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How to enhance the ability of mesenchymal stem cells to alleviate intervertebral disc degeneration**Mesenchymal stem cells alleviate intervertebral disc degeneration**

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Abstract

Intervertebral disc degeneration (IDD) is one of the main causes of chronic low back pain, and degenerative lesions are usually caused by an imbalance between catabolic and anabolic processes in the intervertebral disc. The environment in which the intervertebral disc is located is harsh, with almost no vascular distribution within the disc, and the nutrient supply relies mainly on the diffusion of oxygen and nutrients from the blood vessels located under the endplate. The stability of its internal environment also plays an important role in preventing IDD. The main feature of disc degeneration is a decrease in the number of cells. Mesenchymal stem cells have been used in the treatment of disc lesions due to their ability to differentiate into nucleus pulposus cells in a non-specific anti-inflammatory manner. The main purpose is to promote their regeneration. The current aim of stem cell therapy is to replace the aged and metamorphosed cells in the intervertebral disc and to increase the content of the extracellular matrix. The treatment of disc degeneration with stem cells has achieved good efficacy, with an efficacy rate of almost 70%, and the current challenge is how to improve this efficacy. Here, we review current treatments for disc degeneration, and summarize studies on stem cell vesicles, enhancement of therapeutic effects when stem cells are mixed with related substances, and improvements in the efficacy of stem cell

therapy by adjuvants under adverse conditions. We review the new approaches and ideas for stem cell treatment of disc degeneration, in order to contribute to the development of new therapeutic approaches to meet current challenges.

Key Words: Mesenchymal stem cells; Intervertebral disc degeneration; Extracellular vesicles; Nucleus pulposus cells; Tissue regeneration

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Core Tip: Mesenchymal stem cells have a strong self-renewal capacity and multidirectional differentiation potential, and their secreted vesicles promote regeneration of myeloid cells, increase extracellular matrix production, and alleviate inflammatory status. We review the current relevant targets of stem cell exosomes for the treatment of intervertebral discs and the adjuvant tools used in conjunction with stem cell therapy. This will help to improve the therapeutic efficacy of stem cells and their exosomes, which will also contribute to development of more efficient treatment strategies and approaches for the restoration of disc degeneration.

INTRODUCTION

IDs have a complex structure with a unique internal environment. They contain nucleus pulposus cells, fibrous rings and ECM, which are in a dynamic balance of self-renewal. Stem cell therapy is designed to restore this balance of secreting exosomes, vesicles, mixing other substances to promote their differentiation into nucleus pulposus cells, regulating the content of the ECM, and by treating them accordingly, to resist interference by the harsh environment of the IDs. These synergistic approaches provide new possibilities for stem cell therapy of IDD.

MIRNA IN EXOSOMES

For signal transduction pathways, many miRNAs play a crucial role in the growth and development of IDs. Exosomes of MSCs deliver miRNA-31 to the nucleus pulposus, and upregulate the Wnt/ β -catenin pathway to inhibit apoptosis of nucleus pulposus cells and regulate production of the extracellular matrix (ECM)^[9]. Similarly, under the stimulation of external effects, miRNA-21 can be transfected into mesenchymal cell exosomes, enhancing the ability of MSCs to differentiate into osteoblasts, and promote vascular regeneration^[10]. In other related studies, exosomes derived from bone marrow MSCs inhibited apoptosis and inflammation by upregulating autophagy through the AKT/mTOR signaling pathway^[11]. MSC-derived exosomes can also inhibit apoptosis of nucleus pulposus cells through miRNA-532-5p transport, demonstrating that this transport is also effective in IDD therapy^[12]. Exosome-delivered exogenous miRNA-26a-5p can also be delivered to IDs, as METTL4 is highly expressed in patients with IDD, and the level of NLRP3 regulated by it leads to an increase in pro-inflammatory factors and apoptosis of nucleus pulposus cells. Exogenous miRNA-26a-5p inhibits METTL4 expression and thus treats disc degeneration^[13]. It has also been found that miRNA-15a in exosomes can participate in the protein balance regulated by the PI3K/Akt and Wnt3a/ β -catenin axes of IDD, and downregulate matrix metalloproteinase-3. Therefore, type II collagen and aggrecan levels can be increased, along with differentiation of MSCs to nucleus pulposus cells^[14,15]. BACH1 is a transcription inhibitor of HO-1, and HO-1 activates autophagy in nucleus pulposus cells, and miRNA-155 in exosomes inhibits BACH1 expression by binding to the 3'-UTR of this transcription inhibitor, thereby treating IDD^[16](Table 1).

