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REVIEW ARTICLE

Genome Engineering for Osteoarthritis: From Designer Cells to Disease-Modifying Drugs

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Abstract

BACKGROUND: Osteoarthritis (OA) is a highly prevalent degenerative joint disease involving joint cartilage and its surrounding tissues. OA is the leading cause of pain and disability worldwide. At present, there are no disease-modifying OA drugs, and the primary therapies include exercise and nonsteroidal anti-inflammatory drugs until total joint replacement at the end-stage of the disease.

METHODS: In this review, we summarized the current state of knowledge in genetic and epigenetic associations and risk factors for OA and their potential diagnostic and therapeutic applications.

RESULTS: Genome-wide association studies and analysis of epigenetic modifications (such as miRNA expression, DNA methylation and histone modifications) conducted across various populations support the notion that there is a genetic basis for certain subsets of OA pathogenesis.

CONCLUSION: With recent advances in the development of genome editing technologies such as the CRISPR-Cas9 system, these genetic and epigenetic alternations in OA can be used as platforms from which potential biomarkers for the diagnosis, prognosis, drug response, and development of potential personalized therapeutic targets for OA can be approached. Furthermore, genome editing has allowed the development of "designer" cells, whereby the receptors, gene regulatory networks, or transgenes can be modified as a basis for new cell-based therapies.

Keywords Genetics · Gene editing · Personalized medicine · Osteoarthritis

1 Introduction

Osteoarthritis (OA) is the most prevalent rheumatic disease worldwide, affecting an estimated 10 percent of the world's population over the age of 60 [61]. OA is characterized by pain, stiffness, decreased function, instability, deformity and swelling due to irreversible pathological changes in the joint organ system, including articular cartilage, subchondral bone, synovium, and the infrapatellar fat pad [39, 30]. The pathogenesis of OA involves the development of cartilage degeneration, synovial inflammation, subchondral bone remodeling and sclerosis, degeneration of ligaments and meniscus, and hypertrophy of the joint capsule [9]. At present, there are no disease-modifying OA drugs (DMOADs), and the primary therapies include exercise

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and nonsteroidal anti-inflammatory drugs until total joint replacement at the end-stage of the disease. Clinical risk factors for the development of OA include increasing age, female sex, obesity, repetitive joint overloading, previous joint injury, lower limb deformity, smoking history, and family history of OA [5–18]. This wide array of contributing factors for the initiation and progression of OA supports the notion that OA is not simply one disease, but rather a family of conditions that have similar endpoints that involve a multitude of pathways that lead to joint failure. This diversity in the mechanisms of the etiopathogenesis of OA may contribute to the lack of viable treatment strategies.

While growing evidence suggests a genetic basis for a large proportion of OA incidence [51], it is often difficult to separate causative factors from the combined effects of these environmental contributors, as they frequently happen in combination with each other [7]. However, it is clear that both genetic and environmental risk factors contribute to OA pathogenesis. Epidemiological studies evaluating twin-pair analyses and family-based segregation studies, have demonstrated that genetic susceptibility is also one of key contributors to the development of OA [81, 75, 15–20]. However, while the genetic basis of OA etiology remains an open and active area of investigation, the characterization and analysis of genetic basis of OA pathogenesis provides an exciting platform from which potential biomarkers for the diagnosis, prognosis, drug response and development of potential therapeutic targets for future personalized biological novel treatment strategies for OA can be approached. As such, the purpose of this review is to concisely summarize the state of knowledge in the areas of genetics and genome editing to postulate opportunities for genome engineering for OA applications.

