Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: March 31, 2023

Citation: Tang M. Y., Sowa G., et al., 2023. Gene Therapy for Intervertebral Disc Degeneration: An Overview of Current Strategies and Applications, Medical Research Archives, [online] 11(3). https://doi.org/10.18103/mra.v11i3.3657

DOI: https://doi.org/10.18103/mra.v11i3.3657

ISSN: 2375-1924

REVIEW ARTICLE

Gene Therapy for Intervertebral Disc Degeneration: An Overview of Current Strategies and Applications

Melissa Yunting Tang[1]*, Gwendolyn Sowa[1], Joon Y Lee[1], Nam Vo[1], James Kang[2]

[1]Department of Orthopedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA.
[2]Department of Orthopedic Surgery, Brigham and Women's Hospital, Harvard University, Boston, MA, USA.

*Tangy5@upmc.edu

Abstract

Intervertebral disc degeneration is a pervasive condition contributing to chronic back pain, affecting up to a third of the population, with risk further increasing with age. It is a significant driver of disability for millions of Americans and others worldwide. The standard of care today is reliant on symptomatic treatment rather than addressing the root cause of disease. Surgical interventions alter the structural integrity and biomechanics of the spine, often leading to loss of function and motion, and post-operative complications. This is the basis for innovation in novel biologic treatments, including gene therapy, which aims to reestablish the optimal balance between matrix catabolism and anabolism within pathologically degenerating disc cells. This review will cover the significant advances that have led to identification of target therapeutic genes combined with regulated expression of the therapeutic transgene and successful systems for gene delivery into cells. Recent advances in viral and non-viral vectors for gene transfer, silencing of genes by RNA interference, editing of genes by clustered regularly interspaced short palindromic repeats, and modifying mammalian target of rapamycin signaling pathwayS offer promising treatment avenues. Clinical translation of these approaches, however, will require further investigation of the pathological basis of disc degeneration in addition to systematic safety measures for the adoption of gene therapy.

Introduction

Intervertebral disc degeneration (IDD) is associated with significant morbidity worldwide. In the United States, the lifetime incidence of patients experiencing at least one episode of low back pain (LBP) is over 80%, with a 15-20% chance of LBP within 1 year.[1] IDD presents as a range of symptoms, including LBP, radiculopathy, neuromotor and sensory changes, and even paralysis.[2] [3]Morbidity from IDD presents a significant economic impact to society, with significant health care utilization, office visits and hospital stays.[1] Individuals with LBP incur healthcare expenditures 60% higher than those without back pain. Expenditures are generally higher for patients with disc disorders in addition to those who are older, female, white, and insured.[1] LBP in the United States amount to a total cost of over $100 billion/year from both direct medical costs and indirect costs associated with loss of work and productivity.[4] In patients less than 45 years, back pain is the most common cause of activity limitation.[5]

Current treatments for LBP consist of surgical and non-operative options with unpredictable outcomes. However, surgical treatments attempt only to address clinical symptoms of pain and mechanical instability but not the underlying etiology.[6–8] Non-operative treatments often have limited specificity and variable responses. Novel insight into cellular and molecular biology have found biological targets to arrest further deterioration or to reverse the pathological process in IDD. This article focuses on discussing current state of updates on gene therapy for IDD, including its delivery systems and safety with execution.

Disc Anatomy

The intervertebral disc is the largest avascular structure in body and is largely protected from immune surveillance.[9] It consists of 3 regions, the central nucleus pulposus (NP) surrounded by the annulus fibrosis (AF), and the cartilaginous end plates (CEP). The NP is composed of a gelatinous matrix of proteoglycans encased within a loose network of collagen II fibers. The AF is a stiff fibrous tissue of collagen I fibers surrounding the NP. The AF maintains the swelling hydrostatic pressure created by proteoglycan aggregate within the NP to allow for counteracting compression in axial load. The CEP lies at the superior and inferior surfaces of the disc, is composed of a thin layer of hyaline cartilage, and is the site of peripheral vascularity and nutrient diffusion to the NP.[10] The disc functions in mechanical stress transmission, shock absorption, and importantly, enables vertebral motion.[11] Disc cells have the integral role in the production of extracellular matrix (ECM) components, particularly proteoglycan and collagen.[10] A hallmark of IDD is the loss of ECM proteoglycans, a primary therapeutic target for many biologic treatment approaches, including gene therapy, that aim at restoring disc proteoglycan loss and structural integrity.

Pathophysiology of Intervertebral Disc Degeneration

The pathophysiology of IDD is multifactorial, due to a combination of aging, genetics, and biomechanical contributions. Risk factors for

IDD include chronic abnormal mechanical load, tobacco use, trauma, aging, and lifestyle.[12–15] Structural lumbar IDD is present in up 40% of the population by age 30 and 90% by age 55.[16] The loss of hydrostatic pressure in dehydrated NP cells shunts abnormal forces to the AF, leading to tears of the annulus potentially resulting in herniation of the NP.[17] The hallmark of IDD is the loss of collagen and proteoglycan production resulting in altered ECM structure and histologic changes. Proteoglycan loss is an initial driver of disc degeneration due the dysregulation of anabolism and catabolism within the disc. Upregulation of proteolytic enzymes, such as matrix metalloproteinases (MMPs) and ADAMTS results in the loss of collagen and proteoglycans. In degeneration, catabolic activity outpaces the activity of anti-catabolic inhibitors such as tissue inhibitor of metalloproteinases (TIMP).[18–20] The loss of proteoglycan in the NP cell causes the loss of hydrostatic pressure and disc dehydration.[21] Fibrosis of the NP occurs from the increased ratio of type I compared to type II collagen.[22] Over time, degeneration results in the loss of histological difference between NP and AF, resulting in altered biomechanics of the intervertebral disc and consequently, the spine.[21,23]

The biochemical changes associated with IDD typically result in elevated inflammatory response and altered cell composition and phenotype, including decreasing autophagy and increasing cellular senescence and apoptosis. Inflammation further increases oxygen tension, nutrient deprivation, and acidification within the disc, which has an inherently tenuous blood supply relying on diffusion from the cartilaginous endplates.[2,24,25] Senescence is the irreversible arrest of cellular growth due to aging-associated accumulation of cellular damage. Secretion of senescence-associated secretory phenotype (SASP) induces a pro-inflammation state upregulating cytokine production, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and proteolytic enzymes which galvanizes disc degeneration.[26–29] Apoptosis is also a characteristic of disc degeneration, and is a cause for decrease in the cell population.[16,30,31] Autophagy occurs under nutrient poor conditions, hypoxia, and other forms of cellular stress, in which damaged proteins and organelles are broken down and recycled within the cell for protection against toxic accumulation of defective proteins and organelles. Decreased autophagy has been reported to link to IDD and this cellular change may be extrapolated to be an integral target in modulation of IDD.[32,33]

Pathologic inflammation is thought to be a major driver behind discogenic back pain, which accounts for almost 40% of chronic LBP.[34,35] The pro-inflammatory mediators released by aberrant disc cells triggers pain response and increases pain sensitization.[34,35] Scientific evidence is expanding with regards to identification of key steps in this process involving perturbation in ECM homeostasis, nutrition metabolism, as well as cellular signaling, cellular senescence and apoptosis.[36,37] Gene therapy aims to correct this pathologic biochemical imbalance through upregulation of anabolism and down regulation of catabolism, thereby restoring disc matrix

homeostatic balance and reversing ECM proteoglycan loss, with the goal of altering disease process.[38]

Standard of Care

Treatment for discogenic back pain begins with pain management, through pharmacologic and non-pharmacologic measures, and restoration of function. A variety of medications, oral and injectable, have been used to mitigate the experience of low back pain, but none are effective at addressing or reversing the catabolic/ anabolic imbalance of IDD. Exercise based treatments have the benefit of improving strength and biomechanical loads, with the potential to stimulate mechanobiologic pathways, but lack ability to specifically target pathways responsible for degeneration. Patient who fail non-operative management often undergo surgeries, including discectomy, disc arthroplasty, and/or spinal fusion with insertion of an intervertebral cage. This can result in downstream complications including adjacent segment disease in up to 36.1% at 10 years as well as subsequent return to the operating room for symptomatic disease.[10,39] Up to 30% of patients also continue to have LBP post-operative, known as post spinal surgery syndrome. Some patients subsequently undergo further operative treatment including spinal cord stimulators or revision surgery.[40] However, these approaches do not have an effect on the underlying pathology. To date, there are still no effective biological interventions to stop or slow the progression of disc degeneration or to induce disc regeneration. The need for gene therapy is thus critical to address this current deficiency in treatment. Recent growing advances point to gene therapy as a viable therapeutic option for IDD and discogenic LBP. [4110,3940]

Gene Therapy

The concept of gene therapy stemmed from the treatment of heritable fatal genetic disorders, whereby a faulty gene is replaced by a functional copy to eliminate the associated disease. Gene therapy has since evolved to encompass the transfer of exogenous genes into cells for therapeutic protein production to treat chronic conditions. Once the transgene is transduced into the target cell, it is then transcribed into mRNA, and translated into proteins that modulates the metabolic effects of both the host cell and, depending on the nature of the transgene product, the adjacent cells through paracrine effects. This is crucial given that the exogenous delivery of therapeutic proteins alone cannot produce sufficient long-term treatment in chronic diseases. Thus, the potential advantage of gene therapy is its prolonged efficacy, since incorporation of genetic material into the host cell will result in prolonged production of therapeutic targets. However, this also results in safety concerns due to the potential for undesired effects, which also need to be addressed to facilitate clinical translation.

