

Exosomes derived from stem cells as an emerging therapeutic strategy for intervertebral disc degeneration

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Abstract

Intervertebral disc (IVD) degenerative diseases are a common problem in the world, and they cause substantial social and economic burdens for people. The current methods for treating IVD degenerative diseases mainly include surgery and conservative treatment, which cannot fundamentally restore the normal structure of the disc. With continuous research on the mechanism of degeneration and the development of regenerative medicine, rapid progress has been made in the field of regenerative medicine regarding the use of stem cell-derived exosomes, which are active biological substances used in intercellular communication, because they show a strong effect in promoting tissue regeneration. The study of exosomes in the field of IVD degeneration has just begun, and many surprising achievements have been made. This paper mainly reviews the biological characteristics of exosomes and highlights the current status of exosomes in the field of IVD degeneration, as well as future developments regarding exosomes.

Key words: Exosomes; Intervertebral disc degeneration; Stem cells; MicroRNA; Regenerative medicine; Biological characteristic

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Core tip: This article mainly reviews the brief pathological process of disc degeneration and the biological characteristics and functions of exosomes. We highlight the current status and advancement of exosome research in the field of intervertebral disc

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degeneration, analyze the possible mechanisms, and discuss the future development of exosomes in this field.

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INTRODUCTION

Low back pain (LBP) is a common spinal health problem worldwide^[1]. In a global systematic review, the mean prevalence of LBP at a given time in the general population was approximately 18%, and the 1-year prevalence was approximately 38%^[2]. Therefore, a small reduction in health care or disability rates related to LBP could bring significant social and economic benefits^[3,4].

The causes of LBP are complex^[5,6], and although there is no direct evidence, IVD degeneration is considered a major cause^[5]. The cause of intervertebral disc (IVD) degeneration is still not fully understood, but some factors, such as aging, abnormal mechanical stress, trauma, nutritional deficiencies, and heredity, are considered to be involved in this process^[7]. The pathological process of disc degeneration includes the reduction of nucleus pulposus cells (NPCs)^[8,9] and extracellular matrix (due to decreased synthesis and increased degradation), aging of the annulus fibrosus, and calcification of cartilage endplates^[10].

Current treatments for LBP caused by IVD degeneration include invasive surgery and conservative treatment^[11], which are mainly aimed at relieving symptoms rather than changing pathogenic mechanisms. Therefore, there is an urgent need for new therapies that treat disc degeneration by directly addressing causes and mechanisms to retain and/or restore disc structure and mechanical function.

Recently, an increasing number of studies have focused on degenerated disc regeneration, including studies related to bioactive molecular injection^[12,13], cell-based therapies^[14-16], tissue engineering^[17,18], and gene therapy^[19,20]. Bioactive molecular injection is a biological therapy utilizing chemical molecules with the effect of recruitment of endogenous stem cells into the IVD or stimulation of their proliferation. Although the short-term effect is acceptable, the long-term maintenance of biological activity has become an unavoidable obstacle for this therapy. Cell-based therapies, as the most attractive method among these studies, involve the injection of extracted cells, such as NPCs or various stem cells, into the disc *in vivo* to restore IVD homeostasis following the proliferation, differentiation, and immune regulation of the transplanted cells^[21]. Although some progress has been made, the complex environment of the degenerated IVD causes a low survival rate of stem cells and makes it difficult to accurately control cell viability and differentiation. Additionally, the sources and safety issues of stem cells need to be considered. Gene therapy refers to modification of genomes to increase the expression of effector genes and promote the continuous production of one or more biologically active factors in the IVD to promote cell proliferation, extracellular matrix production, and inhibition of apoptosis.

Studies on the mechanism of stem cell therapy have provided increasing evidence that the factors that play an important role in these treatments are the exosomes that are secreted by stem cells^[22-24]. Exosomes were considered waste products from cells when they were first reported in 1983^[25]. Currently, this nanoscale cell vesicle is known to be an important substance in intercellular communication that can transfer biomolecules such as proteins and nucleic acids from parent cells to recipient cells. Their applications in regenerative medicine are also increasing, including in the regeneration of NPCs and the maintenance of disc homeostasis^[26-28]. This paper reviews the biological characteristics of exosomes and their research status in the field of disc degeneration, and gives outlook on their future applications in this field.

