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Review

Mechanisms of endogenous repair failure during intervertebral disc degeneration

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SUMMARY

Intervertebral disc (IVD) degeneration is frequently associated with Low back pain (LBP), which can severely reduce the quality of human life and cause enormous economic loss. However, there is a lack of long-lasting and effective therapies for IVD degeneration at present. Recently, stem cell based tissue engineering techniques have provided novel and promising treatment for the repair of degenerative IVDs. Numerous studies showed that stem/progenitor cells exist naturally in IVDs and could migrate from their niche to the IVD to maintain the quantity of nucleus pulposus (NP) cells. Unfortunately, these endogenous repair processes cannot prevent IVD degeneration as effectively as expected. Therefore, theoretical basis for regeneration of the NP *in situ* can be obtained from studying the mechanisms of endogenous repair failure during IVD degeneration. Although there have been few researches to study the mechanism of cell death and migration of stem/progenitor cells in IVD so far, studies demonstrated that the major inducing factors (compression and hypoxia) of IVD degeneration could decrease the number of NP cells by regulating apoptosis, autophagy, and necroptosis, and the particular chemokines and their receptors played a vital role in the migration of mesenchymal stem cells (MSCs). These studies provide a clue for revealing the mechanisms of endogenous repair failure during IVD degeneration. This article reviewed the current research situation and progress of the mechanisms through which IVD stem/progenitor cells failed to repair IVD tissues during IVD degeneration. Such studies provide an innovative research direction for endogenous repair and a new potential treatment strategy for IVD degeneration.

Introduction

Low back pain (LBP) is one of the most common diseases in the musculoskeletal system[1–3]. About 70% of adults will suffer low back or neck pain at least once during their lifetime, and the majority of patients will lose their labor capacity[4,5]. Therefore, such disorders cause significant economic losses worldwide each year[6–8]. LBP imposes a great threat on the quality of human life and requires significant medical resources[9–11].

Intervertebral disc (IVD) degeneration is one of the major causes of LBP[12,13]. An important composition to maintain the IVD biomechanical performance is the nucleus pulposus (NP), which undergoes cellular and extracellular matrix (ECM) changes during IVD degeneration at the early pathological stage[4]. Because of the poor self-repair ability of NP, it is difficult to reverse the IVD degeneration[5]. At present, it is a challenge to repair or regenerate the structure and functions of IVD via conservative or surgical treatments, as there are still many complications and problems in terms of long-term curative effects[14,15].

As one of the core elements of biological treatments, stem cells play a significant role in the repair of IVD degeneration[5,16]. The theory of the stem cell based biological treatment is to recruit endogenous IVD stem/progenitor cells and/or supplement exogenous mesenchymal stem cells (MSCs) to the IVD tissues, and then induce the differentiation of stem cells into IVD cells and/or protect the IVD cells, so as to increase the quantity and quality of IVD cells for the repair and regeneration of the degenerative IVD[17]. However, endogenous IVD stem/progenitor cell based endogenous repair processes fail to prevent IVD degeneration, which has been

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indicated as a major cause of IVD degeneration[5,14,18]. The latest research suggests that IVD stem/progenitor cells play a crucial role in endogenous IVD repair, and how to maintain a sufficient number of stem cells with vitality under adverse microenvironments in the IVD, such as compression and hypoxia, is an important issue needing to solve[19,20].

Potential of IVD stem/progenitor cells for IVD degeneration repair

Previous studies have isolated, cultured, and identified IVD progenitor cells in the NP, annulus fibrosus (AF) and cartilage endplate (CEP), and stem cells in stem cell niche areas (perichondrium areas outside of the epiphyseal plate)[21–35], which express a series of MSC markers including OCT3/4, CD90, and stromal cells antigen-1. They are highly homologous, and their migration route from the stem cell niche to the IVD has been reported by several studies[27,36,37] [Fig. 1(A)]. In general regeneration processes, stem cells from stem cell niche areas migrate towards AF and NP to regenerate IVD tissues [Fig. 1(A)]. We found that there were also some progenitor cells in the AF around the CEP (Supplementary Fig. 1), and it suggested that stem cells from stem cell niche areas presumably could migrate towards the AF through CEP (Fig. 1). While in the degenerative IVDs, the migration ability and the number of IVD stem/progenitor cells were negatively affected by the severe adverse IVD microenvironments (Fig. 1(B) and supplementary reference). Therefore, according to the findings described above, these stem/progenitor cells from different parts of IVD could be regarded as the same cells (IVD stem/progenitor cells) under different microenvironments or at different differentiation stages during migration towards the IVD[24,38].