Therapeutic Targets of Gene Therapy in the Intervertebral Disc

Table 1: Transgenes and Pathways in Disc Gene Therapy

Pathways	Transgenes
Inflammatory	NFkB, IL1-β, TNF-α, IL-1Ra
Catabolic	MMP, ADAMTS, TIMP1, TIMP3
Anabolic	BMP2, BMP7, IGF1, TGF-β1
Autophagy	mTOR
Apoptosis	Bcl-2

In the intervertebral disc, it is thought that degeneration occurs due to a disrupted homeostasis between the synthesis and catabolism of key ECM components. Hence, successful delivery of transgenes encoding key regulators of ECM catabolism and anabolism to correct this imbalance would be a viable therapy. This has proven successful in animal models of IDD whereby genes were successfully delivered to host cells.[42] Application of gene therapy to treat IDD is based on the modulation of exogenous proteins that can be upregulated or down regulated to promote a favorable proteoglycan homeostatic balance, via *in vitro* or *in vivo* strategies.[43] The intervertebral disc is an optimal site for gene therapy given that it is immune privileged, avascular and encapsulated. This should theoretically allow for prolonged expression of the transgene given limited immune response to the transgene products or vector components. This is important as NP cells express the Fas ligand, which is the hallmark of other immune privileged tissues such as the testis and retina.[43] In the presence of Fas-positive T-cells, the Fas ligand induces apoptosis and hence limits immune response.

Two broad classes of transgenes have been explored for IDD gene therapy treatment. The first class encompasses growth factors that favor proteoglycan synthesis while the second class includes factors that silence catabolic enzymes that promote matrix degradation. Growth factors including bone morphogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1), and transforming growth factor-β (TGF-β), all have been reported to have therapeutic effects that slow or reverse IDD.[17]

Thompson et al demonstrated that exogenous application of TGF-β1 in a canine disc cell culture increased proteoglycan synthesis.[44] Osada et al reported upregulation of proteoglycan synthesis in a canine disc cell culture with the addition of IGF-1.[45] Takegami et al previously demonstrated an upregulation of proteoglycan synthesis in a rabbit NP cell culture exposed to exogenous osteogenic protein-1, and an increase in proteoglycan loss after exposure to interleukin (IL)-1, an inflammatory cytokine.[46] Li et al showed that exogenous BMP-2, an anabolic factor, increased both aggrecan expression and osteogenic protein-1, further stimulating proteoglycan biosynthesis in an exogenous

rat disc cell culture.[47] Several animal models have also focused on TIMP1, an anti-catabolic factor, which inhibits the activities of most known MMPs and also promotes collagen and aggrecan synthesis in the disc. Introduction of BMP2 and TIMP1 into a validated needle puncture model of rabbit IDD demonstrated delayed disc degeneration on both MRI and histology.[48] Yoon et al further demonstrated in an *in vitro* rat disc culture that BMP-2 augmentation not only increased proteoglycan synthesis and cell proliferation, but also other chondrocyte specific genes including type 2 collagen, aggrecan, and Sox9 mRNA. Sox9 is a transcription factor that induces type II collagen expression, a major component of NP cells.[49] Gruber et al demonstrated that the addition of exogenous IGF-1 and platelet-derived growth factor decreased the number of apoptotic cells in a in a human disc cell culture.[50] These data demonstrate that growth factors have the potential to treat IDD by maintaining the number, phenotype and proteoglycan content of disc cells.

Gene Delivery Strategy

Therapeutic genes are delivered into the target cell using two strategies, *in vivo* and *ex vivo*. The *in vivo* strategy involves transferring one or more therapeutic genes and its vector directly into the target cell population in the host. In the *ex vivo* strategy, target cells are isolated form the host, cultured, then transduced with the therapeutic gene *in vitro*. The genetically modified cells are then reimplanted into the host. Most applications of gene therapy for the treatment of IDD utilize an *in vivo* approach due to the several limitations to *ex vivo* gene delivery. Though in theory, an *in vitro* cell culture for use in an *ex vivo* gene delivery approach is an ideal approach, since cells that respond abnormally after gene transfer can be identified and removed prior to implantation. Nonetheless, the process of cell harvesting and reimplantation may be associated with high cell death and donor tissue morbidity. Removal of the cells themselves may cause permanent alterations in their phenotype making them unsuitable for reimplantation, or may have unintended behaviors if implantation were successful. Hence, most approaches for IDD have utilized an *in vivo* approach.

Vectors

Gene therapy requires successful transfer of the transgene into host cells to allow for production of therapeutic molecules to promote a desired metabolic outcome. Unpackaged molecular material is subject to degradation and generally cannot be delivered successfully. Thus, vectors are integral to allow for efficient transfer into the target cell.

Non-Viral vectors

Non-viral vectors reported in the literature include liposomes, gene guns, DNA-ligand complexes, microbubble enhanced ultrasound, and polyplex micelles. They are advantageous over viral vectors due to their absence of immune reactivity, DNA mutagenesis, and low costs. DNA-ligand complexes and gene guns have a relatively simplistic design and are also cost-effective to produce. However, nonviral vectors are limited by low transfection rates into the host cells, thus allowing for only transient and limited expression of the

transgene. They may have limited efficacy in the treatment of chronic diseases, in which disease modification requires prolonged expression of therapeutic products. Nevertheless, several have been explored for IDD.

Microbubble-Enhanced Ultrasound

Microbubble-enhanced ultrasound is based upon sonoporation, in which transient pores are produced in the target cell surface using ultrasound exposure at a designated intensity.[51] This technique has historically been successful in the transfection of vascular cells, with transgene expression up to ten times the rate of naked DNA alone.[52] Transfection efficacy can be further enhanced through combination with acoustic cavitation. Microbubble echocontrast agents when used in combination with ultrasound exposure increased acoustic cavitation, resulting in 300 times the transgene expression of naked DNA.[53] The delivery of plasmid DNA with microbubble-enhanced ultrasound transfection was proven successful in rat nucleus pulposus *in vivo* by Nishida et al. In this study, plasmids with GFP and firefly luciferase were mixed with microbubbles, then introduced into rat intervertebral discs through direct injection and ultrasound-mediated irradiation. Ultrasound use produced luciferase activity 11 times higher than injection alone. Transgene expression in the ultrasound group was detectable for up to 24 weeks post-transfection.[54] Ultrasound targeted microbubble delivery is thus a practical route for gene delivery into intervertebral discs, through augmentation of the transfection rate.