SPECIAL GENETICS AND MSCS

Microtubules are an important part of the cytoskeleton that can regulate the assembly of proteins and the transport of substances within the cell, and the stability of microtubules may extend their own life and ensure their normal basic functions. A decrease in transforming growth factor (TGF)- β 1 is one of the possible factors involved in ID metamorphosis. Stabilization of microtubules can promote the expression of

collagen type 2 and Sox9 in nucleus pulposus cells^[17], which alleviates and reduces IDD. Sox9 and TGF- β 1 can be used to transfect bone marrow MSCs, so that MSCs can successfully differentiate into chondrocytes, promote formation of ECM, restore ID integrity, improve the inflammatory state, and reduce pain and further IDD. Similarly, MSCs and induced cartilage progenitor cells also increase the level of Sox9 and TGF- β 1 during differentiation into nucleus pulposus cells, with the purpose of regulating inflammatory states and increasing differentiation^[15,18,19].

METABOLITES AND MSCS

MSC-derived exosomes contain MATN3, which regulates the content of TGF- β 1 in the IDs and differentiation of nucleus pulposus cells, promotes production of ECM, and inhibits release of inflammatory mediators^[20]. Urolithin A was able to hinder H₂O₂ in inducing the aging of nucleus pulposus cells and destroying mitochondrial function. The SIRT1/PGC-1 α signaling pathway was activated by Urolithin A *in vitro*, which protects the normal physiological function of mitochondria, links nucleus pulposus cell aging, and can increase the survival time of ECM and prevent further IDD^[21,22]. The compression produced by the degenerated ID can promote ROS production by nucleus pulposus cells and cause oxidative stress, which leads to further apoptosis of nucleus pulposus cells, while MSC-derived exosomes can inhibit apoptosis of myeloid cells caused by excessive oxidative stress and ameliorate compression-induced mitochondrial damage, which relieves the pain caused by IDD^[23].

GEL-LOADED MSCS

Heat-responsive hydrogel can act as a carrier of the extracellular vesicles of MSCs. These vectors deliver vesicles in a continuous box, which contains vasorin (a type I transmembrane glycoprotein regulated by HIF-1) to the disc environment. Vasorin can regulate the expression of relevant matrix metalloenzymes in the nucleus pulposus cells through Notch1 signaling, enhance the ability of nucleus pulposus cells to proliferate, migrate, and anabolize, and inhibit the apoptotic cells to release inflammatory

mediators, preventing further exacerbation of IDD. However, whether vasorin can promote the proliferation and differentiation of MSCs has not been confirmed^[21]. Therefore, the strategy of helping stem cells to treat IDD through gels is worth further study. The combination of heat-sensitive decellularized ECM hydrogels with adipocyte-derived MSC exosomes does not damage the therapeutic activity of MSCs. The heat-sensitive dECM@exo hydrogel system produces gelation *in situ* to help MSCs differentiate into nucleus pulposus cells, and maintains the content of ECM. This hydrogel also creates a suitable environment for the proliferation and differentiation of nucleus pulposus cells^[22]. Previous studies have demonstrated transport of MSCs with hyaluronic acid and platelet-rich hydrogels. This method enhances MSC activity, promotes IDs to increase KRT19 gene expression, induces differentiation of MSCs into nucleus pulposus cells, and maintains the original ID height and normal physiological activity of the ID environment^[23]. This situation leads to increased expression of keratin genes, and it has been demonstrated that porcine nucleus pulposus-rich cell matrix induces differentiation into nucleus pulposus cells by stimulating porcine chordate cells^[24].

