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Original Research Article

Will intervertebral disc diseases be prevented by siRNA or miRNA-based genomic treatment modalities?

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Abstract

In the present study, a systematic review of experimental research carried out by drug delivery systems that allow for drug release in which oligonucleotides are imbrued, such as small interfering-/micro ribonucleic acids, was conducted. The data obtained after an electronic database query with no language limitation were evaluated using statistical methods. Results were shown as frequency. There was no research found that described successfully imbrued oligonucleotides into drug delivery systems that could be used to prevent intervertebral disc degeneration. There is a high possibility that trial target therapies might have a positive effect on nucleus pulposus cell regeneration, which is damaged most in the disc during intervertebral disc degeneration; however, these designs, whose *invitro* release kinetics have been reported, need to be tested *invivo* in living subjects after they have been tested for surgical techniques to fill this gap in the literature.

Key Words: Antisense oligonucleotide therapy, Drug delivery systems, Intervertebral disc degeneration, miRNA; nucleus pulposus cell, Post transcriptional gene silencing, siRNA

INTRODUCTION

Steroid injections and/or myorelaxants combined with non-steroidal analgesic anti-inflammatory drugs are frequently used in current conservative treatment modalities for degenerative disc diseases (Pearce and Moll, 1967). Furthermore, resting, appropriate exercises, and physical rehabilitation are advised (Koes et al., 2006; Saal and Saal, 1989). At the second stage, facet injections, as a minimal interventional symptomatic treatment in patients with no distinct neuromotor deficit, are used for epidural and intradiscal interventions (Buttermann, 2004; Gibson and Waddell, 2005; White et al., 1980).

Surgical operations are performed for patients with significant nerve root pressure, which is diagnosed according to radiological results, failure to respond to conservative treatment, a significant neuromotor deficit accompanied by drop foot syndrome, urinary incontinence, and degenerative disc-related diseases that affect the quality of life (Postacchini, 1996; Weinstein et al., 2006).

A simple discectomy is the most widely used surgical treatment modality. Transpedicular implant applications that continue dynamic activity or result in fusion are other surgical treatment modalities (Putzier et al., 2005; Raj, 2008); however, for these surgical applications, there are some risks in addition to the risks for patients concerning rutin anesthesia: post-op infections, nerve root or dura damage, bleeding, an inability to remove the herniated disc, unsuccessful surgery due to wrong-level surgery, relapse of disease, shift of implant position, and damage in nerve roots due to misplacement or pressure **(**Amstrong**,** 1951; Urban and Roberts**,** 2003). The results are not promising despite conservative and surgical applications (Gou et al., 2014; Li et al.[b], 2016; Tsaryk et al., 2015). Thus, as in all areas of medicine, neurosurgeons and orthopedists have headed towards

the research that could lead to cellular based pharmacologic and pharmacogenomic treatment modalities (Chen et al.[a], 2016; Chen et al.[b], 2016; Hiyama et al., 2013; Jiang et al., 2014; Jiang et al, 2015; Liang et al, 2014; Lijmer et al., 1999 Lin et al., 2016; Liu, et al., 2016; Mavrogonatou et al., 2015; Morigele et al., 2013; Shen et al., 2013; Shen et al., 2016; Sudo et al., 2013; Wang et al.[a], 2016; Wang et al.[b], 2016; Xie et al., 2016; Yamada et al., 2014; Yang et al., 2014; Yu et al., 2013).

Among the research studies that have gained popularity, the approach of silencing the gene at the post-transcriptional stage by repression using a cell mechanism but not gene modification was chosen for this study. The gene silencing process, which is referred to as the interference (RNAi) of ribonucleic acid (RNA), is achieved by inhibiting protein synthesis through precursor mRNA (pre-mRNA). *Small interfering RNA* (siRNA) and micro RNA (miRNA) are transfected into an *RNA-induced silencing complex* (RISC), which is a protein complex, to perform the gene silencing process (Gumustas et al.[a], 2016).

The present study aimed to systematically evaluate antisense oligonucleotide therapies in the treatment of intervertebral disc degeneration (IVD). By analyzing the data, the aim was to investigate probable antisense oligonucleotide strategies that could be used through minimal invasive surgery in the future.

METHODS

Search strategy

The databases of the US National Library of Medicine National Institutes of Health, Embase, OVID, the Cochrane Library, and the references within the retrieved articles were searched to find all relevant antisense oligonucleotide therapy and intervertebral disc degeneration trial studies from January 1942 to August 15, 2016, without any language restrictions. “Antisense oligonucleotide therapy,” “intervertebral disc degeneration,” and “nucleus pulposus cell” AND/OR “miRNA,” “post transcriptional gene silencing,” “siRNA,” “drug delivery systems,” “controlled release,” and “hydrogel” were used as keywords. The percentage distribution of the articles by year was recorded, and the evidence level was determined according to Lijmer et al. (Gumustas et al.[a], 2016; Lijmer et al., 1999). Bibliographies that were potentially overlooked during the database research were examined again. Unpublished grey literature, including articles, comments, letters, editorials, protocols, guides, meta-analyses, and collections, were not included. The most highly cited articles were defined and re-examined to avoid double entries.

