Review

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Navigating the Hurdles of Intra-articular AAV Gene Therapy

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Abstract

Joint diseases represent a significant health burden due to their high prevalence and morbidity, yet current treatments fail to provide comprehensive and long-term relief for all patients. In this context, adeno-associated virus (AAV) gene therapy, emerges as a promising approach, offering advantages such as prolonged efficacy and minimal immunogenicity. AAV has been extensively studied for various medical conditions, with some applications successfully implemented in patient treatments. Currently, there are a few clinical trials utilizing AAV that have been completed for treating arthritis. However, challenges such as transduction efficiency, off-targets, and pre-existing immune response persist. This review provides an overview of the current paradigms of treatment with regards to joint disease, elaborates on the AAV delivery barriers related to application in treating joint diseases, and discusses strategies to improve gene therapy efficacy, including AAV capsid engineering, small molecular-assisted AAV delivery, optimizing tissue-specific or inflammation-inducible promoters, as well as strategies to mitigate immune responses to AAV.

Keywords: adeno-associated virus; joint; barriers; AAV engineering; immune management

Introduction

Joint disease is the leading cause of disability and includes a variety of conditions such as inflammatory and degenerative diseases, injuries, tumors, and others that occur in bone, muscle, tendon, ligament, cartilage, synovium and joint cavity. The impact of joint disease extends beyond physical mobility, significantly restricting patients' daily activities and diminishing their quality of life [1]. Additionally, these patients are usually susceptible to various psychopathologies such as depression and anxiety. Due to the considerable societal and economic impacts, sustained efforts to advance treatment regimens are important to mitigate disease burden.

Patients displaying early to moderate symptoms are frequently prescribed pharmaceutical treatments. In recent years, a sub-class of disease-modifying antirheumatic drugs (DMARDs), known as biologics, have emerged as a promising alternative to conventional DMARDs for the treatment of joint diseases, especially autoimmune forms of arthritis like rheumatoid and psoriatic arthritis. These biologic agents primarily target specific inflammation pathways to alleviate the underlying mechanisms of joint diseases [2]. Despite these advancements, achieving satisfactory outcomes remains a challenge. The efficacy of these treatments often takes a few weeks to manifest, and multiple doses are typically required due to the short half-life of proteins in the joint environment

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[3]. The treatments typically yield a response in less than 50% of patients, and at least 10% of patients still end up with irreversible severe disability [4].

Gene therapy offers a versatile approach to treat diseases, functioning through three primary methods: gene addition, gene silencing, and gene editing. The vectors can be divided into non-viral and viral vectors. Among viral vectors, Adeno-associated viruses (AAV) have gained attention due to their non-pathogenic nature, capability for long-term gene expression, and broad disease application.

This review serves to broadly overview current approaches to the treatment of joint disease, how they potentially benefit from using AAV as an alternative delivery vehicle and examine the specific barriers that AAV faces for the effective, locally-applied treatment of joint diseases, as well as strategies to overcome these barriers.

1. AAV Biology

Unlike other vectors, AAV is episomal, minimizing the risk of insertional mutagenesis, and adverse immune responses. With broad tropism and the ability to efficiently transduce both dividing and non-dividing cells, different AAV serotypes can be utilized to target specific tissues, like the brain, liver, and muscles, making it versatile for treating various diseases. Most importantly, one time administration of AAV vector is able to induce long-term expression of the packaged therapeutic genes, making it an ideal vector for the treatment of joint diseases.

AAV vector was first isolated as a contaminant in the simian adenovirus preparation in 1965. However, it was not until the early 1980s that AAV was found to be replication-defective and non-pathogenic, making it a promising candidate for gene therapy [5]. The earliest clinical trial of AAV for gene therapy was launched in 1993, targeting cystic fibrosis. Despite the trial's limited success due to low gene expression levels, research into AAVs accelerated thereafter.