Polyplex Micelle

Many non-viral vectors have poor solubility as they have a cationic outer shell associated with anionic DNA. Polyplex micelles are designed to bypass this, as they contain a hydrophilic outer layer that surrounds a core composed of the negatively charged DNA associated with cationic fragments.[55] Polyplex micelles can retain their structure producing enhanced gene expression, even in the presence of serum.[56]

Li et al has shown successful delivery of therapeutic microRNA-29 within polyplex micelles into the intervertebral disc, suppressing fibrosis. In the intervertebral disc, progressive fibrosis is due to overexpression of matrix metalloproteinases (MMPs). MicroRNA-29A have demonstrated anti-fibrotic properties but are limited by efficacy of gene delivery into the disc.[57] Feng et al injected hydrogels degradable by MMPs that encapsulated polyplex micelles containing miR-29a into nucleus pulposus cells. Once in the nucleus pulposus, MMPs cleave the MMP soluble hydrogels releasing the micelles. MiR-29a then translocate to the nucleus and successfully silences MMP-2, thereby inhibiting fibrosis and successfully reversing disc degeneration in a rabbit model.[58] Similarly, increase in ECM degrading enzymes and decrease in miRNA-25-3p contribute to the pathogenesis of IDD. Huang et al showed the feasibility of using a polyplex micelle for the delivery of miRNA-25-3p into an *in vitro* NP cell culture and *in vivo* rat model of IDD. The successful delivery of miRNA-25-3p to NP cells inhibited ECM breakdown by inhibiting

expression of catabolic enzymes and increasing the expression of ECM proteins.[59]

Viral Vectors

Viral vectors transduce genetic material by utilizing the inherent ability of viruses to infect the host cell. Viral transduction is efficient and allows for delivery of the transgene into host cells, and is successful even in senescent or slowly replicating cell populations such as the intervertebral disc. Though more efficacious than non-viral vectors, viral vectors pose more risks due to their potential for immune reactivity, cytotoxicity, and DNA mutagenesis if incorporated into the host genome. Moreover, they are also associated with high costs for production, requiring specialized facilities, route of production, and dedicated patient suites for administration.[60]

Viral vectors used in gene therapy include adenovirus, adeno-associated virus (AAV), herpes simplex virus, lentivirus, retrovirus, and poxvirus. Each type of viral vector encompasses its own unique set of risks and benefits, thus appropriate selection of the viral vector is integral to success in gene therapy. Adenovirus and AAV are the most commonly used vectors in disc gene therapy. The intervertebral disc is unique in that it is avascular, encapsulated, hypoxic and poses a challenging environment for cell function and viability. Thus, the most successful mechanism of transduction has been local, direct delivery into the target cells *in vivo*.[38]

Adenovirus

Adenovirus is a double-stranded DNA virus capable of infecting many cell lines. Out of the 47 serotypes in humans, gene therapy makes use of serotypes 2 and 5. They are readily producible at high titers and easy to manipulate. Gene therapy is made possible by removal of the gene responsible for the replication and expression of the virulent gene, envelope E1.[61] The advantage of adenovirus vectors is that they allow for efficient gene transfer and transduction even into slowly replicating, quiescent cell lines such as disc cells. They also pose a lower risk of insertional mutagenesis during transduction since the viral genome stays as an epitome within the nucleus and does not insert into the host cell genome. However, due to this lack of integration, the episome is not replicated with host cell division. Adenovirus vectors are limited by transient gene expression due to activation of the host immune system in response to adenoviral antigens. This results in the degradation of the viral episome, ceasing further protein synthesis.[62,63] The safety profile of adenoviral vectors is also under investigation due to reported fatal anaphylaxis reactions after systemic injection.[64] Viral vectors also pose the risk of infection with the wild-type virus, in which adenoviruses may cause gastrointestinal and upper respiratory pathologies.

The first incidence of gene transfer for IVD using an adenovirus vector was reported by Nishida et al in 1998, whereby the lacZ marker gene was successfully transduced *in vitro* and *in vivo* in a rabbit model. An adenovirus vector encoding the lacZ gene was used to transduce an *in vitro* rabbit NP cell culture. The Ad-lacZ saline was directly injected into rabbit intervertebral discs through an anterior

approach. Transduction was successful via both routes as confirmed by X-Gal staining. Marker gene expression was detectable for up to 12 weeks post-transduction.[65] This same group later successfully transduced transforming growth factor beta 1 (TGF-β1) gene into rabbit intervertebral discs *in vivo*. An adenovirus vector encoding the TGF-β1 gene with saline was directly injected into the lumbar discs of rabbits. Transduction was later confirmed with immunostaining. Compared to controls, the transduced NP cells produced 30 times the amount of active TGF-β1, and 5 times the increase in total (active and latent) TGF-β1. Transduced tissues also demonstrated 100% increase in proteoglycan synthesis, which reached statistical significance ($p < 0.05$).[9]

Yoon et al demonstrated that LMP-1 also upregulates proteoglycan synthesis in IDD cells via upregulation of BMPs. LMP-1 is a regulatory protein that acts within disc cells to increase anabolic activity of BMPS. Human disc cells transduced with an adenovirus vector encoding LMP-1 were injected directly into the discs of rabbits. Both BMP-2 and BMP-7 production increased significantly *in vitro* and *in vivo* as quantified by reverse transcriptase PCR (RT-PCR) and and ELISA.[66]

Paul et al demonstrated Sox9-mediated type 2 collagen production via adenovirus expressing Sox9 in human IDD disc cell culture as well as in rabbit intervertebral discs of rabbits. Increased type 2 collagen production was confirmed using RT-PCR and immunohistochemical staining. The *in vivo* rabbit model also showed preserved histologic architecture of NP cells at 5 weeks compared to controls.[67]

Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a MMP inhibitor and an important regulator of catabolism in NP cells, preventing ECM breakdown. Wallach et al transduced human IVD cell cultures with an adenovirus vector containing either TIMP-1 or BMP-2. Both therapeutic groups demonstrated a significant increase in proteoglycan synthesis with increasing viral concentrations.[68]

Moon et al demonstrated the synergistic upregulation of proteoglycan synthesis with transduction of multiple anabolic genes on a single adenovirus vector, including TGF-β1, BMP-2 and IGF-1. Human NP cell cultures were transduced with one, two, or all three growth factors. Compared to controls, transduction of one growth factor resulted in a proteoglycan increase of 180 to 295%, two growth factors resulted in an increase of 322 to 398%, and three growth factors with an increase of 471%.[69] These studies show that the intervertebral disc is amenable to transduction by adenovirus vectors and can successfully produce the therapeutic protein.

Adeno-Associated Virus

Adeno-associated virus (AAV) is a single-stranded DNA virus in the parvovirus family. It is composed of only two genes, Rep and Cap. It is also able to infect many different cell lines but less efficiently than adenovirus. The AAV vector also poses a lower risk of insertional mutagenesis since it has a specific site of integration on chromosome 19 and does not

cause any damage to the host genome. AAV also triggers a much lower host-cell immune response. On the other hand, this also limits the large-scale production of these vectors. In contrast to adenoviruses, AAVs do not cause disease in humans and have a much more favorable safety profile.[70]

AAV vectors transport around 4.5 kilobases, a much smaller length of therapeutic DNA compared to adenovirus. However, recent advances in AAV vector concatomerization post-transduction have expanded the packaging capacity. In concatomerization, two vectors are simultaneously transduced into cells, one containing the first half and one containing the second half of a protein. The viral genomes are then combined producing a functional gene of a much larger size. Nonetheless, the stability and length of gene expression must be further explored.[71,72] Purification of AAV also require isolation and removal of the helper virus. AAV vectors may also generate an antibody response that limits the transduction efficacy to one trial only. However, this has been bypassed in the literature by changing to a different AAV serotype.[73,74]

Latterman et al have successfully transduced the firefly luciferase (luc) gene using an AAV vector in a rabbit model. The vector was transduced into both human NP cell cultures and directly injected into the intervertebral discs of rabbits using the anterior approach. Transduction was confirmed via luciferase assay, and gene expression *in vivo* was detected for 6 weeks. Significantly decreased transgene expression by over 10-fold was observed in rabbits pre-immunized to AAV from prior exposure. Overall, marker gene expression by the AAV vector resulted in only half the amount produced by adenovirus vectors.[75]

Leckie et al, in a randomized controlled trial of AAV2-BMP2 or AAV2-TIMP1 vector injections into the nucleus pulposus of a validated needle puncture model of rabbit IDD, found that treated rabbits expressed less MRI and histologic evidence of IDD compared to the untreated group. The treated group also expressed levels of collagen type II similar to non-injured discs at 12 weeks, and showed viscoelastic properties distinct from the needle puncture group with biomechanical testing. The success of viral vectors in this *in vivo* animal model again supports the feasibility of AAV vectors in the treatment of human IDD.[48]

The use of AAV in IDD was further demonstrated by Li et al. When AAV vectors encoding AP-2a knockdown and hyperexpression of TGF-β1 were both injected into rat intervertebral discs and transduced to human NP cell cultures, both *in vivo* and *in vitro* populations showed downregulation of MMP-2, MMP-9 and Smad 3 and upregulation of Col-2 and aggrecan. They also showed increased NP cell growth and decreased senescence and apoptosis. Thus, AP-2a knockdown may thus have a therapeutic effect on IDD by increasing NP cell proliferation and decreasing senescence and apoptosis.[76]

Retrovirus

Wehling et al first reported gene transfer using a retrovirus vector for IDD in 1997. Two

genes, LacZ and the cDNA of human interleukin-1 (IL-1) receptor antagonist were introduced into an *in vitro* culture of chondrocyte cells from bovine intervertebral endplates. Transduction of lacZ was confirmed by X-Gal staining and resulted in 1% of cells positive for beta-galactosidease. Transduction of IL-1 receptor confirmed with enzyme-linked immunosorbent assay resulted in 24ng/ml/10(6) cells with the therapeutic protein.[77] Retroviral vectors, however, poses the risk of insertional mutagenesis, and have not been extensively explored for IDD.[60]