2 Genetic variants associated with OA

A number of studies have leveraged the opportunity for investigating the genetic underpinnings of OA, collectively reporting over 80 genetic variants subjected to candidate gene association analysis with the risk of OA [68] (Table 1). Among them, a single nucleotide polymorphism (SNP) rs143383 showed consistent and robust associations with OA after its initial report in 2007 [20–26]. The rs143383 is located in the 3′ untranslated region (3′UTR) of the growth and differentiation factor 5 gene, *GDF5*, which is also known as cartilage-derived morphogenetic protein 1, *CDMP1*, is a member of the transforming growth factor-β (TGF-β) superfamily and an extracellular signaling molecule that participates in the development, maintenance, and repair of bone, cartilage, and other tissues of the synovial joint [65, 44]. The SNP rs143383 is a common C to T transition which mediates reduced *GDF5* transcription relative to the C allele [56, 74, 25]. *GDF5* is an OA-associated locus from which this SNP is found. Decreased mRNA and protein levels for Gdf5 in mice can contribute to OA-like phenotype [19, 59]. More recently, OA susceptibility has been coded by a comparison of the frequency of DNA polymorphisms in individuals with osteoarthritis when compared to those without osteoarthritis (disease-free controls) in the form of candidate gene-based analyses or genome-wide association studies (GWAS). The Arthritis Research UK Osteoarthritis Genetics (arcOGEN) Consortium Study was the first GWAS for knee OA with total joint replacement to report multiple, independent association signals that replicated at a level considered significant after accounting for the multiple tests that are performed in a GWAS (p value $< 5 \times 10^{-8}$) [85]. This analysis identified five novel loci at genome-wide significance (*GLT8D1/GNL3*, *ASTN2*, *FILIP1/SENP6*, *KLHDC5/PTHLH*, and *CHST11*) and three novel loci at near genome-wide significance (*TP63*, *FTO*, and *SUPT3H/CDC5L*). Subsequently, several other large-scale GWAS of hand, hip, and knee OA have been published in European Caucasians, providing a dozen genome-wide significant loci that include *ALDH1A2* for hand OA [78], *DOT1L*, *NCOA3*, *BOP1, LRCH1, STT3B, GADL1, TGFA, PIK3R1, FGFR3, TREH, COMP, and CHADL* for hip OA [31–14], and *LSP1P3*, *GDF5*, *FTO,* mitochondrial DNA variants for knee OA [84, 33].

3 Epigenetics and the pathogenesis of OA

Epigenetics refers to heritable changes in gene expression or phenotype without changes in the DNA sequence [24]. Three primary mechanisms of epigenetic changes have been documented in OA pathogenesis: DNA methylation, histone modification and noncoding RNAs such as microRNAs [71]. Epigenetic changes regulate gene expression either by affecting gene transcription or by acting post-transcriptionally, leading to changes in the levels of the encoded protein. Normally functioning somatic cells, including articular chondrocytes, are subjected to epigenetic mechanisms that aid in stabilizing their phenotype [63]. These epigenetic changes can occur in chondrocytes in response to environmental factors, including diet or aging, which lead to the loss of normal epigenetic control [60]. Epigenetically modified chondrocytes can change their phenotype and function to overexpress cartilage-degenerating proteases and pro-inflammatory mediators. This phenotypic shift is thought to disrupt the homeostatic balance and contribute to the progression of OA through the changes in gene expression of transcription factors (*RUNX2*, *NFAT1*, and *SOX9*), pro- or anti-inflammatory

cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-1 beta [IL-1β], IL-6, inducible nitric oxide synthase [NOS2] and IL-8, matrix-degrading proteinases (matrix metalloproteinase-3 [MMP-3], MMP-9, MMP-13, ADAMTS-4, and ADAMTS-5) and extracellular matrix proteins (COL2A1, COL9A1, and ACAN) in articular cartilage [9, 65, 66, 6].

DNA methylation, the best characterized epigenetic mechanism, is defined by the addition of a methyl group (CH_3) to a cystosine of CpG sites to form methylated cystosine by DNA methyltransferases. Studies assessing DNA methylation arrays have shown that OA and non-OA cartilage have differentially methylated genes which typically harbor CpG sites suggesting that the epigenetic regulation of gene expression via DNA methylation contributes on the pathogenesis of OA [67, 34].

Furthermore, a direct, functional relationship has been demonstrated between epigenetics and genetics at alleles implicated in OA-risk. *GDF5* and *DIO2* (iodothyronine deiodinase 2) has been reported to be subject to epigenetic regulation related to genotype and gene transcription. As described earlier, the *GDF5* functional SNP, rs143383, is a particular risk factor for knee osteoarthritis, and also is thought to effect OA when differentially methylated [26]. Functional analyses using human normal and OA cartilage have showed that differential DNA methylation of rs143383 modulates the binding of SP1, SP3, and DEAF1 repressor proteins and therefore alters the expression differences between the C and T alleles [64]. Furthermore, a CpG site located 4 base pairs upstream of rs143383 showed highly significant demethylation in osteoarthritis knee cartilage compared with osteoarthritis hip cartilage, which correlates with reduced expression of the gene and may be responsible for the specific effect of rs143383 on knee OA [64]. *DIO2* transcribes iodothyronine deiodinase 2 that catalyzes the conversion of intracellular inactive thyroxine (T4) to the bioactive thyroid hormone (T3). A common *DIO2* haplotype composed of the C-allele of SNP rs225014, and the C-allele of SNP rs12885300 has been known to be associated with OA [54]. A recent functional analysis of *DIO2*, including DNA methylation studies, reported that differential methylation of CpGs located upstream of the gene correlated with *DIO2* expression changes, and that these effects were particularly striking for individuals harboring the risk-conferring allele of rs225014 [10].