The distinction between wild-type AAV and recombinant AAV (rAAV) vectors lies in their genetic composition. Both share identical inverted terminal repeats (ITRs), which plays an important role in viral genome replication, encapsidation, transcription and integration [6]. However, in rAAV, the transgene and its promoter take the ~4.7 kb space between the ITRs previously occupied by the rep and cap genes found in the wild-type virus.

The rAAV transduction process [7] is initiated by capsid binding to cell surface receptors and coreceptors. This is followed by their internalization into the cellular milieu via endocytosis. Once inside the acidic environment of endosomes, conformational changes permit the N-termini of VP1 and VP2 proteins to become exposed, which facilitates the escape of the virions to the perinuclear region of the cell. While a subset of these virions is targeted for protease-mediated degradation, the remainder traffics to the nucleus. After rAAV enters the nucleus, the capsid is dismantled and the rAAV genome is released. This single-stranded DNA genome is subsequently converted into a double-stranded form by host cell machinery. The rAAV genetic material may then persist as episomes, often circularized by DNA repair mechanisms, or less commonly, become integrated into the host cell's genome. The episomal or potentially integrated DNA then undergoes transcription and the resulting mRNA is exported from the nucleus, leading to the translation and expression of the encoded therapeutic gene.

2. Clinical Trials

AAV vectors have been through many clinical trials, including several FDA and EU-approved applications for the treatment of inherited retinal dystrophy, spinal muscular atrophy, and hemophilia [8]. Luxturna (voretigene neparvovec), which uses the AAV2 vector, became the first FDA approved AAV gene therapy for an inherited disease in 2017, treating patients with inherited retinal diseases caused by mutations in the RPE65 gene. Zolgensma (onasemnogene abeparvovec-xioi) was approved by the FDA in 2019 for the treatment of patients with spinal muscular atrophy (SMA) using AAV9 vector to deliver a functional copy of the SMN1 gene to motor neurons. In 2022,

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Hemgenix was approved by the FDA to treat hemophilia B, and in 2023, Roctavian was approved by the FDA for treating hereditary hemophilia A. In Europe, Upstaza and Roctavian have been approved for aromatic L-amino acid decarboxylase (AADC) deficiency and hemophilia A, respectively.

In the field of joint gene therapy, while various preclinical studies have been explored using AAV vectors for arthritis with promising results, there are currently no AAV-based gene therapies approved for the treatment of arthritis in patients. Currently, the two major routes for gene therapy administration in joint diseases are systemic and intra-articular injections [9]. Joint defects are good candidates for intra-articular injections because it is possible to treat the joint independently without impacting the other joints, if not damaged, and only a negligible amount of AAV will be circulating in the blood and liver due to the low virus titer required for intra-articular injection relative to other injection routes. Compared to traditional drugs, which face difficulty is maintaining drug pharmacokinetics and stabilizing protein or chemical concentrations, AAV vectors can transduce joint cells to provide sustained potency.

By searching the website of clinicaltrial.gov, currently, there are only seven clinical trials for joint disease that are established or ongoing (Table 1) [10], mostly targeting a single cytokine, such as IL-1Ra and TNFα [11,12]. One such trial is a phase I/II clinical trial of an AAV vector encoding the interleukin-1 receptor antagonist (IL-1Ra) for the treatment of knee osteoarthritis (OA). IL-1Ra is a naturally occurring protein that inhibits the inflammatory cytokine interleukin-1, which has been implicated in the development and progression of OA [13]. The trial evaluated the safety and tolerability of the IL-1Ra gene therapy, as well as its effects on pain and function in patients with knee OA. Another two clinical trials for OA used ICM-203, an AAV vector encoding Nkx3.2, a transcription factor involved in the activity of joint cells, such as chondrocytes and synoviocytes. Based on an interim review, in general, some patients experienced improvements in metrics such as pain, synovitis, and functionality, but with mixed results, with further investigations into higher doses likely necessary for more conclusive findings. For rheumatoid arthritis (RA), two of the phases I clinical trials were AAV vectors encoding the soluble tumor necrosis factor receptor 1 (sTNFR1), while another two were encoding hIFN-β for the treatment of RA, which applied a single intra-articular injection of recombinant AAV type2/5 containing a hIFN-β gene in the carpometacarpal (CMC), metacarpophalangeal (MCP), proximal interphalangeal (PIP), distal interphalangeal (DIP) joint, or wrist joint [8]. However, this clinical trial using hIFN-β was suspended due to issues with tolerability [14]. Overall, the limited benefits observed in these clinical trials indicate the need to explore alternative targets.