Stem cells used in biological therapies for IVD degeneration can be divided into two categories: exogenous MSCs and IVD stem/progenitor cells (endogenous stem/progenitor cells)[1,39], and the theory according to which the stem cells repair IVD degeneration has been clarified above. And many research groups have achieved encouraging progress in IVD degeneration treatment by tissue engineering techniques based on exogenous MSCs[40–42]. However, there is a great challenge for the two methods to maintain the number and viability of stem/progenitor cells in IVDs under adverse IVD microenvironment, including mechanical loading, hypoxia, acidity, hypertonicity and nutrient deficiency[14,43].

Encouragingly, it has been demonstrated that the quantity and quality of stem/progenitor cells in IVDs is maintained by promoting the migration of endogenous stem/progenitor cells into IVDs[37]. Migration of stem cells into damaged tissue is a dynamic process regulated by a series of complicated mechanisms, including cytokines and their receptors, interactions between cells, intracellular signal transduction, and even interactions between intracellular structures such as mitochondria and the cytoskeleton[44]. NP cells can secrete factors that induce MSC chemotaxis, such as hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor, stromal cell derived factor-1 (SDF-1), and chemokine C–C motif ligand (CCL) 5[45–47]. In return, stem cells respond to chemokines and migrate towards the damaged tissue. In 2014, Pattappa *et al.*[47] reported that chemoattractants could be released and active exogenous MSCs migration towards induced degenerative IVDs, which suggests that endogenous IVD stem/progenitor cells could be recruited by chemoattractants and exhibit therapeutic potential for IVD degeneration. However, stem cells in stem cell niche areas and the recruited IVD stem/progenitor cells still have to suffer from the adverse IVD microenvironments (such as compression and hypoxia) and may undergo excessive cell death.

Therefore, further studies are needed to determine how adverse microenvironments affect the cell death and the chemo–attractive activity of IVD stem/progenitor cells, and reveal how stem/progenitor cells interact with other IVD cells and cope with the adverse microenvironments. And these further researches may elucidate a novel strategy and stimulate the potential of IVD stem/progenitor cells to repair degenerative IVDs.

Autophagy, apoptosis, and necroptosis of stem cells under adverse microenvironments

Under adverse microenvironments (such as compression and hypoxia), IVD stem/progenitor cells undergo excessive cell death, which is the major reason for the difficulty to maintain the number and viability of stem/progenitor cells in IVDs[48–50]. Our research group has systematically reported the effect of compression at the magnitude of 1 MPa on autophagy, apoptosis, and necroptosis of NP

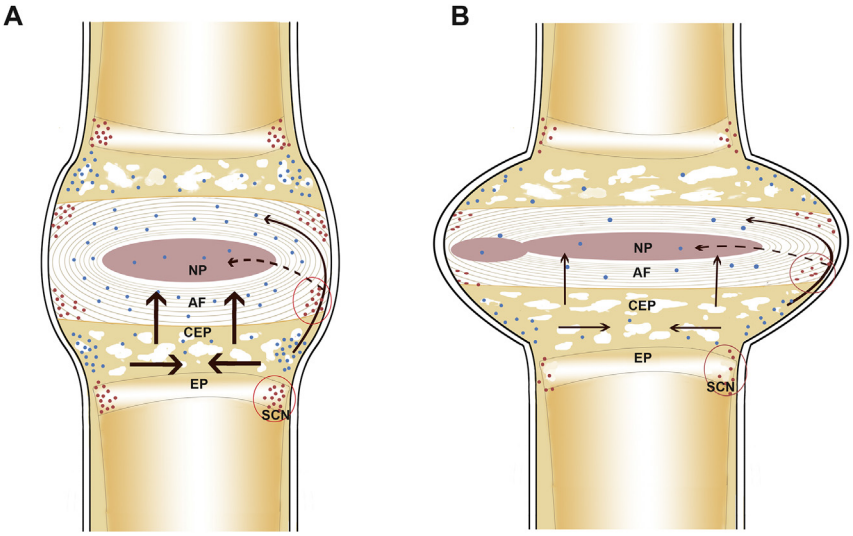

Fig. 1. Diagram of the stem cell niche and migration route of the stem/progenitor cells in an IVD[27,36,37]. (A) Diagram of the non-degenerative IVD. (B) Diagram of the degenerative IVD. (NP: nucleus pulposus; AF: annulus fibrosus; CEP: cartilage endplate; EP: epiphyseal plate; SCN: stem cell niche. The red dots indicated the stem cells in the SCN, the blue dots indicate the progenitor cells outside of the SCN, and the arrows indicate the potential migration routes of stem/progenitor cells).

