms by suppressing leukocyte-derived pro-inflammatory factors.[114]

The features of advanced OA include significant cartilage degradation, disruption of joint architecture, and prominent osteophyte formation.[112] At this stage, structural joint alterations are largely irreversible, which may constrain the efficacy of siRNA therapy. Consequently, the primary therapeutic goal for advanced OA is to alleviate pain and enhance joint function, often necessitating surgical interventions such as joint replacement.[115]

tRNAs in OA

A restricted amount of research exists concerning the function of tRNAs in the regulation of OA, among these, tRFs have gained significant attention. tRFs regulate cartilage degeneration, protect anterior cruciate ligaments (ACLs). Also, tRFs have potential as biomarkers for the diagnosis of OA (Figure 3).

Suppression of Cartilage Degeneration

Long et al[116] investigated the relationship between posttranscriptional gene regulation by tRF-5009A and the processes of autophagy and cartilage degeneration in OA, employing RNA sequencing techniques. In this investigation of human OA knees, it was discovered that tRF-5009A were down-regulated in the cartilage, with a particular emphasis on damaged regions. When its inhibitor was introduced, it led to an up-regulation of mTOR and an inhibition of autophagy, thereby protecting the cartilage from degeneration by disrupting the mTOR pathway. In contrast, a tRF-5009A mimic exhibited the converse effects. Further analysis through a dual-luciferase reporter assay unveiled that tRF-5009A silences mTOR expression by specifically binding to its 3′-untranslated region (3-UTR). In essence, tRF-5009A plays a pivotal

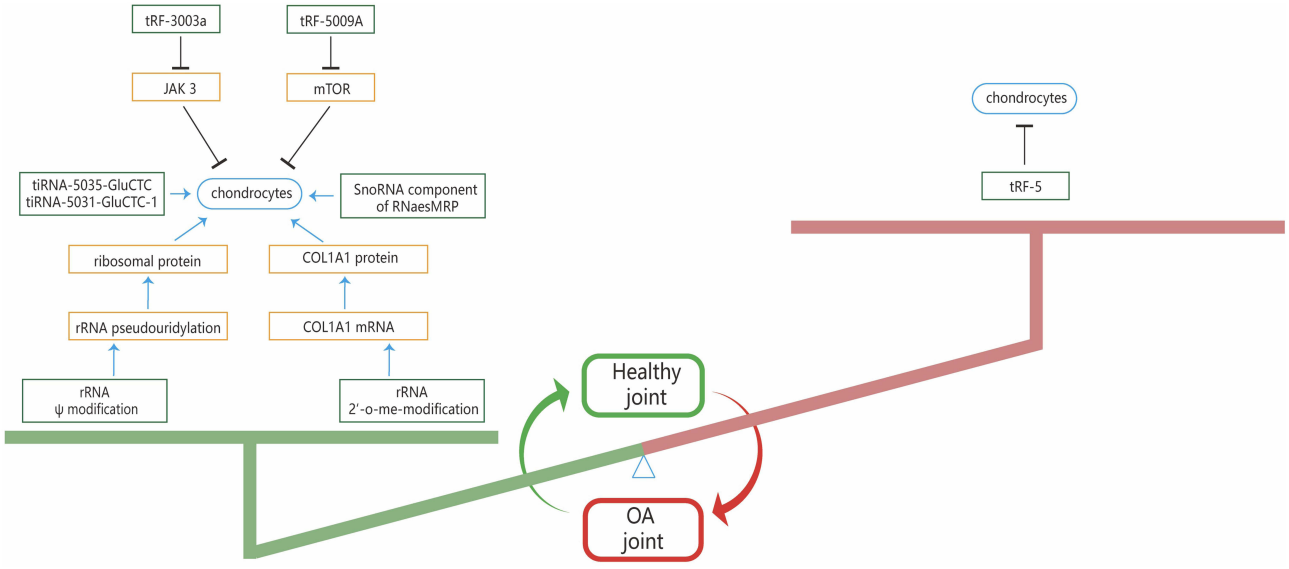

Figure 3 Molecular mechanisms of signalling pathways and RNA modifications in regulating chondrocyte function and impacting joint health and osteoarthritis. tRNAs and rRNAs balance OA via regulating chondrocytes, left side (green side): tRNAs and rRNAs protect chondrocytes, right side (red side): tRNAs impair chondrocytes.
Abbreviations: COL1A, collagen type I alpha 1; IKBKB, inhibitor of nuclear factor kappa B kinase subunit beta; JAK3, Janus kinase 3; mTOR, mammalian target of rapamycin; OA, osteoarthritis; rRNA, ribosomal RNA; snoRNA, small nucleolar RNA; tRF, tRNA-derived fragment.

role in modulating chondrocyte autophagy and halting cartilage degeneration in the context of osteoarthritis by targeting the mTOR pathway.

Green et al[43] focused on tRF-3003a, a type-3 tRF produced from the 3′ end of the tRNA-CysGCA. In the context of post-transcriptional gene regulatory mechanisms, tRF-3003a demonstrated the utmost expression levels in chondrocyte cells following stimulation with IL-1β. Research indicates a notable reduction in the expression levels of tRF-3003a in cartilage derived from OA patients, in contrast to those observed in normal cartilage samples. Besides, tRF-3003a suppresses JAK3 gene expression in chondrocytes via AGO, potentially restoring cell homeostasis by blocking JAK/STAT inflammatory signaling. Consequently, the expression of tRF-3003a was identified to play a crucial role in mediating the suppression of JAK3, which in turn led to a reduction in its downstream target cytokine, IL-6. This finding underscores the potential significance of tRF-3003a in regulating the pathogenesis of OA, thereby contributing to a deeper understanding of its biological functions.

Balaskas et al demonstrated[117] that the tRF-5 fragment (tRF293/294) was induced in high-level OA compared to low-level OA cartilage (older samples of cartilage indicate higher level of OA), and the concentrations of tiRNA-5035-GluCTC and tiRNA-5031-GluCTC-1 exhibited a notable decrease in both human cartilage suffering from high-grade OA and chondrocytes derived from aged equine samples. These findings have demonstrated that the distinctive molecular markers found in aged cartilage hold promise as potential therapeutic targets.

Protection of Anterior Cruciate Ligament Cells

The ACL not only serves as a structural foundation for maintaining knee joint stability but also acts as a critical link between mechanical imbalance and the pathological progression of OA through its cellular metabolic abnormalities and inflammatory responses.[118,119] ACL degeneration triggers a vicious cycle with cartilage and bone damage through activating the NF-κB signaling pathway and promoting the release of inflammatory cytokines, jointly promoting the development of OA.

Long et al[120] aimed to investigate the role and function mechanism of tRFs of ACL cell metabolism in vitro. The expression levels of TRF365 were quantitatively analyzed using quantitative real-time polymerase chain reaction (qPCR) and fluorescence in situ hybridization (FISH) techniques. TRF365 was expressed at a low level in the cells of the ACL in cases of OA and under inflammatory conditions induced by interleukin-1β treatment in ACL cells. On the other hand, the levels of the inhibitor of nuclear factor κB kinase subunit beta (IKBKB) was notably up-regulated in ACL cells isolated

from patients with OA, as well as in ACL cells that had been treated with interleukin-1β. The expression levels of IKBKB and TRF365 exhibited an inverse correlation. The introduction of the TRF365 mimic into cells led to a downregulation of IKBKB expression, whereas the application of the TRF365 inhibitor elicited a contrasting effect, hinting at IKBKB's status as a plausible target gene for TRF365. Furthermore, the outcomes obtained from a dual-luciferase reporter assay demonstrated that TRF365 effectively quenches the expression of IKBKB by specifically binding to its 3′-UTR region. Consequently, this interaction serves as a pivotal mechanism in regulating the metabolism of ACL cells. Overall, the study provides evidence that TRF365 plays a crucial role in ACL cell metabolism by targeting IKBKB.

Serum Biomarker

Recently, a comprehensive methodological validation has been carried out by a study to evaluate the potential of tRF-5022B as a biomarker for OA.[121] The validity of PCR amplification results and assessment of tRF-5022B's molecular attributes were ensured by employing agarose gel electrophoresis and Sanger sequencing. An assessment of the diagnostic performance of tRF-5022B was conducted using receiver operating characteristic (ROC) curve analysis. The study uncovered a marked reduction in the serum levels of tRF-5022B among patients diagnosed with OA. Notably, the Kellgren-Lawrence grading scale correlated closely with serum expression levels of tRF-5022B. Furthermore, the ROC curve analysis confirmed that the serum expression levels of tRF-5022B could distinguish not only between OA cases and healthy individuals but also between OA and rheumatoid arthritis (RA) patients.

rRNAs in OA

Currently, we found that the effect of rRNAs on OA is indirect. Most rRNAs require modification or act through mediators to exert their regulatory influence (Figure 2).