Baculovirus

Baculovirus is an insect virus that has been successfully implemented in the setting of gene therapy. It allows for delivery of exogenous genes into mammalian cells due to its low cytotoxicity and lack of viral replication after transduction.[78]

Liu et al has successfully delivered the green fluorescence protein (GFP) gene via a baculovirus vector to rabbit intervertebral cells *in vitro* and *in vivo*. Six different doses of baculovirus encoding the GFP gene was used to transduce an intervertebral cell culture. GFP expression was confirmed with fluorescence microscopy and flow cytometry. The dose at 200 multiplicity of infection produced the highest rate of transduction at approximately 87% of NP cells and allowed for long term expression without host cell toxicity. The baculovirus vector was then directly injected into the intervertebral discs. Fluorescence microscopy was used to assess for transgene expression in the NP tissues. GFP expression *in vivo* was able to occur without any complications.[79] Although this demonstrated feasibility, one disadvantage of the baculovirus vector is limited of transgene expression due to a host antiviral response in the mammalian cell.[78] The presence of CpG motifs within the viral DNA can induce activation of pro-inflammatory cytokines, interferon, and toll-like receptors.[78]

Lentivirus

Lentivirus has the capability to encode large therapeutic DNA sequences favoring the design of an expression system containing multiple transgenes. Liu et al demonstrated the feasibility of using a lentiviral vector for the transduction of transforming growth factor (TGF)-β3, connective tissue growth factor (CTGF) and tissue inhibitor of metalloproteinase 1 (TIMP1) into an *in vivo* rabbit model of IDD. Intervertebral disc degeneration at the lumbar spine was generated via needle puncture into rabbit AF. The lentiviral vector containing the therapeutic transgenes were directly injected into the lumbar discs. Injection of empty lentiviral vectors served as the control group. MRI assessment of the intervertebral discs of both groups were performed at 16 and 20 weeks post transduction and showed amelioration of IDD in the experimental group. The mRNA levels and protein expression of aggrecan and type II collagen in the NP tissues were quantified via reverse transcription RT-PCR and western blot, respectively. The experimental group had a statistically significant increase in type II collagen and aggrecan ($P < 0.05$).[80]

Zhao et al demonstrated the synergistic role of MMP3 downregulation with Sox9 upregulation inhibited IDD progression using lentiviral vectors in a rabbit model. Lentiviral vectors containing LV-MMP3-shRNA with or without LV-Sox9 were injected into rabbit lumbar discs. Injections of phosphate buffered saline or of the control virus without the transgene were used as controls. MRI evaluation at 8, 12, and 24 weeks showed evidence of IDD in the controls but not in both therapeutic groups, and to a greater extent in the combined MMP and Sox9 group. Both therapeutic groups also demonstrated increased production of type II collagen, aggrecan and proteoglycan, but again to a greater extent in the combined group.[81]

Safety Considerations

Due to the inherent dangers of viral vectors, including immunogenicity, insertional mutagenesis and human disease, future directions in gene therapy will aim to strategize mechanisms for reduced therapeutic viral load, inducible gene expression mechanisms, tissue-specific promoters, and safer AAV vectors. Use of one viral vector to transduce multiple anabolic genes is one strategy to limit viral load while maximizing transgene expression. The transduction of TGF-β1, BMP-2, and IGF-1 with a single adenovirus vector by Moon et al again highlights the feasibility of this mechanism. The transfer of multiple growth factors for the production of ECM components had a synergistic effect.[82] After transduction into the host cell, another risk is the aberrant regulation of transgene expression. Accidental injection of adenovirus vector constructs into a rabbit model have been shown to produce adverse histological changes and bilateral lower extremity paralysis.[83]

Inducible gene expression systems have been introduced to regulate transgene expression, either to simulate therapeutic effect or decrease expression in the event of toxicity. These involve a vector encoding a ligand-activated promoter region linked to the transgene. Transgene expression is turned "on" or "off" based on the presence of an exogenous ligand. For instance, the Tet-on system is activated by an exogenously applied tetracycline derivative. This system has been successful in AAV vectors.[84] Furthermore, the Tet-on system have demonstrated success in the transduction of the lacZ gene into chondrocytes in an *in vivo* rabbit model with the addition of doxycycline.[85] Sowa et al demonstrated control of gene expression using an AAV vector containing a novel control system (AAV-RheoSwitch GFP) in disc cells, whereby ligand-induced gene expression of GFP could be successfully modulated by addition and removal of the ligand.[86] Han et al recently developed a recombinant AAV gene vector, AAV-NFkB-hTIMP1, which only expresses the transgene, human tissue inhibitor of metalloproteinase 1 (hTIMP1), under conditions of stress that activates the NFkB signaling pathway. IL-1β activation of NFkB resulted in upregulation of hTIMP1 expression in rabbit disc cells, demonstrating that AAV-NFkB-hTIMP1 is a novel inducible transgene delivery system for the treatment of IDD, by ensuring that transgene expression only occurs during

pathologic inflammatory conditions. This further minimizes the risk of constitutive transgene expression and subsequent unnecessary accumulation of transgene products.[87] Other systems currently being investigated include heat shock proteins, regulatory promoters with steroid, metallothionine, and the insect ecdysone receptor. Future applications of these inducible systems into the disease modification of IDD will reduce adverse effects of treatment and maximize efficacy.

New Advances in Gene Therapy

Traditional gene therapy involves overexpressing a therapeutic transgene to compensate for a defective gene. Gene therapy is evolving to introduce genes that modify pathways to get the desired metabolic outcome. Newer approach involves use of regulated promoters to control the level of transgene expression. This is accomplished by shutting down or cutting out the gene of interest using Crispr/Cas9 technology or RNAi, both of which are a form of gene therapy. They exist in the form of plasmid vectors that when delivered into cells can turn on or off the gene of interest.

Clustered Regularly Interspaced Short Palindromic Repeats

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 has emerged as an effective tool for genomic editing, allowing for the modulation of precise genomic sequences resulting in gene silencing, repair and expression of target genes. CRISPR/Cas9 is comprised of a Cas9 DNA endonuclease that induces double-strand DNA breaks at target sequences. Cas9 is guided to DNA cleavage sites by CRISPR RNA (crRNA), which are sequence specific and programmable. Downstream DNA repair facilitates the addition, deletion, or substitution of the transgene at the target sequence.[88] This system is highly specific with limited genome-wide off-target effects.[89] CRISPR/Cas9 has been used in both viral and non-viral delivery systems.

Most recently CRISPR-Cas9 has been successfully used in an *in vivo* mouse model to target β-catenin, a potent driver of catabolic responses to disc injury through the upregulation of disc matrix proteases including ADAMTS5 and MMP13.[90] CRISPR-mediated gene ablation was successfully used in a mouse model of disc degeneration to knock out β-catenin using a Ctnnb1-ablating AAV vector.[90] In addition to the successful application of CRISPR-Cas9 *in vivo*, downregulation of β-catenin also proved to attenuate disc degeneration in mice model by slowing pathological remodeling of the disc matrix.[90]

TNF-α and IL-1β are known inflammatory cytokines involved in the progression of IDD. The feasibility of using a lentiviral CRISPR epigenomic editing system via modulation of TNFR1/IL1R1 signaling was demonstrated in an *in vitro* model of human IDD by Farhang et al. A lentivirus was used to deliver Kruppel-Associated Box (KRAB), a transcription repression domain into NP cells from IDD patients. Though transduction was successful, downregulation of TNFR1 but not IL1R1

signaling was observed. Downregulation of TNFR1 was associated with increasing aggrecan and decreasing MMP3, though no such changes were observed in the ILR1 cohort.[91]

CRISPR/Cas9 was used to successfully knock out the transient receptor potential vanilloid type 4 (TRPV4) gene in an *in vitro* cell culture of chronic LBP patients with AF injury. A major contributor of LBP is thought to stem from the mechanical loading and subsequent stretch-induced inflammation in AF cells. TNFR4 silencing successfully prevented the increase of IL-8 mRNA and subsequent IL-6 and IL-8 release normally induced by mechanical stretch.[92]