BIOLOGICAL CHARACTERISTICS AND FUNCTIONS OF EXOSOMES

Exosomes are a type of extracellular vesicle; the other two main types of extracellular vesicles are microvesicles and apoptotic bodies^[29,30]. The characteristics of the three main extracellular vesicles are shown in [Table 1](#).

Exosomes have spheroid membranes of a uniform lipid bilayer with diameters of approximately 30-150 nm^[31]. They typically can be detected in various body fluids, such as blood^[32,33], amniotic fluid^[34-36], breast milk^[37,38], urine^[39,40], synovial fluid^[41,42], and saliva^[43,44]. They can be transported to corresponding target cells through the body fluids to perform a specific function. *In vitro*, exosomes also have been isolated from cell culture supernatant^[45]. We now know that the mechanism of exosome formation involves the inward invagination of the endosomal membrane pathway. At the first stage, the inward budding of the plasma membrane with receptors leads to the formation of an endosome. Then, small vesicles can be formed by further inward budding of the limiting membrane to form a multivesicular body (MVB) with intraluminal vesicles. The vesicular contents in MVBs are finally degraded when MVBs fuse with the lysosome or are released into the extracellular space^[46]. After being released into the extracellular space, exosomes play a biological role when they contact another membrane and are endocytosed into a recipient cell^[46,47].

The function of exosomes mainly depends on their contents. Among the components of exosomes, lipids, proteins, and nucleic acids are the three main substances that determine the biological function of exosomes^[48,49]. Lipids in exosomes are mainly located in the membrane, including cholesterol, phosphatidylserine, sphingomyelin, etc. In addition to maintaining the biological stability of exosomes, lipids are also involved in biological processes such as the formation and release of exosomes^[50]. Exosomes are also rich in a variety of proteins, including cytoskeleton components, tetraspanins, heat-shock proteins, and other types of proteins^[51,52]. Among them, ALIX and tetraspanin proteins, such as CD81, CD9, and CD63, are markers of exosomes^[51]. However, it is difficult to distinguish exosomes from other extracellular vesicles with overlapping size and density based solely on these markers.

Exosomes usually carry nucleic acids, including mRNAs^[53], microRNAs (miRNAs)^[54,55], and long noncoding RNAs (lncRNAs)^[56]. MiRNAs are a class of endogenous noncoding RNAs found in eukaryotes that have a length of approximately 20-25 nucleotides. Mature miRNAs are produced from longer primary transcripts that undergo a series of nuclease-mediated cleavages; then, the miRNAs are assembled into RNA-induced silencing complexes by complementary base pairing to a target mRNA, which guides degradation of targets or suppresses translation of targets, based on the degree of complementarity^[57]. According to the principle of base complementary pairing, a miRNA usually can target hundreds of corresponding genes, which implies that miRNAs carried in exosomes may play an important role in regulating gene transcription in target cells. LncRNAs are a class of RNA molecules longer than 200 bp that do not encode proteins. They are widely involved in the transcription, translation, and posttranslational regulation of genes. LncRNAs can participate in chromatin modification, transcription activation, and transcription interference in cells, or they can act as "bait molecules" that interact with proteins, DNA, and RNA^[58,59]. As messengers of intercellular communication, exosomes are secreted by parent cells and taken up by target cells in the following ways: (1) Transmembrane proteins are fused to target cell membranes by binding to receptor proteins; (2) The exosomal membrane fuses directly with the cell membrane, releasing the contents; and (3) Target cells take up exosomes through endocytosis^[60,61].

THERAPEUTIC APPLICATION OF STEM CELL-DERIVED EXOSOMES IN VARIOUS TISSUES

Recently, an increasing number of studies have shown that exosomes derived from stem cells play an important role in restoring tissue homeostasis and promoting tissue regeneration.