Inhibition of Ageing

Aging stands as a primary risk factor for OA,[122] regulation of the rRNAs is associated with aging and age-related articular cartilage degeneration.[123,124] Studies have shown a decrease in rRNAs expression in bone marrow cells of aging mice.[125] Ren et al[126] demonstrated that CBX4, a component of the polycomb repressive complex 1 (PRC1), is instrumental in antagonizing the aging of human mesenchymal stem cells (hMSCs) by preserving nucleolar heterochromatin organization. Overexpression of CBX4 has been found to mitigate hMSC senescence and the onset of OA. And it functions by maintaining nucleolar homeostasis through the recruitment of nucleolar proteins fibrillin (FBL) and KRAB-related protein 1 (KAP1) to nucleolar ribosomal DNA, thereby restricting the overexpression of rRNAs. Furthermore, age-related differences in gene expression in cartilage have revealed significant alterations in small nucleolar RNAs (snoRNAs), serving as a class of regulatory RNAs, which are responsible for the posttranscriptional maturation of rRNAs.[127] In accordance with conserved sequence elements, snoRNAs are divided into two main classes: the C/D box snoRNAs (SNORDs) and the H/ACA box snoRNAs (SNORAs).[128] Specifically, older donors exhibited significantly higher levels of six SNORAs/SNORDs, while nine SNORAs/SNORDs were found to have lower expression as compared to young cartilage.[129] In an additional investigation, it has been observed that the concentration of SNORD26 is heightened in individuals suffering from OA.[130] Conversely, the quantities of SNORD44 and SNORD78 appear to decrease as the aging process advances.

Regulation of Chondrocytes

(1) Posttranscriptional maturation: snoRNAs, which range from 60 to 300 nucleotides in length, are widely recognized for their critical role in directing nucleotide modifications and processing throughout the maturation process.[127] snoRNAs are involved in the methylation and pseudouridylation of various RNAs, including rRNAs.[128] SNORDs primarily facilitate 2′-O-methylation, while SNORAs guide pseudouridylation of nucleotides.[131]

Ripmeester et al demonstrated a decrease in U3 snoRNA expression in both cartilage and chondrocytes in OA. They discovered that U3 snoRNA plays a regulatory role in the levels of rRNAwithin chondrocytes, suggesting its potential as a novel therapeutic target in the management of OA treatments.[132] Additionally, mitochondrial RNA processing endoribonuclease (RNase MRP) and RNase P represent a unique subset of snoRNAs.[133] RNase MRP has emerged as

a crucial modulator of chondrocyte hypertrophy and exhibits significant functional interplay with chondrogenic signaling pathways.[134]

(2) rRNAs modification: Over the course of approximately six decades following the initial identification of RNA modification in yeast, scientists have subsequently uncovered more than 150 further modifications occurring within diverse RNA species.[135] To a certain degree, the preservation of the rRNA structure hinges upon the occurrence of chemical alterations at particular nucleotides within these rRNA molecules. The alterations include 2'O-ribose methylation (2′-OMe), the process of pseudo-uridylation (ψ), alongside modifications such as base methylation and acetylation.[136] Recent pivotal discoveries indicate that defects in rRNAs modification mechanisms can contribute to the development of OA. Among these modifications, 2′-OMe and ψ stand out as the most prevalent alterations in rRNAs.

The modification of RNA involving the substitution of a methoxy group at the 2′-position of the ribose sugar, thereby altering its hydrophilic hydroxyl moiety, holds a pivotal role in differentiating RNA bases from those found in DNA. This modification can profoundly affect the structure and stability of RNA and even influence ribosome biogenesis.[137] To date, more than 100 2′-OMe sites have been discovered within human rRNAs, overlying the RNA sequence information, expanding its lexicon.[138] Research demonstrated that synovial fluid from human osteoarthritic joints induces specific alterations in the 2′-OMe pattern of rRNA in primary human articular chondrocytes. These modifications are site-specific, impacting particular loci such as 18S-Gm1447, 5.8S-Um14, 28S-Am3739, 28S-Am3846, and 28S-Um4590. They observed differential 2′-OMe levels at five specific sites. Notably, the 2′-OMe status of nucleotide U14 within the 5.8S rRNA, which is one of the identified sites of differential modification, was found to be affected by the reduction in levels of its guiding sRNA, SNORD71. This alteration in 2′-OMe status resulted in a change in ribosome translation mode and promoted the translation of Collagen type I alpha 1 (COL1A1) mRNA, leading to increased levels of the fibrochondrocyte marker COL1A1 protein.[139]

The accessibility of information pertaining to ψ remains significantly constrained, due to the fact that a methodical, high-throughput technique for measuring the quantity of ψ was merely introduced in the recent past, specifically in the year 2020.[140] Chabronova et al[141] identified seven rRNA sites, including 18S-ψ36, 18S-ψ210, 18S-ψ918, and so on, whose ψ-modification levels significantly decreased in response to the microenvironment of chronic OA. For a more thorough functional assessment, they deliberately opted for 28S-ψ4966, employing SW1353 cell pools with knockouts of SNORA22 and SNORA33. It was anticipated that SNORA33, along with SNORA22, would play a crucial role in orchestrating the pseudouridylation process of 28S-U4966. As a result, the depletion of chondrocytic cells specifically targeting SNORA33 led to a decline in the concentration of 28S-ψ4966, subsequently influencing the composition and functionality of ribosomal proteins, and eliciting distinct modifications within the cellular proteome.

(3) rRNAs transcription: The transcription of rRNAs constitutes a primary bottleneck in the formation of ribosomes.[142] The synthesis and processing of rRNA transcripts necessitate a multitude of trans-acting factors and RNA molecules, ultimately leading to the generation of the three principal mature rRNAs that form the structural scaffold of the ribosome.[143] However, the precise regulatory mechanisms that govern these processes remain incompletely characterized. Bone morphogenetic protein 7 (BMP7), currently under investigation as a potential therapeutic agent for disease modification,[144] functions as a morphogen that can counteract the hypertrophic phenotype of OA chondrocytes through the action of NK3 homeobox 2 (NKX3-2). NKX3-2 exhibits the capacity to repress the manifestation of the runt-associated transcription factor, specifically RUNX2, which serves as a crucial regulatory protein in the process of chondrocyte hypertrophic differentiation.[145,146] Ripmeester and colleagues[146] exhibited that BMP7 fosters protein synthesis and elicits rRNA transcription in SW1353 cells and primary chondrocytes derived from humans, utilizing an NKX3-2-directed pathway. This mechanism plays a pivotal role in the therapeutic approach to managing osteoarthritis.

A review of existing literature reveals that the role of rRNAs in the progression and staging of OA has not yet been systematically elucidated. This indicates that research in this area remains at a nascent stage, warranting further investigation in future studies.

Discussion

RNA therapy holds significant potential for the treatment of OA; however, its efficacy is constrained by several factors, such as challenges associated with RNA delivery systems, the stability of the treatment, and potential interactions with concomitant

therapies. Addressing these limitations is essential for the successful advancement and clinical application of RNA-based therapeutic strategies. Despite showing significant therapeutic potential for OA, the clinical application of siRNAs is hindered by in vivo delivery barriers. Primarily, the nonvascular nature and dense ECM of cartilage present significant challenges for effective delivery (the ECM-cell interaction of cartilage extracellular matrix on chondrocytes).[147] Also, unmodified siRNAs are unstable in the presence of nucleases and easily trapped within endosomal vesicles.[79] Therefore, the enhancement of siRNAs' pharmacokinetics and stability necessitates the adoption of sophisticated delivery techniques.[148] To address these challenges, researchers are actively developing a variety of nanodelivery systems, including lipid nanoparticles (LNPs), polymeric nanoparticles, and exosomes. NPs-based delivery systems, such as LNPs and polymeric nanoparticles can be used to improve penetration through the dense ECM.[149] Additionally, conjugating targeting peptides that specifically bind to cartilage components like collagen or aggrecan can guide therapeutic RNA to the cartilage.[68,84] To improve siRNA stability against nucleases, chemical modifications like 2′-OMe, phosphorothioate linkages, or locked nucleic acids (LNAs) can be employed.[150,151] Avoidance of endosomal trapping can be addressed using pH-sensitive lipids or polymers that destabilize the endosomal membrane, or by developing stimuli-responsive nanocarriers that trigger siRNA release in response to intracellular conditions.[71,152] Yan et al[84] created peptide-based nanoparticles (NPs) that exhibit inhibitory effects on the activity of NF-κB via the utilization of siRNAs. They demonstrated the capability of the NPs to permeate through all layers of a significantly thicker human cartilage explant, thereby facilitating the delivery of siRNAs to the chondrocytes. Additionally, the presence of the siRNA signals could be discerned within chondrocytes for a duration spanning up to 21 days, facilitating further research and analysis without the need for frequent re-detection. Duan et al[68] used NF-κB siRNAs with the peptide–siRNAs nanoplatform, and showed that NF-κB knockdown inhibits chondrocyte death and early joint reaction after injury. PLGA polymers have demonstrated the ability to prevent inactivation of loaded drugs or siRNAs, reduce undesirable side effects, and increase the effectiveness of active drug components through increased solubility and bioavailability.[72,81] In addition, a metal-organic framework (MOF) that responds to pH, MIL-101-NH2, protects siRNAs from degradation by lysosome. In the acidic microenvironment characteristic of OA, the pH-responsive nanomaterial MIL-101-NH2 undergoes gradual degradation, leading to the release of CCM payloads.[71] These payloads effectively downregulate pro-inflammatory cytokines. Concurrently, the siRNAs payloads are liberated to target and cleave the HIF-2α mRNAs, enabling gene silencing therapy. The combined effect of silencing HIF-2α genes and inhibiting inflammatory responses and cartilage degeneration in OA results in a potent synergistic therapeutic outcome. Up to now, peptide-siRNAs nanotherapy, Poly (D,L-lactic-co-glycolic acid) (PLGA) NPs and MIL-101-NH2 were used in preclinical studies as important siRNAs carriers (Table 1). Exosomes are nanoscale extracellular vesicles secreted by cells, capable of transporting bioactive molecules such as RNA, proteins, and lipids.[153] These vesicles possess inherent targeting capabilities and excellent biocompatibility, enabling efficient delivery of RNA molecules to specific target cells.[153] However, challenges remain in their production and purification processes, including limited cardiac targeting efficiency and the lack of standardized manufacturing protocols.[154]