Table 1. Clinical trials for AAV gene therapy in arthritis.

AAV serotype	Gene of interest	Indication	Location	Name (Sponsor)	Trial number	Dose	Patient number
AAV5.2	ICM-203	OA	Knee	ICM Co. Ltd.	NCT05454566	6x10e12 vg-	18

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						6x10e13 vg	
AAV5.2	ICM-203	OA	Knee	ICM Biotech Australia Pty Ltd.	NCT04875754	6x10e12 vg-6x10e13 vg	16
AAV5	hIFN-b	RA	wrist	Arthrogen	NCT03445715	2.4x10E12 vg-2.4x10E13 vg	15
AAV5	hIFN-β	RA& OA	carpometacarpal (CMC), metacarpophalangeal (MCP), proximal interphalangeal (PIP), or distal interphalangeal (DIP) joint	Arthrogen	NCT02727764	0.6x10e12 vg-1.2x10e13 vg	12
AAV2.5	IL-1Ra	OA	Knee	Mayo Clinic	NCT02790723	1e11-1e13	9

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AAV2	TNFR	RA	peripheral joint	Targeted Genetics Corporation	NCT00617032	1E10-1E11	15
AAV2	TNFR	RA, Psoriatic arthritis, Ankylosing Spondylitis	peripheral joints	Targeted Genetics Corporation	NCT00126724	1E11-1E13	120

3. Preclinical Targets

Joint diseases usually come with joint pain, stiffness, and swelling, in order to manage those diseases and develop potential strategies for future treatment options, many preclinical trials have been explored and promising targets have been identified. Various joint diseases are characterized by chronic inflammation or other immune dysfunction, including RA, psoriatic arthritis, systemic lupus erythematosus (SLE, lupus), gout and ankylosing spondylitis (AS), and so on. Complex networks have been involved in the abnormal inflammation process, from immune cell dysfunction to small molecules, such as cytokines, chemokines, enzymes, antibodies, to genetic mutations.

One of the main directions to halt disease is to regulate the abnormal signaling pathways. For instance, the delivery of immunomodulatory cytokine genes such as IL-4, IL-10 [15,16], IFN-β [17], and transforming growth factor-beta (TGF-β) via AAV vectors have demonstrated promising results in reducing joint inflammation, pain, and averting joint degradation in animal models. Additionally, an alternative approach involves the use of inhibitors targeting proinflammatory cytokines. For instance, IL-1 [19], IL-6 [20], and IL-17 [21] antagonists have been reported to play a significant role in preventing bone destruction and autoimmunity [22], with Chabaud et al. (2001) demonstrating the ability of an IL17A blockade, via anti-IL17A monoclonal antibody (mAb), to significantly reduce collagen breakdown products in RA synovium explants in vitro. Lastly, signal transduction pathways, including the nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and the janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, have also been implicated as potential treatment targets for inhibition [23].

Another major approach to treating joint disease is dampening the effect of dysfunctional immune cells, which play a direct role in the manifestation of physical symptoms; for instance, cartilage degradation and synovial fibroblast proliferation in rheumatoid arthritis are largely induced by the signaling of pro-inflammatory cytokines released by T cells and other white blood cells,

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including B cells and neutrophils. In particular, the delivery of checkpoint proteins such as Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4) and Programmed death-ligand 1 (PD-L1) have been indicated in multiple autoimmune diseases as a method of reducing dysfunctional T cell activity [18]. Previously, we have demonstrated the potential of a soluble PD-L1 protein, delivered and expressed by AAV6 intra-articularly, to attenuate collagen-induced arthritis (CIA) severity in a mouse model in vivo.