cells in IVDs[51–54]. Although a large number of studies have reported that NP-derived progenitor cells exhibit higher tolerance to acidic and hypertonic microenvironments than exogenous MSCs[14,48,55], few studies have investigated the survival, death, and response mechanism of IVD stem/progenitor cells under compression and hypoxia.

Autophagy is an evolutionarily conserved and lysosome-dependent catabolic pathway essential for the homeostasis of stem cells[56–58]. Our previous study showed that compression-induced NP cell damage could be suppressed by activating autophagy through removing the damaged mitochondria and reducing cell reactive oxygen species (ROS) production, whereas excessive compression (1 MPa lasted for more than 36 h) induced apoptosis and autophagic death through caspase-dependent mitochondrial pathways[51,53]. Furthermore, hypoxia (1% O_2) could balance between cell survival and death by activating autophagy through the SCF/c-kit pathway to improve the self-renewal of MSCs[59]. Regrettably, there have been no studies to reveal the protective effect and regulatory mechanism of autophagy in IVD stem/progenitor cells under compression or hypoxia.

Apoptosis is gene-controlled programmed death that sustains homeostasis[60,61]. Both compression (1 MPa) and hypoxia (2% O_2) stimuli could induce apoptosis of NP-derived progenitor cells[49,62]. For example, it was reported that hypoxia (22–24 mm Hg) could induce apoptosis of MSCs through the mitochondrial pathway[63]. Our previous study also showed that compression at the magnitude of 1 MPa could induce apoptosis of NP cells through caspase-dependent mitochondrial pathways, which correlated with the compression-induced autophagy[51,53]. These results indicate that a correlation between autophagy and apoptosis may exist in IVD stem/progenitor cells, but the mechanism is unclear (Fig. 2).

Necroptosis is a form of programmed cell death, which exhibits a necrosis-like morphology[64–66]. The effect of necroptosis on IVD stem/progenitor cells under adverse microenvironments has not been studied. N-acetylcysteine and ascorbate-2-monophosphate could protect mitochondria to improve the survival rate of transplanted MSCs by decreasing necroptosis[67]. We found that necroptosis was also involved in compression-induced NP cell death by examining compression-induced cell death (at the magnitude of 1 MPa)[54,68]. Whether necroptosis plays the analogous role in IVD stem/progenitor cells needs further investigation.

The survival rate of MSCs can be increased by regulating autophagy and apoptosis[58,69]. Our previous studies showed that autophagy, apoptosis, and necroptosis were all involved in compression-induced NP cell death, and there was a crosstalk among them[53,54]. The inhibitor of necroptosis necrostatin-1 remarkably down-regulated compression-induced NP cell autophagy, while the inhibitor of autophagy 3-methyladenine had no significant effect on the level of necroptosis. There was a waxing and waning phenomenon between autophagy and apoptosis (Fig. 2). These data demonstrate that a single intervention in one of the mechanisms cannot long-lastingly or effectively increase the survival rate of IVD stem/progenitor cells under adverse microenvironments. Hence, it is necessary to comprehensively research the three mechanisms involved in cell death in IVD stem/progenitor cells, so as to seek proper regulatory targets to decrease the number of IVD stem/progenitor cell death under adverse microenvironments.

Recently, increasing evidences have demonstrated that the number of IVD stem/progenitor cells declines significantly with age and the degree of degeneration[13,70,71]. And it was reported that NP-derived progenitor cells harvested from old rats displayed senescent features and presented decreased proliferation ability, compared to the progenitor cells harvested from young rats[33]. These results suggested that the number and viability of IVD stem/progenitor cells could be affected by age, and the cellular senescence might be one of the reasons for the endogenous repair failure and could be used as a novel therapeutic target for IVD degeneration.