Exosomes from bone marrow mesenchymal stem cells (MSCs) can significantly enhance bone regeneration, promote vascular regeneration, and accelerate fracture healing in a rat femur nonunion model^[62]. Exosomes from adipose stem cells promote the vascularization of endothelial cells^[63]. Human umbilical cord MSC exosomes can promote angiogenesis and repair of second-degree burn wounds of the skin^[64]. Exosomes from human stem cells can promote the repair of jaw joints and the synthesis of extracellular matrix in that tissue^[24]. Increasing experimental results show

Table 1 Characteristics of three main types of extracellular vesicles

Types of vesicles	Diameter	Markers	Cargos	Density (g/mL)	Origin	Ref.
Exosomes	30-150 nm	CD63, CD81, CD9, HSP70, Flotillin, TSG101, <i>etc.</i>	mRNA, microRNA, lncRNA, circRNA, DNA lipid, protein, <i>etc.</i>	1.13-1.18	Endosomes pathway	[51,74-77]
Microvesicles	50-1000 nm	Integrins, selectins, CD40 ligand	mRNA, microRNA, other non-coding RNA, protein, <i>etc.</i>	1.16-1.19	Plasma membrane; outward budding	[51,75]
Apoptotic bodies	500-2000 nm	Phosphatidylserine, genomic DNA	Nuclear fractions, cell organelles, <i>etc.</i>	1.16-1.28	Plasma membrane	[47,51,78]

the potential regenerative ability of stem cell-derived exosomes through their promotion of cell proliferation, enhancement of angiogenesis, promotion of extracellular matrix homeostasis recovery, inhibition of inflammation, and other unknown effects. Some of these beneficial mechanisms can also be achieved in the repair of disc degeneration.

THERAPEUTIC EFFECTS OF STEM CELL-DERIVED EXOSOMES ON IVD DEGENERATION

Stem cell transplantation for treatment of IVD degeneration has made great progress. *In vitro* and *in vivo* studies have revealed the great advantages of stem cells as seed cells for cell-based therapies. However, because of the complex and harsh *in vivo* environment of the IVD, there are obstacles to be overcome by IVD degeneration stem cell therapy approaches^[65]. With continued research into stem cell therapies, it has been found that the exosomes secreted by stem cells play an important role in their therapeutic effect^[26,66]. Therefore, exosomes have attracted more and more attention in some preclinical studies of promoting IVD regeneration (Table 2).

When exosomes derived from bone marrow MSCs were cocultured with NPCs from degenerated IVDs, cell proliferation was significantly accelerated by extending the incubation time with exosomes. Additionally, the expression of the extracellular matrix synthesis and protection genes *ACAN*, *COL2A1*, *SOX-9*, and *TIMP-1* increased with incubation time, while the degradation-related genes *MMP-1* and *MMP-3* were decreased. Therefore, it seems to indicate that MSC-derived exosomes promote the proliferation and extracellular matrix homeostasis of NPCs^[26].

Stem cell-derived exosomes not only promote the proliferation of NPCs but also inhibit their apoptosis. In a study by Cheng *et al.*^[28], human bone marrow MSCs and fibroblast-derived exosomes were used to treat TNF- α -induced apoptotic NPCs. The cells treated with the exosomes derived from the bone marrow MSC group had a significantly lower apoptotic rate than those of the other groups. *In vivo* experiments showed that the MSC-derived exosome treatment group had significantly lower Pfirrmann grade, histological grade, and apoptotic rate than the noninjection groups. Another *in vitro* study also confirmed the anti-apoptotic effect of stem cell exosomes. Liao *et al.*^[27] co-incubated exosomes from MSCs with advanced glycation end products-induced NPCs and confirmed that the levels of apoptosis-related markers caspase-3 and caspase-12 decreased significantly. With the increase in exosomal concentration, the declining trend was greater. The above studies confirmed that exosomes have significant anti-apoptotic effects both *in vivo* and *in vitro*.

The accumulation of a large number of inflammatory factors and extracellular matrix-degrading enzymes in the IVD is an important cause of NPC apoptosis and loss of the extracellular matrix^[67,68]. Xia *et al.*^[69] collected the normal nucleus pulposus from trauma patients and the degenerated nucleus pulposus and then screened 150 proteins by gene ontology and KEGG analysis, of which 69 proteins were downregulated and 81 were upregulated. Most of the proteins were associated with inflammatory responses, showing enhanced inflammatory responses in degenerative discs. By adding MSC-derived exosomes to apoptotic NPCs, the expression of IL-1 β , iNOS, COX-2, IL-6, MMP3, MMP13, and other inflammation- and extracellular matrix degradation-related enzymes was significantly reduced. *In vivo* experiments also demonstrated that the exosome injection group had significantly lower MMP13 expression at 2, 4, and 8 wk than the control group^[69].