Therapies such as siRNA and CRISPR/Cas9 systems may bind to unintended genomic targets, potentially resulting in adverse off-target effects.[155,156] For example, siRNA can regulate non-target mRNA through sequence-dependent off-target mechanisms that resemble the activity of miRNA.[157] Similarly, mismatches between sgRNA and the intended target sequence in CRISPR/Cas9 gene editing can lead to off-target effects.[158] Emerging approaches, such as DNA nanotechnology, offer promising strategies to minimize hybridization-dependent off-target effects associated with oligonucleotide-based therapies.[159] To address these challenges, further improvements in sgRNA design and predictive modelling for off-target effects are essential.[160] The CRISPR off T database serves as a comprehensive resource by integrating CRISPR/Cas off-target data, thereby supporting the advancement of gene editing technologies and enhancing the accuracy of off-target prediction algorithms.[161] It is essential to establish a comprehensive screening approach to effectively identify and quantify potential off-target effects associated with ncRNA therapy.[156] This strategy may encompass the application of bioinformatics tools, high-throughput screening techniques, and in vivo experimental models to assess off-target interactions and refine ncRNA sequences for improved specificity.[161]

Limited studies have explored the use of tRNAs and rRNAs in treating OA. Most research on tRNAs has focused on tRFs, we found only one study using tiRNAs.[117] Regarding rRNAs, studies have focused on their indirect roles in regulating OA progression, such as through modulating ribosomal biogenesis and protein synthesis, which may influence cellular functions related to OA pathology.[162] Despite the scant literature, concerns similar to those for siRNAs exist, particularly regarding the need for effective delivery systems to target these molecules accurately. Although there is

evidence of chemical modifications of rRNAs in OA to improve stability and reduce immune response,[136] advanced delivery methods such as NPs, liposomal delivery systems, hydrogel-based systems, and exosome-mediated delivery, which have shown promise in enhancing the stability, targeting, and controlled release of siRNAs, have yet to be extensively utilized for the delivery of tRNAs and rRNAs in the treatment of OA. To date, we have not found a study reported the successful rRNA delivery, which may be attributed to the inherent challenges associated with large molecular size, structural complexity, and susceptibility to degradation of rRNA molecules.

Safety is a cornerstone in the advancement of RNA-based therapies, encompassing siRNAs, tRNAs, and rRNAs. We have discussed the targeted pathways and tissues, however, the research on off-target effects that RNA molecules interfere with unintended pathways or tissues is necessary to avoid potentially harmful effects.[163] Future studies can integrate CRISPR-Cas9 technology to knock out potential off-target genes in model systems, potentially mitigating off-target effects.[156] Introduce chemical modifications such as 2′-OMe or LNAs to the RNA backbone in order to increase the RNA's binding affinity for the target sequence while reducing the likelihood of unintended interactions with non-target sites.[150,151] Moreover, the immune responses triggered by RNA molecules can lead to inflammation which may worsen OA.[164] Incorporate modified nucleotides like ψ or 2-thiouridine into RNA molecules to reduce activation of toll-like receptors (TLRs) which are responsible for recognizing RNA as foreign and triggering immune responses.[165] LNPs not only protect RNA from degradation but reduce immune recognition by shielding RNA from TLRs, thus incapsulating RNA molecules in LNPs can reduce immune responses.[166] The long-term effects of RNA-based therapies are not fully understood, as some effects may only become apparent after extended use.[167] Although currently no clinical studies have been found related to siRNAs, tRNAs, and rRNAs in treating OA, conducting long-term preclinical studies in animal models is essential to assess the chronic effects of RNA therapies.[168] Besides, we can learn from the clinical application of these RNA molecules in other diseases by establishing patient registries and pharmacovigilance systems to track the long-term safety of RNA therapies in the general population.[169,170] Also, RNA-based therapies should be evaluated for potential interactions with other medications or treatments that OA patients may be receiving.

In conclusion, ncRNAs have recently become a widely studied area in OA research. This indicates that treatment methods for arthritis based on ncRNA bring us tremendous opportunities. Nonetheless, investigations into the modulation of siRNAs, tRNAs, and rRNAs within the framework of various diseases, and their probable utility as diagnostic tools or therapeutic biomarkers for OA, are still in their nascent phases. The challenges inherent in ncRNA-based therapies encompass the delivery of molecules to the joint's target cells and ensuring that effective concentrations reach the appropriate tissues. It is imperative to control specificity and duration to avoid off-target effects and preserve efficacy. The intricacies of ncRNA biology, as well as the interactions between ncRNA species and their targets, also pose significant challenges. Nevertheless, the potential of ncRNA therapies for treating arthritis remains promising and merits further investigation. This review examines the general functions of siRNAs, tRNAs, and rRNAs and their application in OA. We found that the primary focus has been on regulating cartilage degradation, a key aspect of OA pathogenesis. No clinical trials were conducted yet, as challenges such as effective delivery systems, immune responses, long-term effects, and interactions between therapies need to be addressed. Future research should focus on the development of more effective RNA delivery systems, enhancing the stability and biocompatibility of RNA molecules, and acquiring a deeper understanding of the interactions between RNA-based therapies and other treatment modalities for OA. Furthermore, it is essential to further investigate the application of siRNA in OA treatment, such as targeting specific genes in chondrocytes, synovial cells, and immune cells. Addressing these challenges may enable RNA therapy to emerge as a promising therapeutic strategy for managing OA.

Abbreviations

2′-OMe, 2′O-ribose methylation; 3-UTR, 3′-untranslated region; ACLs, Anterior cruciate ligaments; ACLT, Anterior cruciate ligament transection/tear; ADAMTs, A disintegrin and metalloproteinase with thrombospondin-like motifs; AGO, Argonaute; ALS, Amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; ANG, Angiogenin; BHQ3, Black hole quencher-3; BMP7, Bone morphogenetic protein 7; BMSCs, Bone marrow mesenchymal stem cell; CCM, Curcumin; circRNAs, Circular RNAs; COL10A1, Collagen type X alpha 1; COL1A1, Collagen type I alpha 1; COX-2, Cyclooxygenase-2; Cy5, cyanine 5; DMM, Destabilization of medial meniscus; dsRNAs, Double-stranded RNAs; ECM, Extracellular matrix; FBL, Fibrillin; FISH, Fluorescence in situ hybridization; FLSs, Fibroblast-like synoviocytes; HIF-2α, Hypoxia-induced factor 2α; hMSCs, Human mesenchymal stem cells; IKBKB, Inhibitor of nuclear factor kappa B kinase subunit beta; IL-1β, Interleukin 1β; IL-6, Interleukin

