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Narrative Review

Gene Therapy for Lumbar Disc Disease; An Overview of Animal and Human Studies

Majid Rezvani[1], Laleh Beigi[2], Masih Saboori[1], Sheida Ebrahimi[2], Mohammad Reza Akrami[1], Saeed Oraee-Yazdani[3], Soheil Tahani[2], Shima Shadabi[2], Leila Dehghani[4*]

[1]Department of Neurosurgery, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Isfahan Neurosciences Research Center, Alzahra Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Functional Neurosurgery Research Center, Shohada Tajrish Neurosurgical Comprehensive Center of Excellence, Shahid Beheshti University of Medical Sciences,Tehran,Iran
[4]Department of Tissue Engineering and Regenerative Medicine, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Leila Dehghani, PhD, Department of Tissue Engineering and Regenerative Medicine, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: l_dehghani2002@yahoo.com

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Abstract

Low back pain (LBP), a common disorder involving the muscles, nerves, and bones of the back, is associated with lumbar disc degeneration. It is a complex phenomenon, likely the result of a combination of biochemical and biomechanical factors that are known to occur in discs. However, the findings of previous studies have suggested that disc degeneration may be explained primarily by genetic influences. For this reason, scientists are interested in the use of genes/proteins for the treatment of disc degeneration. Protein-based therapies involve the administration of biologic factors into the intervertebral disc to enhance matrix synthesis, postpone degeneration, and prevent inflammation. These factors can be delivered by an intradiscal injection, alone or in combination with cells or tissue scaffolds, and by gene therapy. A systematic search for articles dated from 1990 to the present was performed to identify pertinent articles related to the topic of the role of gene therapy in treating intervertebral disc degeneration. Twenty-seven studies reported the use of at least one gene in treating this disease through gene therapy. Researchers have been performing phases I/II of a clinical trial on the treatment of disc degeneration with gene therapy since 2008. Recent studies have shown that gene therapy may have promise as a method of slowing down or preventing some of the changes seen in intervertebral discs. However, because the clinical trial is not complete and therefore the results are indeterminate, this method cannot be proffered as a replacement for surgery. It is hoped that definitive results of the possible effects of gene therapy on the human body will be acquired soon.

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Introduction

Low back pain (LBP) is one of the most disabling conditions in the world and is associated with social, economic, and health problems.[1,2] This disorder leads to a decrease in physical activity, loss of quality of life, and the development of mental disorders. Although LBP has many definitions, lumbar spine degeneration is clearly an effective factor.[3]

Disc degeneration is characterized by biochemical and morphological changes in a disc. MRI is the most common standard method for evaluating the integrity of intervertebral discs.[4] Degenerating changes to imaging are generally based on reduced signal integrity, dropped disc height, the presence of a gap in other layers of the disc, or the displacement of the disc material outside its original location.

Disc degeneration is a multifactorial disorder, and factors such as age, mechanical load, gender, trauma, obesity, and other factors that have a detrimental effect on disc material are effective in creating it.[5,6] Based on the results of studies on the disorder conducted at the end of the 20th century, the heredity factor has been proposed as the largest cause of the spread of this disorder, it has been shown that non genetic factors may have less effect.[5,7]

As soon as the process of degeneration begins, preventing or reversing its process is difficult. Methods commonly used to treat diseases related to disc degeneration often include discectomy with or without fusion. Another technique involves the stabilization of semi-rigid functional spinal units[8] or the insertion of an artificial intervertebral disc[9,10] in the

desired location. Each of these methods, however, has its own specific problems. Therefore, biological methods today focus on the reuse of degenerated discs and are divided into three large groups: (1) Injection of growth factors with or without the use of vectors; (2) Use of cells, cell therapy, with or without scaffold; and (3) Genetic modification of gene expression in the disc through gene therapy.[11]

Gene therapy involves the transfer of genetic material through vectors. This method is a direct injection of genetic material into a living organism (in vivo) or, after removing the disc cells, inserting the vectors into the laboratory environment and eventually returning the modified cells to the disc (ex vivo).

The vectors used in this method are divided into viral and non-viral groups. Viral vectors are genetically modified viruses that lack genetic pathogens but retain their genetic information to enter the disc cells. The viral vectors used in this case include retroviruses, adenoviruses, adeno-associated viruses, and baculoviruses.[11]

This review article focuses on gene therapies for the treatment of disease caused by disc degeneration and genetic studies and animal samples for lumbar disc disease from 1999 to 2016.