To directly target specific immune cells, another solution is to apply antibodies or inhibitors to block surface markers or receptors on immune cells. Specifically, antibodies or inhibitors against CD19 or CD20 for B cells, CD3 or CD4 for T cells [24], CD64, GM-CSF, for macrophage repolarization, [25] and CR3 and DEC-205 for dendritic cells, are all potential strategies [26].

Besides immunological targets, research can also focus on cartilage regeneration, angiogenesis, and pain relief. For example, while AAV-delivered Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) have been applied to treat glaucoma and tumors [27], overexpression of MMPs is also associated with cartilage degradation, and serves as a potential indicator of the onset of OA and RA [28]. Besides, various growth factors have also demonstrated promise in chondral and vascular regeneration [29,30], for example, AAV delivered TGF-β was proved to efficiently repair cartilage explants [29].

4. Barriers and Solutions to AAV Gene Therapy

While there are common complications associated with AAV gene therapy, such as the low capacity of transgene size, toxicity and immunogenicity, there are three main obstacles that should be considered when using AAV vectors for joint gene delivery: the transduction efficiency in the joint; the potential risk of AAV vectors entering the bloodstream and inducing off target transduction into unrelated tissues which may lead to unwanted complications; as well as the presence of pre-existing neutralizing antibodies (Nabs) against AAV in joint fluid, as Nabs against AAV are prevalent in human populations and can largely diminish the therapies effectiveness.

4.1. Transduction Efficiency

The efficiency of gene therapy is largely determined by transduction in the target tissue. For systemic transduction-methods, such as intravenous injection, a significant portion of the transgene is often expressed in the liver, leading to largely decreased transgene expression in the joint. When local injections are administered, more transgene expression will be located at the injected sites, which are typically the primary areas of symptoms, however, there is still plenty of room to improve the general transduction efficiency. Specifically, innovations in AAV technology can be used to address three main issues with general transduction efficiency. First, the range of cell types being transduced is limited, as certain cells such as lymphocytes and myogenic stem cells are difficult to transduce. Secondly, the percentage of transduced cells typically reaches only 20-30% [18], leaving substantial potential for increasing protein expression. Last but not least, the amount of protein produced per transduced cell unit can also be enhanced.

Solutions

Despite the predominant early application of wild-type AAV serotypes, clinical trials have exposed numerous limitations and undesirable effects associated with these serotypes, many of which can be attributed to the wild-type viral capsids. As such, capsid engineering presents a promising opportunity to overcome these obstacles and develop a more satisfying vector for cell targeting with minimal immunogenicity or other side effects.

Rational design involves using techniques like peptide sequence fusion and domain swapping to create novel capsids. For instance, exchanging capsid variable regions between different serotypes. These methods could explore the properties of novel capsids, with advantages from parent capsids while eliminating undesirable characteristics. The resultant novel vectors could exhibit specific

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tropisms, and enhanced transduction efficiency, among other benefits. For instance, one study used a "provector" approach where the AAV with a matrix metalloproteinase (MMP) cleavage sequence insertion [31] that was protease-activated in MMP rich areas, a common hallmark for various inflammatory conditions including arthritis. This method also overcomes temporal and spatial limitations, enabling AAV expression solely in inflamed tissues, thereby achieving targeted delivery.

Even small changes to the capsid can yield significant effects on transduction efficacy. Single amino acid alterations can impact the capsid structure for receptor binding and antibody escape, hydrogen bonding which changes the stability of the capsid, or the post-translational modifications process, including acetylation, phosphorylation, ubiquitination, SUMOylation and so on [32], which decrease the proteasome-mediated degradation and enhance the trafficking to Golgi [33]. For instance, previous studies showed that deletion of threonine in VRI increased transduction efficiency and antibody escape in mice joints [34].

