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Review Article

The effects of microenvironment in mesenchymal stem cell–based regeneration of intervertebral disc

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Abstract

BACKGROUND CONTEXT: Recent studies have demonstrated new therapeutic strategy using transplantation of mesenchymal stem cells (MSCs), especially bone marrow–derived MSCs (BM-MSCs), to preserve intervertebral disc (IVD) structure and functions. It is important to understand whether and how the MSCs survive and thrive in the hostile microenvironment of the degenerated IVD. Therefore, this review majorly examines how resident disc cells, hypoxia, low nutrition, acidic pH, mechanical loading, endogenous proteinases, and cytokines regulate the behavior of the exogenous MSCs.

PURPOSE: To review and summarize the effect of the microenvironment in biological characteristics of BM-MSCs for IVD regeneration; the presence of endogenous stem cells and the state of the art in the use of BM-MSCs to regenerate the IVD in vivo were also discussed.

STUDY DESIGN: Literature review.

METHODS: MEDLINE electronic database was used to search for articles concerning stem/progenitor cell isolation from the IVD, regulation of the components of microenvironment for MSCs, and MSC-based therapy for IVD degeneration. The search was limited to English language.

RESULTS: Stem cells are probably resident in the disc, but exogenous stem cells, especially BM-MSCs, are currently the most popular graft cells for IVD regeneration. The endogenous disc cells and the biochemical and biophysical components in the degenerating disc present a complicated microenvironment to regulate the transplanted BM-MSCs. Although MSCs regenerate the mildly degenerative disc effectively in the experimental and clinical trials, many underlying questions are in need of further investigation.

CONCLUSIONS: There has been a dramatic improvement in the understanding of potential MSC-based therapy for IVD regeneration. The use of MSCs for IVD degeneration is still at the stage of preclinical and Phase 1 studies. The effects of the disc microenvironment in MSCs survival and function should be closely studied for transferring MSC transplantation from bench to bedside successfully.

Keywords: Mesenchymal stem cell; Intervertebral disc; Degeneration; Microenvironment; Regeneration

Introduction

Low back pain (LBP) affects approximately 70% of the adult population at least once in their lives and has become a major cause of disability and suffering worldwide [1]. There are obviously many causes of LBP, but intervertebral disc (IVD) degeneration is one of the major reasons [2,3]. Currently, there is no satisfactory way of prevention or reversal of degeneration, and treatment approaches are mostly salvage strategies.

The IVD comprises three regions: the bony and cartilaginous end plates (EPs) sandwiching the central nucleus

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pulposus (NP) and the peripheral annulus fibrosus (AF). Human EP is about 0.6 mm in thickness and resembles articular cartilage with the function to transport nutrition from the vascular vertebral body to the IVD [4,5]. The NP lies between the adjacent EPs and forms the hydrogel-like core of the disc, whose primary component is proteoglycans (PGs), in particular aggrecan [6,7]. There are also randomly organized collagen II, collagen VI, and elastic fibers [8]. The AF surrounds the NP and comprises between 15 and 25 concentric rings of highly organized collagen fibers that are mainly collagen I (up to 80%), collagen II, and collagen III [8].

The normal IVD is a relatively acellular tissue with the average cell density of 5.8×10^3 cells/mm^3 (NP is 4×10^3 cells/mm^3 and AF is 9×10^3 cells/mm^3, respectively) that decreases significantly with age [9,10]. The morphology of NP cells is round, phenotypically similar to articular chondrocytes, and that of the AF is thin, morphologically and phenotypically similar to fibroblasts [11]. Interestingly, the phenotypes of these two cell types are interchangeable depending on the culture system that strongly affects the gene expression of aggrecan and collagen II [12]. Many researches have focused on identifying specific markers that could distinguish NP cells from their closely related articular chondrocytes, such as hypoxia-inducible factor (HIF)-1α [13], keratin 19 (KRT19), glypican 3 (GPC3) [14], paired box 1, and forkhead box F1 [15]. Further comprehensive investigations are still required to explore specific and universally accepted markers for NP cells.

In a healthy IVD, there is a balance between the anabolic and the catabolic processes that are regulated by mechanical loading, enzymes, cytokines, and endogenous cells [16–18]. During the course of IVD degeneration that is believed to be caused by aging, trauma, excessive mechanical loading, and other diseases, changes in IVD morphology, matrix composition, and microenvironment have been observed. These changes include increased cell senescence and death, less production of PGs and collagen II, increased proteinases and cytokines, and acidic pH [19,20]. These events when combined with the biochemical and biophysical changes in a degenerating IVD will present an unfavorable microenvironment for the endogenous cells and cells to be introduced into the disc in cell-based therapy.