6; IPFP, Infrapatellar fat pad; JAK3, Janus kinase 3; KAP1, KRAB-related protein 1; KL, Kellgren-Lawrence; LGALS1, Galectin 1; LNAs, Locked nucleic acids; LNPs, Lipid nanoparticles; lncRNAs, Long non-coding RNAs; MCLT, Medial collateral ligament transection; MIA, Monosodium iodoacetate; MIF, Macrophage migration inhibitory factor; miRNAs, MicroRNAs; MMPs, Matrix metalloproteinases; MOF, Metal-organic framework; mRNAs, Messenger RNAs; mTOR, Mammalian target of rapamycin; MSCs, Marrow mesenchymal stem cells; ncRNAs, Non-coding RNAs; NF-κB, Nuclear factor-kappa B; NKX3-2, NK3 homeobox 2; NO, Nitric oxide; NOS-2, Nitric oxide synthase 2; NP, Nanoparticle; OA, Osteoarthritis; OARSI, Osteoarthritis Research Society International; p16INK4a, Cyclin-Dependent Kinase Inhibitor p16; p16 si_NP, p16INK4a siRNAs-loaded PLGA nanoparticles; PD, Parkinson's disease; PLGA, Poly (D,L-lactic-co-glycolic acid); pMMx, Partial medial meniscectomy; PRC1, Polycomb repressive complex 1; pre-tRNAs, Precursor tRNAs; PTOA, Post-traumatic osteoarthritis; RA, Rheumatoid arthritis; qPCR, Quantitative real-time polymerase chain reaction; rDNA, Ribosomal DNA; RISC, RNA-induced silencing complex; RNAi, RNA interference; RNAse MRP, Mitochondrial RNA processing endoribonuclease; RNAse P, Ribonuclease P; ROS, Reactive oxygen species; rRNAs, Ribosomal RNAs; RUNX2, Runt-related transcription factor 2; siM13, siRNA silencing MMP13; siNP-μPLs, siRNAs loaded nanoparticles-based microplates; siRNAs, Small interfering RNAs; SNORAs, The H/ACA box snoRNAs; SNORDs, The C/D box snoRNAs; snoRNAs, Small nucleolar RNAs; SOX6, SRY-box transcription factor 6; STAT, Signal transducer and activator of transcription; tiRNAs, tRNA-derived stress-induced RNAs; TLRs, Toll-like receptors; TNF-α, Tumour necrosis factor-alpha; tRFs, tRNA-derived fragments; tRNAs, Transfer RNAs; tsRNAs, tRNA-derived small RNAs; YAP, Yes-associated protein; ψ, Pseudo-uridylation.

Acknowledgments

This study was supported by China National Natural Science Foundation (grant number: 82205246; 82274642; 82074547), Beijing Hospital Management Centre "Peak" Talent Training Plan Team (DFL20241001), The Fifth Batch Of National TCM Clinical Outstanding Talents Project, The sixth batch of Beijing Municipal Traditional Chinese Medicine Expert Academic Experience Inheritance Work Project, National Famous Traditional Chinese Medicine Expert Inheritance Studio Construction Project (National TCM Education Letter [2022] No. 75). This paper has been uploaded to [Social Science Research Network] as a preprint: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4917809.

Disclosure

The authors declare no competing interests.

References

1. Yao Q, Wu X, Tao C, et al. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther*. 2023;8(1):56. doi:10.1038/s41392-023-01330-w
2. Motta F, Barone E, Sica A, et al. Inflammaging and osteoarthritis. *Clin Rev Allergy Immunol*. 2023;64(2):222–238. doi:10.1007/s12016-022-08941-1
3. Steinmetz JD, Culbreth GT, Haile LM. Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Rheumatol*. 2023;5(9):e508–e522. doi:10.1016/S2665-9913(23)00163-7
4. Ghafouri-Fard S, Poulet C, Malaise M, et al. The emerging role of non-coding RNAs in osteoarthritis. *Front Immunol*. 2021;12:773171.
5. De Roover A, Escribano-Núñez A, Monteagudo S, et al. Fundamentals of osteoarthritis: inflammatory mediators in osteoarthritis. *Osteoarthritis Cartilage*. 2023;31(10):1303–1311. doi:10.1016/j.joca.2023.06.005
6. Yunus MHM, Nordin A, Kamal H. Pathophysiological Perspective of Osteoarthritis. *Medicina*. 2020;56(11).
7. Damerau A, Rosenow E, Alkhoury D, et al. Fibrotic pathways and fibroblast-like synoviocyte phenotypes in osteoarthritis. *Front Immunol*. 2024;15:1385006. doi:10.3389/fimmu.2024.1385006
8. Panichi V, Costantini S, Grasso M, et al. Innate immunity and synovitis: key players in osteoarthritis progression. *Int J Mol Sci*. 2024;25(22):12082. doi:10.3390/ijms252212082
9. Yue S, Zhai G, Zhao S, et al. The biphasic role of the infrapatellar fat pad in osteoarthritis. *Biomed Pharmacother*. 2024;179:117364. doi:10.1016/j.biopha.2024.117364
10. Pereira Herrera B, Emanuel K, Emans PJ, et al. Infrapatellar fat pad as a source of biomarkers and therapeutic target for knee osteoarthritis. *Arthritis Res Ther*. 2025;27(1):81. doi:10.1186/s13075-025-03517-8
11. Zhang Y, Li S, Jin P, et al. Dual functions of microRNA-17 in maintaining cartilage homeostasis and protection against osteoarthritis. *Nat Commun*. 2022;13(1):2447. doi:10.1038/s41467-022-30119-8
12. Delplace V, Boutet M-A, Le Visage C, et al. Osteoarthritis: from upcoming treatments to treatments yet to come. *Joint Bone Spine*. 2021;88(5):105206. doi:10.1016/j.jbspin.2021.105206
13. Chen D, Shen J, Zhao W, et al. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res*. 2017;5:16044. doi:10.1038/boneres.2016.44

