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Antisense oligonucleotide-based therapies for the treatment of osteoarthritis: Opportunities and roadblocks

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ABSTRACT

Osteoarthritis (OA) is a debilitating disease with no approved disease-modifying therapies. Among the challenges for developing treatment is achieving targeted drug delivery to affected joints. This has contributed to the failure of several drug candidates for the treatment of OA. Over the past 20 years, significant advances have been made in antisense oligonucleotide (ASO) technology for achieving targeted delivery to tissues and cells both in vitro and in vivo. Since ASOs are able to bind specific gene regions and regulate protein translation, they are useful for correcting aberrant endogenous mechanisms associated with certain diseases. ASOs can be delivered locally through intra-articular injection, and can enter cells through natural cellular uptake mechanisms. Despite this, ASOs have yet to be successfully tested in clinical trials for the treatment of OA. Recent chemical modification to ASOs have further improved cellular uptake and reduced toxicity. Among these are locked nucleic acid (LNA)-based ASOs, which have shown promising results in clinical trials for diseases such as hepatitis and dyslipidemia. Recently, LNA-based ASOs have been tested both in vitro and in vivo for their therapeutic potential in OA, and some have shown promising joint-protective effects in preclinical OA animal models. In order to accelerate the testing of ASO therapies in a clinical trial setting for OA, further investigation into delivery mechanisms is required. In this review article, we discuss opportunities for viral-, particle-, biomaterial-, and chemical modification-based therapies, which are currently in preclinical testing. We also address potential roadblocks in the clinical translation of ASO-based therapies for the treatment of OA, such as the limitations associated with OA animal models and the challenges with drug toxicity. Taken together, we review what is known and what would be useful to accelerate translation of ASO-based therapies for the treatment of OA.

1. Introduction

Osteoarthritis (OA) is a degenerative disease of the joints, affecting approximately 10% of men and 18% of women over the age of 60 worldwide [1]. It is the most common type of arthritis, yet there is no cure. Characteristic features of OA include cartilage degeneration, synovial inflammation, subchondral sclerosis and osteophyte formation. OA is associated with persistent pain in articulating joints including the knees, hips, hands and spine, among other joints. This significantly impacts mobility and independence, with 25% of those affected by OA being unable to perform major daily activities [1]. Treatment options are limited, as there are no approved disease-modifying OA drugs (DMOADs). Symptom-modifying drug therapies provide only short-term relief from pain. Often the disease continues to progress leading to joint replacement surgery as the only viable option [2]. This is an invasive and expensive treatment, and is not a solution for those with generalized OA affecting multiple joints throughout the body. There is an outstanding need in the OA field for drugs which can not only attenuate symptoms but prevent disease progression.

Over recent decades, there have been several attempts to identify

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DMOADs, including clinical trials with biologics targeting pro-inflammatory cytokines [3–5], catabolic enzymes [6] and growth factors [7]. An ideal agent would target the major features of OA by halting cartilage degeneration, synovitis and subchondral bone sclerosis, or even promoting tissue regeneration. However, none of the trials have been able to adequately demonstrate disease modification in OA patients [3,8,9]. Reasons for this may include inefficacy of the drug itself, inadequate drug targeting and penetration in the local tissue, or rapid physiological turnover, among others. Given that OA affects the entire joint, drugs are required to be beneficial, or at least not harmful, to multiple tissues including the cartilage, bone, synovium and ligaments. These are among the barriers, in addition to safety, for developing novel DMOAD candidates with the potential for clinical translation.

Over 40 years ago, a breakthrough discovery was made in antisense technology with major implications for disease therapy. Antisense oligonucleotides (ASOs) were described in 1978 by Zamecnik and Stepheson, who reported that a 13-nucleotide-long oligodeoxynucleotide with complementarity to the target sequence in sarcoma virus RNA was able to block viral replication and protein translation in vitro [10]. Since this first generation of ASOs, several modifications have been made to enable clinical application where disease outcomes are improved and toxicity is minimized. These second and third generation ASOs are currently available in the market or are undergoing clinical trials, showing beneficial outcomes in refractory diseases such as amyloidosis, muscular atrophy and lymphoma [11–14].

One of the first reports showing the impact of ASOs on cartilage was in 1994 [15]. Nietfeld et al. showed interleukin (IL)-6-ASOs could inhibit IL-1-induced production of IL-6 and prevent IL-1-induced inhibition of proteoglycan synthesis in ex-vivo human articular cartilage [15]. Subsequently, Fibbi et al. proposed ASOs as a potential new class of drugs for OA, where ASOs targeting urokinase-typeplasminogen activator (u-PA) were used to inhibit u-PA-dependent cell proliferation and chemo-invasion of synoviocytes [16]. Since then, ASOs have been used to modulate therapeutic targets in vitro and in vivo, some of which have shown promising effects in preventing or alleviating OA features [17–20]. Targeting specific RNAs through small interfering RNAs (siRNAs) [21] and microRNAs (miRNAs) [19,20,22] has shown multiple beneficial effects in cartilage, including reductions in inflammation, catabolism and apoptosis, and induction in anabolic activity. Despite accumulating evidence over the past 25 years showing the therapeutic potential of ASOs in OA, to the best of our knowledge, none have proceeded to clinical trial testing. This review focuses on opportunities and roadblocks in the development of ASOs as a potential therapeutic strategy for OA treatment. We summarize what is known from previous studies and what may be required to accelerate clinical translation.