Eligibility criteria

The drug delivery system that could be used for IVD were examined to determine whether:
- it contains an antisense oligonucleotide treatment method;
- it uses a delivery system in which siRNA/miRNA is imbued;
- it was preclinically tested *in-vitro* and/or *in-vivo*;
- it would to lead to a controlled release of oligonucleotide in the design;
- the effects of NP and AF cells on proliferation and apoptosis were studied on a molecular basis;
- the design was tested clinically.

All studies not containing the above information were excluded. The study inclusion process is summarized in Figure 1.

Data collection and evaluation

The authors selected the included studies independently, and to minimize selection bias, the studies were revised by all authors. In the event of conflicting results, the final decision was made by NK, YA, and TC, who have a higher level of experience regarding pharmaco-molecular orthopedic device design. Finally, the experienced authors (DYS and IY) were consulted, and the topics were revised accordingly if necessary.

Statistical analysis

It was found that the obtained data were not based on the fact that they were collected from the sources witha probability distribution function. Therefore, non-pragmatic statistical methods were used; however, given the lack of common findings, statistical analyses could not be performed, and thus complementary statistical methods were applied. Microsoft Office Excel (2010) was used, and the results were shown as mean ± standard deviation or frequency (%).

RESULTS

After searching for the antisense oligonucleotide therapy keyword, 7,030 studies were found. When intervertebral disc degeneration, nucleus pulposus cell, post-transcriptional gene silencing, siRNA, or miRNA were searched, 5,760, 1,503, 58,005, 77,993, and 52,080 studies were found, respectively. However, when nucleus pulposus cell and intervertebral disc degeneration words were searched together with siRNA or miRNA, 38, 22, 32 and 31 studies were found, respectively (Figure 2). Among these studies, none with a high evidential value met the research criteria.

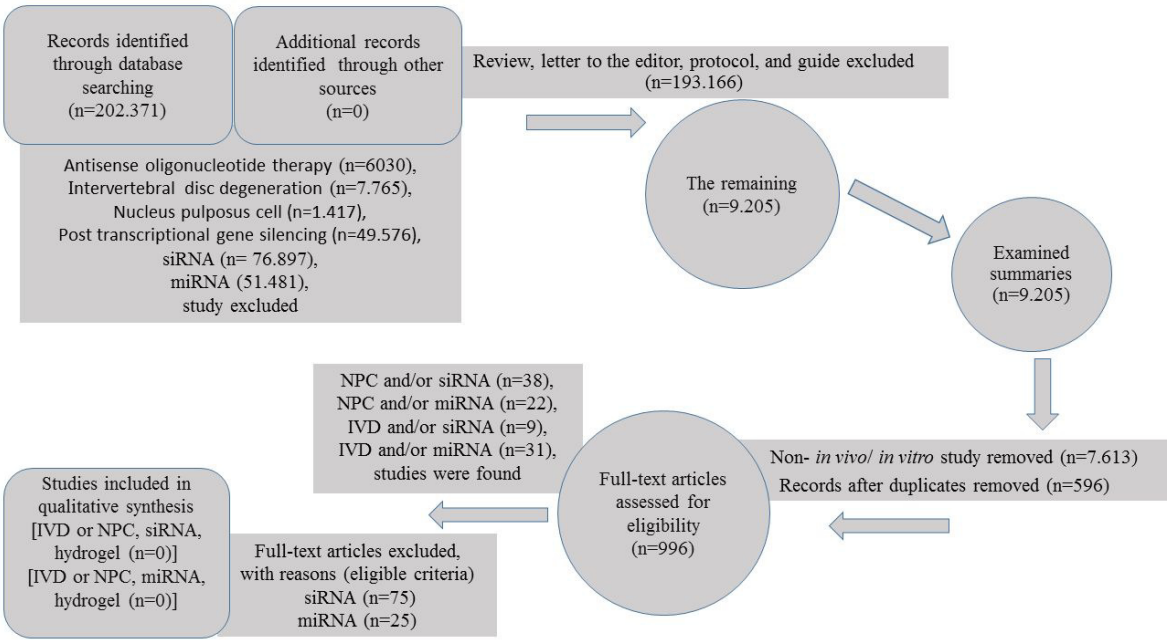

Figure 1. The flow chart of literature identification.