The decrease of viable cells is a key factor in the process of disc degeneration;

Table 2 Studies on exosomes for intervertebral disc degeneration

Ref.	Experimental objective	Cargo analysis	Animal model	<i>In vitro</i> appraisalment	<i>In vivo</i> appraisalment	Inhibition test	Research type
HBMSCs; Lu <i>et al</i> ^[26]	To detect the role of exosomes derived from BM-MSCs in NPCs	Not mentioned	None	(1) Promoted proliferation; and (2) Increased synthesis of extracellular matrix and decrease in degradation	None	None	Cell experimentation
HBMSC; Cheng <i>et al</i> ^[28]	To explore the protective effect of MSC-exosomes on NPCs in a cell and rat model	Highly enrichment in miR-21	SD rat model of IVD degeneration by needle puncture	(1) Decreased apoptosis rate; and (2) Decreased cleaved caspase-3	(1) IVD degeneration score lower; (2) Decreased apoptosis rate; and (3) Lower histologic score	MiR-21 antagonist enhanced cell apoptosis	Cell and animal experimentation
Rat nucleus pulposus; Moen <i>et al</i> ^[79]	To study the role of extracellular miRNA in lumbar radicular pain	Increased miR-223	Lewis rat IVD herniation	None	MiR-223 increased after disc herniation	None	Animal experimentation
Porcine notochordal cells; Bach <i>et al</i> ^[80]	To explore the biologic effect of the NCCM-derived EVs on canine and human CLCs from degenerated IVDs <i>in vitro</i>	None	None	Increased glycosaminoglycan (GAG) deposition	None	None	Cell experimentation
HBMSCs; Liao <i>et al</i> ^[27]	To prove that the delivery of MSC-exos could modulate ER stress and inhibit excessive NP cell apoptosis during IDD	None	SD rat model of IVD degeneration by needle puncture	(1) Western blot and TUNEL assays indicated decreased apoptosis rate; and (2) Western blot and qPCR data indicated decreased reticulum stress	(1) Higher DHI; (2) Lower Pfirrmann grade; (3) Lower histological grades; and (4) Decreased apoptosis rate	Akt inhibitor LY294002; ERK inhibitor PD98059	Cell and animal experimentation
C57BL/6 mice BMSCs; Xia <i>et al</i> ^[69]	To investigate the therapeutic effect of exosomes for use as IVDD therapeutics	None	Rabbit model of IVD degeneration by needle puncture	(1) Decreased apoptosis rate; (2) Western blot and qPCR data indicated recovery of matrix homeostasis; (3) Decreased inflammatory marker expression; (4) Suppressed inflammasome; and (5) Recovery of mitochondrial-related proteins and attenuated mitochondrial dysfunction	(1) Higher DHI; (2) Lower Pfirrmann MRI grade; (3) Lower histological grades; and (4) Decreased apoptosis rate	None	Cell and animal experimentation

BMSC: Bone marrow stromal cells; IVDD: Intervertebral disc degeneration; MRI: Magnetic resonance imaging; IVD: Intervertebral disc; IDD: Intervertebral disc degeneration; MSC: Mesenchymal stem cell; HBMSC: Human bone marrow stromal cells; CLC: Cardiomyoblast-like cells; NPC: Nucleus pulposus cells.

conversely, in the process of disc regeneration, the recovery of cell numbers is the most important issue. These studies have confirmed that stem cell-derived exosomes could enhance cell proliferation and inhibit apoptosis, especially for stem cells remaining in the disc. Moreover, exosomes could also enhance the expression of the extracellular matrix in NPCs and inhibit the expression of matrix protein degrading enzymes, which is beneficial for maintaining the homeostasis of the extracellular matrix. During IVD degeneration, a large number of cytokines participate in and accelerate the degeneration of the IVD, leading to apoptosis and senescence of NPCs^[70]. Exosomes have a significant inhibitory effect on inflammation, which induces the restoration of the microenvironment for the surviving cells and reduces the disturbance of the intracellular environment. Therefore, stem cell-derived exosomes have the potential to treat disc degeneration.