Next, directed evolution can be used to isolate promising candidates from many random variants. This could be explored by screening large mutant capsid libraries through random mutagenesis of the capsid, via DNA shuffling or high throughput approaches [35], and then selecting for variants with desired properties, such as specific tissue, cell type or enhanced transduction. A study [35] created a library with cap genes from parent AAV serotypes, and then ran the error prone PCR to generate random cap chimeras. The virus, after a few circles of selections to identify the highest viral titer and further verify the other properties, was then cultured in specific cell plates that were relevant to the targeting cell types. The selection can also be done by in vivo screening, for example by testing which mutated variant was mostly found in the AAV injected mice or primates [36]. This method requires a large mutant library and significant labor if fully relied upon. Nevertheless, with advancements in bioinformatics technology, the efficiency of the process has been greatly accelerated, leading to the identification of a few promising candidates from thousands of options.

AAV genome engineering includes modifying the transgene cassette that will be encapsulated in the AAV capsid, it involves codon optimization [37] or self-complementary ITR to enhance transduction in certain cells [38]. ITR engineering has the potential to improve various AAV characteristics. One classic method to modify ITR to enhance transduction and reduce the onset time is the development of self-complementary AAV [39], it is generated by mutating one of the terminal resolution site sequences from one terminal repeat so that the ITR cannot generate the essential ssDNA nick [39], and instead form a dimeric inverted repeat, which can be used as the starting primer for gene transcription and thus, results in decreased the initiation time. Altering the CpG content in the ITR could be another strategy. One study developed a CpG-free ITR based on the fact that unmethylated CpG motifs trigger potent host immune response [40]. Additionally, ITR modification might facilitate the dual vector heterodimerization and episomally stable concatemers. However, the gene expression from the current dual delivery system is relatively low, since only the heterodimers formed in the same orientation can be recombined into the full transgene [41].

Other elements in the process of transgene expression, including introns, post-transcriptional regulatory elements, polyadenylation signal sequences, enhancers, and so on are also related to the therapeutic efficiency of AAV-delivered material and have been adopted as targets for optimization. For example, the incorporation of scaffold/matrix attachment elements into AAV cassettes may enhance vector efficiency. Scaffold/matrix attachment regions are A/T-rich DNA sequences that attach to the nuclear matrix. It is reported that scaffold/matrix attachment region [42] helps to maintain the AAV concatemers in an open chromatin, which guarantees the transgene expression. However, the issues of increased vector size, unintended epigenetic change on host genome, among others, increase the risk associated with using this system.

Finally, small molecules or biomaterials have also been reported to enhance AAV trafficking or uncoating, and hijacking the function of AAV binding proteins or peptides to increase AAV transduction. For example, it is well-known that proteasome inhibitors, such as bortezomib, can enhance AAV transduction by inhibiting the degradation of ubiquitinated AAV capsids. In addition,

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histone deacetylase inhibitors (HDACi) including FK228 [43] and depsipeptide [44] have also been reported to enhance the AAV transduction, this process is associated with accumulation of acetylated histones, which affect transcription of specific genes. Another biomaterial that has been explored is hydrogel. Johanna et. al used 3% alginate in PBS, and mixed the AAV with alginate solution, followed by crosslinking in calcium chloride, they showed that alginate-mediated AAV/ IGF-I gene delivery successfully repaired the chondral defect [45]. Recently, studies have found that engineered nucleases such as zinc finger nucleases (ZFNs) and transcription activator–like effector nucleases (TALENs) could be applied to induce double-stranded breaks (DSBs) into the cellular genome. This action prompts cellular DNA repair mechanisms, creating opportunities for the uptake and recombination of transgenes from AAV with the cellular DNA [46]. This strategy could potentially enhance the gene targeting in cells by 25% [35]. However, it also increases the risk to host genome stability.

4.2. AAV Toxicity

Potential risks following AAV administration include unintended gene expression in non-targeted tissues and more severe tissue damage, including malignancies, acute inflammatory responses, or even fatal outcomes. Various factors contribute to AAV toxicity, including the overexpression of the transgene or capsid, production issues such as unpurified AAV or contamination with wild-type AAV, high doses, and the potential impact of the host's immune system and genetic background.