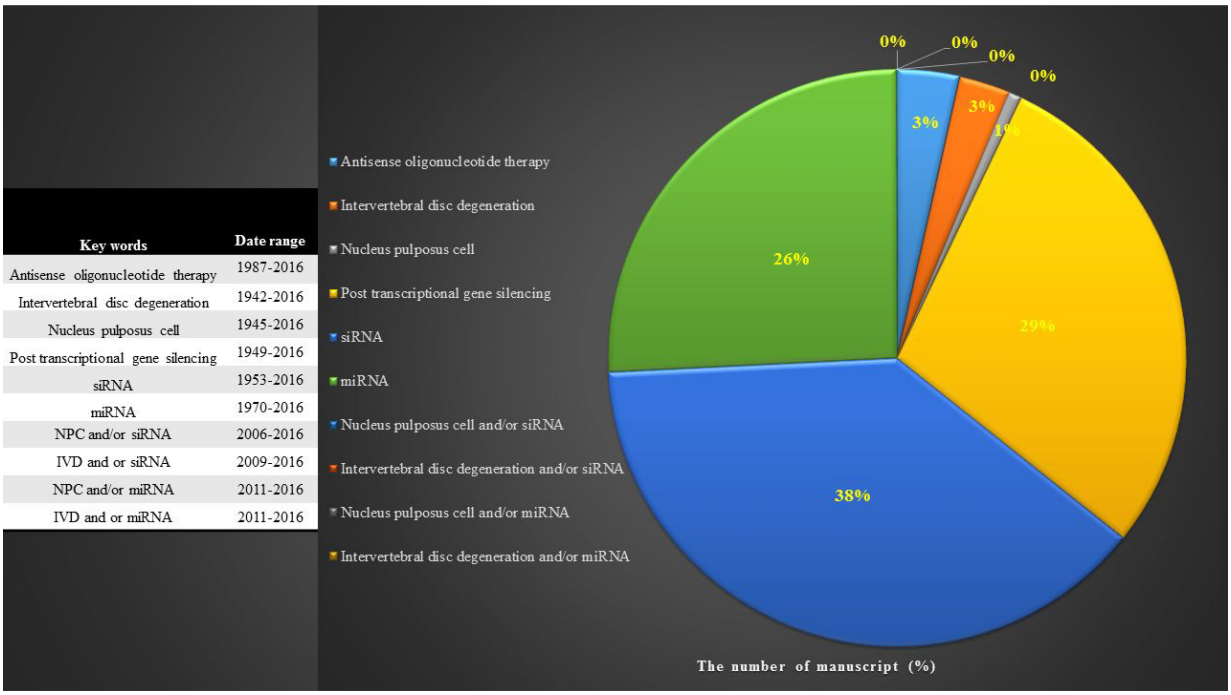

Figure 2. The frequency of the studies by years

DISCUSSION

The number of research studies has increased, and an enormous amount of information has been provided by these independent research studies. In these studies, researchers have investigated small samples or group sizes due to restrictions pertaining to economic and research periods. Similar studies may provide different results. Consequently, it was difficult to determine whether the findings would be similar. These results are consistent with other researchers' results who reviewed the literature. For this reason, it is more efficient to combine similar studies (Gumustas et al.[a], 2016).

The present study aimed to systematically evaluate

antisense oligonucleotide therapies that are currently being tested in the treatment of IVD. While evaluating these studies against IVD and studies including pharmaco-molecular orpharmacogenomic-based analyses, whether hydrogels that allow for a controlled release were used in researches was also determined. Following the evaluation of the data, it was concluded that this study could pioneer hydrogel designs containing oligonucleotides such as SiRNA and miRNA, which are promising designs in IVD treatment. Thus, the present study will be beneficial to designing both comprehensive and reliable research.

Intervertebral disc degeneration limits movement and negatively affects quality of life. It commonly occurs immediately after nucleus pulposus (NP) damage (Ehlicke et al., 2010). Although both conservative and surgical treatment modalities are applied during IVD treatment, the results of the treatment are not promising due to complications after application (Gou et al., 2014; Pearce and Moll, 1967; Tsaryk et al., 2015). Therefore, neurosurgeons and orthopedists have tended towards treatment modalities that aim to regenerate damaged tissues or to prevent damage to tissues (Dogan et al., 2016; Gumustas et al.[b], 2016; Guzelant et al., 2017; Isyar et al[a]., 2015; Isyar et al.[b], 2016; Isyar et al.[c], 2015; Isyar et al.[d], 2016).