Genes Involved in the Disease

In studies conducted to date, various kinds of genes have been discovered that can be helpful in treating the disease. These genes are divided into three categories as described below.

Extracellular Matrix Synthesis Enhancer Factors

Certain biomolecules can stimulate matrix biosynthesis at the protein or gene level. These biomolecules include growth and anabolic factors. Therefore, disc treatment with these factors is appropriate for patients who are in the initial stage of the disease. Researchers have discovered that BMP family proteins or bone morphogenic proteins increase proteoglycan synthesis in animal models.[12,13] BMP2 and BMP7 or OP-1 (osteogenic protein) increase proteoglycan production in disc cells. The use of recombinant human BMP2 and BMP12 on human disc cells increases collagen and proteoglycan production in nucleus pulposus cell types.[14] RhBMP7 injection has been shown to strengthen disc height in rabbit discs,[15] and when it is injected with rhBMP7-expressing nucleus pulposus cells and then bound to the spinal cord of dogs, it is able to prevent the development of allogeneic discs in the twenty-fourth week.[16] The administration of growth differentiation factor 5 (GDF5) into rat cells resulted in increased synthesis of proteoglycan and collagen type 2.[17] This increase in synthesis was observed in vitro in intervertebral discs of cows. MRI findings and histological characteristics of intervertebral discs were restored.[18] RhGDF5 applied in intervertebral discs treated with glycolic acid polylactic microsphere led to the restoration of disc height, returned GAG levels to normal, and increased the number of collagen type 2 mRNAs.[18] BMP2 in NP and AF cells is anti-catabolic, and its effects are partly due to stimulation of the catabolic effect of IL-18. However, BMP2 competes with IL-18, resulting in the setting of aggrecan, collagen type 2, and SOX9 levels, and leads to a reversal of disc degeneration by mediating IL-18.[20]

In 10 studies, the effects of OP-1 on disc reconstruction with direct injections of OP-1 were discovered.[21-30]

In 4 studies on allogeneic methods of cell-based gene transfer[30-33] and a study using a vector-based gene transfer method[34] the effect of OP-1 on disc reconstruction was evaluated. OP-1 and GDF5 were used in the first phase of a clinical trial by the US Food and Drug Administration on human degeneration.[35]

In addition, the use of beta transforming growth factor or TGF-β[36] and epidermal growth factor[37] (EGF) in rat cells increased aggrecan expression and type 1 and type 2 collagen. An increase in the expression of mRNA-encoded matrix-related proteins in the cervical nucleus pulposus and lumbar cells in degenerated discs were reported in vitro with TGF-β and rhBMP2 treatment.[38]

The use of TGF and insulin–like growth factor (IGF) in the treatment of degenerating disc disorder is restricted by the presence of their receptors in the blood vessels of the intervertebral disc.[39] Therefore, injecting them may stimulate angiogenesis and, as a result, stimulate nerve growth that could indicate an exacerbation of symptoms in the dislocation of intervertebral discs.[40] In other words, BMP receptors are not detected by the blood vessels of the intervertebral discs; therefore, BMP family growth factors are more effective in treating an intervertebral degenerating disc disorder.[39]

The effects of growth factor injections in a degenerated intervertebral disc singly suggested the administration of platelet-rich plasma (PRP), which includes most growth factors.[41] PRP increases the expression of the matrix gene, stimulates cell proliferation of the nucleus pulposus and swollen annulus fibrosis, and activates the key regulator of the disc cells' chondrogenic phenotype in vitro.[42,43] In addition, PRP stimulated the return of the disc to its initial position in a rabbit which was operated on using a surgical knife.[44]

Apart from the direct injection of growth factors, gene therapy has been used to strengthen matrix synthesis in intervertebral discs. The cDNA encoding an incomplete collagen type 2 gene was cloned into the intervertebral discs of a rabbit. Also, restorative activity has been shown to be effective against the degenerative process in damaged fibrocartilage.[45]

Transfection of the TGFβ gene using an adenoviral vector significantly increased the proteoglycan synthesis in nucleus pulposus cells under in vivo conditions.[46] In one study, GDF5 was successfully transmitted to rabbit discs as an ex vivo gene therapy, resulting in the proteins forming an extracellular matrix.[47]