CIRCULAR RNA AND EXOSOMES

Circular RNA, such as circ_0050205 and circ_0072464, is transmitted through exosomes into nucleus pulposus cells to promote cell proliferation and ECM synthesis^[24,25]. Endogenous long non-coding RNA (lncRNA) Colorectal Adenocarcinoma HyperMethylated (CHAM) delivery by MSC exosomes inhibits M1-type macrophage polarization, which reduces nucleus pulposus apoptosis, the release of inflammatory factors, and a reduction in ECM, which prevent further IDD^[26]. In the MSCs exosomes, miRNA-217 is transferred to the ID, and binds to the forkhead box (FOX)O3 promoter by targeting Enhancer of Zeste Homolog (EZH)2. This maintains ID homeostasis and stimulates autophagy, thereby promoting collagen II and **aggrecan** content, and inhibiting degradation of nucleus pulposus cells^[15,27,28]. In other vesicles, overexpression of miRNA-194-5p inhibits the occurrence of disc degeneration by targeting TRAF6.

Lentiviral vectors have been shown to transport the *GDF5* gene and integrate it into the chromosomal genome of nucleus pulposus MSCs, which then express *GDF5* along with the MSC genes. *GDF5* can also increase the level of proteoglycan and type II collagen^[12]. If vesicles are subjected to hypoxia, miRNA-17-5p regulates the proliferation and differentiation of human nucleus pulposus cells and the proliferation and synthesis of ECM through the TLR4 pathway, alleviating progression of IDD. This is more effective than the direct injection of MSCs into the ID^[29]. During disc degeneration, the release of inflammatory factors also leads to a decrease in the uptake of extracellular vesicles. Vesicles prevent excess death of nucleus pulposus cells by delivering peroxidase 2, and reversing the decline in therapeutic effectiveness of MSCs due to tumor necrosis factor- α damage^[30]. miRNA-129-5p in vesicles increases MSC proliferation and differentiation into nucleus pulposus cells by blocking the *lrg1*-mediated p38 MAPK pathway and polarizing macrophages to the M1 phenotype, ultimately alleviating disc degeneration^[31]. The combination of electromagnetic radiation and tissue engineering can stimulate the BMP/Smad and mitogen-activated protein kinase (MAPK)-related p38 signaling pathway of ID stem cells, to regulate differentiation of MSCs and increase their osteogenic capacity for the treatment of IDD^[32]. Vesicles can also transmit exogenous miRNA-105-5p to revitalized nucleus pulposus cells, and transmit miRNA-140-3p to regulate the KLF5/N-cadherin/MDM2/Slug axis; all of which can slow down progression of IDD and restore normal ID physiological function^[33,34]. miRNA-cahama also exists in MSC exosomes, which targets GREM1 down-regulation of the TGF- β pathway to prevent apoptosis of nucleus pulposus cells and inhibit progression of IDD^[35].

MSC MIXTURE

MSC therapy alone has been shown to be effective in IDD, but its efficacy still falls short of expectations^[36]. A recent study has shown that MSCs can be mixed with cell-free bioresorbable ultra-purified alginate gel for the treatment of IDD^[37]. Such mixed preparations inhibit cell necrosis in the IDs and the release of inflammatory factors, and

consolidate the therapeutic effect of MSCs. Similarly, the combination of MSCs with *in situ* bioresorbable gel (dMD-001) produced the above therapeutic effects in IDD, and was also used after discectomy to prevent IDD. Similarly, when MSCs are combined with coenzyme Q10 for the treatment of most ID lesions, it reduces oxidative stress in the ID, inhibits degradation of nucleus pulposus cells, and steadily improves the efficacy of IDD treatment. Gelatin microparticles can also be mixed with MSCs, which can regulate the release of TGF- β 1 and bone morphogenetic protein-2 to promote regeneration of osteochondral tissue^[38]. When bone marrow MSCs are injected into the human body, leakage and decreased viability of MSCs occur, and gelatin colloidal hydrogels using nanostructures can effectively prevent these conditions. Under the load of gelatin colloid, MSCs promote regeneration of IDs, increase the number of nucleus pulposus cells between the IDs, ECM content and the ID height^[39]. Selective cell retention technology can concentrate MSCs and then the gelatin vector described above is used, which has been shown to enhance the effect of carrier materials on MSCs in the treatment of IDD^[40]. A novel amphiphilic copolymer, polyethylene glycol-PAPO, fused with lipophilic kartogenin into a complex, is an esterase-reactive micelle that can carry MSCs and maintain their activity. When this combination is injected into IDs, it protects them from oxidative stress, activates autophagy of MSCs, regulates gene expression in the ECM, promotes ECM production, and increases ID height and hydration between the IDs^[41]. Previous studies have found that collagen hydrogel can promote differentiation of MSCs^[42]. A more recent study found that biocompatible earthworm gel fused with MSCs induced their differentiation into nucleus pulposus cells in a targeted manner, and improved their differentiation ability and efficiency^[43]. For the expression of special genes such as *SOX9*, *ACAN* and *COL2* in nucleus pulposus cells, the study found that MSCs increased the expression of appeal genes after superoxide dismutase 2 and catalase processing, thereby reducing the deterioration of inflammatory states and promoting repair of ID tissue^[44]. MSCs can also fuse with connective tissue growth factor and TGF- β 3, and transport polydopamine nanoparticles to the corresponding positions between the nuclei of the IDs, and finally induce MSCs