POTENTIAL MECHANISM OF STEM CELL-DERIVED EXOSOMES FOR IVD DEGENERATION

With an increasing understanding of the mechanisms behind disc degeneration and with in-depth studies of exosomes, the application of exosomes in disc degeneration has achieved some new progress. This progress clearly shows the tremendous potential of exosomes in disc repair. However, the exact mechanism of how exosomes affect disc repair is still unclear.

In a study of Cheng *et al.*^[26], miRNA array hybridization and data analysis was performed to compare normal NPCs and TNF-induced apoptotic NPCs; five miRNAs (miR-18a, miR-21, miR-106b, miR-217, and miR-26a) were found at significantly lower levels in the TNF-induced NPC group than in the control group. Furthermore, only miR-21 was present in MSC-derived exosomes at higher levels than it was in fibroblast-derived exosomes. MiR-21 also decreased apoptosis and suppressed the expression of PTEN. Based on the above results, the researchers believe that the PTEN-PI3K-Akt pathway is a potential target of exosomal miR-21-mediated apoptosis protection in NPCs. However, they believe that there are still other extracellular vesicles or other components in exosomes that are involved in this procedure^[28].

Liao *et al.*^[27] proposed another possible mechanism. The endoplasmic reticulum stress-related markers GRP78 and CHOP were significantly increased in degenerated discs, and their expression positively correlated with Pfirrmann classification. Then, exosomes were added to induce NPCs, and the expression of endoplasmic reticulum-related pathways and apoptosis markers was inhibited. This means that exosomes inhibit endoplasmic reticulum stress-mediated apoptosis through AKT and ERK signaling pathways by reducing the levels of CHOP, the key molecule of endoplasmic reticulum stress^[27].

In addition to the above studies, Xia *et al.*^[69] found that 150 proteins differentially expressed in degenerative discs are closely related to enhanced inflammatory responses. Exosomes can significantly inhibit the inflammatory response of apoptotic NPCs and the formation of inflammatory bodies. The proteins found in bone marrow MSC exosomes mainly recovered the damage to mitochondria in NPCs, restored the normal structure of mitochondria, and reduced oxidative stress in mitochondria. The results indicate that exosomes can play a role in inhibiting disc degeneration by restoring mitochondrial homeostasis and the antioxidative response and inhibiting formation of inflammatory bodies^[69].

These potential mechanisms have mainly been studied in terms of promoting extracellular matrix production, inhibiting matrix degradation, promoting an anti-inflammatory response, and inhibiting apoptosis and other aspects of exosome-based promotion of IVD repair. Unfortunately, there is no study on how exosomes promote the proliferation mechanism of NPCs. One of the most important reasons for the degeneration of the disc is the decrease in the number of cells, and how to restore the number of cells in the disc is a key question in treatment. Alternatively, in the process of IVD degeneration, the senescence of NPCs is also an important factor. Some molecules have also been found in exosomes that can inhibit the senescence of cells. Previous research has mainly focused on specific miRNAs in exosome-mediated apoptosis, but are there additional miRNAs in exosomes that promote the proliferation and inhibit the aging of NPCs? Furthermore, how do exosomes inhibit inflammation and promote mitochondrial homeostasis, and how are other molecules in exosomes, including lipids, proteins, mRNAs, and lncRNAs, involved?

DISCUSSION

The unique double-layered membrane structure of exosomes makes their contents difficult to degrade by various enzymes in body fluids. The unique shape, size, and density range of exosomes, as well as the special molecular markers on their surface, enable their identification and isolation. Animal experiments have confirmed that exosomes are more efficient at delivering effective content into cells and cause a lower immune response in recipients than other methods. By overexpressing miRNAs targeting specific mRNAs in donor cells, exosomes promote cell proliferation, inhibit apoptosis, and promote the production of the extracellular matrix^[28]. Engineered exosomes can also be loaded with miRNA synthesized *in vitro* by electroporation and can then be injected into tissues to achieve therapeutic goals^[71]. Exosomes may also be combined with scaffold material to promote IVD regeneration. In a study by Liu *et al.*^[72], photoinduced imine crosslinking hydrogel glue combined with stem cell-

derived exosomes promoted defective cartilage repair and regeneration^[72]. Therefore, an increasing number of studies have shown that exosomes are a promising method for the treatment of disc degeneration. However, there are still many challenges and disadvantages.