It has been suggested that a certain amount of nucleus pulposus cells of a specific quality would be needed, which gained momentum in research on cell-based treatments (Ehlicke et al., 2010;Fernandez et al., 2016; Li et al.[a], 2016; Pohl et al., 2016). Consequently, studies that investigate cell-based pharmaco-molecular and pharmacogenomic treatment modalities have become popular(Chen et al.[a], 2016; Chen et al.[b], 2016; Hiyama et al., 2013; Jiang et al., 2014; Jiang et al, 2015; Liang et al, 2014; Lijmer et al., 1999 Lin et al., 2016; Liu, et al., 2016; Mavrogonatou et al., 2015; Morigele et al., 2013; Shen et al., 2013; Shen et al., 2016; Sudo et al., 2013; Wang et al.[a], 2016; Wang et al.[b], 2016; Xie et al., 2016; Yamada et al., 2014; Yang et al., 2014; Yu et al., 2013).

Chen et al. (2016) studied programmed cell death 4 (PDCD4), a tumor suppressor, and examined the effect of microRNA-21 (miR-21) on the reproduction of degenerated human nucleus pulposus (NP). They also examined miR-21 expression on nucleus pulposus tissues, which was obtained from 20 cases with a disc disease and five cases with traumatic vertebra fractures. They divided NP cells with disc disease into six groups: bare control group, negative control group, which was transfected with an miR-21 negative sequence, miR-21 inhibitor group, miR-21 mimic group, and PDCD4 siRNA group. They applied Cell Counting Kit-8 during the cell reproduction analyses. Western Blot was used to determine c-Jun and p-c-Jun expressions. They found that miR-21 and PCD4 values increased in disc degeneration patients. They reported that miR-21 expression was negatively related to the mRNA of PDCD4. They also found that MMP-2 and MMP-9 mRNA expressions and p-c-Jun protein expressions were lower in the miR-21 inhibitor group, whereas PDCD4 mRNA and protein expressions were higher than for the other groups. Based on these findings, they concluded that miR-21 increase degenerated NP cells by targeting PCD4. They underlined that this effect was the result of c-jun protein phosphorylation and ap-1 dependent MMP transcription. Thus, they concluded that miR-21 could be an important biomarker in degenerative disc disease (Chen et al., 2016).

Liu et al. (2016) suggested that IVD, which is one cause of backaches, is characterized by increasing apoptosis, and they aimed to investigate the effect mechanism of stromal cell-derived factor-1 (SDF-1)/C-X-C motif chemokine receptor 4 (CXCR4) on degenerated NP cells. They observed SDF-1 and CXCR4 expressions using a Western Blot analysis and an immunohistochemical assay. They analyzed NP apoptosis using Annexin V/propidium iodide fluorescence. They then performed gene silencing for CXCR4 via SDF-1 stimulation. They examined the connection provided by the Nuclear factor-κB (NF-κB) signaling pathway through CXCR4-siRNA and the NF-κB inhibitor via pyrrolidine dithiocarbamate (PDTC) treatment. They observed that SDF-1 and its receptor CXCR4 expression increased in generative disc disease when compared to the control group. They also reported that SDF-1 stimulation increased NP apoptosis. On the other hand, they indicated that CXCR4 receptors suppressed apoptosis in the group in which siRNA was transfected. They reported that SDF-1 treatment resulted in a phosphorylated NF-κB subunit P65 increase, and downregulation occurred following CXCR4-siRNA and PDTC treatment. Moreover, they observed that P65, which was inducted by SDF-1, inhibited nuclear translocation in the CXCR4-siRNA and PDTC group. Consequently, they underlined that SDF-1-mediated apoptosis could be suppressed using PDTC through NF-κB inhibition. They deduced that SDF-1/CXCR signaling could be a new target in treatments as a result of the induction of cell apoptosis through the SDF-1/CXCR4; NF-κB pathway in degenerated discs. They concluded that the SDF-1/CXCR4 axis promoted cell apoptosis in human degenerative NPCs via the NF-κB pathway, which suggests that SDF-1/CXCR signaling could be a therapeutic target for the treatment of degenerative IVD diseases (Liu et al., 2016).

Wang et al. (2016) used rat NP cells in their study in which they asserted that NP cell apoptosis, the main cause of an intervertebral disc, occurs as a result of the relation between caveolin-1 and cytokine-induced apoptosis. They treated Rat NP cells with interleukin (IL)-1β or tumor necrosis factor alpha (TNF-α). They then generated a knockdown of interleukin (IL)-1β or tumor necrosis factor alpha (TNF-α) via specific siRNA. They