4 Genome and epigenome engineering tools

Genome engineering has at least two roles in elucidating the natural history of disease states: (1) identify the responsible genes for a particular disease and (2) to facilitate the functional validation of the identified genes and the development and study of disease models. The ability to precision-edit the genome of mammalian cells allows for a deeper mechanistic understanding while minimizing off-target effects [80, 35]. The era of genome editing began in the late 1970s with the successful exchange of pieces of yeast DNA via the homologous recombination system [38, 70]. With this technique, single and multiple knockouts were generated for applications in functional characterization [38, 70]. In the late 1980s, gene-targeting technologies using embryonic stem cells were created that were proficient in homologous recombination (HR) [53]. However, gene targeting was only applicable to homologous recombination-proficient cells and therefore the application to other cell types and eukaryotic systems was limited. The efficacy of homologous recombination is improved by the discovery of engineered nucleases, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), that can generate site-specific double-strand breaks (Fig. 1) [16].

There are two major repair pathways for double strand breaks; non-homologous end-joining and homologous recombination (Fig. 2) [15]. The non-homologous end-joining pathways are active throughout the cell cycle and are therefore the most predominant repair mechanism in mammalian cells, despite being error-prone and resulting in insertions or deletions of nucleotides (indels), which may cause a frameshift and, effectively, a functional gene knockout when occurring in the coding region [17]. The homologous recombination pathways are highly precise because they utilize a DNA template with ends that are homologous to the break site. These techniques have been

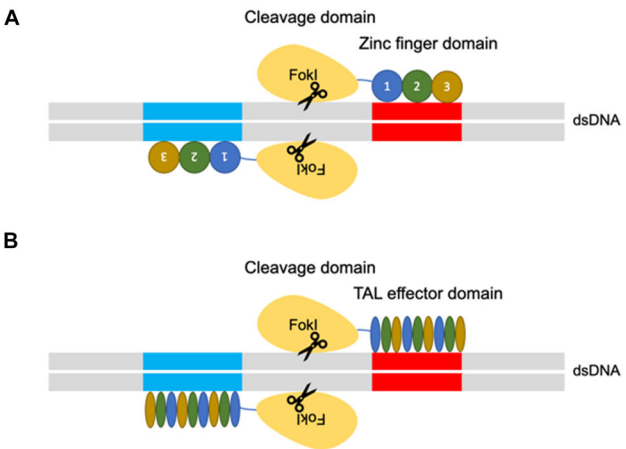

Fig. 1 **A** Zinc finger nucleases (ZFNs) and **B** transcription activator-like effector nucleases (TALENs) genome engineering platform. The ZFNs and TALENs are engineered to bind a desired sequence, and then fused to the FokI endonuclease to create targeted double strand breaks