4.2.1. Integration

Even though integration is less common compared to lentivirus and other types, it is not a rare occurrence. AAV integration primarily occurs at chromatin 19, mediated by the essential cis and trans elements, AAV REP78/68, and their binding sequences. Integration has the potential to facilitate long-term protein expression and expansion [47], although, it has been reported that transgene presence and expression in the episome may also persist [48]. However, integration also brings concern of tumors and other adverse events by random insertional mutagenesis, which can activate oncogenes or deactivate tumor suppressor genes. Although, so far there is no direct evidence of tumor formation that has been associated with AAV vectors in over 200 clinical trials [49], leaving the risk of tumorigenesis from AAV unclear. Russell et. al suggested that normal newborn mice injected intravenously with AAV showed a higher ratio of hepatocellular carcinomas development [50], while other reports showed a relatively safe profile. Greig et. al followed up on 12 macaques over multiple years after they received AAV-based gene therapies targeting liver cells—15 years after AAV treatment, they found no evidence of marked clonal expansion that was linked to liver cancer [47].

4.2.2. Off Target

Off-target events are often associated with factors such as delivery methods, dosage, and serotype. Systemic delivery methods typically pose a higher vulnerability to off-target occurrences. Almost all of the AAV will be mostly expressed in the liver, the primary off target organ, when AAV is injected intravenously. However, studies focusing on liver targeting have revealed transgene mRNA exclusively identified in the liver, without any indication of chronic liver disease or malignancy, with the efficacy of the gene correction lasting decades [51]. In the context of local administration, such as intra-articular AAV delivery, certain serotypes exhibit nearly full retention of AAV at the injection site, while in others, they may cross the blood barrier and migrate to the liver. Based on a previous study, among AAVs 1-9, AAV1-6 were exclusively observed in the injected joint, but AAV7, 8, and 9 displayed robust expression not only in the injected joint but also in the liver [34]. Therefore, depending on the capsid characteristics, the administration of high vector doses may still pose a risk of unintended distribution beyond the targeted site for local administration.

4.2.3. Immunogenicity

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The immune response toward capsid or transgene is serotype-specific, species-specific [52] as well as transgene-specific. Several factors can influence the development of immune responses to AAV vectors, including the dose of vector, CpG content within the genome, properties of transgene product, and the patient's immune status, the route of administration, age as well as the HLA type of the patients [53]. Recent reports applied a high dose AAV of 2x1014 viral genomes (vg) per kg in non-human primates demonstrated severe hepatotoxicity, with side effects in the dorsal root ganglia [54].

Recognition pathways include Toll-like receptor 2 (TLR2), which is activated when AAV binds to the cell surface, and TLR 9, which is activated by non-methylated CPG dinucleotides when AAV traffics through endosomes. Other pathways include DNA sensing pathways including cGAS/STING, as well as RNA sensing through RIG-1 for single RNA sensing, and MDA5 for double RNA sensing respectively. Recognition by AIM2 or IFI16 could also trigger inflammasome activation. The activated dendritic cells could further activate CD8+ and CD4+ lymphocytes and eliminate the AAV transduced cells that are displayed on the MHC I and MHC II respectively [55].

Besides the capsid, transgene products also elicit different levels of immune response. Both secreted and non-secreted transgene proteins could potentially induce a strong immune response, secreted transgenes having a greater impact by generating both peripheral and central immune response. The transgene product can be recognized by circulating immune cells, leading to the production of antibodies from B cells that work to neutralize the protein. The complement pathway plays a role in assisting antibodies by clearing pathogens as well as damaged cells. Antibodies bound to AAV particles are recognized by C1, which can activate C3 and C5, leading to the formation of the membrane attack complex (MAC). This process further induces phagocytes or cell lysis [56].