2. Methods

We performed a search for original articles in PubMed using key words “antisense oligonucleotide” and “osteoarthritis” and identified 39 papers before December 2019. Among these, we found 26 original research articles which reported the effects of ASOs as potential therapeutics in OA. The details of the included studies are shown in Table 1, highlighting various characteristics of the ASOs that were described.

3. Antisense oligonucleotides

3.1. History of chemical modification

Since 1978 when ASOs were described as a short fragment of unmodified DNA that was used in cell culture [10], remarkable advances have been made in ASO-based drug development. Typical ASOs possess phosphorothioate (PS) linkages as their backbone, with ribose substitution. To render the nucleotide bonds resistant to nucleases, the non-bridging oxygen atom of the phosphodiester backbone in the ASO is replaced with a sulphur atom [23]. This structure is representative of first generation ASOs. Most of these ASOs failed to reach their primary endpoints in clinical trials due to rapid turnover and low affinity to targets [24,25]. Rigorous modifications in their chemical structures yielded the second generation of ASOs, with a backbone incorporating 2’-O-methyl (2’-OMe) and 2’-O-methoxyethyl (2’-O-MOE) [26]. These ASOs had substantially improved structural stability to resist nuclease degradation and achieve greater affinity to their targets [27,28]. This led to second generation ASOs being tested in clinical trials [13,14]. Some of these have been approved by the US Food and Drug Administration for neurological disorders, including Eteplirsen for Duchenne muscular dystrophy [29] and Nusinersen for spinal muscular atrophy [30].

Representing further improvement, third generation ASOs have locked nucleic acid (LNA) technology, in which the ribose ring in the backbone is connected by a methylene bridge between the 2’-O and 4’-C atoms [31]. While third generation ASOs still have a PS backbone, the LNA technology markedly increases binding to the target RNA. LNA oligonucleotides contain modified RNA nucleotides with an extra bridge linking the 2′-O and 4′-C atoms thus “locking” the ribose ring. This leads to an increased affinity for complementary RNA targets, without loss of sequence specificity [32]. The superior performance of single-stranded LNA-ASOs, especially for in vivo applications, is becoming widely recognized in various diseases including cancer, neurodegenerative disease and systemic infection [33,34]. Notably, some of these LNA-ASOs were tested in clinical trials with encouraging results for future clinical approval [35].

3.2. ASOs: mechanism of action

ASOs are designed as “anti-sense” against specific sequences to target RNAs, including encoded mRNAs. By binding to and interfering with the function of target mRNAs, ASOs modulate the expression of proteins encoded by mRNAs. Among the multiple mechanisms through which ASOs can impact protein synthesis, the most important is activation of the RNase H enzyme [36]. Modified ASOs leave a central nucleotide-phosphorothioate gap (gapmer) that allows RNase H to cleave the target mRNA [37]. Specifically, after binding to the target mRNA, ASOs form a RNA-DNA hybrid that becomes a substrate for RNase H, leading to RNA degradation (Fig. 1). This mechanism is highly efficient and can result in 85–95% downregulation of mRNA relative to control levels [37]. Other mechanisms of downregulating or destabilizing target mRNAs include inhibition of RNA-binding protein, polyadenylation and splicing (Fig. 1). ASOs are also able to increase translational activity by blocking upstream open reading frames (uORFs) that typically inhibit the expression of the primary ORFs (Fig. 1). Therefore, ASOs can both positively and negatively regulate expression depending on their precise mechanism of action.

3.3. ASOs: advantages as a therapeutic

Current antibody-based therapies for inflammatory arthritis such as rheumatoid arthritis (RA) and spondyloarthritis (SpA) target specific protein ligands and/or receptors of pro-inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor (TNF). Therefore, antibody-based therapies can only regulate their targets after the protein is translated and secreted outside of the cell. In contrast, ASOs target specific mRNAs before protein translation. This ability to modulate expression of targets at an upstream level is a major advantage of ASOs, as functional protein products are not made. Thus, ASOs can act to inhibit the synthesis of specific proteins within cells such as chondrocytes, fibroblasts, and osteocytes, in addition to lowering levels of secreted inflammatory cytokines or catabolic enzymes in blood or synovial fluid. Demonstrating this, a study investigating cholesterol profiles in hepatocytes confirmed a reduction of triglyceride content in both hepatocytes and serum of mouse models with ASOs targeting Angiopoietin-like protein-3

Table 1
Reported use of antisense oligonucleotides in osteoarthritis research.