Intervertebral disc degeneration commonly involves changes in disc morphology and composition of extracellular matrix (ECM) as well as a loss of disc cells [19]. The decrease in NP cells seems to be the trigger of disc degeneration and, therefore, replenishing autologous cells could be a possible solution to decelerate further disc degeneration in vivo [21]. However, invasive procedures are usually used to obtain NP tissues for cell expansion, which has only a small number of endogenous cells [9]; additionally, it has been found that removal of NP tissues could induce degenerative diseases [22]. These main limitations balk this regenerative strategy with autologous NP cells, and it is more prone to select ethically conducive and easily accessible sources of graft cells for disc regeneration. Because mesenchymal stem cells (MSCs) provide a nearly unlimited cell source with self-renewal capability and multilineage differentiation potential, they have become the excellent graft cells for IVD regeneration [23] and those isolated from bone marrow (bone marrow–derived MSCs [BM-MSCs]) are currently the most popular candidate stem cells with encouraging results.

However, how the complex microenvironment regulates the survival and function of BM-MSCs is not clearly understood; most of the current studies focused on the outcomes of MSC transplantation. Additionally, the endogenous stem cells inside the IVD have been regarded as attractive seed cells and may perform better than BM-MSCs for IVD regeneration, but whether true stem cells exist in the IVD remains controversial. Therefore, the purpose of this review is threefold: to discuss the presence of stem cells inside the IVD; to elaborate how the components of microenvironment affects MSCs, especially those from bone marrow; and to summarize current advances regarding mechanisms of BM-MSC transplantation for IVD degeneration in vivo as well as their clinical application.

Methods

The electronic databases of MEDLINE were searched for English-language articles published from inception to May 2012 by Y-CH: multiple and specified terms to structure and function of IVD, to stem/progenitor cells harvested from NP and EP, to the role of components of IVD microenvironment in MSCs' behavior, and to use BM-MSCs for IVD regeneration. This resulted in a total number of 137 included articles.

Results

Are there resident stem cells in the IVD?

Most of the stem cells used for IVD regeneration experiments are from non-IVD tissues, such as bone marrow, adipose tissue, and synovium [24–26]. In the recent years, it is suggested that there are stem or progenitor cells residing in the IVD with the following supporting reasons. First, the intact IVD is generally avascular and aneural, but fibrocartilage-like tissue [27], calcification [28], and nerve and blood vessel growth [29,30] were often found in the degenerated disc. They suspected that these pathological tissues could have originated from resident stem or progenitor cells. Second, during the process of degeneration, cell cluster formation was evident inside the NP, which also indicated the existence of a replicating progenitor cell population in the IVD [31]. Another reason, application of autologous disc cells was beneficial for the disc regeneration [21]; this regenerative effect may be related to stimulate the

resident disc progenitor cells to assist the regeneration and the function maintenance of the IVD [24].

Some researches have demonstrated that IVD contained an endogenous stem cell population. Risbud et al. [32] described that human degenerated IVD (both NP and AF) contained a heterogeneous population of skeletal progenitor cells that were positive for CD105, CD166, CD63, CD49a, CD90, CD73, and CD133 and negative for CD34; when stimulated by the specific mediums, they displayed osteogenic, adipogenic, and chondrogenic characteristics. Moreover, these progenitor cells that are isolated from the IVD shared the majority of the characteristics with BM-MSCs [33]. Recently, progenitor/stem cells were further determined from the degenerative cartilaginous EP; although they shared similar phenotype and morphology with BM-MSCs, these cells possessed better osteogenic and chondrogenic differentiation capacity [34]. On the contrary, there are other factors that argue against the possibility of their being true MSCs. First, the cells isolated from the degenerated IVD have defective adipogenic differentiation ability; second, if MSCs could be isolated from the degenerated IVD, then they should also be retrievable from the normal IVD; and unfortunately, the supporting evidence is lacking. Meanwhile, in the AF border to ligament zone, cells expressing stem cell markers were detected [35], and recently reports emerged that AF cells obtained from the nondegenerated IVD of adolescent patients with idiopathic scoliosis could differentiate into adipocytes, osteoblasts, chondrocytes, neurons, and endothelial cells in vitro, and they expressed several phenotypes similar to that of MSCs [36]. This suggests that normal AF may be a source of stem cells.