measured the apoptosis level of NP cells and caveolin-1/β-catenin signaling expression and activation using flow cytometry, qRT-PCR, and Western Blotting. They examined the relation between the Mitogen-activated protein kinase (MAPK) pathway and caveolin-1 promoter activity using a luciferase assay. They increased IL-1β via TNF-α mediated apoptosis and reported that it activated Wnt/β-catenin signaling. They observed that this effect was reversed when they transfected the caveolin-1 and β-catenin pathways. Due to Caveolin overexpression, they underlined that rat NP cell apoptosis and β-catenin nuclear translocation were reversed using β-catenin siRNA. They reported that the p38 MAPK inhibitor, or dominant negative-p38, and caveolin-1/β-catenin blocked the induction of cytokine dependent induction, expression, and activity. According to these results, they showed that p38/caveolin-1/β-catenin plays a role in cytokine-induced apoptosis, and correspondingly, p38/caveolin-1/β-catenin activity regulates IL-1β- and TNF-α-induced apoptosis (Wang et al.[a], 2016).

In another study by Chen et al. (2016), it was reported that NP was the most important disc component in intervertebral disc disease. They argued that although carbonic anhydrase 12 (CA12) has been viewed as an important marker, the physiological mechanism is not clear concerning the CA12 effect. Thus, they carried out analyses using molecular analyzation methods on 81 degenerated NP samples to determine the CA12, hypoxia-inducible factor 1α (HIF-1α), and HIF-2α expression levels. They cultured rat NP cells in a hypoxic environment and examined hypoxia-induced CA12 expression. They also treated rat np cells with HIF-1α siRNA or prolyl hydroxylase (PHD) inhibitor dimethyloxalylglycine (DMOG) and attempted to determine the regulation effect of PHD/HIF on CA12 expression. They reported that CA12 levels were downregulated significantly due to mRNA and protein. In addition, they reported that the CA12 level increased up to 30 times in a hypoxic environment. They noted that there was a positive correlation between decreasing HIF-1α levels and CA12, and they stated that HIF-1 was knockdown under hypoxia, which resulted in a decrease of CA12, mRNA, and protein levels. They deduced that if CA12 was downregulated in degenerated discs, expression might be regulated through PHD/HIF-1, and the decreased level of CA12 might lead to the progression of degenerative intervertebral disc disease due to the decrease of the matrix synthesis (Chen et al., 2016).

Wang et al. (2016) carried out a similar research study. They aimed to identify hypoxic reactions and vitality in NP and annulus fibrosus (AF) cells. They applied small ubiquitin-like modifier (SUMO) molecules onto the intervertebral disc cells of rats along with SAE1, SAE2, and SUMO-E2, which are SUMO E1 activator enzymes, enzyme UBC9, which is a SUMO-E conjugated enzyme, and de-SUMOylation enzyme sentrin/SUMO-specific proteases (SENP). Next, they cultured NP and AF cells in a hypoxic environment. While they evaluated vitality, quantifying cell proliferation, cellular senescence, apoptosis of cells, and cell cycle distribution, they also analyzed the hypoxic regulation of SUMOylation pathways and transcription and the expression of sumo enzymes. They used SENPI-siRNA transfection to determine the tolerance effect of hypoxic reactions. They reported that NP and AF cells increased SENP1 and preserved vitality under hypoxic conditions. In addition, they concluded that while hypoxia increased SUMO-1, SUMO-2/3, SAE2, and UBC9 temporarily in NP cells, it increased SUMO-1 in AF cells but decreased SUMO-2/3, SAE1, SAE2, and UBC9. They explained that even if the downregulation of SENP1 diminished transcriptional activity, there was no significant change in cell vitality. They reported that NP and AF cells showed an equal tolerance, but they regulated the pathways in different ways (Wang et al.[b], 2016).

Xie et al. (2016) argued that in previous studies, it was reported that aquaporin 3 (AQP3), which provides water transport across cells and increases glycerol permeability, decreases in degenerative lumbar disc disease; however, the role of the AQP3 molecule in the pathogenesis of IVD had not been explained. Thus, they aimed to identify the effects of AQP3 on cell proliferation and extracellular matrix degradation by gain-of-function and loss-of-function experiments and to determine whether it affected the Wnt/β-catenin signaling pathway. To this end, they used siRNA transfection. They transfected NP cells by AQP3-pcDNA3.1 plasmid or AQP3-siRNA. With the MTT cell viability, toxicity and proliferation analysis, they observed that AQP3 promoted proliferation of NP cells. They evaluated the effects of this molecule on extracellular matrix degradation using a Western Blot analysis via disintegrin and MMP. As a result, they reported that AQP3 decreased degradation and suppressed Wnt/β-catenin signaling. Moreover, they noted that they reversed the effects of AQP3 on human NP cells through lithium chloride, which is the signaling activator of Wnt/β-catenin. Correspondingly, they deduced that the effect of AQP3, which can degrade an extra cellular matrix, was partially realized through the Wnt/β-catenin signaling pathway (Xie et al., 2016).