Target gene (s)	Backbone	Sample species	Target cells or tissues	Administration	Outcome	Reference
IL-6	PS	human	cartilage	ex vivo	Suppression of IL-1-induced production of IL-6 Prevention of IL-1-induced PG synthesis	[15]
uPA	NA	human	FLS, chondrocytes	in vitro	Suppression of cell proliferation and chemo-invasion	[16]
CD44	PS	bovine	cartilage	ex vivo	Loss of PG production	[107]
Notch-1	NA	human	FLS	in vitro	Inhibition of TNFa-induced synoviocyte proliferation	[108]
Alpha5	PS	bovine	chondrocytes, cartilage	in vitro, ex vivo	Inhibition of PG degradation	[109]
Bcl-3	PS	human, rabbit	SW-1353 cells, FLS	in vitro	Represses IL-1β–induced MMP-1 gene expression	[110]
FLIP	PS	human	FLS	in vitro	Enhancement of Fas-mediated apoptosis	[111]
GlcAT-I	PS	rat	cartilage	ex vivo	Reduction of matrix PG synthesis	[112]
caveolin 1	PS	human	chondrocytes	in vitro	Inhibition of chondrocyte senescence	[113]
Rev-ErbAα	NA	human	chondrocytes	in vitro	Reduction in IL-1–induced aggrecanase activity	[114]
c-Fos, c-Jun	PS	human	chondrocytes	in vitro	Decrease in SDF-1a-induced expression of MMP13	[115]
hGas7	PS	human	hMSCs	in vitro	Delay in the chondrogenic differentiation of hMSCs	[21]
OBRl	PS	human	FLS	in vitro	Attenuation of leptin-induced IL-8 production	[116]
Dkk-1	end-capped PS	rat		in vitro and in vivo (IP)	Attenuation of surgery-induced cartilage degeneration and subchondral damage. Decreased cell apoptosis in chondrocytes and osteoblasts	[17]
Dkk-1	end-capped PS	rat	FLS	in vivo (IP)	Reduction in the expression of angiogenic factors and proteinases Amelioration of synovial vascularity and cartilage deterioration	[117]
SHP-2	morpholino	human	FLS	in vivo (IP)	Reduction in the invasion, migration, adhesion, spreading, and survival of RA FLS.	[118]
OBRl	PS	human	FLS	in vitro	Abolishment of the leptin-mediated increase of IL-6 expression	[119]
OBRl	PS	human	osteoblasts	in vitro	Abolishment of the leptin-mediated increase of OSM (oncostatin M) expression	[120]
NA	morpholino	mouse	chondrocytes	in vivo (IA-injected PEG-SWCNTs)	IA-injected PEG-SWCNTs showed long-lasting resident time in the joint cavity, enter the cartilage matrix, and deliver gene inhibitors into chondrocytes	[78]
miR-15a	NA	human	chondrocytes	in vitro	Decrease the aggregation of proteoglycan and the collagen content, but increase the release of proteoglycan and collagen	[121]
COX-2	LNA	human	chondrocytes (in hydrogel)	in vitro	Exhibiting the effect of COX-2 silencing over 14 days	[18]
miR-320a	NA	human	chondrocytes	in vitro	Reverse the IL-1b-induced increase of MMP13 and decrease of type II collagen and aggrecan	[122]
miR-29a	PS	human	bone marrow MSCs	in vitro	Decrease in the expression of Wnt3a expression and osteogenic differentiation	[123]
miR-128	NA	rat	chondrocytes/ cartilage	in vivo (IA with lentivirus assist) in vitro	Stabilization of chondrocyte autophagy and slowed ACLT-mediated articular tissue destruction	[47]
miR-181a-5p	LNA	mouse, rat, human	chondrocyte/ cartilage	in vitro in vivo (IA)	Attenuation of surgery-induced cartilage degeneration Decrease in the expression of MMP13 and apoptosis markers	[19]
ADAMTS5	LNA	human	chondrocyte	in vitro	LNA-ASOs targeting ADAMTS5 incorporated with fibrin-HA hydrogel exhibited sustained release profile up to 14 days	[82]

Abbreviation: IL-6, interleukin-6; PS, phosphorothioate; IL-1, interleukin-1; PG, proteoglycan; uPA, urokinase plasminogen activator; FLS, fibroblast-like synoviocyte; CD44, cluster of differentiation 44; TNFα, tumor necrosis factor alpha; Bcl-3, B-cell lymphoma-3; IL-1β, interleukin-1 beta; MMP-1, matrix metalloproteinase-1; FLIP, FLICE-inhibitory protein; GlcAT-1, glycosaminoglycan (GAG)-synthesizing enzyme, β1,3-glucuronosyltransferase-I; Rev-Erbα, Rev-ErbA-alpha; MMP-13, matrix metalloproteinase-13; SDF-1α, stromal cell-derived factor-1-alpha; hGas-7, human growth-arrest-specific-7; hMSCs, human mesenchymal stem cells; OBRl, obese gene receptor (long); IL-8, interleukin-8; DKK-1, Dickkopf WNT signaling pathway inhibitor-1; IP, intraperitoneal injection; SHP-2, SH2 domain-containing phosphatase-2; RA, rheumatoid arthritis; IA, intra-articular injection; PEG-SWCNTs, polyethylene-glycol-modified single-walled carbon nanotubes; miR, microRNA; COX-2, cyclooxygenase-2; ACLT, anterior cruciate ligament transection; LNA-ASO, locked nucleic acid (LNA)-based antisense oligonucleotides; HA, hyaluronic acid; ADAMTS-5, a disintegrin and metalloproteinase with thrombospondin motifs-5; NA, not available.