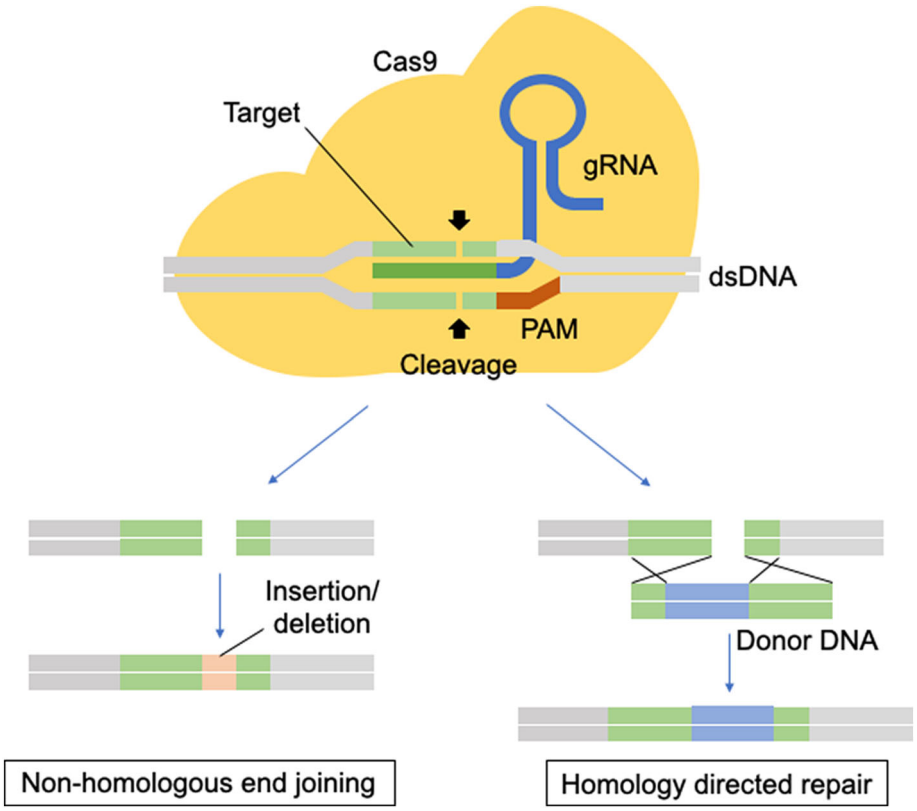

Fig. 2 CRISPR-Cas9 Genome Engineering Platform. Cas9 is guided by a guide RNA (sgRNA) to induce double-strand DNA breaks at a desired genomic locus, the damaged DNA can be repaired by non-homologous end-joining or homologous recombination, which results in controlled editing of the genome

used mainly in genome editing to rewrite the DNA sequence and generate gene or protein variants. Researchers generated hybrid proteins by fusion of a DNA-binding module by altering the DNA-binding domains of transcription factors (TFs), zinc finger proteins (ZFPs) or TAL effectors with the DNA-cleaving module from the restriction endonuclease, *Fok*1 [45, 55]. In those nuclease platforms, a pair of ZFNs or TALENs must target adjacent sites in the genome to bring the two *Fok*1 domains together because the endonucleolytic domain of *Fok*1 acts like a dimer [45, 55]. However, protein engineering is required to obtain site specificity. The process of engineering and optimizing specific combinations of ZFNs or TALENs modules for new sequences is not trivial and is an extensive process [57].

5 CRISPR-Cas9 genome editing platform

One of the most recently discovered nuclease platforms is the clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated (Cas) endonuclease system. The CRISPR-Cas9 genome editing platform has allowed for rapid and efficient precision edits in mammalian genomes [23, 46]. The CRISPR-Cas systems are essential in the prokaryote adaptive immune system. CRISPR is a family of DNA sequences in prokaryotes which contain short segments of DNA from viruses which are used to detect and destroy DNA from similar viruses at subsequent attacks as a defense mechanism. The CRISPR-Cas system has six different major types (type I–VI) based on their genetic content and structural differences of their effector proteins. Among them, type II is the most studied and consists of two main components of the system: an operon (codes for Cas9 protein) and CRISPR, which provide a template for synthesizing CRISPR RNA (crRNA) to direct nuclease activity [21]. To achieve site-specific DNA recognition and cleavage, crRNA has to bind directly to Cas9 through an accessary trans-activating CRISPR RNA (tracrRNA) [41]. To use this mechanism in genome editing, a single synthetic guide RNA was developed by combining crRNA and tracrRNA to direct the Cas9 endonuclease to its specific site in the genome in a sequence-specific manner (Fig. 1) [63–52]. Once the sgRNA hybridize to the target DNA, Cas9 is activated and cleave the targeted site [52]. A protospacer adjacent motif (PAM) which is a 5′-NGG-3′ trinucleotide sequence at the target sequence is essential for Cas9 binding to DNA [76]. While off-target effects are one key concern of Cas9 binding [5], there have been efforts to reduce off-target activity of Cas9 using gene-engineering [3]. There are two groups of gene-engineered Cas9, mutant proteins and split Cas9 proteins. Catalytically inactive, nuclease-deficient Cas9 (dCas9) is a form of mutant Cas9 and various fusions

with transcriptional activator, repressor and recruitment domains have been used to modulate gene expression at targeted loci without introducing irreversible mutations to the genome [29, 62].