Solution

The promoter is an essential regulatory element for gene expression. The selection or design of a promoter may be able to control the transgene expression level based on the disease condition, specifically the spatial and temporal dynamics of arthritis. Most clinical studies currently use high-expressing ubiquitous promoters such as variants of the human cytomegalovirus (CMV) or chicken beta-actin (CBA) promoters with CMV enhancer (CAG). However, to achieve precise cell targeting, including targeting various subsets of chondrocytes, synoviocytes, or newly formed inflamed cells that develop during the disease progression is critical. The turnover rate of chondrocytes is relatively low, which makes them a good candidate for AAV transduction and sustained long-term expression. In order to target chondrocytes, the use of cartilage-specific promoters has been explored. These promoters are active specifically in chondrocytes, or even more specifically, only effective in specific chondrocyte subsets. The most commonly explored promoters are collagen type II, collagen type XI, and aggrecan promotors. The Col2a1 promoter is shown to have high expression in all chondrocytes, while Col10a1 regulates specifically in hypertrophic chondrocytes [57]. Bennett et.al found cartilage-specific promoters within intron 2 of the alpha 2(I) collagen gene [58]. Another approach is the use of genome editing to guide AAV delivery and insert the therapeutic gene directly into the endogenous locus with its own endogenous promoter, thereby avoiding the need for exogenous promoters [59]. However, tissue-specific promoters usually come with lessened transduction; as the AAV package size is limited to approximately 4.7kb and many good tissue-specific promoters are over the size of 1kb.

Another innovation to regulate transgene activity is designing the vector with RNA molecular. For example, microRNA (miRNA) as single stranded noncoding RNA that binds to the target receptors or sequence has been applied to assist AAV internalization and regulate transgene transduction in specific cell types [60]. Nevertheless, this method requires careful optimization to mitigate unintended silencing of genes and enhance the stability of vector.

Besides regulating genes in the manner of tissue specificity, another concern is controlling gene expression in a timely manner. Arthritis comes with flare-ups and remission, if the treatment matches the disease progression, it will largely decrease the potential side effects. It was reported that inflammation itself could potentially enhance the transgene expression regardless of other factors

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such as AAV serotypes, the promoter, or the single strand or self-complementary design of AAV used [61]. One study reported that stronger and longer persistence of AAV transduction was observed in RA compared to healthy joints [62]. The potential mechanisms are likely attributed to the alternation of cellular metabolism and physiology [63], such as enhanced blood flow and vascular permeability in the affected area. Oxidative stress may also play an important role. It was reported that oxidative stress induces ROS, which has been reported to enhance viral infection by assisting endosome escape and facilitating the trafficking process [64].

One strategy to regulate gene expression during inflammation is by applying the inflammation-inducible promoters derived from those genes into therapeutic genes. In this context, when the arthritis exacerbates, the promoter will sense the signal and enhance transgene expression. The most commonly reported inflammation responsive promoters include IL-1, IL-6, NFκB-responsive promoters [61], MMP, Activator protein 1 (AP-1), chemokine (C-X-C motif) ligand 1 (CXCL1), and complement C3–based promoter, a cyclooxygenase (COX)-2–based promoter. However, most of those studies were done in vitro in different cell lines, meaning animal data is limited.

4.3. Preexisting Immune Response in Joint

In both serum and synovial fluid, preexisting Nabs have been reported in many species. The level is species and serotype specific [67]. >90% of humans have been infected with AAV, and ~50% of humans may have neutralizing Abs [68]. Similar to serum, synovial fluid also contains all kinds of immune cells and Nabs, although the titer of Nab from the synovial fluid does not typically correlate to the serum level [69]--one study measuring the anti-AAV2.5 antibody in serum and synovial fluid of OA patients found that serum titer was higher than synovial fluid [69].

T cells were also reported to be extracted from the synovial fluid of arthritis patients, as T cells often infiltrate 30-50% of the synovial lining in RA [70,71]. However, T cell level also does not necessarily correlate with the Nab prevalence [72]. Anita et. al reported a similar amount of T cell response against various AAV serotypes despite different Nabs [72]. Hildegund et. al reported that about 50% of healthy human adults have detectable circulating CD8+ and/or CD4+ T cells against AAV capsids [73]. In preimmunized patients, effector T cells are localized to the joint tissues, and are able to be activated once the antigen comes up again; for instance, the CD8+ T cells will be activated into cytotoxic T lymphocytes (CTL) and induce CTL-mediated cell lysis. It was reported that severe cytotoxic T cell response could lead to acute severe hepatitis.