In addition to targeting the anabolic/catabolic balance, CRISPR technology has also been used to target pain signaling. Pain from IDD is in part due to the hyperactivity of dorsal root ganglion (DRG) neurons. AKAP150 is an important target in this pathway as it triggers IL-6 mediated increases in neuronal activity. A lentiviral CRISPR/Cas-9 system has been successfuly used to silence the AKAP150 gene in a rat model via targeted promotor histone methylation. The delivery of these epigenomic editing vectors to DRG neurons inhibited the elevated neuronal activity attributed IDD but spared the non-pathologic activity of the neurons.[93] Such an approach is novel as it is distinct from modulation of the disc structure. Though it is not without limitations, advances in the use of CRISPR/Cas 9 in IDD has demonstrated its potential as an accurate and powerful tool in the attenuation of IDD pathogenesis, with promise for future translational therapies.[94]

RNA Interference

RNA interference (RNAi) is a novel approach that can be used to suppress catabolic drivers of matrix breakdown through targeted gene silencing. In RNAi, double-stranded RNA (dsRNA) is used to silence post-transcriptional gene expression at specific sequences, inducing mRNA degradation or translational suppression. Short interfering RNA (siRNA) has been used to successfully induce silencing in mammalian cells via transfection through electroporation and lipid-based reagents. In the intervertebral discs, catabolic genes can be targeted and silenced through this mechanism.[95] When dsRNA is delivered to the host cell, Dicer, a RNAase II family of ribonucleases recognizes and cleaves it into siRNA oligonucleotides, comprised of 21 to 23 base pair segments. The siRNA is then incorporated into the RNA-induced silencing complex (RISC) and directs RISC to the target RNA. This nuclease complex then cleaves the target RNA sequence resulting in gene silencing.[96] Incorporation into RISC further ensures the siRNA's stability within the cell.[97] These siRNA oligonucleotides can be chemically synthesized in the laboratory or transcribed *in vitro* via T7 RNA polymerase.[98,99] The commercial production of these siRNAs paved the way for the possibility of gene therapy in intervertebral disc cells. However, one limitation to the use of siRNA is its short-half life, and hence short-term modulation of expression of the therapeutic gene.

The successful use of siRNA technology in the disc has been demonstrated, both *in vivo* and *in vitro*. Katutani et al demonstrated the

feasibility of siRNA-mediated gene silencing in rat and human NP cells *in vitro*. Two reporter luciferase plasmids (Firefly and Renilla) were co-transfected with siRNA targeting firefly luciferase degradation in rat and human NP cells. Firefly luciferase expression was significantly downregulated in both cell lines, 94.7% in rats and 93.7% in humans. This inhibitory effect was detected for 3 weeks post-transfection.[100]

The efficacy of relatively prolonged gene silencing by siRNA in rat discs *in vivo* was later demonstrated by Suzuki et al. This study further demonstrated the feasibility of siRNA downregulation of not only of the exogenous reporter gene, but also of the endogenous gene. The same two reporter luciferase plasmids (Firefly and Renilla) and siRNAs targeting firefly luciferase were co-transfected via ultrasound gene-transfer into the intervertebral discs of rats *in vivo*. SiRNAs targeting the endogenous Fas Ligand (FasL) in rat discs were transfected in the same fashion. SiRNA-mediated downregulation of firefly luciferase was apparent, with the transfected group demonstrating 80% inhibition compared to controls and lasted for 24 weeks post-transfection. Endogenous FasL expression was also down regulated, expressed at 53% of that of controls.[101]

Another successful implementation of siRNA technology in the disc involves modulation of ADAMTS (a metalloprotease with thrombospondin-like repeats), a proteolytic enzyme and known driver of disc degeneration. An *in vivo* annular puncture model in rabbits demonstrated the efficacy of direct injection of siRNA targeting ADAMTS5 in rabbit disc cells, resulting in downregulated expression of ADAMTS5 mRNA. The injected discs also demonstrated improved MRI and histological scores.[102]

The silencing of caspase-3, an apoptosis inducing enzyme in the pathogenesis of IDD, via siRNA targeting caspase-3 was also observed to have similar efficacy. Both transfection of rabbit NP cells *in vitro* and direct injection into the intervertebral discs of rabbits *in vivo* through an annular puncture model resulted in down regulation of caspase-3 mRNA. Improved MRI and histological scores were again observed at 4 and 8 weeks after injection.[103] Similarly, transfection with caspase-3 siRNA significantly improved not only MRI and histological scoring, but also limited the number of apoptotic NP cells in response to compressive loading. This demonstrated that inhibition of caspase-3 allowed for attenuation of IDD secondary to mechanical load.[104]

Bi et al used RNAi silencing to elucidate the negative feedback loop between toll-like receptor 4 (TLR5) and antiaging protein klotho in both an *in vitro* NP cell culture and *in vivo* rat NP via needle puncture. Klotho is a key anti-inflammatory modulator in NP cells that is rapidly decreased after injury to the intervertebral disc. Upregulation of Klotho inhibits H_2O_2-mediated acute inflammation by inhibiting toll-like receptor 4 (TLR4). In both *in vitro* and *in vivo* models, RNAi-induced silencing of Klotho increased proinflammatory NF-kB signaling and cytokine upregulation,

while RNAi silencing of TLR4 results in loss of the inhibitory effect of H_2O_2 in NP cells.[105]

Mammalian target of rapamycin (mTOR), another target for IDD, is a serine/threonine kinase that modulates cell metabolism through activation of catabolism or anabolism depending on the extracellular environment. Yurube et al found mTOR expression and phosphorylation to be upregulated in intermediately degenerated discs and to be decreased with age.[106]

Activation of the Akt signaling pathway and subsequent mTOR activation has been found to downregulate the expression of catabolic proteins including matrix metalloproteinases (MMPs) and to inhibit apoptosis by downregulating caspase-3 activity. This is significant given that the loss of ECM secondary to upregulation of MMPs is the cornerstone of IDD.[107] RNAi of mTORC1 in an *in vitro* culture of human NP cells under stimulation of proinflammatory interlueukin-1 β (IL1-β) has demonstrated protection against inflammation-induced apoptosis, senescence, and matrix catabolism, representing another potential approach with gene silencing.[33]

Conclusions

IDD is a pervasive disease affecting up to a third of the population in the US and is associated with significant morbidity worldwide. The standard of care has traditionally been reactive in treating the symptoms when they present rather than to correct the underlying pathophysiology. Gene therapy seeks to restore the balance between ECM catabolism and anabolism to halt the process of degeneration with the goal to restore or maintain the native anatomy of the intervertebral disc. Recent advances in elucidating the mechanism of IDD, identification of new therapeutic transgenes, inducible and regulated transgene expression vectors, and novel viral and non-viral vectors for gene delivery have all pushed gene therapy towards a foreseeable future in clinical applications. The future direction of gene therapy research in the treatment of intervertebral disc degeneration will be to maximize safety, minimize adverse events and to prolong the duration of gene expression to restore disc cell and matrix function.

Corresponding author:

Melissa Yunting Tang

Department of Orthopedic Surgery

University of Pittsburgh

Pittsburgh, PA, USA.

Email: Tangy5@upmc.edu

Funding:

None.

Acknowledgements:

None.

Conflicts of interests:

None.

References

1. Luo X, Pietrobon R, Sun SX, Liu GG, Hey L. Estimates and patterns of direct health care expenditures among individuals with back pain in the United States. *Spine (Phila Pa 1976)*. 2004;29(1):79-86. doi: 10.1097/01.BRS.0000105527.13866.0F

2. Urban JPG, Roberts S. Degeneration of the intervertebral disc. *Arthritis Res Ther*. 2003;5(3):120-130. doi: 10.1186/ar629

3. Saal JA, Saal JS. Nonoperative treatment of herniated lumbar intervertebral disc with radiculopathy. An outcome study. *Spine (Phila Pa 1976)*. 1989;14(4):431-437. doi: 10.1097/00007632-198904000-00018

4. Katz JN. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. *J Bone Joint Surg Am*. 2006; 88 Suppl 2:21-24. doi: 10.2106/JBJS.E.01273

5. Andersson GB. Epidemiological features of chronic low-back pain. *Lancet*. 1999; 354(9178):581-585. doi: 10.1016/S0140-6736(99)01312-4

6. Bono CM, Lee CK. Critical analysis of trends in fusion for degenerative disc disease over the past 20 years: influence of technique on fusion rate and clinical outcome. *Spine (Phila Pa 1976)*. 2004;29(4):455-463; discussion Z5. doi: 10.1097/01.brs.0000090825.94611.28

7. Hwang SL, Hwang YF, Lieu AS, et al. Outcome analyses of interbody titanium cage fusion used in the anterior discectomy for cervical degenerative disc disease. *J Spinal Disord Tech*. 2005;18(4):326-331. doi: 10.1097/01.bsd.0000164198.30725.2d