14. Yang K, Gao Y, Yang M, et al. Creating conditional dual fluorescence labeled transgenic animals for studying function of small noncoding RNAs. *Connective Tissue Res*. 2017;58(1):103–115. doi:10.1080/03008207.2016.1247834
15. Wei J-W, Huang K, Yang C, et al. Non-coding RNAs as regulators in epigenetics (Review). *Oncol Rep*. 2017;37(1):3–9. doi:10.3892/or.2016.5236
16. Chen J, Yu X, Zhang X. Advances on biological functions of exosomal non-coding RNAs in osteoarthritis. *Cell Biochem Funct*. 2022;40(1):49–59. doi:10.1002/cbf.3679
17. Razmara E, Bitaraf A, Yousefi H, et al. Non-coding RNAs in cartilage development: an updated review. *Int J Mol Sci*. 2019;20(18):4475. doi:10.3390/ijms20184475
18. Sun H, Hu S, Zhang Z, et al. Expression of exosomal microRNAs during chondrogenic differentiation of human bone mesenchymal stem cells. *J Cell Biochem*. 2019;120(1):171–181. doi:10.1002/jcb.27289
19. Zhou Y, Ming J, Li Y, et al. Exosomes derived from miR-126-3p-overexpressing synovial fibroblasts suppress chondrocyte inflammation and cartilage degradation in a rat model of osteoarthritis. *Cell Death Discov*. 2021;7(1):37. doi:10.1038/s41420-021-00418-y
20. Yan L, Liu G, Wu X. The umbilical cord mesenchymal stem cell-derived exosomal lncRNA H19 improves osteochondral activity through miR-29b-3p/FoxO3 axis. *Clin Transl Med*. 2021;11(1):e255. doi:10.1002/ctm2.255
21. Mao G, Xu Y, Long D, et al. Exosome-transported circRNA_0001236 enhances chondrogenesis and suppress cartilage degradation via the miR-3677-3p/Sox9 axis. *Stem Cell Res Ther*. 2021;12(1):389. doi:10.1186/s13287-021-02431-5
22. Cavallaro G, Sardo C, Craparo EF, et al. Polymeric nanoparticles for siRNA delivery: production and applications. *Int J Pharm*. 2017;525(2):313–333. doi:10.1016/j.ijpharm.2017.04.008
23. DeJulius CR, Walton BL, Colazo JM, et al. Engineering approaches for RNA-based and cell-based osteoarthritis therapies. *Nat Rev Rheumatol*. 2024;20(2):81–100. doi:10.1038/s41584-023-01067-4
24. Guo S, Zhang M, Huang Y. Three 'E' challenges for siRNA drug development. *Trends Mol Med*. 2024;30(1):13–24. doi:10.1016/j.molmed.2023.10.005
25. Chen X, Rechavi O. Plant and animal small RNA communications between cells and organisms. *Nat Rev Mol Cell Biol*. 2022;23(3):185–203. doi:10.1038/s41580-021-00425-y
26. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. *Nat Rev Mol Cell Biol*. 2007;8(1):23–36. doi:10.1038/nrm2085
27. Charbe NB, Amnerkar ND, Ramesh B, et al. Small interfering RNA for cancer treatment: overcoming hurdles in delivery. *Acta Pharm Sin B*. 2020;10(11):2075–2109. doi:10.1016/j.apsb.2020.10.005
28. Finan C, Gaulton A, Kruger FA, et al. The druggable genome and support for target identification and validation in drug development. *Sci Transl Med*. 2017;9(383). doi:10.1126/scitranslmed.aag1166
29. Wu SY, Lopez-Berestein G, Calin GA, et al. RNAi therapies: drugging the undruggable. *Sci Transl Med*. 2014;6(240):240ps7. doi:10.1126/scitranslmed.3008362
30. Zogg H, Singh R, Ro S. Current Advances in RNA Therapeutics for Human Diseases. *Int J Mol Sci*. 2022;23(5):2736. doi:10.3390/ijms23052736
31. Suzuki T. The expanding world of tRNA modifications and their disease relevance. *Nat Rev Mol Cell Biol*. 2021;22(6):375–392. doi:10.1038/s41580-021-00342-0
32. Orellana EA, Siegal E, Gregory RI. tRNA dysregulation and disease. *Nat Rev Genet*. 2022;23(11):651–664. doi:10.1038/s41576-022-00501-9
33. Goodarzi H, Nguyen HCB, Zhang S, et al. Modulated expression of specific tRNAs drives gene expression and cancer progression. *Cell*. 2016;165(6):1416–1427. doi:10.1016/j.cell.2016.05.046
34. Ishimura R, Nagy G, Dotu I, et al. RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science*. 2014;345(6195):455–459. doi:10.1126/science.1249749
35. Liu F, Clark W, Luo G, et al. ALKBH1-mediated tRNA demethylation regulates translation. *Cell*. 2016;167(7):1897. doi:10.1016/j.cell.2016.11.045
36. Zong T, Yang Y, Zhao H, et al. tsRNAs: novel small molecules from cell function and regulatory mechanism to therapeutic targets. *Cell Prolif*. 2021;54(3):e12977. doi:10.1111/cpr.12977
37. Xie Y, Yao L, Yu X, et al. Action mechanisms and research methods of tRNA-derived small RNAs. *Signal Transduct Target Ther*. 2020;5(1):109. doi:10.1038/s41392-020-00217-4
38. Kim HK, Yeom JH, Kay MA. Transfer RNA-derived small RNAs: another layer of gene regulation and novel targets for disease therapeutics. *Mol Ther*. 2020;28(11):2340–2357. doi:10.1016/j.ymthe.2020.09.013
39. Hoagland MB, Stephenson ML, Scott JF, et al. A soluble ribonucleic acid intermediate in protein synthesis. *J Biol Chem*. 1958;231(1):241–257. doi:10.1016/S0021-9258(19)77302-5
40. Zhang X, Yang F, Zhan X, et al. Structural basis of pre-tRNA intron removal by human tRNA splicing endonuclease. *Mol Cell*. 2023;83(8):1328–1339.e4. doi:10.1016/j.molcel.2023.03.015
41. Kumar P, Anaya J, Mudunuri SB, et al. Meta-analysis of tRNA derived RNA fragments reveals that they are evolutionarily conserved and associate with AGO proteins to recognize specific RNA targets. *BMC Biol*. 2014;12:78. doi:10.1186/s12915-014-0078-0
42. Krishna S, Raghavan S, DasGupta R, et al. tRNA-derived fragments (tRFs): establishing their turf in post-transcriptional gene regulation. *Cell Mol Life Sci*. 2021;78(6):2607–2619. doi:10.1007/s00018-020-03720-7
43. Green JA, Ansari MY, Ball HC, et al. tRNA-derived fragments (tRFs) regulate post-transcriptional gene expression via AGO-dependent mechanism in IL-1β stimulated chondrocytes. *Osteoarthritis Cartilage*. 2020;28(8):1102–1110. doi:10.1016/j.joca.2020.04.014
44. Lu S, Wei X, Tao L, et al. A novel tRNA-derived fragment tRF-3022b modulates cell apoptosis and M2 macrophage polarization via binding to cytokines in colorectal cancer. *J Hematol Oncol*. 2022;15(1):176. doi:10.1186/s13045-022-01388-z
45. Szaflarski W, Leśniczak-Staszak M, Sowiński M, et al. Early rRNA processing is a stress-dependent regulatory event whose inhibition maintains nucleolar integrity. *Nucleic Acids Res*. 2022;50(2):1033–1051. doi:10.1093/nar/gkab1231
46. Elkordy A, Mishima E, Niizuma K, et al. Stress-induced tRNA cleavage and tiRNA generation in rat neuronal PC12 cells. *J Neurochem*. 2018;146(5):560–569. doi:10.1111/jnc.14321

47. Tao EW, Cheng WY, Li W-L, et al. tiRNAs: a novel class of small noncoding RNAs that helps cells respond to stressors and plays roles in cancer progression. *J Cell Physiol*. 2020;235(2):683–690. doi:10.1002/jcp.29057
48. Sarais F, Perdomo-Sabogal A, Wimmers K, et al. tiRNAs: insights into their biogenesis, functions, and future applications in livestock research. *Noncoding RNA*. 2022;8(3). doi:10.3390/ncrna8030037
49. Denisenko O. Epigenetics of Ribosomal RNA Genes. *Biochemistry*. 2022;87(Suppl 1):S103–s31. doi:10.1134/S0006297922140097
50. Sharifi S, Bierhoff H. Regulation of RNA Polymerase I transcription in development, disease, and aging. *Annu Rev Biochem*. 2018;87:51–73. doi:10.1146/annurev-biochem-062917-012612
51. Schneider B, Morávek Z, Berman HM. RNA conformational classes. *Nucleic Acids Res*. 2004;32(5):1666–1677. doi:10.1093/nar/gkh333
52. Feng S, Manley JL. Beyond rRNA: nucleolar transcription generates a complex network of RNAs with multiple roles in maintaining cellular homeostasis. *Genes Dev*. 2022;36(15–16):876–886. doi:10.1101/gad.349969.122
53. Tafforeau L. About the ribosomal biogenesis in human. *Med Sci*. 2015;31(6–7):622–628. doi:10.1051/medsci/20153106015
54. Hori Y, Engel C, Kobayashi T. Regulation of ribosomal RNA gene copy number, transcription and nucleolus organization in eukaryotes. *Nat Rev Mol Cell Biol*. 2023;24(6):414–429. doi:10.1038/s41580-022-00573-9
55. Ghoshal K, Majumder S, Datta J, et al. Role of human ribosomal RNA (rRNA) promoter methylation and of methyl-CpG-binding protein MBD2 in the suppression of rRNA gene expression. *J Biol Chem*. 2004;279(8):6783–6793. doi:10.1074/jbc.M309393200
56. Kaliatsi EG, Giarimoglou N, Stathopoulos C, et al. Non-coding RNA-driven regulation of rRNA biogenesis. *Int J Mol Sci*. 2020;21(24):9738. doi:10.3390/ijms21249738
57. Tuorto F, Parlato R. rRNA and tRNA bridges to neuronal homeostasis in health and disease. *J Mol Biol*. 2019;431(9):1763–1779. doi:10.1016/j.jmb.2019.03.004
58. Harold CM, Buhagiar AF, Cheng Y, et al. Ribosomal RNA transcription regulation in breast cancer. *Genes*. 2021;12(4):502. doi:10.3390/genes12040502
59. Gaviraghi M, Vivori C, Tonon G. How cancer exploits ribosomal RNA biogenesis: a Journey beyond the boundaries of rRNA transcription. *Cells*. 2019;8(9):1098. doi:10.3390/cells8091098
60. Oie S, Matsuzaki K, Yokoyama W, et al. Hepatic rRNA transcription regulates high-fat-diet-induced obesity. *Cell Rep*. 2014;7(3):807–820. doi:10.1016/j.celrep.2014.03.038
61. Zheng L, Zhang Z, Sheng P, et al. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. *Ageing Res Rev*. 2021;66:101249. doi:10.1016/j.arr.2020.101249
62. Salucci S, Falcieri E, Battistelli M. Chondrocyte death involvement in osteoarthritis. *Cell Tissue Res*. 2022;389(2):159–170. doi:10.1007/s00441-022-03639-4
63. Li L, Li J, Li J-J, et al. Chondrocyte autophagy mechanism and therapeutic prospects in osteoarthritis. *Front Cell Dev Biol*. 2024;12:1472613. doi:10.3389/fcell.2024.1472613
64. Lee YT, Mohd Yunus MH, Yazid MD, et al. Unraveling the path to osteoarthritis management: targeting chondrocyte apoptosis for therapeutic intervention. *Front Cell Dev Biol*. 2024;12:1347126. doi:10.3389/fcell.2024.1347126
65. Bedingfield SK, Colazo JM, Yu F, et al. Amelioration of post-traumatic osteoarthritis via nanoparticle depots delivering small interfering RNA to damaged cartilage. *Nat Biomed Eng*. 2021;5(9):1069–1083. doi:10.1038/s41551-021-00780-3
66. Zhou D, Wei Y, Sheng S, et al. MMP13-targeted siRNA-loaded micelles for diagnosis and treatment of posttraumatic osteoarthritis. *Bioact Mater*. 2024;37:378–392. doi:10.1016/j.bioactmat.2024.04.010
67. Bedingfield SK, Colazo JM, Di Francesco M, et al. Top-down fabricated microPlates for prolonged, intra-articular matrix metalloproteinase 13 siRNA nanocarrier delivery to reduce post-traumatic osteoarthritis. *ACS Nano*. 2021;15(9):14475–14491. doi:10.1021/acsnano.1c04005
68. Duan X, Cai L, Pham CTN, et al. Amelioration of posttraumatic osteoarthritis in mice using intraarticular silencing of periostin via nanoparticle-based small interfering RNA. *Arthritis Rheumatol*. 2021;73(12):2249–2260. doi:10.1002/art.41794
69. Yan H, Duan X, Pan H, et al. Suppression of NF-κB activity via nanoparticle-based siRNA delivery alters early cartilage responses to injury. *Proc Natl Acad Sci U S A*. 2016;113(41):E6199–e6208. doi:10.1073/pnas.1608245113
70. Zhang H, Yan W, Wang J, et al. Surface functionalization of exosomes for chondrocyte-targeted siRNA delivery and cartilage regeneration. *J Control Release*. 2024;369:493–505. doi:10.1016/j.jconrel.2024.04.009
71. Zhang ZJ, Hou Y-K, Chen M-W, et al. A pH-responsive metal-organic framework for the co-delivery of HIF-2α siRNA and curcumin for enhanced therapy of osteoarthritis. *J Nanobiotechnology*. 2023;21(1):18. doi:10.1186/s12951-022-01758-2
72. Shin HJ, Park H, Shin N, et al. p66shc siRNA nanoparticles ameliorate chondrocytic mitochondrial dysfunction in osteoarthritis. *Int J Nanomed*. 2020;15:2379–2390. doi:10.2147/IJN.S234198
73. Lianxu C, Hongti J, Changlong Y. NF-kappaBp65-specific siRNA inhibits expression of genes of COX-2, NOS-2 and MMP-9 in rat IL-1beta-induced and TNF-alpha-induced chondrocytes. *Osteoarthritis Cartilage*. 2006;14(4):367–376. doi:10.1016/j.joca.2005.10.009
74. Chen LX, Lin L, Wang HJ, et al. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. *Osteoarthritis Cartilage*. 2008;16(2):174–184. doi:10.1016/j.joca.2007.06.006
75. Chen X, Liu Y, Wen Y, et al. A photothermal-triggered nitric oxide nanogenerator combined with siRNA for precise therapy of osteoarthritis by suppressing macrophage inflammation. *Nanoscale*. 2019;11(14):6693–6709. doi:10.1039/C8NR10013F
76. Park H, Lee H-R, Shin HJ, et al. p16INK4a-siRNA nanoparticles attenuate cartilage degeneration in osteoarthritis by inhibiting inflammation in fibroblast-like synoviocytes. *Biomater Sci*. 2022;10(12):3223–3235. doi:10.1039/D1BM01941D
77. Zhou HW, Lou SQ, Zhang K. Recovery of function in osteoarthritic chondrocytes induced by p16INK4a-specific siRNA in vitro. *Rheumatology*. 2004;43(5):555–568. doi:10.1093/rheumatology/keh127
78. Chu X, You H, Yuan X, et al. Protective effect of lentivirus-mediated siRNA targeting ADAMTS-5 on cartilage degradation in a rat model of osteoarthritis. *Int J Mol Med*. 2013;31(5):1222–1228. doi:10.3892/ijmm.2013.1318
79. Pi Y, Zhang X, Shao Z, et al. Intra-articular delivery of anti-Hif-2α siRNA by chondrocyte-homing nanoparticles to prevent cartilage degeneration in arthritic mice. *Gene Ther*. 2015;22(6):439–448. doi:10.1038/gt.2015.16
80. Conte R, Finicelli M, Borrone A, et al. MMP-2 silencing through siRNA loaded positively-charged nanoparticles (AcPEI-NPs) counteracts chondrocyte de-differentiation. *Polymers*. 2023;15(5):1172. doi:10.3390/polym15051172