If progenitor or stem cells are truly resident in the normal and degenerated IVD, then they will provide a new tool for IVD regeneration. But whether these progenitor cells can be helpful in reconstructing a normal IVD in vivo is still unknown. In the past decade, exogenous stem cells, especially BM-MSCs, have commonly used for IVD regeneration with promising results; more recently, clinical application of BM-MSCs for IVD regeneration has been reported. Nevertheless, for the purpose of regenerating a degenerating IVD with BM-MSCs, the first question to address is whether the BM-MSCs can survive in the inhospitable microenvironment and stay active in proliferation, differentiation, production of PGs to contribute to the final improvement of biomechanics, and kinematics of the spinal segments.

BM-MSCs in the microenvironment of IVD

Translation of BM-MSC therapy into a multimodal protocol for IVD degeneration requires not only the survival of these cells but also their ability to function normally amidst the harsh microenvironment of hypoxia, low nutrition, acidic pH, high mechanical loading, high osmolarity, and a complicated protease and cytokine network [37,38]. Furthermore, the resident NP cells play an interactive role in regulating the behavior of exogenous BM-MSCs.

"NP-like" differentiation and coculture with NP cells

The major purpose of MSC-based therapy is to repair, maintain, and enhance the function of a particular cell type of the disc in the long term. It was shown that BM-MSCs could differentiate into cells with an ''NP-like'' phenotype, and complementary DNA array revealed that many genes relevant for ECM components were expressed equally both in native IVD tissue and induced BM-MSCs [39]. Hypoxia (2% O_2) further synergized this transforming growth factor (TGF)-β–induced NP-like differentiation of BM-MSCs via the activation of mitogen-activated protein kinase pathway [40]. Ehlicke et al. found that in addition to TGF-β3, insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), and platelet derived growth factor BB also possessed the ability to induce differentiation of human BM-MSCs into NP-like cells [17]. Despite the lack of specific NP marker proteins to discriminate between NP cells and chondrocytes, this differentiation capacity made BM-MSCs the attractive graft cells for IVD regeneration.

Coculture of BM-MSCs with disc cells or the whole IVD in vitro was used to investigate the compatibility of BM-MSCs in IVD. Richardson et al. [41] showed sharp increase in the expression levels of genes encoding SOX9, aggrecan, collagen I, collagen II, and collagen VI in BM-MSCs after a 7-day cell-cell contact with NP cells at 75:25 ratio, and this coculture system yielded the greatest increase in ECM production on Day 14 [42]. In addition, direct cell-cell contact with autologous human BM-MSCs enhanced the proliferation and DNA and PG synthesis of NP cells [43]. Moreover, budding and spontaneously forming satellite pellets were observed after coculture of human BM-MSCs and bovine NP cells, in which BM-MSCs stayed inside the satellite pellets whereas NP cells stayed outside [44]. More recently, bidirectional membrane transfer between BM-MSCs and NP cells during direct coculture was noted that may provide a new mechanism for the interaction of these two cells [45]. These results suggested that paracrine signaling and interaction between these two cells benefited both the biological activities of NP cells and the differentiation ability of BM-MSCs [46]. Furthermore, when human BM-MSCs were inserted into the bovine caudal NP tissue, they differentiated spontaneously into chondrocyte-like cells that were positive for the expression of SOX9, collagen II, and aggrecan [47]. In vitro evidence also showed that rat BM-MSCs had the ability to differentiate into NP-like cells after coculture with intact IVD tissue [48]. These coculture systems highlighted that NP tissue and intact IVD tissue were sufficient to induce the chondrogenesis of BM-MSCs. All these evidences suggest that coculture system is a powerful tool for IVD regeneration, not only to differentiate the graft cells into NP-like cells but also to generate new ECM.