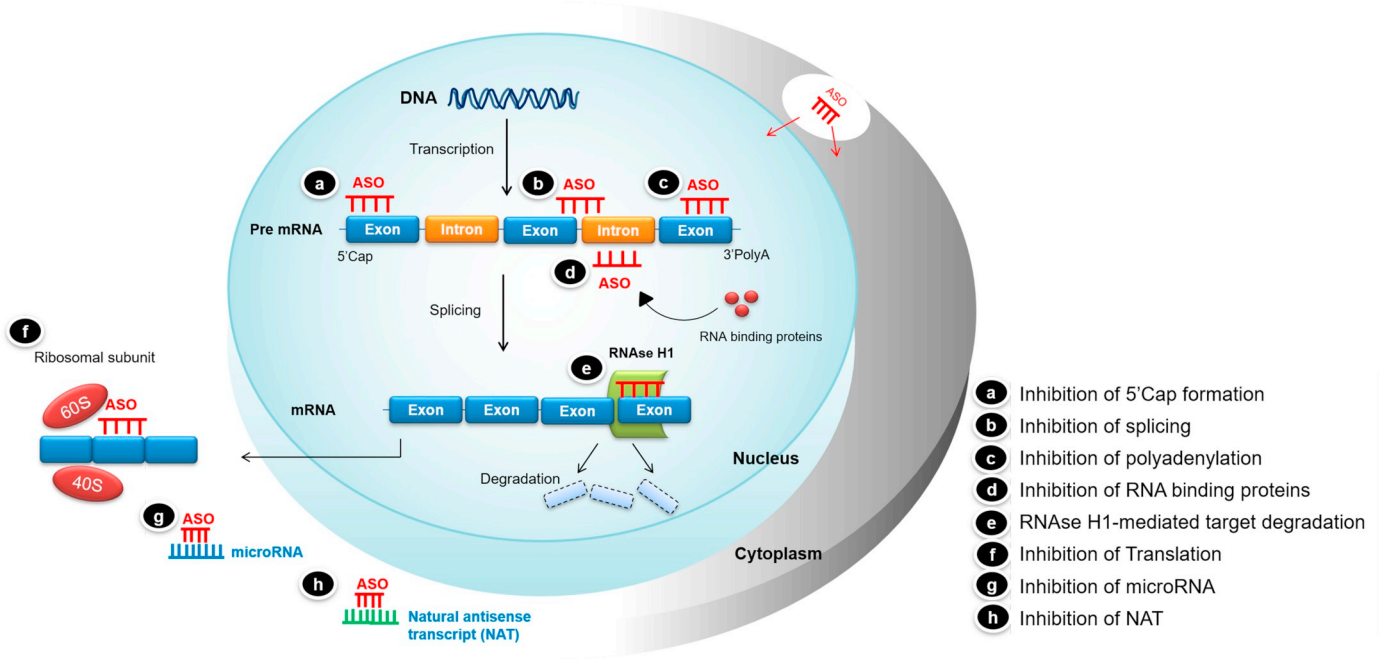

Fig. 1. Functional mechanisms of antisense oligonucleotides (ASOs) in the regulation of targets. When ASOs enter the nucleus, they can directly bind to immature mRNA (pre mRNA) and inhibit (a) formation of the 5′ cap, (b) splicing, (c) polyadenylation, and (d) attachment of RNA binding proteins to mRNA. (e) ASOs also induce degradation of ASO-targeted mRNA by RNase H1. (f) ASOs in the cytoplasm can block the binding of ribosomal subunits, which inhibits the translation of target mRNA. (g) ASOs can directly bind to microRNA sequences (h) as well as natural antisense transcripts (NATs). Thus, ASOs prevent protein synthesis by interfering with various steps of mRNA synthesis.

(ANGPTL3) [38]. This intracellular lipid reduction was not observed with anti-ANGPTL3 antibodies [39], suggesting that regulation of target genes in the desired cells via ASO treatment was able to achieve a robust effect.

A second major advantage of ASOs as a therapeutic is their ability to enter cells via natural cellular uptake mechanisms. Unlike siRNAs that are delivered to cells with lipid nanoparticles via macropinocytosis pathways [40,41], PS-based ASOs can enter cells without additional modification or formulation. In fact, one of the major mechanisms of cellular uptake is endocytosis, with ASOs binding to cell-surface proteins [42]. Several cell-surface receptors have been reported to bind ASOs, including the toll-like receptor family and scavenger receptors [43–46]. These natural uptake mechanisms enable effective ASO delivery without the disadvantages associated with other delivery mechanisms (e.g. viral delivery).

The third major advantage of ASOs as a therapeutic is that they can be delivered or injected both systemically and locally. Previous studies have demonstrated the efficacy of systemic ASO injections in preclinical animal models as well as in clinical trials [34,35]. For OA treatment, a local injection may be beneficial over a systemic injection in order to increase efficiency of drug delivery to target joint tissues. To the best of our knowledge, there have been no clinical trials conducted using an intra-articular injection of ASOs; however, recent preclinical OA studies have showed some encouraging results as described in the sections below [19,22,47].

3.4. Pharmacokinetic features of ASOs

For ASOs to be effective therapeutic agents, it is critical to maximize bioavailability and promote exposure of ASOs to the target tissues or biofluids. Drug distribution is strongly influenced by the administration route. For ASOs, routes for systemic drug administration have been well-studied, including intravenous, intraperitoneal and subcutaneous injections [38,48]. Following systemic drug administration, ASOs rapidly bind to serum proteins such as albumin and α-2 macroglobulin, which prevent ASOs from being excreted via glomerular filtration in kidneys and allow ASOs to stay in tissues and be absorbed in cells [49].

The distribution of intravenously-injected first generation ASOs was previously tested in rodent tissues in vivo [43]. ASOs were detectable in the extracellular matrix of organ tissues (e.g. liver and kidney) at 2 h post-injection, and became more prominent intracellularly 24 h post-injection, suggesting that ASOs are taken up by cells within 24 h of intravenous injection [43]. Interestingly, this study also tested the distribution of ASOs in the joint tissues. While connective tissues including bone, muscle, synovium and joint capsule were positive at 24 h post-injection, positive cells were not detected in the cartilage, which is an avascular tissue [43]. Given that the pharmacokinetic properties of ASOs are similar across species [50], it is very likely that human articular cartilage would also be the most difficult joint tissue to target by systemic injection of ASOs. Whereas pharmacokinetic studies following systemic injection are well-documented, studies on local injection of ASOs are limited. However, some recent studies show encouraging therapeutic potential of locally injected LNA-based ASOs in spinal cord injury [51], lung fibrosis [52] and OA [19,20,22].