6 Applications of genome-engineering technologies to osteoarthritis treatments

One potential therapeutic application of genome editing technology is the engineering of cell-based anti-cytokine therapies under endogenous promoter sequences by editing in key transcripts for anti-inflammatory molecules. Using the successful framework of biologics in rheumatoid arthritis (RA), where a variety of protein therapies have been developed and applied, key receptors for inflammatory mediators can be inserted into cells under endogenous promoters [72]. While advancements have led to the development of several effective RA treatment modalities for RA pathogenesis, which is understood to be a systemic inflammatory disease, treatments fail or cease to benefit patients up to 50% of the time [72]. With growing appreciation that both systemic and local inflammatory mediators may also play a role in the pathogenesis of OA, there has been increasing investigation into therapeutics that may be beneficial in this context [49, 37], such as a cell-based anti-cytokine therapy that senses and responds to endogenous levels of inflammatory mediators in OA, i.e., TNF-α or IL-1 [47, 48]. However, while it is important to note that a better understanding of the individual roles for IL-1, TNF-α, and other cytokines is required in OA [42], evaluating new approaches to delivering these therapies could fill a meaningful gap in treatment for the prevention of other inflammatory conditions. A comprehensive review of genome editing for personalized arthritis therapies can be found here [1].

Genome-edited cell-based anti-cytokine therapy also may overcome several important limitations of existing biologic drugs. Continuous use of conventional disease-modifying anti-rheumatic drugs (DMARDs) and the newer biologics can lead to significant adverse effects for RA patients due to continued high-level delivery of DMARDS [79]. As the severity of symptomatic arthritic diseases and pain can fluctuate over time [39], development of specific therapeutic strategies that can sense and respond to varying degrees of endogenous inflammatory mediators in OA may mitigate unwanted adverse effects. In recent studies, CRISPR-Cas9 gene editing was used to develop an autoregulated anti-cytokine stem cell system in mouse induced pluripotent stem cells (iPSCs), which provides the opportunity to theoretically differentiate them into a variety of cell types [11, 12]. These cells were designed to transcribe the soluble receptor for TNF-α (sTNFR1), interleukin-1 receptor antagonist (IL1Ra), or luciferase (control) in a feedback-controlled manner driven by the endogenous macrophage chemoattractant protein-1 (*Ccl2*) promoter (Fig. 3). Given that *Ccl2* and NF-kB signalling are implicated in the onset and progression of pain and structural damage OA [40], this genome-edited artificial gene circuit is an attractive therapeutic approach for evaluation in both *in vitro* and *in vivo* OA model systems. *In vitro* characterization of Ccl2-based genome-edited cells reveals that these cells can reduce RA-relevant inflammatory mediators when challenged with an inflammatory stimulus. Ongoing work is characterizing these cells *in vivo* in reponse to supra-physiological levels of inflammation, and to disease-relevent stimuli in murine models of both RA and OA.

7 Disease modeling and drug discovery

Despite the discovery of multiple risk alleles for OA through GWAS and twin studies (as described earlier), it is difficult to separate the influence of environmental factors from genetics on OA predisposition *in vivo*. The use of genome-editing in iPSCs now opens up the possibility for functional interrogation of causative genetic elements, such as coding/noncoding SNPs, as the basis for more targeted—even "personalized"—drug discovery for OA. For example, recent studies have developed *in vitro* tissue-engineered models of human and mouse iPSC chondrogenesis that show responsiveness to OA-associated cytokines such as IL1 and TNF-α similar to native cartilage [82–83]. These *in vitro* modeling platforms demonstrate the potential application of iPSCs and gene editing systems

Table 1 Summary of genetic variants in OA-related GWAS studies

Involved joint	Genetic variants	References
Hip	*GLT8D1/GNL3*, *ASTN2*, *FILIP1/SENP6*, *KLHDC5/PTHLH*, *CHST11, DOT1L*, *NCOA3*, *BOP1, LRCH1, STT3B, GADL1, TGFA, PIK3R1, FGFR3, TREH, COMP, and CHADL*	[85, 31–14]
Hand	*ALDH1A2*	[78]
Knee	*LSP1P3*, *GDF5*, *FTO,* mitochondrial DNA variants	[84, 33]