Correlation of stem cell migration with IVD microenvironments

Decreased migration of IVD stem/progenitor cells into IVDs may be another important reason for the declining number of IVD stem/progenitor cells under adverse microenvironments. However, migration ability of stem cells can be affected by different conditions and even by the same conditions on different scale under altered microenvironments.

For example, hypoxia (2.2% O_2) could promote proliferation as well as migration of MSCs through the HIF-1α/FASN/mTORC1 pathway[72]. In contrast, the migration ability of MSCs dramatically decreased in a severe hypoxic microenvironment (1% O_2) via the

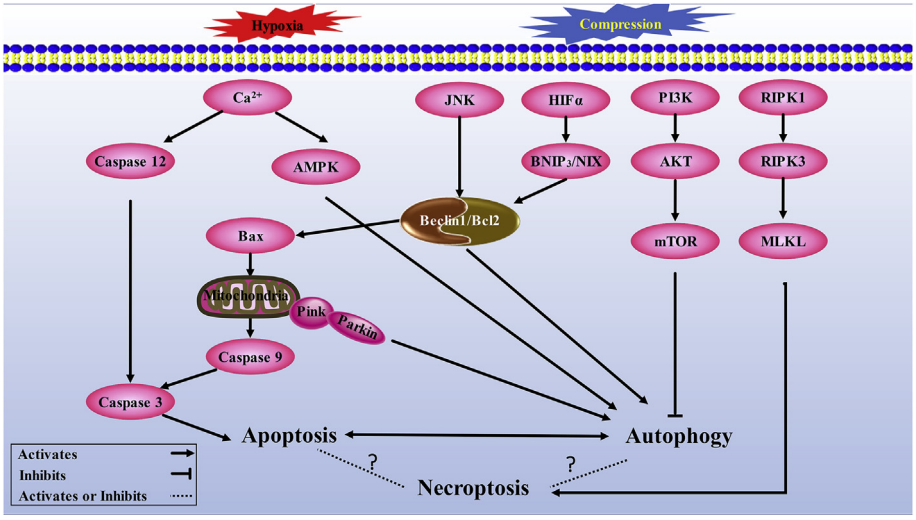

Fig. 2. The possible signaling pathways of IVD stem/progenitor cells death under compression and hypoxia. Under adverse microenvironments (such as compression and hypoxia), NP cells and other IVD cells underwent cell death (apoptosis, autophagy or necroptosis), and several signaling pathways involved in it, including caspase-dependent apoptosis pathway[51], JNK pathway[109], BNIP3/NIX pathway[109], PI3K/AKT pathway[110], RIPK1/RIPK3/MLKL pathway[54] and cross-talks among them[52,53]. Therefore, it can be speculated that the same adverse microenvironments may induce IVD stem/progenitor cells apoptosis, autophagy or necroptosis through these signaling pathways. And further studies are needed to verify the hypotheses, which contribute to better understanding of endogenous repair failure during IVD degeneration.

regulation of GTPase RhoA activity[73]. In IVDs, oxygen concentrations fall towards the disc center due to the avascular structure and the distribution of oxygen are very variable in the center of nucleus[74]. Therefore, IVD stem/progenitor cells in different anatomic regions are exposed to different levels of oxygen concentration, and their migration abilities may be affected by hypoxia in varying degrees. However, there has been no study to clarify the correlation between hypoxia and migration in IVD stem/progenitors.

The BrdU labeling technique has been adopted to study the effect of continuous pressure on stem cell migration in a controllable axial loading-induced rabbit lumbar disc degeneration model (Supplementary reference). It revealed that the number of stem cells migrating to the IVD increased dramatically in the mild pressure group (19.6 N), but decreased distinctively in the severe pressure group (78.4 N). These studies indicate that stem cells may migrate to the IVD to repair the IVD under mild adverse microenvironments, such as mild hypoxia and compression microenvironments. And the endogenous repair behaviors can be interrupted or even fail when stem cells are exposed to severe hypoxia and excessive compression[37]. Hence, studying the recruitment mechanisms of stem cells in degenerative IVDs and the interference mechanisms of stem cell migration under adverse microenvironments may contribute to better understanding of endogenous repair failure during IVD degeneration.