81. Shin HJ, Park H, Shin N, et al. p47phox siRNA-loaded PLGA nanoparticles suppress ROS/oxidative stress-induced chondrocyte damage in osteoarthritis. *Polymers*. 2020;12(2):443. doi:10.3390/polym12020443
82. Gong Y, Li S-J, Liu R, et al. Inhibition of YAP with siRNA prevents cartilage degradation and ameliorates osteoarthritis development. *J Mol Med*. 2019;97(1):103–114. doi:10.1007/s00109-018-1705-y
83. Akagi R, Sasho T, Saito M, et al. Effective knock down of matrix metalloproteinase-13 by an intra-articular injection of small interfering RNA (siRNA) in a murine surgically-induced osteoarthritis model. *J Orthop Res*. 2014;32(9):1175–1180. doi:10.1002/jor.22654
84. Yan H, Duan X, Pan H, et al. Development of a peptide-siRNA nanocomplex targeting NF- κB for efficient cartilage delivery. *Sci Rep*. 2019;9(1):442. doi:10.1038/s41598-018-37018-3
85. Hoshi H, Akagi R, Yamaguchi S, et al. Effect of inhibiting MMP13 and ADAMTS5 by intra-articular injection of small interfering RNA in a surgically induced osteoarthritis model of mice. *Cell Tissue Res*. 2017;368(2):379–387. doi:10.1007/s00441-016-2563-y
86. Li LL, Li X-F, Zhang J-Y, et al. Repair of osteoarthritis in animal model with exosomes derived from BMSCs transfected by the siRNA -Piezo1 through CT navigation. *Zhongguo Gu Shang*. 2021;34(12):1171–1178. doi:10.12200/j.issn.1003-0034.2021.12.015
87. Legendre F, Ollitrault D, Hervieu M, et al. Enhanced hyaline cartilage matrix synthesis in collagen sponge scaffolds by using siRNA to stabilize chondrocytes phenotype cultured with bone morphogenetic protein-2 under hypoxia. *Tissue Eng Part C Methods*. 2013;19(7):550–567. doi:10.1089/ten.tec.2012.0508
88. Shi Y, Hu X, Cheng J, et al. A small molecule promotes cartilage extracellular matrix generation and inhibits osteoarthritis development. *Nat Commun*. 2019;10(1):1914. doi:10.1038/s41467-019-09839-x
89. Sondergaard BC, Schultz N, Madsen SH, et al. MAPKs are essential upstream signaling pathways in proteolytic cartilage degradation--divergence in pathways leading to aggrecanase and MMP-mediated articular cartilage degradation. *Osteoarthritis Cartilage*. 2010;18(3):279–288. doi:10.1016/j.joca.2009.11.005
90. Zou ZL, Sun M-H, Yin W-F, et al. Avicularin suppresses cartilage extracellular matrix degradation and inflammation via TRAF6/MAPK activation. *Phytomedicine*. 2021;91:153657. doi:10.1016/j.phymed.2021.153657
91. Guo Y, Min Z, Jiang C, et al. Downregulation of HS6ST2 by miR-23b-3p enhances matrix degradation through p38 MAPK pathway in osteoarthritis. *Cell Death Dis*. 2018;9(6):699. doi:10.1038/s41419-018-0729-0
92. Stanton H, Rogerson FM, East CJ, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature*. 2005;434(7033):648–652. doi:10.1038/nature03417
93. Wang M, Sampson ER, Jin H, et al. MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther*. 2013;15(1):R5. doi:10.1186/ar4133
94. Fei J, Liang B, Jiang C, et al. Luteolin inhibits IL-1β-induced inflammation in rat chondrocytes and attenuates osteoarthritis progression in a rat model. *Biomed Pharmacother*. 2019;109:1586–1592. doi:10.1016/j.biopha.2018.09.161
95. Nieuwstraten J, Riester R, Hofmann UK, et al. Matrix metalloproteinases accelerate pericellular matrix breakdown and disrupt mechanotransduction in osteoarthritis. *Acta Biomater*. 2025;195:73–82. doi:10.1016/j.actbio.2025.02.034
96. Olivotto E, Otero M, Marcu KB, et al. Pathophysiology of osteoarthritis: canonical NF-κB/IKKβ-dependent and kinase-independent effects of IKKα in cartilage degradation and chondrocyte differentiation. *RMD Open*. 2015;1(Suppl 1):e000061. doi:10.1136/rmdopen-2015-000061
97. Zhang FJ, Luo W, Lei GH. Role of HIF-1α and HIF-2α in osteoarthritis. *Joint Bone Spine*. 2015;82(3):144–147. doi:10.1016/j.jbspin.2014.10.003
98. Deng Y, Wu A, Li P, et al. Yap1 regulates multiple steps of chondrocyte differentiation during skeletal development and bone repair. *Cell Rep*. 2016;14(9):2224–2237. doi:10.1016/j.celrep.2016.02.021
99. Savadipour A, Nims RJ, Rashidi N, et al. Membrane stretch as the mechanism of activation of PIEZO1 ion channels in chondrocytes. *Proc Natl Acad Sci U S A*. 2023;120(30):e2221958120. doi:10.1073/pnas.2221958120
100. Lee W, Nims RJ, Savadipour A, et al. Inflammatory signaling sensitizes Piezo1 mechanotransduction in articular chondrocytes as a pathogenic feed-forward mechanism in osteoarthritis. *Proc Natl Acad Sci U S A*. 2021;118(13). doi:10.1073/pnas.2001611118
101. Brylka LJ, Alimy A-R, Tschaffon-Müller MEA, et al. Piezo1 expression in chondrocytes controls endochondral ossification and osteoarthritis development. *Bone Res*. 2024;12(1):12. doi:10.1038/s41413-024-00315-x
102. Damman W, Liu R, Bloem JL, et al. Bone marrow lesions and synovitis on MRI associate with radiographic progression after 2 years in hand osteoarthritis. *Ann Rheum Dis*. 2017;76(1):214–217. doi:10.1136/annrheumdis-2015-209036
103. Felson DT, Niu J, Neogi T, et al. Synovitis and the risk of knee osteoarthritis: the MOST Study. *Osteoarthritis Cartilage*. 2016;24(3):458–464. doi:10.1016/j.joca.2015.09.013
104. Li YH, Tavallaee G, Tokar T, et al. Identification of synovial fluid microRNA signature in knee osteoarthritis: differentiating early- and late-stage knee osteoarthritis. *Osteoarthritis Cartilage*. 2016;24(9):1577–1586. doi:10.1016/j.joca.2016.04.019
105. Li Y, Zhao J, Guo S, et al. siRNA therapy in osteoarthritis: targeting cellular pathways for advanced treatment approaches. *Front Immunol*. 2024;15:1382689. doi:10.3389/fimmu.2024.1382689
106. Shan Y, Zhao J, Wei K, et al. Multi-target RNA interference: a disruptive next-generation strategy for precision treatment of rheumatoid arthritis. *Int Immunopharmacol*. 2025;159:114890. doi:10.1016/j.intimp.2025.114890
107. Mahmoudian A, Lohmander LS, Mobasheri A, et al. Early-stage symptomatic osteoarthritis of the knee - time for action. *Nat Rev Rheumatol*. 2021;17(10):621–632. doi:10.1038/s41584-021-00673-4
108. Hu S, Mao G, Zhang Z, et al. MicroRNA-320c inhibits development of osteoarthritis through downregulation of canonical Wnt signaling pathway. *Life Sci*. 2019;228:242–250. doi:10.1016/j.lfs.2019.05.011
109. Iqbal Z, Xia J, Murtaza G, et al. Targeting WNT signalling pathways as new therapeutic strategies for osteoarthritis. *J Drug Target*. 2023;31(10):1027–1049. doi:10.1080/1061186X.2023.2281861
110. Huang X, Yu J, Gou S, et al. CRISPR/CasRx-mediated RNA knockdown targeting β-catenin and Ihh signaling alleviates osteoarthritis. *Genes Dis*. 2025;12(4):101468. doi:10.1016/j.gendis.2024.101468
111. Zhou X, Wang M, Liu C, et al. Chondrocyte apoptosis in early osteoarthritis inhibited by silencing microRNA-34a. *Chin J Exp Surg*. 2014;31(7):1413–1415.
112. McKinney JM, Pucha KA, Bernard FC, et al. Osteoarthritis early-, mid- and late-stage progression in the rat medial meniscus transection model. *J Orthop Res*. 2025;43(1):102–116. doi:10.1002/jor.25969