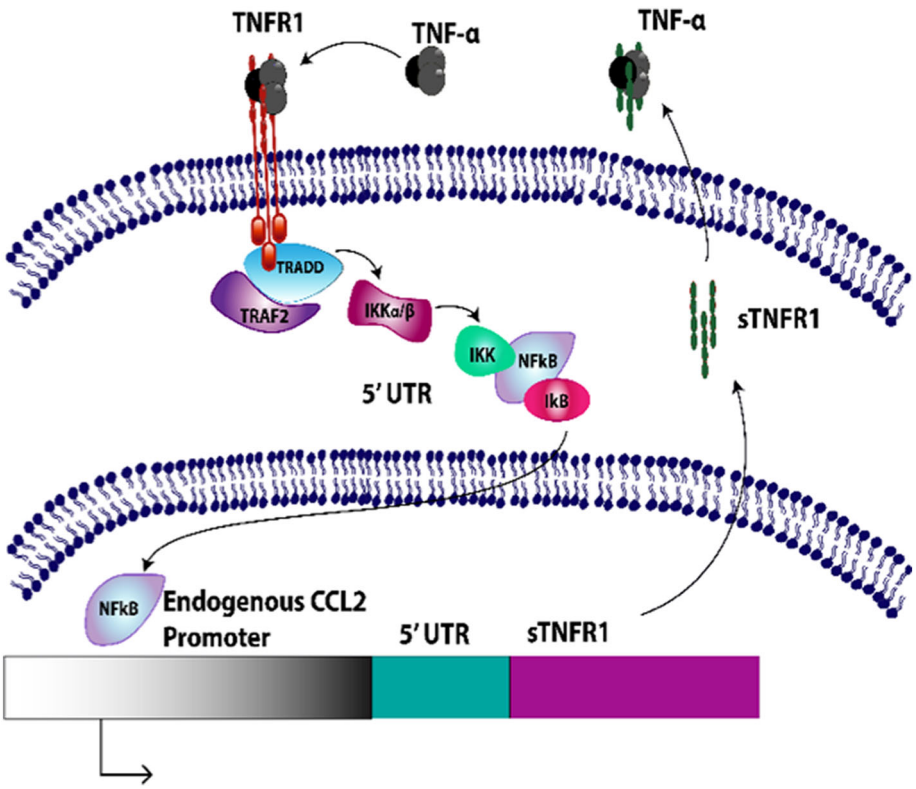

Fig. 3 CRISPR-Cas9 Ccl2-edited Gene Circuit Transcribing Soluble Tumor Necrosis Factor-alpha Receptor (sTNFR1) Under the Macrophage Chemoattractant-1 Locus. The transcription and synthesis of sTNFR1 in response to TNF-α-induced expression of *Ccl2* results in a negative feedback system that reduces TNF-α driven inflammation. Similar systems have been developed to produce either interleukin-1 antagonist (IL1Ra) to inhibit IL-1 signaling, or produce luciferase as a control reporter system under the *Ccl2* endogenous promoter

such as CRISPR-Cas9 to high-throughput screening of novel DMOADs that account for various genetic backgrounds and risk alleles [50, 73].

8 Conclusions and applications

Due to fundamental developments in the field of genome engineering, an array of novel therapeutic options for OA are now accessible and available to explore. Specifically, the development of targeted and safe cell-based therapies that can sense and respond to endogenous levels of inflammation may provide a tremendous opportunity for the development of new therapies for OA. These cells can be differentiated into chondrocytes, tested in *in vitro*, and delivered *in vivo* for characterization and testing. Evaluating, refining and translating this potentially highly responsive therapeutic strategy for OA treatment could overcome the limitations of current anti-cytokine therapies or biologic drugs more broadly, ultimately posing less risk of adverse events to patients when compared to conventional pharmacological or biologic therapy. This approach may increase the effectiveness for OA compared to previous efforts to use biologics in this context. In addition to OA, many chronic diseases (rheumatoid arthritis, psoriatic arthritis, psoriasis, metabolic disease, diabetes) involve increased fluctuating levels of TNF-α and IL-1-mediated signaling. Long term, the scale up and applicability of this approach to other musculoskeletal disease models that could benefit from anti-cytokine therapy can be tested. The ability to deliver an auto-regulated anti-cytokine system with tunable sensitivity will allow for a range of applications in a wide variety of inflammatory conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

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