Increased cell death and decreased migration into IVDs are the two main mechanisms of declining number of IVD stem/progenitor cells under adverse microenvironments, and there may be a positive correlation between them. The intimate connection between migration and autophagy in stem cells has been wildly confirmed in other tissues[75,76]. For example, an autophagy activator facilitates the migration of dental pulp stem cells, while an inhibitor significantly suppresses SDF-1α-induced migration[77]. Moreover, noncoding RNAs have been reported to participate in both migration and cell death in other tissues and have been recognized as important regulators of IVD degeneration in recent years[76,78–80]. Then it can be speculated that miRNAs may play a crucial role in the regulatory mechanisms of the correlation between cell death and migration of IVD stem/progenitor cells. Therefore, studies to clarify the correlation between cell death and migration of IVD stem/progenitor cells, as well as the corresponding signaling pathways and miRNAs involved should be performed, which may provide new therapeutic targets for IVD degeneration.

Interactions between exogenous MSCs and NP cells

IVD stem/progenitor cells, which migrate into IVDs, should not only adapt to the IVD microenvironment, but also communicate with NP cells inside the IVD. But, there are few studies related to the interactions between IVD stem/progenitor cells and NP cells. Previous study showed that NP cells induced MSC differentiation into NP-like cells in a co-culture system[81]. And some studies indicated that adipose-derived MSCs could communicate with NP cells under mechanical loading *in vitro*, and NP cells promoted adipose-derived MSCs to differentiate into NP-like cells and synthesize more collagen, proteoglycans, cytokines and enzymes[82,83]. And it was demonstrated that MSCs influenced NP cells via paracrine manner, cell contact, and cell fusion, promoting NP cells to secrete cytokines that induced the differentiation of stem cells to NP-like cells, and these cytokines could also facilitate NP-like cells to repair IVD degeneration[84,85]. It was also reported that NP cells could secrete exosomes to induce bone marrow-derived MSCs differentiation to NP-like cells and promote bone marrow-derived MSCs migration[86].

Currently, how to guide stem cells in their original location, the stem cell niche, to migrate into the IVD and differentiate to NP-like cells has been a research hotspot. There are many factors related to chemotaxis, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, SDF-1, monocyte chemotactic protein-1 (MCP-1), insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β, platelet derived growth factor (PDGF), fibroblast growth factor-2 and macrophage-derived protein[87]. TNF-α can enhance the effects of PDGF, TGF-β, IGF-1, SDF-1, MCP-1 and CCL5/RANTES on MSCs, and induce stem cell migration into damaged tissues[88–90]. And chemokine receptors on the cell surface can be classified into two categories: CXC and CC receptors. These chemokine receptors interact with various chemokines, such as the interactions between CXCR4 and SDF-1, and CCR1/3/4/5 and CCL5, to induce stem cell migration[6,46]. It is still unclear which kinds of chemokine receptors are expressed on the surface of IVD stem/progenitor cells. An *in vitro* study showed that IVD cells might express chemokines that induced MSC migration into IVD tissues[91]. Another study showed that NP cells expressed more CCL5 during degenerative changes of IVDs[92]. Therefore, CCL5 may play an important role in the early stage of IVD degeneration, and increased secretion of CCL5 from NP cells in IVDs may attract more stem/progenitor cells[47]. However, the approach to regulate the secretion function of NP cells to increase the number of stem/progenitor cells needs further study.

It was generally accepted that MSCs repaired the damaged tissues mainly by differentiating into specific IVD cell types and replacing the lost IVD cells. But some studies demonstrated that stem cells secreted cytokines and growth factors that could influence NP cells, such as TGF-β and IGF-1 to inhibit NP cell senescence[93], IGF-1 and bone morphogenetic protein (BMP)-7 to prevent NP cell apoptosis[50], and TGF-β and BMP-7 to reduce decomposition of the cell matrix and inhibit inflammation in IVDs[85,94]. And it was reported that adipose-derived MSCs inhibited the apoptosis of NP cells by down-regulating the expression of caspase-3 and -9 in NP cells, and promoted NP cells to produce more ECM and suppressed the expression of matrix metalloproteinases, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) as well as pro-inflammatory factors, which could induce NP cells to express tissue inhibitors of metalloproteinases (TIMPs), cytokeratin 8, and other cytokines[40,81,95]. These studies showed a brand new mechanism of cellular interaction between MSCs and IVD cells, and suggested that MSCs might repair the degenerative tissue by protecting damaged IVD cells. Whether IVD stem/progenitor cells play a similar role in degenerative IVD requires further studies in the future. Based on these studies, we put forward two hypotheses: (1) The interaction between IVD stem/progenitor cells and NP cells has an important regulatory function in IVD stem/progenitor cell migration and differentiation. (2) The interaction between IVD stem/progenitor cells and NP cells can be regulated. Regulating the secretion of CCL5 and other chemotactic factors may increase the number of stem/progenitor cells migrating into the IVD. Regulating the secretion of TGF-β and other factors may induce IVD stem/progenitor cells to differentiate to NP-like cells.