Hypoxia and low nutrition

To a large extent, the therapeutic potential of MSCs is always investigated under a traditional 20% O_2 (normoxia) condition in vitro. Actually, the average physiological oxygen tension in human is about 4% to 7% [49,50] and falls to 1% (hypoxia) in some pathological ischemic tissues [51], as well as in the degenerated IVD [52]. Research conducted with BM-MSCs has been directed toward their biology in hypoxia (1–5% O_2). Compared with normoxia, BM-MSCs proliferated faster, exhibited greater colony-forming units (CFU) formation ability [53,54], and maintained better stemness in hypoxia through the downregulation of E2A-p21 by HIF-1α-Twist pathway [55]. These expanded BM-MSCs showed normal telomerase activity, karyotyping, and intact genetic integrity and did not form a tumor [55]. Furthermore, exposure of BM-MSCs to hypoxia exhibited a negative impact on osteogenic differentiation [56,57]. Nevertheless, it has been well documented that when rat BM-MSCs were exposed in normoxia, stimulated with chondrogenic growth factors, followed by hypoxia, the chondrogenic differentiation was obliviously improved through HIF-1α and protein kinase B (AKT) pathways [58]. Similar positive results were observed in porcine BM-MSCs after continuous exposure in hypoxia [59]. Moreover, Risbud et al. [40] found that 2% O_2 and 10 ng/ml TGF-β could stimulate rat BM-MSC differentiation to acquire phenotypes similar to that of NP cells. Difference in BM-MSC isolation, culture, and oxygen tension makes it difficult to draw a broad conclusion regarding the role of hypoxia in BM-MSCs, but hypoxia is undoubtedly an important regulator for BM-MSCs.

In vitro, hypoxia and serum deprivation have received increasing recognition in simulating ischemia or low nutrition. It was reported that hypoxia and serum deprivation induced apoptosis via the caspase-dependent signaling in transplanted BM-MSCs within 24 hours [60], which could be inhibited by lovastatin, lysophosphatidic acid, and heat shock protein 90 via phosphatidylinositol 3-kinase (PI3K)/AKT and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) pathways [61–63]. Longer exposure (120 hours) to hypoxia and serum deprivation resulted in complete cell death [64]. Interestingly, there was a contradictory report demonstrating that BM-MSCs were metabolically flexible to survive under ischemic conditions (serum and glucose deprivation) while retaining their multipotency [65], but like placenta-derived MSCs, BM-MSCs may lose their CFU formation ability [66,67].

Beyond oxygen tension and serum, glucose is another source of energy that markedly affects proliferation, apoptosis, and differentiation as well as replicative senescence of BM-MSCs. In rat BM-MSCs, low glucose concentration (1.39 mM) better stimulated proliferation, enhanced CFU formation ability, and decreased apoptosis compared with high glucose concentration (25 mM) [37,68]. However, unlike rat BM-MSCs, human BM-MSCs were resistant to the short-term (24 or 48 hours) exposure of high glucose (30 mM) without changes in their growth factor production and proliferative capacity [69,70]. The underlying mechanisms of high glucose resistance of BM-MSCs are yet to be unraveled.

Acidic pH

The oxygen tension and serum and glucose concentration are easy to be kept precisely to mimic a degenerative disc's microenvironment in vitro; the biggest obstacle for BM-MSCs should be the matrix acidity, ranging from 6.8 to 6.2 in degenerated IVD [71], whereas that in a normal disc is between 7.0 and 7.2 [72]. This is mainly caused by increased lactic acid production transformed from glucose by cytokines to maintain the ATP level in the IVD [4,73]. Findings from Wuertz et al. [37,74] demonstrated that acidity induced a decrease in proliferation and viability, a change in rat BM-MSC morphology, and an inhibition of aggrecan, collagen I, and tissue inhibitors of metalloproteinase-3 (TIMP-3) expression; additionally, this descending tendency was enlarged within the lower pH values, ranging from 7.4 to 6.5. However, both these investigations are for rat BM-MSCs cultured in monolayer, and the response of human BM-MSCs to acidic environment in degenerated disc is still unknown.

Mechanical loading and osmotic conditions

Mechanical loading is a natural constituent of IVD, and several publications showed that mechanical loading stimulates the chondrogenic differentiation of MSCs. The effect of mechanical loading on the biosynthetic activities of chondrocytes has been extensively studied in three-dimensional culture, especially on the formation of cartilaginous matrix [75,76]. Similarly, it was found that cyclic hydrostatic pressure upregulated the ECM deposition of human BM-MSCs [77]. Cyclic compressive loading could improve the chondrogenesis of rabbit BM-MSCs in agarose by promoting TGF-β1 production and some responsive genes expression involved in early chondrogenic differentiation [78,79]. Similar results were observed in human BM-MSCs [80–82]. Furthermore, Mouw et al. [83] demonstrated that during the processes of chondrogenic differentiation, ECM gene expression and matrix synthesis of bovine BM-MSCs remained nearly unchanged on Day 8 but were significantly upregulated on Day 16 under mechanical loading. It is hard to make a quantitative evaluation regarding the choice of the optimal parameter for chondrogenic differentiation of BM-MSCs because of the difference in bioreactor and mechanical loading model, but these studies highlight that appropriate mechanical loading benefits the chondrogenesis of BM-MSCs.