The pharmacokinetic properties of ASOs are driven by the chemistry of the backbone. Pharmacokinetic investigation using second and third generation ASOs showed rapid drug distribution to tissues with a longstanding half-life of > 2 weeks [50]. For instance, 2'-OMe-based ASOs have been shown to have > 2 weeks tissue retention time after systemic administration [53]. Moreover, third generation LNA-based ASOs exhibit remarkable knockdown efficacy of the target gene in various tissues even 5 weeks after systemic injection [54].

4. Opportunities for clinical application

4.1. Targeted ASO delivery: intra-articular injection

The concept of ASOs may seem straightforward: a target mRNA with therapeutic relevance is selected, a complementary ASO sequence is synthesized, the ASO is administered to the relevant tissue(s) and

protein expression is modulated. Yet there are several obstacles to achieving this, one of which is successful delivery of the ASO to the target tissue and cells. These hurdles must be cleared for ASOs to reach their functional sites intracellularly, in particular the chondrocytes in the cartilage, the most commonly affected tissue in OA [55,56].

Articular cartilage exhibits a formidable biological barrier to drug delivery. Because cartilage is an avascular, alymphatic and aneural tissue, drug penetration only occurs through limited mechanisms such as diffusion and electrostatic interaction [57]. When a free (non-modified) drug is administered into the synovial fluid by intra-articular injection, it is rapidly cleared out. Small molecules may diffuse through the sub-synovial capillaries, whereas macro-molecules and particles may leave through the lymphatic vessels in the synovium. Drug diffusion through cartilage is generally slower than the rate of clearance out of the joint [56]. This means that drug penetration to chondrocytes must be adequately efficient to reach therapeutic levels before the drug is cleared from the joint space.

ASOs show potential in targeting articular cartilage, but not without challenges due to their size and anionic charge. At approximately 20 bases, ASOs are much larger than traditional small molecule drugs such as steroids [58,59]. Their anionic nature makes diffusion across the negatively-charged cell membrane difficult. Previous studies using intra-articular injection of ASOs suggest there is potential for ASOs to penetrate cartilage and reach intracellular sites, as decreased expression of target genes in preclinical mouse and rat models have been observed [22,47].

4.2. Viral-mediated gene therapy

Intra-articular injection provides a mode for local administration of viral vectors carrying ASOs. These vectors may include lentiviruses, adenoviruses, adeno-associated viruses (AAV) and retroviruses. Preclinical evidence from animal studies supports the use of viral vectors for ASO delivery. Lian et al. demonstrated that lentivirus-shuttled ASOs could be delivered to the cartilage of the mouse knee joint by intra-articular administration to effectively reduce target gene expression of *Atg12*, an autophagy regulator [47]. Others used adenovirus and successfully showed that inhibition of miR-101 with ASOs prevented cartilage degeneration by targeting SOX9 in a monoiodoacetate-induced rat model of OA [60]. Previous clinical trials with viral-mediated gene therapy showed encouraging results [61,62], suggesting that this system may be effective for ASO delivery in future clinical trials.

AAV is now widely used for gene therapy because of its ability to transduce different cell types and tissues, and its low risk of immunogenicity [63]. Retrovirus was used in the world's first gene therapy product TissueGene C (Invossa), which was approved for the treatment of OA in South Korea in 2017 [64]. Invossa is a drug containing allogenic chondrocytes that have been transduced with retrovirus containing transforming growth factor-beta 1 (TGF-β1). Although structural modification is still unclear, Invossa was successfully shown to reduce pain [64,65]. A Phase III clinical trial in the United States is currently suspended to recruit participants (Clinical Trial ID: NCT03203330) due to CMC (chemistry, manufacturing and control) concerns.

Although viral-mediated therapies have been successfully used in gene therapy for their superior transfection efficacy, there are several challenges to overcome. Viral-mediated therapies can lead to cytotoxicity, immunogenicity and oncogenecity. It is difficult to functionalize the virus with carrying agents. Finally, there is a significant monetary cost associated with this delivery method [66]. To overcome some of these concerns, non-viral gene delivery systems such as particle-based therapies present viable alternatives [67].

4.3. Particle-based strategy

For particle-based drug delivery, particles such as polymeric micelles, liposomes and dendrimers are commonly used. Micron-scale (microgels, microparticles, etc.) biomaterials made from synthetic and/or natural polymers are also used to reduce the range of clearance and control the release of drugs. This is particularly useful for achieving targeted and sustained drug delivery to the joint, and in fact particle-based drug delivery has been previously explored for intra-articular injection in OA. For instance, intra-articular injections of liposomes carrying hyaluronan or collagen-based surface-anchored ligand into knee joints showed the anti-inflammatory efficacy of diclofenac and dexamethasone in a rat model of OA induced by monosodium iodoacetate [68].

Recently, nanoparticulated drugs (nanodrugs) have gained recognition for their strengths in drug delivery. Systemic administration of a nanodrug was shown to localize in the synovial joint of preclinical animal models of rheumatoid arthritis [69]. Following systemic injection, nanodrugs exploit synovial capillary fenestrations to accumulate within the synovium, and subsequently enter the synovial cavity through the gaps between synovial cells [70,71]. However, unlike the abundant angiogenic capillaries with fenestrations in RA synovial tissue, OA synovial tissue has fewer fenestrated capillaries, and this hampers the efficacy of systemic injection in targeting the joint [72]. Therefore, intra-articular injection of nanodrugs would be required to target delivery to the OA joint.