Constructing a tissue engineering matrix material with a suitable structure and biological activity for NP cells and IVD stem/progenitor cells

To maintain the number of stem/progenitor cells in the IVD and improve the efficiency of IVD stem/progenitor cells in IVD degeneration treatment, creating the microenvironment that is conducive to the stem/progenitor cells growth is needed. Therefore, constructing a tissue engineering matrix material with a suitable structure and biological activity, and providing an appropriate stromal environment for IVD stem/progenitor cells are two important methods of effective treatments for IVD degeneration[96–100]. Polypeptide self-assembled nanofiber gels are one of the latest materials in tissue engineering[42,99,101]. They have been

used for three-dimensional culture of cells, repair of defective tissue, and drug/cytokine release. Studies have shown that functional materials possessing similar structure/properties to the target tissues, such as mechanical strength and the ultrastructure, are more beneficial for the biological functions of cells[42,100]. In addition to the advantages of traditional materials (such as modeling of natural ECM nanomaterials, high-density functional epitopes, biodegradability, non-toxicity and immunogenicity), peptide hydrogels have the same mechanical strength as NP tissue[102]. Therefore, self-assembled nanofiber hydrogels with polypeptides may be the most promising scaffold material for NP tissue engineering. Recent studies have shown that the peptide self-assembly hydrogel intensity and its molecular skeleton sequence are closely related[35]. Whether the skeleton sequence of self-assembled peptide molecules can be changed to increase the strength of self-assembled hydrogels or not and the construction of polypeptide biomimetic materials that are more suitable for NP tissue engineering are the focuses of future research.

Although stem cells themselves can migrate into the IVD, their migration efficiency and rate are relatively low, and they have to suffer the adverse IVD microenvironments. The bottleneck of endogenous repair for IVD degeneration is that it is difficult to provide continued sufficient numbers of IVD stem/progenitor cells with high activity. Studies have shown that cytokines can regulate stem cell migration[103,104]. SDF-1α is a growth factor secreted by stromal cells with excellent biological functions[105–107]. SDF-1α can not only induce stem cell migration, but also can interact with proteoglycans to positively regulate SDF-1α functions[108]. Therefore, more IVD stem/progenitor cells can be recruited into the IVDs to repair early disc degeneration by transplanting hydrogel material loaded with chemokines or other active molecules, which may help them adapt to the IVD environment better and maintain a healthy condition and high activity. Hydrogel-loaded with chemokines and other signaling molecules may be the future trend of tissue engineering.

Conclusion

In summary, IVD degeneration is a complex process and endogenous stem/progenitor cell based treatment for IVD degeneration is a promising approach. Further studies are needed to clarify the mechanisms of endogenous repair failure by studying the correlations among IVD stem/progenitor cell death, migration and IVD microenvironments, and the interactions between IVD stem/progenitors and NP cells. Then, biomimetic peptide biomaterials loaded with signaling molecules related to the mechanisms can be designed to improve the migration and survival of IVD stem/progenitor cells, promote the regeneration of NP tissue *in situ*, and repair the degenerative IVDs. It can be expected that these studies will provide a theoretical basis for early treatment of IVD degeneration and create new treatment strategies.

Authors' contributions

Zengwu Shao and Liming Xiong contributed to the conception and design of this review article. Kaige Ma and Sheng Chen performed searches, analyses, and interpretations. Zhiliang Li and Xiangyu Deng drafted the paper, Donghua Huang substantially revised the paper. Zengwu Shao and Liming Xiong gave final approval of the version to be submitted.

Conflicts of interest

None.

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Supplementary data

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