8. Karasek M, Bogduk N. Twelve-month follow-up of a controlled trial of intradiscal thermal anuloplasty for back pain due to internal disc disruption. *Spine (Phila Pa 1976)*. 2000;25(20):2601-2607. doi: 10.1097/00007632-200010150-00010

9. Nishida K, Kang JD, Gilbertson LG, et al. Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenovirus-mediated transfer of the human transforming growth factor beta 1 encoding gene. *Spine (Phila Pa 1976)*. 1999; 24(23):2419-2425. doi: 10.1097/00007632-199912010-00002

10. Dowdell J, Erwin M, Choma T, Vaccaro A, Iatridis J, Cho SK. Intervertebral Disk Degeneration and Repair. *Neurosurgery*. 2017;80(3S):S46-S54. doi: 10.1093/neuros/nyw078

11. Hubert MG, Vadala G, Sowa G, Studer RK, Kang JD. Gene therapy for the treatment of degenerative disk disease. *J Am Acad Orthop Surg*. 2008;16(6):312-319. doi: 10.5435/00124635-200806000-00003

12. Sambrook PN, MacGregor AJ, Spector TD. Genetic influences on cervical and lumbar disc degeneration: a magnetic resonance imaging study in twins. *Arthritis Rheum*. 1999; 42(2):366-372. doi: 10.1002/1529-0131(199902)42:2<366::AID-ANR20>3.0.CO;2-6

13. Noponen-Hietala N, Kyllönen E, Männikkö M, et al. Sequence variations in the collagen IX and XI genes are associated with degenerative lumbar spinal stenosis. *Ann Rheum Dis*. 2003;62(12):1208-1214. doi: 10.1136/ard.2003.008334

14. Kelsey JL, Githens PB, Walter SD, et al. An epidemiological study of acute prolapsed cervical intervertebral disc. *J Bone Joint Surg Am*. 1984;66(6):907-914.
doi: 10.2106/00004623-198466060-00011

15. Pluijm SMF, van Essen HW, Bravenboer N, et al. Collagen type I alpha1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. *Ann Rheum Dis*. 2004;63(1):71-77. doi: 10.1136/ard.2002.002287

16. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine (Phila Pa 1976)*. 2002;27(23):2631-2644.
doi: 10.1097/00007632-200212010-00002

17. Roh EJ, Darai A, Kyung JW, et al. Genetic Therapy for Intervertebral Disc Degeneration. *Int J Mol Sci*. 2021;22(4).
doi: 10.3390/ijms22041579

18. Pockert AJ, Richardson SM, le Maitre CL, et al. Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. *Arthritis Rheum*. 2009;60(2):482-491.doi: 10.1002/art.24291

19. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. *Spine (Phila Pa 1976)*. 2000;25(23):3005-3013. doi: 10.1097/00007632-200012010-00007

20. Kanemoto M, Hukuda S, Komiya Y, Katsuura A, Nishioka J. Immunohistochemical study of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 human intervertebral discs. *Spine (Phila Pa 1976)*. 1996;21(1):1-8.
doi: 10.1097/00007632-199601010-00001

21. Urban JP, McMullin JF. Swelling pressure of the inervertebral disc: influence of proteoglycan and collagen contents. *Biorheology*. 1985;22(2):145-157.
doi: 10.3233/bir-1985-22205

22. Roberts S, Evans H, Trivedi J, Menage J. Histology and pathology of the human intervertebral disc. *J Bone Joint Surg Am*. 2006;88 Suppl 2:10-14.
doi: 10.2106/JBJS.F.00019

23. Butler D, Trafimow JH, Andersson GB, McNeill TW, Huckman MS. Discs degenerate before facets. *Spine (Phila Pa 1976)*. 1990; 15(2):111-113.
doi: 10.1097/00007632-199002000-00012

24. Buckwalter JA. Aging and degeneration of the human intervertebral disc. *Spine (Phila Pa 1976)*. 1995;20(11):1307-1314.
doi: 10.1097/00007632-199506000-00022

25. Colombini A, Lombardi G, Corsi MM, Banfi G. Pathophysiology of the human intervertebral disc. *Int J Biochem Cell Biol*. 2008;40(5):837-842.
doi: 10.1016/j.biocel.2007.12.011

26. Johnson ZI, Schoepflin ZR, Choi H, Shapiro IM, Risbud M v. Disc in flames: Roles of TNF-α and IL-1β in intervertebral disc degeneration. *Eur Cell Mater*. 2015;30:104-116; discussion 116-7.
doi: 10.22203/ecm.v030a08

27. Patil P, Niedernhofer LJ, Robbins PD, Lee J, Sowa G, Vo N. Cellular senescence in

intervertebral disc aging and degeneration. *Curr Mol Biol Rep*. 2018;4(4):180-190. doi: 10.1007/s40610-018-0108-8

28. le Maitre CL, Freemont AJ, Hoyland JA. Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. *Arthritis Res Ther*. 2007; 9(3):R45. doi: 10.1186/ar2198

29. Gruber HE, Ingram JA, Norton HJ, Hanley EN. Senescence in cells of the aging and degenerating intervertebral disc: immunolocalization of senescence-associated beta-galactosidase in human and sand rat discs. *Spine (Phila Pa 1976)*. 2007;32(3):321-327. doi: 10.1097/01.brs.0000253960.57051.de

30. Gruber HE, Hanley EN. Analysis of aging and degeneration of the human intervertebral disc. Comparison of surgical specimens with normal controls. *Spine (Phila Pa 1976)*. 1998; 23(7):751-757. doi: 10.1097/00007632-199804010-00001

31. Fuchs Y, Steller H. Programmed cell death in animal development and disease. *Cell*. 2011;147(4):742-758. doi: 10.1016/j.cell.2011.10.033

32. Kakiuchi Y, Yurube T, Kakutani K, et al. Pharmacological inhibition of mTORC1 but not mTORC2 protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction. *Osteoarthritis Cartilage*. 2019;27(6):965-976. doi: 10.1016/j.joca.2019.01.009

33. Ito M, Yurube T, Kakutani K, et al. Selective interference of mTORC1/RAPTOR protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism with Akt and autophagy induction. *Osteoarthritis Cartilage*. 2017; 25(12):2134-2146. doi: 10.1016/j.joca.2017.08.019

34. Fujii K, Yamazaki M, Kang JD, et al. Discogenic Back Pain: Literature Review of Definition, Diagnosis, and Treatment. *JBMR Plus*. 2019;3(5):e10180. doi: 10.1002/jbm4.10180

35. Mosley GE, Evashwick-Rogler TW, Lai A, Iatridis JC. Looking beyond the intervertebral disc: the need for behavioral assays in models of discogenic pain. *Ann N Y Acad Sci*. 2017;1409(1):51-66. doi: 10.1111/nyas.13429

36. Kang JD, Stefanovic-Racic M, McIntyre LA, Georgescu HI, Evans CH. Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. *Spine (Phila Pa 1976)*. 1997;22(10):1065-1073. doi: 10.1097/00007632-199705150-00003

37. Kang JD, Georgescu HI, McIntyre-Larkin L, Stefanovic-Racic M, Donaldson WF, Evans CH. Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. *Spine (Phila Pa 1976)*. 1996;21(3):271-277. doi: 10.1097/00007632-199602010-00003

38. Nishida K, Suzuki T, Kakutani K, et al. Gene therapy approach for disc degeneration and associated spinal disorders. *Eur Spine J*. 2008;17 Suppl 4:459-466. doi: 10.1007/s00586-008-0751-5

39. Ghiselli G, Wang JC, Bhatia NN, Hsu WK, Dawson EG. Adjacent segment degeneration in the lumbar spine. *J Bone Joint Surg Am*. 2004;86(7):1497-1503.
doi: 10.2106/00004623-200407000-00020

40. Palmer N, Guan Z, Chai NC. Spinal Cord Stimulation for Failed Back Surgery Syndrome -- Patient Selection Considerations. *Transl Perioper Pain Med*. 2019;6(3):81-90.