Previous studies have shown that when small (< 15 nm) cationic nanocarriers are injected intra-articularly, they can overcome the biological barriers of the joint by binding and penetrating anionic cartilage tissue faster than the carriers can be cleared from the joint space [73–76]. Geiger et al. showed that nanodrugs composed of polyamidoamine dendrimers (generation 4 and 6) carrying IGF-1 successfully penetrated bovine cartilage of similar thickness to human cartilage within 2 days. This enhanced therapeutic IGF-1 residence time in rat knees by 10-fold for up to 30 days [77]. Furthermore, a single intra-articular injection of dendrimer-IGF-1 rescued surgically-induced OA changes to cartilage and bone in rat knee joints. Not only did the single injection reduce the total area and width of medial tibial cartilage degeneration, it also reduced the total volume of osteophytes compared to that of rats with no treatment following surgery [77].

Regarding the use of nanocarriers to administer ASOs in OA, Sacchetti et al. utilized intra-articular injection of polyethylene glycol (PEG) chain-modified single-walled carbon nanotubes (SWCNTs) as a drug delivery system to chondrocytes [78]. PEG-SWCNTS are 1D nanoparticles with diameters smaller than 10 nm and lengths ranging from tens to several hundreds of nanometers [79]. The authors showed that intra-articularly injected PEG-SWCNTs displayed long-lasting residence time within the joint cavity of both healthy and OA mice, and efficiently entered chondrocytes residing in the upper zone of the cartilage. More importantly, intra-articularly injected PEG-SWCNTs successfully delivered morpholino ASOs into chondrocytes of both healthy and OA cartilage without discernible toxicities in the mice.

Although nanocarriers have the demonstrated ability to deliver cargo into cartilage, their safety profile is still fragmental and needs to be confirmed before testing in clinical settings. Indeed, not all nanocarriers that enter the joint infiltrate the cartilage [80], and drug accumulation in cells of surrounding joint tissues may result in adverse events. Clinical trials using particle-based therapy are ongoing (NCT02837094 and NCT04120194), and these results will provide further insight into the potential for clinical application.

4.4. Chemical modification-based strategy

Since the safety profiles of viral-based delivery and nanocarriers have not yet been confirmed, various natural drug delivery strategies have been investigated. To circumvent the use of viruses or nanocarriers, chemical modifications to ASOs have been made to facilitate in vivo delivery. The LNA-modified ASO delivery system is one of the most advanced in vivo delivery systems [81]. LNA can interact with

complementary RNA with high affinity, neutralizing the targeted RNA [31]. In 2018, our group showed the therapeutic potential of LNA-miR-181a-5p ASO in facet and knee OA using preclinical animal models across joints and species [19]. Specifically, we demonstrated that blocking miR-181a-5p with intra-articularly injected LNA-miR-181a-5p ASOs decreased the severity of OA in both injury-induced spine OA in rat and trauma-induced knee OA in mice, accompanied by decreased expression of cartilage catabolic and cell death markers. Baek et al. also showed the efficacy of LNA-miR-449a ASO in a rat preclinical model of knee OA [22]. They demonstrated that LNA-miR-449a ASOs were successfully delivered to cartilage defect sites and performed dual positive roles, regenerating damaged cartilage and preventing OA progression by targeting LEF1 and SIRT1. These studies demonstrate that intra-articular injection of LNA-based ASOs resulted in high stability and cellular uptake in rat facet joints and mouse/rat knee joints to suppress disease progression in surgically-induced OA. While these results are promising, showing that ASOs are able to have an effect in cartilage, pharmacokinetic studies with labeled-LNA-ASOs (e.g. fluorescence-labeled ASOs) are warranted to visualize cellular uptake and trafficking in chondrocytes and confirm that the observed effects are indeed the result of ASO activity.

4.5. Biomaterial-based therapy

Biomaterial carriers such as hydrogels are able to sustain drugs in the joint with slow release. Recently, Garcia et al. reported a drug delivery strategy targeting ADAMTS5 with LNA-ASOs and fibrin-hyaluronic acid (HA) using a hydrogel-based scaffold to deliver the ASOs to human OA chondrocytes [82]. This hydrogel-based platform displayed a 14-day sustained release of the incorporated LNA-ASOs, and allowed for LNA-ASO uptake by primary human OA chondrocytes after diffusion through the hydrogel. Furthermore, knockdown of ADAMTS5 was observed 14 days post-drug administration [82]. The same group also showed that high-molecular weight HA can be used to tune nanoparticle targeting to specific epitopes [83]. The authors demonstrate increased CD44-dependent chondrocyte binding and controlled release of ASOs in a hydrogel where effective silencing of COX2 was observed over 14 days in OA chondrocytes [18]. This suggests that high-molecular weight HA-based drug delivery systems have the potential of specifically targeting cartilage by offering a good scaffold for chondrocyte binding. These studies demonstrate that chemical modifications to ASOs combined with HA-based delivery strengthens bioavailability in the synovial cavity, and therefore promotes ASO uptake and function in local tissues over longer periods.

4.6. Targeting a gene family

Typically, ASOs are used to target one specific gene (sequence). However, this strategy may not be sufficient to achieve optimal outcomes in some instances. Recent studies have demonstrated a strategy for using ASOs to target a group (family) of genes, especially for miRNAs. Obad et al. developed an approach for inhibiting miRNA families using ultra-short LNA (termed “tiny LNA”) ASOs that complementarily bind to the common seed region of miRNA family members [84]. The greatest advantage of this method is the ability to inhibit all miRNA family members that may have overlapping or redundant roles in disease. Indeed, Hullinger et al. showed that tiny LNA-ASOs with complementarity to the seed region of the miR-15 family were more potent in eliciting de-repression of downstream targets than the regular LNA ASOs which targeted a specific family member, even though both ASOs showed comparable uptake to cardiac tissue [85]. Moreover, tiny LNA-ASOs targeting miR-34 family members (−34a, −34b, and −34c) were effective in inhibiting all three members in two different cardiac stress models and attenuated cardiac remodeling and atrial enlargement, while inhibition of a specific miRNA member (miR-34a) alone with regular LNA-ASOs did not show these effects [86].