Shen et al. (2016) reported that the sirtuin 1 (SIRT1) molecule plays a role in lumbar disc degeneration cytokines or in the control mechanism of some age-associated diseases; however, they stated that the mechanisms and pathways through which this molecule causes intervertebral disc degeneration are unclear. Thus, they aimed to investigate the effects of SIRT1 on the signal transduction pathway and pro-inflammatory stress induced by IL-1β in human NP cells. They evaluated the anti-apoptotic and anti-catabolic effects of SIRT1 on IL-1β in NP cell cultures. They achieved SIRT1 overexpression by transfection using the lentiviral vector or SIRT1 inhibition by transfection via siRNA. They

reported that there was a relation between diminished SIRT1 and intervertebral discs and that the extracellular matrix synthesis was regulated by SIRT1 expression. In contrast, they reported that the knockdown of the SIRT1 gene increased MMP expression, so they suggested that IL-1β-mediated cell apoptosis. They also observed that NF-κB could repress inflammation by inhibiting nuclear translocation and deacetylating SIRT1 RelA/p65, and IL-1β could downregulate by activating TLR2. They deduced that SIRT1, which showed an anti-inflammatory effect, inhibited NP cell degeneration through TLR2/SIRT1/NF-κB via IL-1β (Shen et al., 2016).

In another study, they showed that the surviving molecule in NP cells, which is used as a proliferation reagent in tumor cells, was not examined in any studies concerning the degeneration of NP cells. They compared the expressions of the surviving molecule obtained from patients under 25 years old with vertebra fractures with patients over 45 years old with degenerative disc disease using siRNA in vitro experiment samplings. They indicated that expressions were observed at mRNA levels in normal discs; however, proliferation concerning NP cells following siRNA transfection and surviving suppression decreased, and escalated sensitivity was also observed against a pro-apoptotic stimulus. In light of these results, they emphasized that the surviving molecule plays an important role in preventing apoptosis of proliferation and degenerative NP cells (Lin et al., 2016).

Mavrogonatou et al. (2015) continuously exposed intervertebral disc cells to a hyperosmotic environment, and proliferation inhibition was observed due to p38 MAPK and p53 activation following the stress. To identify the biochemical pathways under hyperosmotic stress, they used whole-genome arrays to examine high osmolality-induced transcriptional changes in cattle NP cells. They reported that they found >100 and >200 gene expression after 5 h and 24 h treatments, respectively. Among these expressed genes were LC4A11, SLC5A3, ATP1A1, SLC38A2, KCNK17, KCTD20, KCTD11, SLC7A5, and CLCA2 transporter genes. Using microarray analyses, they tested the transcriptional differences of these genes using two and three-dimensional cell culture samples containing hyperosmolar salt in a sorbitol environment. They reported that p38 MAPK and p53 play roles in the regulation of transporters. Consequently, they concluded that “the inhibition of ATP1A1 had the most prominent effect on the transcription of the rest of the transporters and was found to enhance the anti-proliferative effect of hyperosmotic conditions through an increased G2/M cell cycle block, ascribing to this pump a central role in the osmoregulatory response of nucleus pulposus cells” (Mavrogonatou et al., 2015).

In another study carried out in a hyperosmotic environment, they investigated how NP cells could survive after being exposed to a stressful environment. They evaluated rat NP cells in a hyperosmotic stress environment to determine whether they had cell adaptations, such as autophagy, using aSQSTM1/P62 expression level measurement. They reported that under stress, it was observed temporarily intracellular calcium stimulation in intracellular storage sites and extracellular areas. They confirmed that P70S6K was inhibited along with AMPK activation and that lc3-II was inhibited by intracellular calcium chelation. Moreover, they reported that hyperosmotic stress decreased cell vitality and increased apoptosis, and the inhibition of autophagy led to SQSTM1/P62 accumulation. In line with these data, they emphasized that NP cells activated the calcium-dependent AMPK/mTOR pathway in a hyperosmotic environment, thus increasing autophagy (Jiang et al., 2015).

One study reported that SIRT1 could protect NP cells from apoptosis. In this study, whose aim was to identify the relation between apoptosis and autophagy, it was found that there was a close relationship between apoptosis and autophagy. The researchers concluded that it was necessary to determine whether autophagy played a role in the mechanism in whichSIRT1 prevents apoptosis. Based on the results, they reported that the auto-phagosomes number, the mRNA levels of LC3 and Beclin-1, and the protein expressions of LC3-II/I and Beclin-1 decreased. In addition, they concluded that resveratrol increased LC3-II/I and Beclin-1 protein expressions and decreased apoptosis and that nicotinamide or SIRT1 -siRNA transfection showed a counter effect. They reported that the incidence of Cleaved Caspase-3 and apoptosis increased bafilomycin significantly whether or not resveratrol was added, and as a result, it played an important role in the intervertebral disc degeneration of autophagy. They also concluded that SIRT1 decreased apoptosis in degenerated NP cells by increasing autophagy (Jiang et al., 2014).