41. Knezevic NN, Mandalia S, Raasch J, Knezevic I, Candido KD. Treatment of chronic low back pain - new approaches on the horizon. *J Pain Res*. 2017;10:1111-1123.
doi: 10.2147/JPR.S132769

42. Sobajima S, Kim JS, Gilbertson LG, Kang JD. Gene therapy for degenerative disc disease. *Gene Ther*. 2004;11(4):390-401.
doi: 10.1038/sj.gt.3302200

43. Takada T, Nishida K, Doita M, Kurosaka M. Fas ligand exists on intervertebral disc cells: a potential molecular mechanism for immune privilege of the disc. *Spine (Phila Pa 1976)*. 2002;27(14):1526-1530.
doi: 10.1097/00007632-200207150-00009

44. Thompson JP, Oegema TR, Bradford DS. Stimulation of mature canine intervertebral disc by growth factors. *Spine (Phila Pa 1976)*. 1991;16(3):253-260.
doi: 10.1097/00007632-199103000-00001

45. Osada R, Ohshima H, Ishihara H, et al. Autocrine/paracrine mechanism of insulin-like growth factor-1 secretion, and the effect of insulin-like growth factor-1 on proteoglycan synthesis in bovine intervertebral discs. *J Orthop Res*. 1996;14(5):690-699.
doi: 10.1002/jor.1100140503

46. Takegami K, Thonar EJMA, An HS, Kamada H, Masuda K. Osteogenic protein-1 enhances matrix replenishment by intervertebral disc cells previously exposed to interleukin-1. *Spine (Phila Pa 1976)*. 2002; 27(12):1318-1325.
doi: 10.1097/00007632-200206150-00014

47. Li J, Yoon ST, Hutton WC. Effect of bone morphogenetic protein-2 (BMP-2) on matrix production, other BMPs, and BMP receptors in rat intervertebral disc cells. *J Spinal Disord Tech*. 2004;17(5):423-428.
doi: 10.1097/01.bsd.0000112084.85112.5d

48. Leckie SK, Bechara BP, Hartman RA, et al. Injection of AAV2-BMP2 and AAV2-TIMP1 into the nucleus pulposus slows the course of intervertebral disc degeneration in an in vivo rabbit model. *Spine J*. 2012;12(1):7-20.
doi: 10.1016/j.spinee.2011.09.011

49. Tim Yoon S, Su Kim K, Li J, et al. The effect of bone morphogenetic protein-2 on rat intervertebral disc cells in vitro. *Spine (Phila Pa 1976)*. 2003;28(16):1773-1780.
doi: 10.1097/01.BRS.0000083204.44190.34

50. Gruber HE, Norton HJ, Hanley EN. Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro. *Spine (Phila Pa 1976)*. 2000;25(17):2153-2157.
doi: 10.1097/00007632-200009010-00002

51. Newman CM, Lawrie A, Brisken AF, Cumberland DC. Ultrasound gene therapy: on the road from concept to reality. *Echocardiography*. 2001;18(4):339-347.
doi: 10.1046/j.1540-8175.2001.00339.x

52. Lawrie A, Brisken AF, Francis SE, et al. Ultrasound enhances reporter gene expression after transfection of vascular cells

in vitro. *Circulation*. 1999;99(20):2617-2620. doi: 10.1161/01.cir.99.20.2617

53. Lawrie A, Brisken AF, Francis SE, Cumberland DC, Crossman DC, Newman CM. Microbubble-enhanced ultrasound for vascular gene delivery. *Gene Ther*. 2000; 7(23):2023-2027. doi: 10.1038/sj.gt.3301339

54. Nishida K, Doita M, Takada T, et al. Sustained transgene expression in intervertebral disc cells in vivo mediated by microbubble-enhanced ultrasound gene therapy. *Spine (Phila Pa 1976)*. 2006; 31(13):1415-1419. doi: 10.1097/01.brs.0000219945.70675.dd

55. Harada A, Kataoka K. Polyion complex micelle formation from double-hydrophilic block copolymers composed of charged and non-charged segments in aqueous media. *Polym J*. 2018;50(1):95-100. doi: 10.1038/pj.2017.67

56. Itaka K, Yamauchi K, Harada A, Nakamura K, Kawaguchi H, Kataoka K. Polyion complex micelles from plasmid DNA and poly(ethylene glycol)–poly(l-lysine) block copolymer as serum-tolerable polyplex system: physicochemical properties of micelles relevant to gene transfection efficiency. *Biomaterials*. 2003;24(24):4495-4506. doi: 10.1016/S0142-9612(03)00347-8

57. Li Z, Yu X, Shen J, Chan MT v, Wu WKK. MicroRNA in intervertebral disc degeneration. *Cell Prolif*. 2015;48(3):278-283. doi: 10.1111/cpr.12180

58. Feng G, Zha Z, Huang Y, et al. Sustained and Bioresponsive Two-Stage Delivery of Therapeutic miRNA via Polyplex Micelle-Loaded Injectable Hydrogels for Inhibition of Intervertebral Disc Fibrosis. *Adv Healthc Mater*. 2018;7(21):e1800623. doi: 10.1002/adhm.201800623

59. Huang Y, Huang L, Li L, et al. MicroRNA-25-3p therapy for intervertebral disc degeneration by targeting the IL-1β/ZIP8/MTF1 signaling pathway with a novel thermo-responsive vector. *Ann Transl Med*. 2020;8(22):1500-1500. doi: 10.21037/atm-20-6595

60. Takeoka Y, Yurube T, Nishida K. Gene Therapy Approach for Intervertebral Disc Degeneration: An Update. *Neurospine*. 2020; 17(1):3-14. doi: 10.14245/ns.2040042.021

61. Robbins PD, Ghivizzani SC. Viral vectors for gene therapy. *Pharmacol Ther*. 1998; 80(1):35-47.

62. Yang Y, Nunes FA, Berencsi K, Furth EE, Gönczöl E, Wilson JM. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci U S A*. 1994;91(10):4407-4411. doi: 10.1073/pnas.91.10.4407

63. Tripathy SK, Black HB, Goldwasser E, Leiden JM. Immune responses to transgene-encoded proteins limit the stability of gene expression after injection of replication-defective adenovirus vectors. *Nat Med*. 1996; 2(5):545-550. doi: 10.1038/nm0596-545

64. Somia N, Verma IM. Gene therapy: trials and tribulations. *Nat Rev Genet*. 2000;1(2):91-99. doi: 10.1038/35038533

65. Nishida K, Kang JD, Suh JK, Robbins PD, Evans CH, Gilbertson LG. Adenovirus-mediated gene transfer to nucleus pulposus

cells. Implications for the treatment of intervertebral disc degeneration. *Spine (Phila Pa 1976)*. 1998;23(22):2437-2442; discussion 2443. doi: 10.1097/00007632-199811150-00016

66. Yoon ST, Park JS, Kim KS, et al. ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs in vitro and in vivo. *Spine (Phila Pa 1976)*. 2004;29(23):2603-2611. doi: 10.1097/01.brs.0000146103.94600.85

67. Paul R, Haydon RC, Cheng H, et al. Potential use of Sox9 gene therapy for intervertebral degenerative disc disease. *Spine (Phila Pa 1976)*. 2003;28(8):755-763.

68. Wallach CJ, Sobajima S, Watanabe Y, et al. Gene transfer of the catabolic inhibitor TIMP-1 increases measured proteoglycans in cells from degenerated human intervertebral discs. *Spine (Phila Pa 1976)*. 2003; 28(20):2331-2337. doi: 10.1097/01.BRS.0000085303.67942.94

69. Moon SH, Nishida K, Gilbertson LG, et al. Biologic response of human intervertebral disc cells to gene therapy cocktail. *Spine (Phila Pa 1976)*. 2008;33(17):1850-1855. doi: 10.1097/BRS.0b013e31817e1cd7

70. Lai CM, Lai YKY, Rakoczy PE. Adenovirus and adeno-associated virus vectors. *DNA Cell Biol*. 2002;21(12):895-913. doi: 10.1089/104454902762053855

71. Nakai H, Storm TA, Kay MA. Increasing the size of rAAV-mediated expression cassettes in vivo by intermolecular joining of two complementary vectors. *Nat Biotechnol*. 2000;18(5):527-532. doi: 10.1038/75390

72. Yan Z, Zhang Y, Duan D, Engelhardt JF. Trans-splicing vectors expand the utility of adeno-associated virus for gene therapy. *Proc Natl Acad Sci U S A*. 2000;97(12):6716-6721. doi: 10.1073/pnas.97.12.6716

73. Halbert CL, Rutledge EA, Allen JM, Russell DW, Miller AD. Repeat transduction in the mouse lung by using adeno-associated virus vectors with different serotypes. *J Virol*. 2000;74(3):1524-1532. doi: 10.1128/jvi.74.3.1524-1532.2000

74. Chirmule N, Xiao W, Truneh A, et al. Humoral immunity to adeno-associated virus type 2 vectors following administration to murine and nonhuman primate muscle. *J Virol*. 2000;74(5):2420-2425. doi: 10.1128/jvi.74.5.2420-2425.2000

75. Lattermann C, Oxner WM, Xiao X, et al. The adeno associated viral vector as a strategy for intradiscal gene transfer in immune competent and pre-exposed rabbits. *Spine (Phila Pa 1976)*. 2005;30(5):497-504. doi: 10.1097/01.brs.0000154764.62072.44