The IVD is an osmotic system just like other load-bearing cartilage, but the extracellular osmolarity is not constant and varies directly as a result of disc loading, mobile ions transportation, and tissue hydration [84]. Change in osmotic condition is a vital component of the IVD, and all the resident cells have to adapt by alterations in

gene expression and cellular volume [85,86]. Previous studies have demonstrated that the hyperosmotic media (450 mOsm/kg H_2O) modified the gene expression in human disc cells in central NP region, which were related to cytoskeleton remodeling and stabilization and osmolyte transportation [85], and finally led to regulate cellular volume [86]. Nevertheless, only two publications illustrated the influence of osmolarity on BM-MSCs. It was found that the proliferation and viability of rat BM-MSCs were strongly inhibited after cultured in an IVD-like osmolarity (485 mOsm) for 2 weeks; meanwhile, the levels of aggrecan and collagen I messenger RNA were significantly decreased [37]. Interestingly, when osmolarity dropped from 370 to 250 mOsm, BM-MSCs showed no significant difference in viability in 24 hours [87], but whether the viability can remain in the previous varying osmolarities for longer period remains as a question.

Proteinases and cytokines

At molecular level, a number of endogenous proteases and cytokines and those upregulated during the degeneration process create another complicated microenvironment for transplanted MSCs. In this respect, matrix metalloproteinases (MMPs) seem to be the major proteolytic enzymes for IVD matrix degradation. MMPs represent a family of zinc-dependent endoproteinases including at least 25 members that are distinguished based on the substrate specificity and structure [88]. The interaction between MMPs and TIMPs is biologically crucial for tissue remodeling [89], including morphogenesis, cell proliferation, and apoptosis [88] as well as the pathogenesis of diseases [90]. In the progress of IVD degeneration, an increase in the expression of MMPs, including MMP-1, -2, -3, -7, -8, -9, -10, -13, and -14 and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) [73,91,92], has been repeatedly observed, and some of them were associated with the severity of degeneration [93]. Several types of MMPs genes have been determined in uncommitted BM-MSCs [94,95]. Although it has been hypothesized that the balance between MMPs and TIMPs plays an essential role in MSC differentiation [96], there are few direct evidences. The activity of MMPs (MMP-2, -3, and -13) was an essential link for delivering mechanical signals into BM-MSCs and responses arising, such as change in proliferation and migration [94]. Furthermore, MMP-2, MT1-MMP, and TIMP-2 were upregulated as mediators when inflammatory cytokines were used to promote the invasive capacity of BM-MSCs in a reconstituted basement membrane model [97], and Wnt signaling might involve in this process with MT1-MMP as the target gene [98]. Controversially, Ho et al. [99] reported that MMP-1 acted as a critical determinant for the migration activity of BM-MSCs in monolayer through the MMP1/PAR1 axis, but MMP-2 and -9 and MT1-MMP were not correlated. More recently, MMP-1, -2, and -13, MT1-MMP, and MT3-MMP were found responsible for MSC-mediated Type I collagenolysis in vitro, but only MT1-MMP played a dominant and direct role in the invasive activity and intravasation of MSCs; moreover, MT1-MMP controlled the osteogenic potential [100]. During skeletal formation, MMPs were involved in bone formation and remodeling and promoted chondrogenesis; it has been further hypothesized that MMPs might regulate chondrogenic differentiation of BM-MSCs [101]. But there is a paucity of information except for the control of migration activity discussed previously. Therefore, it is not fully understood how MMP/TIMP balance modulates the fate of MSCs, especially the chondrogenic differentiation.