to differentiate into nucleus pulposus cells and fibrous ring cells, reconstructing the mechanical environment of the IDs^[45](Figure 1).

STIMULATION INDUCTION WITH MSCS

Sustained mechanical stimulation is an innovative method of induced differentiation in MSCs, which can be continuously stimulated in a special microgel attached to them^[46]. Repeated continuous stretching regulates TRPV4 and Piezo1 channel proteins, regulates the value-added differentiation of MSCs into chondrocytes, regenerates the intercellular matrix, increases the water content in the IDs, and restores the normal structure of degenerated IDs^[47]. In addition, formation of ECM has been found to correlate with tissue specificity. Therefore, the isolated nucleus pulposus cells and fiber rings are processed into corresponding hydrogels, which are specific in composition and space structure. Viscous fibrinogen-thrombin-genipin gels act specifically through the RhoA/LATS/YAP1 signaling pathway to direct differentiation of MSCs into nucleus pulposus cells or fibrous rings. This helps patients with IDD to reduce the number of nucleus pulposus cells and fibrous ring damage, which may provide a new direction for the treatment of IDD^[46,48]. In the treatment of IDD and recovery of ID height, by using bleomycin to induce MSC fibrosis, by stimulating the TGF β -SMAD2/3 signaling pathway, the gene expression of related collagen and extracellular matrix is maintained, thereby maintaining the height of the ID and increasing its ability to resist wear^[49].

HARSH ENVIRONMENT OF THE ID

The treatment of IDD with MSCs has been widely applied, and the role of MSCs in delaying ID lesions has been proven^[50], but the stability and efficacy of MSCs entering the ID are affected by the microenvironment of the ID. The IDs are located in an environment of nutritional deficiency, high tension, low pH, hypoxia, and high mechanical load^[51]. In this environment, with progression of IDD, most of the nucleus pulposus cells begin to die, resulting in a series of malignant chain reactions that lead to

aggravation of oxidative stress, secretion of inflammatory substances, and aggravation of pain^[5](Figure 2).

Many studies have reported that the microenvironment in which cells are located has an important impact on their biological activity^[52]. The low pH of IDs significantly inhibits ¹ acid-sensitive ion channels (ASICs), which are key receptors for extracellular protons in central and peripheral neurons, related to IDD^[53]. Due to the decreased activity of ASICs, MSCs differentiate into nucleus pulposus cells. When the number of nucleus pulposus cells decreases, and production of ECM decreases, this increases the short-function peptide fragments that can recognize ASIC blockers, as ASICs can activate cell aging pathways, such as p53-p21/p27 and p16-Rb1 signaling factors, to induce apoptosis of nucleus pulposus cells^[54]. ASIC blockers can significantly help stem cells overcome the acidic environment during IDD treatment, improve the ability of MSCs to proliferate and differentiate into nucleus pulposus cells, and help restore the normal state of IDs. 1,25(OH)₂D₃-treated nucleus pulposus MSCs have better tolerance to the hypertonic and acidic microenvironment in which the IDs are located^[55]. This reduces apoptosis of nucleus pulposus mesenchymal cells, restores the height between the IDs, and delays occurrence of IDD.

The large changes in pressure between the degenerative IDs leads to a decrease in the survival and differentiation of MSCs^[56]. Medullary pulposus-cell-derived hydrogels help MSCs differentiate into nucleus pulposus cells^[57]. A recent study found that the current methanipine cross-linked decellularization NP hydrogel-like cell delivery system can transport MSCs to the IDs^[58]. The changes in ID pressure ensure that MSCs differentiate into nucleus pulposus cells, repair the reduced ECM, and maintain ID height to delay IDD.