Regarding OA, and to the best of our knowledge, no study to date has tested the inhibition of co-expressed miRNA family members, at least in in vivo animal models. This represents a promising novel therapeutic strategy. While our group showed the therapeutic potential of inhibiting miR-181a-5p in facet and knee OA animal models [19,20], others independently demonstrated that inhibiting miR-181b had an effect in cartilage protection in a surgery-induced OA mouse model [87]. Furthermore, miR-29 family members (−29a, −29b, and −29c) have been reported as potential therapeutic targets in OA, as their expression increases upon surgical induction in a mouse cartilage injury model [88]. Therefore, using tiny LNA ASOs to target miRNA families such as miR-181 and miR-29, and other miRNAs involved in OA pathogenesis, should be further explored.

5. Roadblocks for clinical application

5.1. Animal models

Use of preclinical OA animal models is essential for testing new drug candidates prior to clinical trials. The vast majority of studies use mouse or rodent models to test new agents, including intra-articular injection of ASOs [19,20,22,47]. Although rodent models have enhanced our understanding of OA pathogenesis, they may not be sufficient to understand drug delivery and uptake mechanisms. Pharmacokinetics must be assessed with larger animals such as dogs, sheep, or horses, as their joint anatomy (e.g. cartilage thickness) has more similarity to that of humans and thus is more clinically relevant [89]. The thickness of cartilage in the joint increases with animal size [90]. For instance, the average mature cartilage thickness is up to 40 times less in mouse (around 50 μm) or rats (100–150 μm) than in humans (1.5–2.0 mm) [90,91]. This difference could hinder translation across species when investigating drug uptake, diffusion-based transport kinetics and retention in the joint. For example, whereas drug carriers can penetrate rapidly into 50 μm-thick mouse cartilage, in larger animals or humans, these drug carriers are easily cleared from the joint before adequate penetration. Conversely, once a drug is delivered to the cartilage, retention might be prolonged with thicker cartilage, which would enable adequate time to function at the target cells. Therefore, given that any disease-modifying drug targeting chondrocytes ought to penetrate 1 to 2 mm of cartilage in humans to access resident chondrocytes [92], demonstrating penetration through thicker tissues in large animal models is necessary for translation of cartilage drug delivery technology intended to target human chondrocytes.

In addition to the species, the method used to induce OA is also critical in the evaluation of new drugs. In animal models of knee OA, surgical methods such as destabilization of the medial meniscus (DMM [19,93,94]) and anterior cruciate ligament transection (ACLT [95,96]), are commonly used. These surgical models represent post-traumatic OA, where phenotypic features of disease tend to appear much faster than is typical for human OA. The shorter overall disease trajectories may not truly reflect the disease course in humans during primary OA. Furthermore, the severity of OA that develops can depend on surgical skill and harvest time points. These factors could lead to different outcomes between animal OA models and human OA. For this reason, models of spontaneous OA may be an appropriate alternative, such as the life-long voluntary joint loading mouse model [97], despite the greater time investment that is required to age animals and assess outcomes.

5.2. Targeting a distinct stage (severity) of OA

Animal models can be used to capture different types and stages of OA. Post-traumatic OA is best represented by surgical models of OA, as described above. Primary (idiopathic) OA is best represented by aging models of OA, where animals are allowed to age and joints are assessed for signs of OA using gene expression, histological features, and

radiographic features. Mice that are aged 18–24 months are considered old and roughly equivalent in age to humans between 56 and 69 years [98]. Given that the incidence of OA increases with age, the aging animal model is a strategy for more closely representing the natural history of the disease. Different stages of OA can be captured by examining mice at different ages. For example, a 1-year-old mouse is predicted to be roughly equivalent in age to a 42.5-year-old person [98]. Careful examination of the joints at this stage may reveal early features of OA, making this model appropriate for testing ASOs with preventative effects in OA.

With regard to clinical assessment, detecting early stages of OA in patients is not straightforward due to the lack of congruence between symptoms and radiographic features [99]. Currently, Kellgren-Lawrence (KL) grading [100] of radiographic features of OA, including joint space narrowing and osteophyte formation, is used to determine the severity of OA based on 5 grades ranging from 0 (radiographically undetectable OA) to 4 (most severe OA). However, it is not uncommon for patients to experience pain while not showing any radiographic features of OA. Pain is often assessed through self-report, using scales such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [101]. In particular, WOMAC scores capture pain, stiffness and physical function for people with knee or hip OA. A relevant clinical outcome for testing the effectiveness of ASO therapy is the change in WOMAC score over time, where responders to ASO therapy could be differentiated from non-responders. This outcome may show more responsiveness than structural changes reflected by KL grade, which would likely require longer treatment durations. The choice of outcome that is used in OA clinical trials is critical, as the mechanism of the ASO therapy must be known (symptom-modifying versus structure-modifying), and the duration of the ASO therapy must be appropriately captured in the trial (short-acting to improve pain versus long-acting to prevent structural progression).