Liang et al. (2014)explained that in NP cells, the function of extracellular-signal-regulated kinase 5 (ERK5), a mitogen-activated protein kinase family that regulates many cell processes, such as proliferation, necrosis, apoptosis, and degeneration, was unclear, so they examined the differential expression of ERK5 in normal and degenerated human NP cells using a Western Blot analysis and immunohistochemical methods. They indicated that pro-inflammatory cytokine Tumor Necrosis Factor Alfa (TNF-α) decreased both ERK5 and specific NP markers, which are aggrecan and type II collagen. They found that the suppression of ERK5 gene expression with ERK5-siRNA after transfection resulted in a decrease in the collagen and aggrecan levels and proved the existence of ERK5 expression in NP degenerated cells (Liang et al., 2014). There is a research reporting that in the majority of the population, there is still no clear treatment due to the uncertain pathogenesis of the lumbar pain which is caused by IVD. In this research, it has been reported that

there is apoptosis which is characteristic in NP cells (Yamada et al., 2014).

Using *in vitro* and *in vivo* models, whether compressive loading causes IVD was investigated, and whether apoptosis could be a method of treatment due to the inhibition of apoptosis was also determined. It was observed that time-dependent apoptosis increased in NP cell-agarose three-dimensional composite cultures by uniaxial, unconfined, static, and compressive loading using a tunnel assay. In addition, it was reported that there was an increase in enzymes, which degrade the extracellular matrix, whereas there was a decrease in tissue inhibitory matrix metalloporteinases-1. These data were obtained through inhibition after transfection via caspase-3'ü siRNA. It was found that there was a significant decrease in IVD in the compressive loading model of a single dose of caspase-3-siRNAinjections according to the magnetic resonance image and the histological and tunnel assay for in vivo model (Yamada et al., 2014).

In a study that examined a molecule called an aberrant in the pathogenesis of IVD and the proliferation of NP cells (Yu et al., 2013), it was reported that miRNA and small noncoding RNA classifications regulated cell proliferations in many pathologies. When the miRNA-10b level was compared with NP, which was obtained from idiopathic scoliosis patients, it was found that there was a significant increase in degenerative NP cells. It was also proven that there was a relation between miRNA-10b and the disc degeneration grade and homeboxD10 downregulation. It was shown that NP cell proliferation was stimulated depending on the overexpression of miRNA-10b in culturized NP cells. Thus, it was observed that homeboxd10 caused translation inhibition. It was also reported that miRNA-10b mediated HomeboxD10 and caused ras homologue gene family member-C expression and an increase in akt phosphorylation. They reported that upregulation of miRNA-10b from *Aberan* caused abnormal NP cell proliferation by targeting homeBoxD10 through Ras and Akt pathway.(Yu et al., 2013). It is well-known thatRNA interference (RNAi) is a new treatment method used for degenerative cartilage tissue (Yang et al., 2014).

Yang et al. (2014) applied the cationic lipid-based commercial reagents Lipofectamine RNAiMAX and polyethylenimine (PEI), which are synthetic transfection agents, and chitosan and hyaluronic acid, which are natural transfection agents. They used these agents to achieve siRNA transfection into primary mesenchymal cells and cells containing articular cartilage and NP. The inhibition activity caused by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or siRNA were evaluated on the third and sixth days after post-transfection. They found that the commercial liposome-based agent was the most effective agent. It was shown that siRNA, which is used in cell vitality and transfection, varies depending on dosage and cell type in cases where cationic liposomes, chitosan, and PEI are used. Moreover, it was found that there was a decrease in DNA content independent from GAPDH knockdown, which was not related to cell vitality. Cell cycle marker genes, such as p21 and pcna, could not account for the decrease in DNA content. Interestingly, it was reported that there was a significant increase in the GAPDH levels of articular cartilage cells (Yang et al., 2014).