In 2016, Luo et al introduced the GDF 5 gene with an adenoviral vector into the nucleus pulposus of the human degenerated disc, suggesting that Ad-GDF 5 gene therapy is a potential cure for degenerating disc disorders, since the activity of the intervertebral disc is reversed by enhancing the production of extracellular matrix in these cells.[43] The reversal in activity of the injured disc was shown by the transfer of the GDF 5 gene by the adenovirus in rat lumbar disc cells in 2010.[48] The transfer of hBMP7 gene by the viral vector rAAV to a dog's nucleus pulposus cells increased the levels

of mRNA, aggrecan protein, and type 2 collagen, but it did not affect the expression and protein measure of collagen type 1.[35] Moreover, the transfer of a combination of IGF 1, BMP 2, TGFβ, and BMP-12 genes produced a significant anabolic effect in nucleus pulposus cells.[49] Gene therapy in the future may include a combination of different growth factors that increase matrix synthesis. Recent studies have shown that the intradiscal injection of the pharmacologic agents simvastatin and lovastatin in rabbits strengthens cartilage and increases the expression of the encoding gene of aggrecan and collagen type 2.[50,51] Another group of biomolecules that are potentially capable of enhancing matrix synthesis are transcription factors. The transfer of membrane by an adenoviral vector leads to increased levels of BMP 2 and BMP 7, thereby increasing aggrecan production by nucleus pulposus cells in vitro.[51] The adenovirus vector is used to transfer the LMP 1 gene into the nucleus pulposus and chondrocytes as reported above, which increases the production of proteoglycan and collagen.[52] Transformation of human degenerated intervertebral disc cells with an SOX9-expressing adenovirus vector led to an increase in the synthesis of collagen type 2.[53] Furthermore, when OP1 was administered as a single gene therapy, the height of a rabbit intervertebral disc was restored to normal, and expression of proteoglycan and collagen type 2 was increased.[34] Another group of scientists introduced SOX9 and BMP7 by AAV virus into nucleus pulposus cells located on a human intervertebral disc and reported that the expression level of the mRNA related to the type 2 collagen gene in these specimens was similar to that of the sample.[54]

One of the characteristics of SOX9 is its effect on bone marrow mesenchymal stem cells (BMSCs) and the differentiation into nucleus pulposus-like cells in vitro. The transfer of this gene to the BMSCs by adenovirus and the expression of SOX9 in these cells resulted in their differentiation, and then these cells were injected into the intervertebral disc of rabbit. Transformed BMSCs with SOX9 resulted in the repair of the degenerated disc, due to which the extracellular matrix production was enhanced.[55]

In one recent study, human telomerase enzymes reverse transcriptase, or hTERT, were transmitted to the human nucleus pulposus by the lentivirus. The study reported increased collagen type 2 and aggrecan expression.[56]

The vitamin D receptor (VDR) gene is the first reported gene to probably be related to the risk for degenerated disc. This gene is located on chromosome 12 [12qvq14], the length of which is about 100 kb, and it has more than 100 cut sites. VDR is one of the nuclear steroid receptor superfamily members which mainly manages 1, 25 dihydroxy vitamin D3 transcription activity. VDR FOK I polymorphism is an independent polymorphic site in exon 2 and provides an alternate translation initiator site which develops a long VDR isoform that is expected to be less active.

Previous studies have investigated the relationship between polymorphism in the VDR FOK I polymorphism and disc degeneration. However, a significant increase in the VDR FOK I genotype and the frequency of f allele in degenerated disc patients was observed in comparison with the control group. VDR mRNA and vitamin D were significantly reduced in patients with degenerating disc compared with the control group. There was a positive correlation between vitamin D level and expression of VDR mRNA in patients with degenerating disc.[57,58]

2) Factors That Delay Degeneration

Considering intervertebral disc degeneration as an imbalance between the synthesis and degrading of the components, discontinuing/halting the catabolic cascade can be a reasonable therapeutic goal. Although most researchers focus on growth factors, a few studies have tracked the potential role of catabolic factors in the treatment of disc degeneration. In the case of disc degeneration, increased expression of proteases such as matrix metalloproteases (MMPs) and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) will result to degrade varied components of extracellular matrix. These degrading activities are mediated by cytokines, such as interlukin1 (IL1) and tumor necrosis factor α (TNFα). ADAMTS 5 siRNA, after being injected into a rabbit degenerated disc, inhibited the matrix degeneration and corrected the histological grade of the nucleus pulposus tissue.[59]