5.3. Potential toxicity associated with ASOs

Each class of third-generation ASO has a stereotypic toxicity profile. PS-based ASOs are reported to have potential acute toxicity. ISIS2303, an ASO of intercellular adhesion molecule 1 (ICAM-1), was shown to cause remarkable changes in blood pressure, lethargy, periorbital edema and increase of circulating neutrophils and cytokines such as IL-6 and IL-12 in monkey [102]. In most cases, these changes recovered within 15–30 s, but in rare cases, more severe effects occurred [102]. The underlying mechanisms remain elusive but complement activation is one pathway that may contribute to toxicity [102]. Furthermore, ASOs can occasionally bind to proteins such as toll-like receptors in a sequence-dependent manner, which can also cause unexpected side effects [103].

As described above, LNA ASOs have relatively high potency with favorable binding affinities in part due to their short sequence. Since LNAs work primarily though RNase H, they exhibit lower immunostimulatory activity relative to earlier generations of ASOs. Despite this, LNA ASOs have been reported to have greater potential for hepatotoxicity through several mechanisms that are largely independent of immunostimulatory activity [104]. First, while high affinity is a huge advantage in suppressing target genes, intracellular drug accumulation is also increased, and that may increase associated toxicities. Second, ASOs may bind to off-target RNAs that share a high degree of homology with the target sequence, thereby inducing toxicity via multiple tissues [105]. Third, since the liver and kidney are major drug clearance organs, the issue of hepatotoxicity and nephrotoxicity remain as challenges in ASOs-based treatment [106].

6. Conclusion

Recent in vitro and in vivo animal models of OA exploring ASO-based therapies have provided promising proof of concept data. Further tailoring this approach for administration in humans, including targeted drug delivery systems, will promote the feasibility of clinical application (Fig. 2). The stage is set with the ongoing surge in human genomic and proteomic data that will enable identification of promising RNA targets for ASOs. Validation of these targets, accompanied by optimized ASO structure, delivery and safety profile, will position ASO-based drugs as a promising therapeutic strategy for OA treatment.

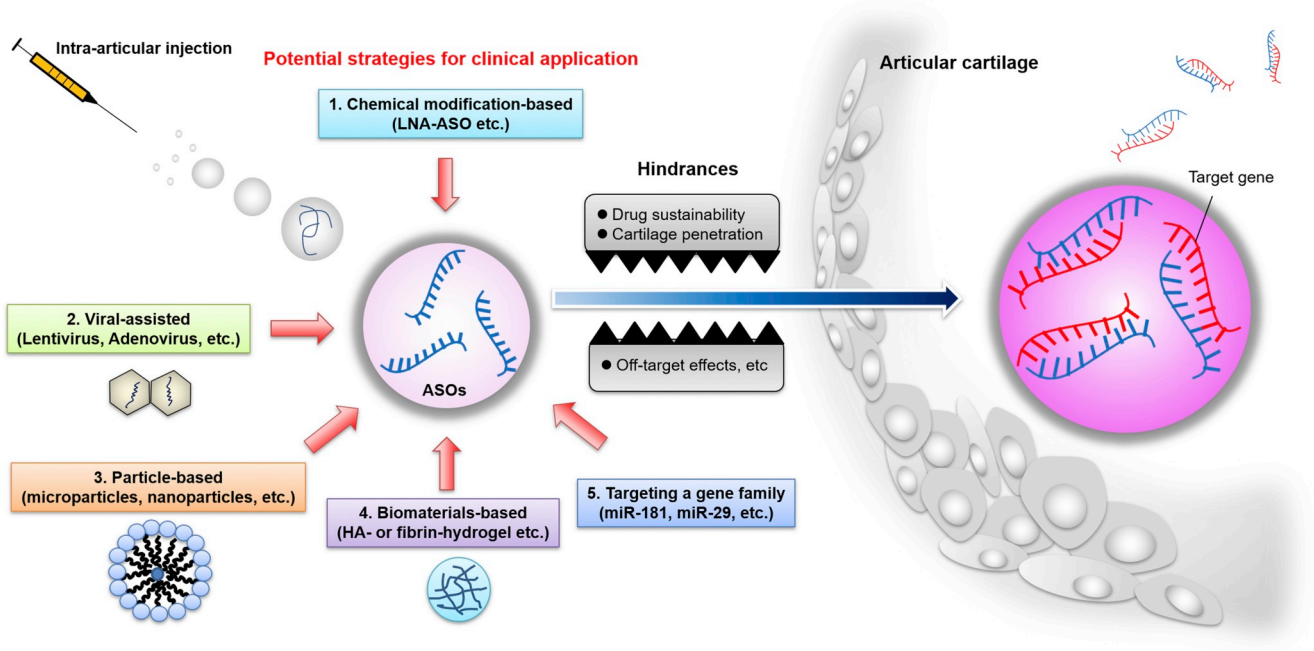

Fig. 2. Overview of potential therapeutic strategies with antisense oligonucleotides (ASOs) in osteoarthritis (OA). Intra-articular (IA) injections deliver the modified ASOs to the joint space. The ASOs subsequently penetrate cartilage to bind to the target mRNA. Potential strategies with ASOs include (1) chemical modification-based, (2) viral-assisted, (3) particle-based, (4) biomaterial-based, and (5) targeting gene family-based approaches, which may overcome the difficulties toward clinical applications such as drug sustainability, cartilage penetration and off-target effects.

CRediT authorship contribution statement

Akihiro Nakamura: Conceptualization, Writing - original draft, Writing - review & editing. **Shabana Amanda Ali**: Conceptualization, Writing - original draft, Writing - review & editing. **Mohit Kapoor**: Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

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