MSCs pretreated with lithium chloride (LiCl) can increase the adaptability of MSCs^[59], as LiCl helps MSCs antagonize oxidative stress and protect nucleus pulposus cells by activating more extracellular signal-regulated kinase (ERK) 1/2^[60]. ERK1/2 plays a vital role in fighting inflammation, which is one of the main ways it works in the harsh environment of the IDs. This conduction pathway ultimately also helps to

improve the ability of MSCs to reduce nucleus pulposus cell death, increase ECM production, and improve inflammatory status and IDD. 1,25(OH)₂D₃-treated nucleus pulposus MSCs have better tolerance to the hypertonic and acidic microenvironment in which the IDs are located^[55]. It can reduce the apoptosis of nucleus pulposus mesenchymal cells, restore the height between the IDs, and delay the occurrence of IDD.

Significant progress has been made in the treatment of IDD by MSCs, but due to the harsh environment of the IDs^[5], the therapeutic effect of MSCs is reduced due to the lack of oxygen in the environment^[61]. However, it has been found that the differentiation of MSCs to chondrocytes under hypoxic conditions can be helped by the addition of leptin^[62], which provides energy for cell differentiation through its dependent glycolysis.

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FUTURE DIRECTIONS FOR STEM-CELL-BASED THERAPY

Stem-cell-based therapy offers promise for disc degeneration. Related studies have applied exogenous stem cells such as MSCs to treat disc degeneration with promising results^[63]. As the field of stem cells continues to be researched^[64], the histology of the IDs becomes clearer, and studies targeting the way in which stem cells restore disc structure are becoming more advanced. How to improve the efficiency of stem cell therapy for disc degeneration and how to resist the harsh disc environment for stem cell therapy are the focus of research^[65]; including how to help stem cells restore the normal physiological structure of the IDs under hypoxia and lack of blood supply^[5]. This is a new direction that needs to be developed in the field of stem cells.

Sometimes it is not the stem cell therapy that is ineffective, as the way MSCs are administered similarly affects their behavior once inside the body. Overall, factors such as injection point site, syringe, carrier material, and buffer can affect the therapeutic efficacy of stem cells. Different injection sites may lead to variations in reflux of the cellular injection fluid^[66], and the syringe (needle size/shape) may lead to variations in the shear rate and shear stress of the cellular injection fluid, which can affect the

viability of the injected cells. There are various challenges in the clinical application of stem cells, both for local administration and circulatory system administration^[65]. So does the appropriate choice of drug delivery prescription necessarily guarantee the clinical outcome of MSCs? Obviously not. While performing stem cell therapy, we not only need to choose the appropriate delivery method, but also need to consider the individualization of the patient at the same time. We need to consider all these factors together in order to make the most appropriate stem cell treatment plan, thus improving the efficiency of MSC treatment^[67]. Different sources of MSCs have their own advantages and disadvantages in terms of therapeutic efficacy, and one of the major dilemmas that we are facing now is the production of stable MSCs at the production site, which is one of the major issues that we need to explore further^[68]. Current research allows us to understand the current potential of MSCs for cell transplantation, tissue engineering and cell-based therapies to improve the lives of those affected by disc injuries^[65]. This requires us to deepen our understanding of MSCs, refine therapeutic approaches, and address the challenges of translating research findings into clinical practice. We can do this by further optimizing the sources of MSCs^[69], delivery methods and timing of interventions, as well as standardized protocols for isolation, expansion and characterization. Conducting well-designed clinical trials will help evaluate the safety, efficacy and long-term outcomes of MSC-based therapies^[70]. As the clinical application of MSCs continues to be researched both at home and abroad, stem cell therapeutic drugs are gradually being introduced, and more research teams and medical institutions are involved, this will gradually deepen the clinical application of stem cell therapy and bring hope to the majority of lumbar disc patients.

CONCLUSION

IDs have a complex structure with a unique internal environment. They contain nucleus pulposus cells, fibrous rings and ECM, which are in a dynamic balance of self-renewal. Stem cell therapy is designed to restore this balance of secreting exosomes, vesicles, mixing other substances to promote their differentiation into nucleus pulposus cells,

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