113. Bedingfield SK, Yu F, Liu DD, et al. Matrix-targeted nanoparticles for MMP13 RNA interference blocks post-traumatic osteoarthritis. *bioRxiv*. 2020.
114. Zhang X, Chen W, Lan S, et al. Stem cell-derived small extracellular vesicles containing miR-27b-3p attenuated osteoarthritis through inhibition of leukaemia inhibitory factor. *Fundam Res*. 2023.
115. Dumenci L, Perera RA, Keefe FJ, et al. Model-based pain and function outcome trajectory types for patients undergoing knee arthroplasty: a secondary analysis from a randomized clinical trial. *Osteoarthritis Cartilage*. 2019;27(6):878–884. doi:10.1016/j.joca.2019.01.004
116. Deng Z, Long D, Liu H, et al. tRNA-derived fragment tRF-5009A regulates autophagy and degeneration of cartilage in osteoarthritis via targeting mTOR. *Oxid Med Cell Longev*. 2022;2022:5781660. doi:10.1155/2022/5781660
117. Balaskas P, Green JA, Haqqi TM, et al. Small non-coding RNAome of ageing chondrocytes. *Int J Mol Sci*. 2020;21(16):5675. doi:10.3390/ijms21165675
118. Magnussen RA, Duthon V, Servien E, et al. Anterior cruciate ligament reconstruction and osteoarthritis: evidence from long-term follow-up and potential solutions. *Cartilage*. 2013;4(3 Suppl):22s–6s. doi:10.1177/1947603513486559
119. Barenius B, Ponzer S, Shalabi A, et al. Increased risk of osteoarthritis after anterior cruciate ligament reconstruction: a 14-year follow-up study of a randomized controlled trial. *Am J Sports Med*. 2014;42(5):1049–1057. doi:10.1177/0363546514526139
120. Long D, Xu Y, Mao G, et al. tRNA-derived fragment TRF365 regulates the metabolism of anterior cruciate ligament cells by targeting IKBKB. *Cell Death Discov*. 2022;8(1):19. doi:10.1038/s41420-021-00806-4
121. Ni Y, Wu A, Li J, et al. Evaluation of the serum tRNA-derived fragment tRF-5022B as a potential biomarker for the diagnosis of osteoarthritis. *J Orthop Surg Res*. 2023;18(1):800. doi:10.1186/s13018-023-04273-8
122. Varela-Eirin M, Loureiro J, Fonseca E, et al. Cartilage regeneration and ageing: targeting cellular plasticity in osteoarthritis. *Ageing Res Rev*. 2018;42:56–71. doi:10.1016/j.arr.2017.12.006
123. D'Aquila P, Montesanto A, Mandalà M, et al. Methylation of the ribosomal RNA gene promoter is associated with aging and age-related decline. *Aging Cell*. 2017;16(5):966–975. doi:10.1111/acel.12603
124. Ugalde AP, Roiz-Valle D, Moledo-Nodar L, et al. Noncoding RNA contribution to aging and lifespan. *J Gerontol a Biol Sci Med Sci*. 2024;79(4). doi:10.1093/gerona/glae058
125. Watada E, Li S, Hori Y, et al. Age-dependent ribosomal DNA variations in mice. *Mol Cell Biol*. 2020;40(22). doi:10.1128/MCB.00368-20
126. Ren X, Hu B, Song M, et al. Maintenance of nucleolar homeostasis by CBX4 alleviates senescence and osteoarthritis. *Cell Rep*. 2019;26(13):3643–3656.e7. doi:10.1016/j.celrep.2019.02.088
127. Wajahat M, Bracken CP, Orang A. Emerging functions for snoRNAs and snoRNA-derived fragments. *Int J Mol Sci*. 2021;22(19):10193. doi:10.3390/ijms221910193
128. Dieci G, Preti M, Montanini B. Eukaryotic snoRNAs: a paradigm for gene expression flexibility. *Genomics*. 2009;94(2):83–88. doi:10.1016/j.ygeno.2009.05.002
129. Peffers M, Liu X, Clegg P. Transcriptomic signatures in cartilage ageing. *Arthritis Res Ther*. 2013;15(4):R98. doi:10.1186/ar4278
130. Peffers MJ, Chabronova A, Balaskas P, et al. SnoRNA signatures in cartilage ageing and osteoarthritis. *Sci Rep*. 2020;10(1):10641. doi:10.1038/s41598-020-67446-z
131. Massenet S, Bertrand E, Verheggen C. Assembly and trafficking of box C/D and H/ACA snoRNPs. *RNA Biol*. 2017;14(6):680–692. doi:10.1080/15476286.2016.1243646
132. Ripmeester EGJ, Caron MMJ, van den Akker GGH, et al. Impaired chondrocyte U3 snoRNA expression in osteoarthritis impacts the chondrocyte protein translation apparatus. *Sci Rep*. 2020;10(1):13426. doi:10.1038/s41598-020-70453-9
133. Mattijssen S, Welting TJ, Pruijn GJ. RNase MRP and disease. *Wiley Interdiscip Rev RNA*. 2010;1(1):102–116. doi:10.1002/wrna.9
134. Chabronova A, van den Akker GGH, Meekels-Steinbusch MMF, et al. Uncovering pathways regulating chondrogenic differentiation of CHH fibroblasts. *Noncoding RNA Res*. 2021;6(4):211–224. doi:10.1016/j.ncrna.2021.12.003
135. Ontiveros RJ, Stoute J, Liu KF. The chemical diversity of RNA modifications. *Biochem J*. 2019;476(8):1227–1245. doi:10.1042/BCJ20180445
136. Jaafar M, Paraqindes H, Gabut M, et al. 2'O-ribose methylation of ribosomal RNAs: natural diversity in living organisms, biological processes, and diseases. *Cells*. 2021;10(8):1948. doi:10.3390/cells10081948
137. Jiapaer Z, Su D, Hua L, et al. Regulation and roles of RNA modifications in aging-related diseases. *Aging Cell*. 2022;21(7):e13657. doi:10.1111/acel.13657
138. Jonkhout N, Tran J, Smith MA, et al. The RNA modification landscape in human disease. *Rna*. 2017;23(12):1754–1769. doi:10.1261/rna.063503.117
139. Chabronova A, van den Akker GGH, Housmans BAC, et al. Ribosomal RNA-based epitranscriptomic regulation of chondrocyte translation and proteome in osteoarthritis. *Osteoarthritis Cartilage*. 2023;31(3):374–385. doi:10.1016/j.joca.2022.12.010
140. Marchand V, Pichot F, Neybecker P, et al. HydraPsiSeq: a method for systematic and quantitative mapping of pseudouridines in RNA. *Nucleic Acids Res*. 2020;48(19):e110. doi:10.1093/nar/gkaa769
141. Chabronova A, van den Akker G, Housmans BAC, et al. Depletion of SNORA33 abolishes ψ of 28S-U4966 and affects the ribosome translational apparatus. *Int J Mol Sci*. 2023;24(16):12578. doi:10.3390/ijms241612578
142. Moss T. At the crossroads of growth control; making ribosomal RNA. *Curr Opin Genet Dev*. 2004;14(2):210–217. doi:10.1016/j.gde.2004.02.005
143. Huffines AK, Engel KL, French SL, et al. Rate of transcription elongation and sequence-specific pausing by RNA polymerase I directly influence rRNA processing. *J Biol Chem*. 2022;298(12):102730. doi:10.1016/j.jbc.2022.102730
144. Ripmeester EGJ, Caron MMJ, van den Akker GGH, et al. BMP7 reduces the fibrocartilage chondrocyte phenotype. *Sci Rep*. 2021;11(1):19663. doi:10.1038/s41598-021-99096-0
145. Caron MMJ, Ripmeester EGJ, van den Akker G, et al. Discovery of bone morphogenetic protein 7-derived peptide sequences that attenuate the human osteoarthritic chondrocyte phenotype. *Mol Ther Methods Clin Dev*. 2021;21:247–261. doi:10.1016/j.omtm.2021.03.009
146. Ripmeester EGJ, Welting TJM, van den Akker GGH, et al. BMP7 increases protein synthesis in SW1353 cells and determines rRNA levels in a NKX3-2-dependent manner. *PLoS One*. 2022;17(2):e0263430. doi:10.1371/journal.pone.0263430
147. Yang C, Chen R, Chen C, et al. Tissue engineering strategies hold promise for the repair of articular cartilage injury. *Biomed Eng Online*. 2024;23(1):92. doi:10.1186/s12938-024-01260-w
148. Shim MS, Kwon YJ. Efficient and targeted delivery of siRNA in vivo. *Febs J*. 2010;277(23):4814–4827. doi:10.1111/j.1742-4658.2010.07904.x