In another randomized and controlled study, resveratrol (RES), impregnated with alginate microspheres, was applied to both cultures as both monolayer and three-dimensional (3-D), after human NP cell cultures from six patients with burst limb vertebrae were prepared (Hiyama et al., 2013). They also investigated whether pathologic TNF-α expression was inhibited using Dickkopf (DKK) isoforms and Sclerostin (SOST) during the inhibition of TNF-α and Wnt-β-catenin signals. In accordance with the data, they concluded that there was an increase in TNF-α via Wnt-β-catenin signal expression and promotor activity. In addition, they reported that there was an increase in TNF-α expression depending on Wnt-β-catenin activation and 6-bromoindirubin-3'-oxime (BIO), a selective glycogen synthase kinase 3 (GSK-3) inhibitor. Following the transfection through DKK-3 and Dkk-4, a Wnt-β-catenin signaling inhibitor, or SOST, it was observed that TNF-α activation was blocked. They also found that Wnt-β-catenin signaling regulated TNF-α and created a positive feedback loop. It was deduced that the degeneration of NP cells could be prevented by blocking the pathway (Hiyama et al., 2013). The mRNA levels related to SIRT1 were analyzed by Western Blot, and the aggrecan and collagen type-II expression levels were evaluated by a qRT-PCR (Shen et al., 2013).

In a molecular-based study where Western Blot and qRT-PCR techniques were used, proteoglycan and collagen type-II expressions were compared. In this study, siRNA and chromobox homolog 8 (CBX8) were developed on rat IVD cells. At the post-trans functional stage, whether the damaged DNA of NP cells would be repaired was investigated. Hydrogen peroxide was used to form a DNA oxidative damage model. In this group, CBX8 expression was silenced by siRNA, and it was reported that type-II collagen and proteoglycans were diminished, and that cell proliferation was inhibited. Moreover, it was concluded that the cell cycle was decelerated and arrested in the G0/G1 phase. The researchers deduced that this effect was caused by the INK4A-ARF pathway and that CBX8 could be used in future gene treatments, as CBX8 plays a key role in the DNA damage of CBX8 (Zhou et al., 2013).

In a research study in which a knockdown and transforming growth factor (TGF)-β1 combined treatment was studied, New Zealand rabbits were used as the living mammal subject for culturization. The Notch siRNAs were synthesized, transfection was provided temporarily, and TGF-β1 was added. Using qRT-PCR and Western

Blot analyses, the values of the protein expressions were measured. It was reported that there was increased proteoglycan and collagen type-II synthesis after knockdown and TGF-β1 were added. Based on the data results, the researchers deduced that the Notch 1 knockdown method could be used in IVD treatment as a new treatment method (Morigele et al., 2013).

Sudo et al. carried out a study in which they reported that IVD was a highly frequent disease and that NP cells caused degeneration on a large scale. They aimed to research the genes that were affected in a short-starved environment. Thus, they aimed to determine whether a short-starved environment using NP cells obtained from rats could be used as a treatment strategy (Sudo et al., 2013). They tested global gene expression using a microarray analysis and examined the functional relations between the cyclin-dependent kinase inhibitor p21(CIP1) and caspase-3. They underlined the fact that the expressions of DNA damage checkpoints were affected by a significant cell cycle in the case of serum starvation. Furthermore, they reported that both p27(Kip1) and p53 expression values increased. They observed that the expression level of NP cells p21(Cip1), which were transfected by caspase-3-siRNA, remained unchanged, and G1 arrest and apoptosis were inhibited. As a nutritional deficiency in NP cells in signal pathways results in the activation of DNA damage checkpoint genes, it was concluded that a nutritional deficiency should be used as a new strategy in IVD treatment (Sudo et al., 2013).

While evaluating data from various studies, it was noted that all data should be converted into the same sphere of influence; thus, calculations are highly important during the conversion process. In addition, the calculation and inclusion of the research results with similar characteristics regarding their sphere of influence are crucial (Berman and Parker, 2002; Bernard et al., 2004; Cohen, 1992; Gumustas et al.[a], 2016).

Upon reviewing the articles that met the criteria, common data were not found, so homogeny or heterogeneity could not be applied. Therefore, the assumption that there is only one underlying reason could not be justified statistically. As it was not possible to identify a common finding, a graphic drawing for certainty measurements, such as sample size or variance reciprocal, could not be produced. In the present study, all the articles were retrospectively designed, and most had small sample sizes subject to systematic and random bias, which comprise the limitations of this study. Consequently, upon reviewing the literature, a high evidential indexed study related to a drug delivery system application for oligonucleotide for an IVD damage disease was not found. (Berman and Parker, 2002; Bernard et al., 2004; Cohen, 1992; Gumustas et al.[a], 2016).

CONCLUSION

It should be taken into consideration that, in the IVD, especially where the NP cells are damaged, the drug delivery systems to be used for transporting the oligonucleotides, such as siRNA / miRNA, which are still in the experimental stage, to the site of injury are promising. Following pharmacokinetics and bio mechanic analyses, scaffolds loaded with oligonucleotides, whose convenience for surgical techniques has been tested, should be applied to living mammals, and this gap in the literature should be filled.

Competing Interests

All authors certify that they, or a member of their immediate family, have no funding or commercial associations (e.g., consultancies, stock ownership, equity interest, patent/ licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

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Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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