In arthritis, there is an increased presence of antibodies and T cells locally compared to normal joints. The impact of this heightened local immunity on the level of immune response towards AAV and transgene products is debatable. It remains unclear whether it leads to an increased or a weakened immune response towards AAV, as there is an imbalanced immune system skewed towards arthritis-related factors, but not necessarily a greater immune reaction towards AAV. One fact is clear, if patients are concurrently taking immunosuppressant drugs, the overall immune response will be decreased, thus facilitating AAV transduction.

Solution

When the immune response towards AAV is robust and imminent, it results in the elimination of transduced cells or the neutralizing antibodies toward transgene products, thus reducing the long term efficacy of gene therapy treatments [74], and may require higher doses of AAV vectors to achieve therapeutic levels of gene expression, which can further increase the risk of toxicity or immune responses.

To extend the indication and effective duration, specifically in seropositive patients, briefly blocking the immune response during the AAV transduction window is critical. Methods such as plasmapheresis [44] or immunosuppressive drugs, including corticosteroids, rapamycin, anti-thymocyte globulin (ATG), CSP and MMF [75] or calcineurin inhibitors, depletion of circulating antibodies by IdeS [76], utilization of decoys that mimic the antigen and thus block the antibody binding [77], could be applied before and after AAV gene therapy. The antibodies associated with

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disease progression could also indirectly damage tissues by complement system. Therefore, employing complement inhibitors is another approach [78].Several downstream of inflammation response could also be altered through this process, such as TNF-α signaling, MyD88, IKK-γ or NF-κB [79]. These drugs could temporarily suppress the immune response, including antibodies and T cell response, therefore, giving the time window for AAV to cross the vessel barrier and enter the cells. However, for intra-articular injection, plasmapheresis [44] or immunosuppressive drugs through blood or other systemic alteration may not be efficient, as synovial antibodies were reported to not always consistent with serum level. Direct intra-articular application of these treatments may therefore be more effective.

Careful selection of the AAV capsid with lower doses is another strategy to reduce the immune response against both the capsid and the transgene product. This can be achieved by capsid engineering as discussed above to avoid cross-linking reactions caused by pre-existing antibody binding.

The delivery route also plays an important role. For example, intravenous administration and intramuscular injection of AAV vectors have been associated with a higher incidence of humoral immune responses than intra-articular administration, likely due to differences in the distribution and clearance of the vector [80]. Literature suggests that liver targeting or oral mucosal administration has been reported to induce immune tolerance, perhaps due to decreased immune response towards transgene, through the activation of tolerating DC and Treg cells, as well as anti-inflammatory cytokines such as IL10 and TGF-β [81]. Isolated limb perfusion administration [82] was reported as another safe way, it enhanced the gene transduction with a lesser dosage of viral particles needed, as the AAV is mostly limited in the local, resulting in relatively mild T cell responses.

5. Future Perspectives and Conclusion Remarks

While gene therapy has been applied in quite a few clinical trials, and some of them have even entered the market, the application of AAV in treating non-lethal or complex multigenic disorders, like arthritis, remains elusive due to potential off-target effects, immunogenicity, and toxicity. Overall, local treatment poses relatively fewer risks compared to systemic approaches, and thus, might be a safer alternative in the current landscape of gene therapy.

In conclusion, AAV-based gene therapy offers substantial potential for treating various joint diseases. Yet, several challenges persist. The efficacy of this approach relies on the precise delivery of therapeutic genes to the affected joint during the active disease phase, minimizing off-target effects and toxicities, and ensuring sustained and regulated expression of the therapeutic genes. Future research should prioritize optimizing vector capsid and transgene designs, investigating the ideal targets, and circumventing the AAV immune response. Finally, consideration of ethical situation and biosafety is essential for achieving a greater success in joint gene therapy.

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