149. Mitchell MJ, Billingsley MM, Haley RM, et al. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 2021;20(2):101–124. doi:10.1038/s41573-020-0090-8
150. Hagedorn PH, Persson R, Funder ED, et al. Locked nucleic acid: modality, diversity, and drug discovery. *Drug Discov Today*. 2018;23(1):101–114. doi:10.1016/j.drudis.2017.09.018
151. Abou Assi H, Rangadurai AK, Shi H, et al. 2′- O -methylation can increase the abundance and lifetime of alternative RNA conformational states. *Nucleic Acids Res*. 2020;48(21):12365–12379. doi:10.1093/nar/gkaa928
152. Ali Zaidi SS, Fatima F, Ali Zaidi SA, et al. Engineering siRNA therapeutics: challenges and strategies. *J Nanobiotechnology*. 2023;21(1):381. doi:10.1186/s12951-023-02147-z
153. Zhu H, Luo H, Chang R, et al. Protein-based delivery systems for RNA delivery. *J Control Release*. 2023;363:253–274. doi:10.1016/j.jconrel.2023.09.032
154. Gil-Cabrerizo P, Simon-Yarza T, Garbayo E, et al. Navigating the landscape of RNA delivery systems in cardiovascular disease therapeutics. *Adv Drug Deliv Rev*. 2024;208:115302. doi:10.1016/j.addr.2024.115302
155. Hueso M, Mallén A, Suñé-Pou M, et al. ncRNAs in therapeutics: challenges and limitations in nucleic acid-based drug delivery. *Int J Mol Sci*. 2021;22(21):11596. doi:10.3390/ijms222111596
156. Guo C, Ma X, Gao F, et al. Off-target effects in CRISPR/Cas9 gene editing. *Front Bioeng Biotechnol*. 2023;11:1143157. doi:10.3389/fbioe.2023.1143157
157. Jackson AL, Linsley PS. Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. *Nat Rev Drug Discov*. 2010;9(1):57–67. doi:10.1038/nrd3010
158. Sun H. The challenge facing CRISPR/Cas9 system: off-target effects and their optimization. *Highlights Sci Eng Technol*. 2023;74:782–787. doi:10.54097/psd28z73
159. Li X, Hu H, Wang H, et al. DNA nanotechnology-based strategies for minimising hybridisation-dependent off-target effects in oligonucleotide therapies. *Mater Horizons*. 2025;12(5):1388–1412. doi:10.1039/D4MH01158A
160. Wu J, Yin H. Engineering guide RNA to reduce the off-target effects of CRISPR. *J Genet Genomics*. 2019;46(11):523–529. doi:10.1016/j.jgg.2019.11.003
161. Wang G, Liu X, Wang A, et al. CRISPRoffT: comprehensive database of CRISPR/Cas off-targets. *Nucleic Acids Res*. 2025;53(D1):D914–d924. doi:10.1093/nar/gkae1025
162. van den Akker GGH, Caron MMJ, Peffers MJ, et al. Ribosome dysfunction in osteoarthritis. *Curr Opin Rheumatol*. 2022;34(1):61–67. doi:10.1097/BOR.0000000000000858
163. Bartoszewski R, Sikorski AF. Editorial focus: understanding off-target effects as the key to successful RNAi therapy. *Cell Mol Biol Lett*. 2019;24:69. doi:10.1186/s11658-019-0196-3
164. Han D, Xu MM. RNA modification in the immune system. *Annu Rev Immunol*. 2023;41:73–98. doi:10.1146/annurev-immunol-101921-045401
165. Liu A, Wang X. The pivotal role of chemical modifications in mRNA therapeutics. *Front Cell Dev Biol*. 2022;10:901510. doi:10.3389/fcell.2022.901510
166. Landesman-Milo D, Peer D. Altering the immune response with lipid-based nanoparticles. *J Control Release*. 2012;161(2):600–608. doi:10.1016/j.jconrel.2011.12.034
167. Saw PE, Song E. Advancements in clinical RNA therapeutics: present developments and prospective outlooks. *Cell Rep Med*. 2024;5(5):101555. doi:10.1016/j.xcrm.2024.101555
168. Huang CK, Kafert-Kasting S, Thum T. Preclinical and clinical development of noncoding RNA therapeutics for cardiovascular disease. *Circ Res*. 2020;126(5):663–678. doi:10.1161/CIRCRESAHA.119.315856
169. McGettigan P, Alonso Olmo C, Plueschke K, et al. Patient registries: an underused resource for medicines evaluation: operational proposals for increasing the use of patient registries in regulatory assessments. *Drug Saf*. 2019;42(11):1343–1351. doi:10.1007/s40264-019-00848-9
170. Chen Y, Luo X, Kang R, et al. Current therapies for osteoarthritis and prospects of CRISPR-based genome, epigenome, and RNA editing in osteoarthritis treatment. *J Genet Genomics*. 2024;51(2):159–183. doi:10.1016/j.jgg.2023.07.007