76. Li H, Li W, Liang B, Wei J, Yin D, Fan Q. Role of AP-2α/TGF-β1/Smad3 axis in rats with intervertebral disc degeneration. *Life Sci*. 2020;263:118567. doi: 10.1016/j.lfs.2020.118567

77. Wehling P, Schulitz KP, Robbins PD, Evans CH, Reinecke JA. Transfer of genes to chondrocytic cells of the lumbar spine. Proposal for a treatment strategy of spinal disorders by local gene therapy. *Spine (Phila Pa 1976)*. 1997;22(10):1092-1097. doi: 10.1097/00007632-199705150-00008

78. Ono C, Okamoto T, Abe T, Matsuura Y. Baculovirus as a Tool for Gene Delivery and Gene Therapy. *Viruses*. 2018;10(9):510. doi: 10.3390/v10090510

79. Liu X, Li K, Song J, Liang C, Wang X, Chen X. Efficient and Stable Gene Expression in Rabbit Intervertebral Disc Cells Transduced With a Recombinant Baculovirus Vector. *Spine (Phila Pa 1976)*. 2006;31(7):732-735. doi: 10.1097/01.brs.0000206977.61305.43

80. Liu Y, Yu T, Ma XX, Xiang HF, Hu YG, Chen BH. Lentivirus-mediated TGF-β3, CTGF and TIMP1 gene transduction as a gene therapy for intervertebral disc degeneration in an in vivo rabbit model. *Exp Ther Med*. 2016; 11(4):1399-1404. doi: 10.3892/etm.2016.3063

81. Zhao Z, Li S, Huang H, Fang J, Wei H, Xi Y. In vivo delivery of MMP3-shRNA and Sox9 lentivirus cocktail enhances matrix synthesis to prevent lumbar disc degeneration. *Advances in Clinical and Experimental Medicine*. 2020; 29(6):639-647. doi: 10.17219/acem/121509

82. Moon SH, Nishida K, Gilbertson LG, et al. Biologic response of human intervertebral disc cells to gene therapy cocktail. *Spine (Phila Pa 1976)*. 2008;33(17):1850-1855. doi: 10.1097/BRS.0b013e31817e1cd7

83. Wallach CJ, Kim JS, Sobajima S, et al. Safety assessment of intradiscal gene transfer: a pilot study. *Spine J*. 6(2):107-112. doi: 10.1016/j.spinee.2005.05.002

84. Chtarto A, Bender HU, Hanemann CO, et al. Tetracycline-inducible transgene expression mediated by a single AAV vector. *Gene Ther*. 2003;10(1):84-94. doi: 10.1038/sj.gt.3301838

85. Ueblacker P, Wagner B, Krüger A, et al. Inducible nonviral gene expression in the treatment of osteochondral defects. *Osteoarthritis Cartilage*. 2004;12(9):711-719. doi: 10.1016/j.joca.2004.05.011

86. Sowa G, Westrick E, Pacek C, et al. In vitro and in vivo testing of a novel regulatory system for gene therapy for intervertebral disc degeneration. *Spine (Phila Pa 1976)*. 2011; 36(10):E623-8. doi: 10.1097/BRS.0b013e3181ed11c1

87. Han Y, Ouyang Z, Wawrose RA, et al. ISSLS prize in basic science 2021: a novel inducible system to regulate transgene expression of TIMP1. *Eur Spine J*. 2021;30(5):1098-1107. doi: 10.1007/s00586-021-06728-0

88. Zhang F, Wen Y, Guo X. CRISPR/Cas9 for genome editing: progress, implications and challenges. *Hum Mol Genet*. 2014; 23(R1):R40-6. doi: 10.1093/hmg/ddu125

89. Kleinstiver BP, Pattanayak V, Prew MS, et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*. 2016;529(7587):490-495. doi: 10.1038/nature16526

90. Fan Y, Zhao L, Lai Y, Lu K, Huang J. CRISPR-Cas9-mediated loss of function of β-catenin attenuates intervertebral disc degeneration. *Mol Ther Nucleic Acids*. 2022; 28:387-396. doi: 10.1016/j.omtn.2022.03.024

91. Farhang N, Ginley-Hidinger M, Berrett KC, Gertz J, Lawrence B, Bowles RD. Lentiviral CRISPR Epigenome Editing of Inflammatory Receptors as a Gene Therapy Strategy for Disc Degeneration. *Hum Gene Ther*. 2019; 30(9):1161-1175. doi: 10.1089/hum.2019.005

92. Cambria E, Arlt MJE, Wandel S, et al. TRPV4 Inhibition and CRISPR-Cas9 Knockout Reduce Inflammation Induced by Hyperphysiological Stretching in Human Annulus Fibrosus Cells. *Cells*. 2020;9(7). doi: 10.3390/cells9071736

93. Stover JD, Farhang N, Berrett KC, Gertz J, Lawrence B, Bowles RD. CRISPR Epigenome Editing of AKAP150 in DRG Neurons Abolishes Degenerative IVD-Induced Neuronal Activation. *Mol Ther*. 2017; 25(9):2014-2027. doi: 10.1016/j.ymthe.2017.06.010

94. Krupkova O, Cambria E, Besse L, Besse A, Bowles R, Wuertz-Kozak K. The potential of CRISPR/Cas9 genome editing for the study and treatment of intervertebral disc pathologies. *JOR Spine*. 2018;1(1):e1003. doi: 10.1002/jsp2.1003

95. Han H. RNA Interference to Knock Down Gene Expression. *Methods Mol Biol*. 2018; 1706:293-302. doi: 10.1007/978-1-4939-7471-9_16

96. Sontheimer EJ. Assembly and function of RNA silencing complexes. *Nat Rev Mol Cell Biol*. 2005;6(2):127-138. doi: 10.1038/nrm1568

97. Brummelkamp TR, Bernards R, Agami R. A system for stable expression of short interfering RNAs in mammalian cells. *Science*. 2002;296(5567):550-553. doi: 10.1126/science.1068999

98. Yu JY, DeRuiter SL, Turner DL. RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. *Proc Natl Acad Sci U S A*. 2002;99(9):6047-6052. doi: 10.1073/pnas.092143499

99. Micura R. Small interfering RNAs and their chemical synthesis. *Angew Chem Int Ed Engl*. 2002;41(13):2265-2269. doi: 10.1002/1521-3773(20020703)41:13<2265::AID-ANIE2265>3.0.CO;2-3

100. Kakutani K, Nishida K, Uno K, et al. Prolonged down regulation of specific gene expression in nucleus pulposus cell mediated by RNA interference in vitro. *J Orthop Res*. 2006;24(6):1271-1278. doi: 10.1002/jor.20171

101. Suzuki T, Nishida K, Kakutani K, et al. Sustained long-term RNA interference in nucleus pulposus cells in vivo mediated by unmodified small interfering RNA. *Eur Spine J*. 2009;18(2):263-270. doi: 10.1007/s00586-008-0873-9

102. Seki S, Asanuma-Abe Y, Masuda K, et al. Effect of small interference RNA (siRNA) for ADAMTS5 on intervertebral disc degeneration in the rabbit anular needle-puncture model. *Arthritis Res Ther*. 2009; 11(6):R166. doi: 10.1186/ar2851

103. Sudo H, Minami A. Caspase 3 as a therapeutic target for regulation of intervertebral disc degeneration in rabbits. *Arthritis Rheum*. 2011;63(6):1648-1657. doi: 10.1002/art.30251

104. Yamada K, Sudo H, Iwasaki K, et al. Caspase 3 silencing inhibits biomechanical overload-induced intervertebral disk degeneration. *Am J Pathol*. 2014;184(3):753-764. doi: 10.1016/j.ajpath.2013.11.010

105. Bi F, Liu W, Wu Z, Ji C, Chang C. Antiaging Factor Klotho Retards the Progress of Intervertebral Disc Degeneration through

the Toll-Like Receptor 4-NF-κB Pathway. *Int J Cell Biol*. 2020;2020:8319516. doi: 10.1155/2020/8319516

106. Yurube T, Ito M, Kakiuchi Y, Kuroda R, Kakutani K. Autophagy and mTOR signaling during intervertebral disc aging and degeneration. *JOR Spine*. 2020;3(1):e1082. doi: 10.1002/jsp2.1082

107. Ouyang ZH, Wang WJ, Yan YG, Wang B, Lv GH. The PI3K/Akt pathway: a critical player in intervertebral disc degeneration. *Oncotarget*. 2017;8(34):57870-57881. doi: 10.18632/oncotarget.18628