First, the physiological environment of the IVD is complex. As the largest avascular tissue in the body, long-term internal high pressure, high permeability, low pH, low nutrition, and low oxygen make it not suitable for cell proliferation^[73]. In the degenerated IVD, the complex inflammatory environment, the decrease in the number of cells, and fibrosis may affect *in vivo* as *in vitro* results being the same. Additionally, IVD degeneration is a pathological process involving multiple factors, and the exact mechanism has not yet been determined. Therefore, choosing the appropriate exosomes for specific causes is very important.

Second, the exact mechanism of exosomal biogenesis needs to be further investigated. Exosomes are derived from endosomes after cell endocytosis. After processing, they may join multivesicular bodies (MVBs) and may contain proteins, nucleic acids, lipids, cholesterol, and other biologically active molecules; further, they may be secreted by exocytosis or may be encountered by a lysosome and become degraded^[29]. This is a complex set of biological process, and more research needs to be done to determine the specific mechanisms. An increasing number of studies have demonstrated that exosome-mediated effects are mainly due to the contents of the exosomes, such as miRNAs, lncRNAs, and other molecules. Understanding how cells assemble these molecules in exosomes will enable additional exosomes to be harvested. The isolation of exosomes also has limitations regardless of the current methods being used, such as ultracentrifugation, ultrafiltration, and chromatography. Therefore, new methods need to be developed to improve the isolation and purity of exosomes.

Moreover, exosomes, as a collection of various biologically active molecules, are also affected by various factors, such as the source of cells, the status of cell growth, the conditions of culture, and even the consistency and reproducibility of their effects. All of those factors need to be considered. As a special carrier in the treatment of diseases, the application of exosomes still faces a series of problems, such as dosage, mode of administration, and evaluation of the efficacy.

CONCLUSION

Exosomes are attracting increasing attention because of their unique structures and diverse properties. Exosomes have shown favorable possibilities during the repair of IVD, since they can promote the proliferation of NPCs, promote the homeostasis of the extracellular matrix, and inhibit cell apoptosis (Figure 1). However, the detailed mechanisms behind these activities are still unclear, so further research is needed to explore the complex regulation mechanisms, optimize the culture and transplant conditions, and perform more preclinical trials to verify the safety of exosomes.

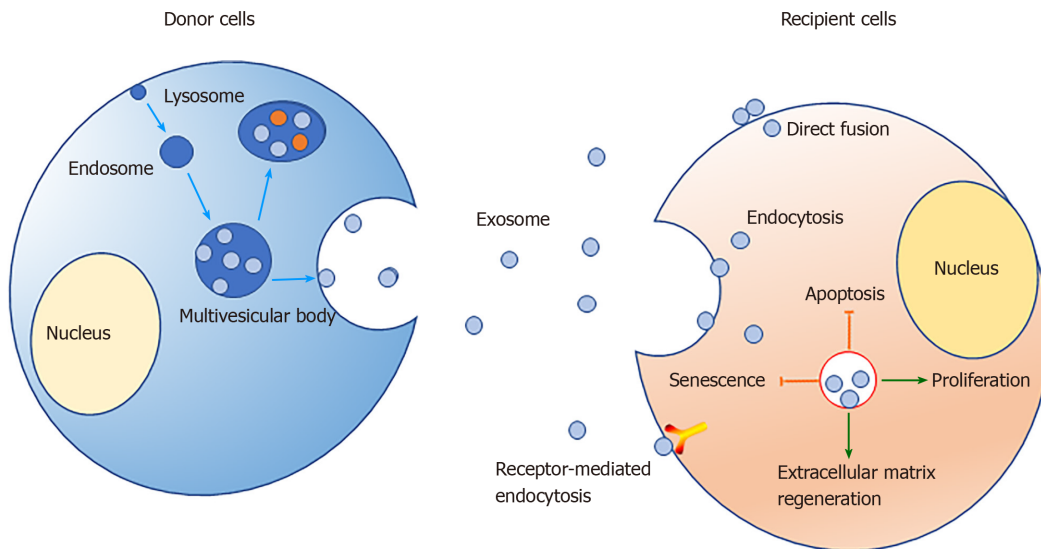


Figure 1 Exosome-mediated mechanism of stem cells regulating the activities of nucleus pulposus cells.

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