OP-1 has an additional antic-catalytic effect. This finding was discovered when this protein blocked ADAMTS4 and ADAMTS5 induced by TNFα. This effect leads to reversed degradation activity of aggrecan and collagen type 2 through the TNFα in human intervertebral discs in vitro.[60] Etanercept, an antagonist of TNFα, corrects the symptoms of patients with persistent discogenic pain,[61] and TNFα receptors neutralize the effects of this protein on human intervertebral disc cells in vitro.[62] When IGF1 and platelet-derived growth factor (PDGF) were used in serum-free annulus fibrosis cells, a significant reduction in the percentage of apoptotic cells was observed.[63] Transformation of nucleus pulposus cells with adenoviruses expressing rhIGF1 reduced the percentage of apoptotic disc cells under in vitro conditions.[64]

In a recent study, treatment with rhPDGF significantly inhibited apoptotic cells, proliferated cells, produced matrix, and enhanced the expression of the mRNA for the genes encoding the extracellular matrix components in human disc cells under in vitro conditions.[65] Furthermore, it was discovered that PDGF, IGF1, and basic fibroblast growth factor (bFGF) stimulate the proliferation of bovine nucleus pulposus cells.[66,67] Human annulus fibrosis cells stimulated with TGFβ 3 and FGF 2 in vitro enhanced the expression of matrix and MMP13 molecules in an enriched cartilage matrix.[68] Transformation of these cells that are located in the intervertebral degenerated disc through an AAV viral vector carrying the human vascular endothelial growth factor 165 (hVEGF 165) and TGFβ 1 gene during therapy enhances the expression of collagen type 1 protein.

Originally, the hVEGF165 in collaboration with TGFβ1 enhanced the expression of this type of collagen and reversed the process of disc degeneration.[69] The introduction of connective tissue growth factor (CTGF) and tissue inhibitor metalloprotease1 (TIMP 1) with the help of the AAV viral vector in the process of ex vivo gene therapy to nucleus pulposus cells in rabbit intervertebral discs helped maintain

disc height, increased the biosynthesis of collagen type 2 and proteoglycan, and reversed the process of intervertebral disc degeneration.[70] The effect of these two genes on the expression of collagen type 2 and proteoglycan was initially tested by the same group of researchers who transfected cells belonging to rhesus monkey intervertebral discs.[71] The treatment of rabbit intervertebral discs with AAV 2 BMP2 or AAV2 TIMP 1 vector during the vivo gene therapy process caused a delay in disc degeneration.[72]

Injection of synthetic peptide N-Link (which has a growth factor specific) in rabbit degenerated disc, after the formation of a circular hole, decreased the expression of MMP3 and ADAMTS4 genes in both annulus fibrosis and nucleus pulposus cells, and decreased ADAMTS5 gene expression in annulus fibrosis tissues.[68] N-Link increased the expression of the SOX9, aggrecan, and collagen type 2 transcription factor, whereas this protein enhanced the expression of BMP4 and BMP7 in rabbit disc cells ex vivo.[73] In adult human discs, N-Link can enhance aggrecan synthesis and reduce the expression of MMP3 and MMP13, ADAMTS4, and ADAMTS genes in a dose-dependent manner.[74] The administration of vectors with adenovirus serotype 2 (AAV2) which has tissue inhibitor metalloproteinase1 (TIMP1) in perforated rabbit intervertebral discs delayed degenerative changes.[72]

Antiapoptotic gene therapy can slow down or reverse the process of degeneration. Survivin is an apoptotic inhibitor. The introduction of this gene into nucleus pulposus-derived human degenerated disc cells with the help of the lentivirus vector has led to a clear change in cell morphology, but the amount of apoptosis has not changed. Further studies are needed to determine whether survivin is a good candidate for gene therapy.[75]

Inflammatory Inhibitory Factors

TNFα and IL1 are two pre-inflammatory cytokines; their expression is increased in the process of intervertebral disc degeneration. Increased TNFα and IL1β will increase the expression of nerve growth factor (NGF), which will result in the proliferation and penetration of nerve fibers in the degenerated disc.[76] Blocking IL1 can be a possible target to prevent the inhibition of matrix synthesis. Wehling et al were the first to introduce ex vivo gene therapy to the interlukin-1 receptor antagonist gene by retrovirus into the bovine chondrocytic end plates of vertebrae cells. They suggested that gene therapy can reverse the disc degeneration process.[77] Stimulation of the human intervertebral disc in vitro with TNFα and IL1 increases the expression of MMP3 and MMP9 genes; this finding reinforces the hypothesis that TNFα is involved in the onset of matrix degeneration.[78] Fibrotic cells lead to a significant increase in MMP1 levels.[79] IL1 inhibition revealed excellent results that were comparable with the control of TNFα and degenerated discs.[80] There is sufficient knowledge about the administration of IL1 receptor antagonists in the case of rheumatoid arthritis where it is injected subcutaneous[81]; however, in the case of intervertebral degenerated disc disease, IL1Ra should be injected directly into the disc, because IL1Ra cannot be transmitted through the systemic blood stream due to the nature of avascular intervertebral discs.[82] Because of the short life span of IL1Ra, repeated injections are required to achieve clinical outcomes. Therefore, gene therapy inhibits IL1Ra gene transfer to the degenerated disc cells to stimulate degenerating enzymes which may be a solution to achieving long-term clinical outcomes. In the case of ex vivo IL1Ra gene therapy, in an intervertebral human implant, adeno-associated virus vectors have been used to significantly inhibit the activity of degrading enzymes in the degenerated discs.[82,83] Incubating human disc cells with IL1Ra or TNF inhibitors significantly reduces all MMP3 levels and eliminates MMP1 in vitro.[84] Mouse intervertebral discs show loss of proteoglycan, the normal structure of collagen, and enhanced expression of matrix-degrading enzymes including MMP3, MMP7, and ADAMTS4 after the removal of IL1Ra.[85]

Administration of PRP in combination with TNFα and IL1 to human nucleus pulposus cells in vitro suppressed the inflammatory degrading enzymes of MMP3 and cyclooxygenase-2 (COX-2), which are stimulated by cytokine, and reduced collagen type 2 and aggrecan expression to original state.[86] Inhibition of MMPs can be another goal for treating intervertebral disc degeneration. Although TIMP gene therapy is promising, it does not show an increase in the expression of ADAMTS, which is an important biomolecule for the pathogenicity of disc degeneration.[87] Finally, resveratrol has anti-inflammatory and anti-catalytic effects on the levels of protein and mRNA related to MMP3, MMP1, IL8, IL6, and MMP13 genes in vitro. When administered in vivo to a rodent model of resveratrol disease, radiculopathy significantly reduces pain by applying nucleus pulposus in the back root of the ganglion more than 14 days.[88]

Conclusion

The transfer of genes that have the potential to interfere with disc degeneration or to stimulate disc reconstruction has been recently addressed by researchers. This strategy requires identifying the particular genes that play a role in the cascading pathway of disc degeneration and developing better methods for transferring these genes into disc cells. One method of gene transfer is the direct injection of the gene, but this method requires repeated injections due to the chronic nature of the disease and the short lifespan of these factors. To overcome this problem, gene therapy seems appropriate. The majority of concerns with gene therapy are its safety, efficiency, and persistence in gene expression. Another prime concern is with the ex vivo method is the patient's acceptance of virus-mediated methods and the selection of genes. Immunogenicity of the vectors and long-term survival of the transfected gene are two key issues that need to be resolved before gene therapy is used to treat disc degeneration in humans. Retroviral vectors are ideal in ex vivo gene transfer, but have limited value in treating disc degeneration, because retroviruses transmit genes that repeat. Many studies have examined the use of adenovirus vectors in gene therapy for this disease. Since adenoviruses do not enter the genome, the risk of oncogenesis decreases, and the cellular turnover in the intervertebral disc resolves the problem of diluting the amount of gene expression with each cell

division. Adenoviruses can infect cells that are not dividing, but they cause severe immune responses in the body. Another type of vector is adeno-associated viruses (AAVs). This type of vector has the least immune responses, but their efficiency in cell transformation is low. One study investigated the transformation efficiency of multiple serotypes of AAVs in the transformation of human NP cells.

The advantage of using AVVs over other viral vectors can be found in: (1) the low immune response and high safety during use in the body; (2) achievement of a period of dormant and hidden infection in the absence of a helper virus; (3) entrance into the genome in a specific location, avoiding the risk of mutations in the host cell genome and unwanted activation of oncogenes during random entrance in the genome; (4) a wide range of host cells including dividing cells and silent cells; and (5) long and stable expression of exogenous genes transmitted by these vectors. Despite studies on disc degeneration gene therapy, there are limitations to using these methods for treating this disease.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Not applicable.

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