Proinflammatory tumor necrosis factor (TNF) is a homotrimer of 157 amino acid subunits; the TNF signals are mediated by two distinct cell surface receptors (a 55-kDa receptor [TNFR1] and a 75-kDa receptor [TNFR2]) mediate while TNFR1 plays a major role [102]. TNF-α involves the pathogenesis of various human diseases including IVD degeneration, and previous investigation confirmed that it expressed in the NP and AF cells of degenerated IVD [103,104]. Additionally, NP tissues were exquisitely sensitive to exogenous TNF-α, and even low level of TNF-α treatment caused a decreased expression of collagen II and aggrecan; also decreased ECM formation by activation of MMP-2 gelatinase correlated with induction of MT1-MMP [105,106]. TNF-α has been linked to the NP-induced nerve root injury, as systemic specific TNF-α antibodies could prevent the reduction of nerve conduction velocity and limit nerve fiber injury and thrombus and edema formation [107]. Besides, TNF-α has been reported to impair adipocytes differentiation [108], as well as the adipogenesis of BM-MSCs, but had no effect on the cell viability [109]. In addition, TNF-α is crucial in bone remodeling as a Cbfa1/Runx2 inhibitor [110]. It was shown that TNF-α (from 0.1 to 10 ng/ml) reduced TAZ (transcriptional coactivator with PDZ-binding motif, a Runx2/Cbfa1 transcriptional coactivator) expression and consequently impaired osteogenesis of BM-MSCs from patients with multiple myeloma [111]. TNF-α also involves in the regenerative function of BM-MSCs. BM-MSCs from knockout mice with ablation of TNFR1 provided significant protection against myocardial ischemic insult that was associated with decreased levels of TNF-α, IL-1, and IL-6 and an increased level of vascular endothelial growth factor (VEGF) [112].

Mediated via interleukins (IL-6 and IL-8), degenerated NP is a biologically active tissue capable of responding to proinflammatory factors and produces cytokines [113]. Apart from the effects of MMPs and TNF on BM-MSCs, interleukins and other cytokines in IVD also regulated the behavior of BM-MSCs. First, interleukins (IL-1α and IL-1β) played an important role in inducing the immunosuppressive ability of BM-MSCs [114]. IL-6 regulated the stemness and paracrine function of BM-MSCs; it was shown that IL-6 maintained the proliferation and the undifferentiated state by an ERK1/2-dependent mechanism

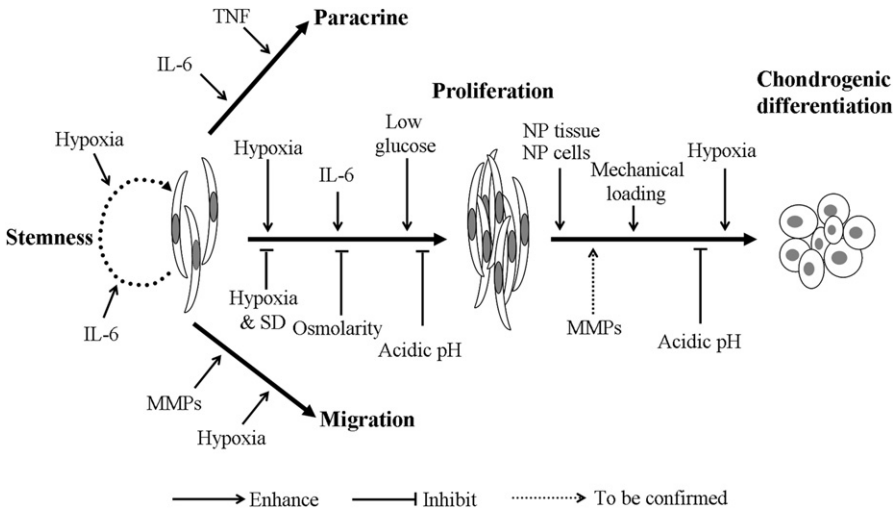

Figure. Possible regulation of the microenvironment of the intervertebral disc in the biological characterization of mesenchymal stem cells. SD, serum deprivation; NP, nucleus pulposus.

[115]. More recently, IL-6 was found to stimulate VEGF production via ERK-, JNK-, and PI3K-mediated pathways [116]. Furthermore, the receptors of various growth factors were found to be expressed in both normal and degenerated discs, such as TGF-βRII, FGFR3, IGFRI, and bone morphogenetic protein receptor II (BMPRII) [117]; because of the complicated molecular network in the degenerated IVD, the synergistic and antagonistic effects on the BM-MSCs required further investigation.

As summarized in the Figure, all the current data indicated that only NP cells, appropriate hypoxia, low glucose concentration, and mechanical loading, as well as some specific types of proteinases and cytokines, could be beneficial for BM-MSCs in the IVD-like microenvironment. It has been reported that 60% of bovine BM-MSCs survived after initial injection in to a cryopreserved IVD tissue in vitro, but only 20% of cells remained alive after 7 days [118]. Although it was evidenced that BM-MSCs improved the production of ECM and differentiated into disc-like cells, and remained viable in the IVD for more than 6 months [24,119,120], the survival rate of BM-MSCs after transplantation into the degenerated IVD has not been fully elucidated. Similar to the ischemic microenvironment of infracted heart, where more than 99% of transplanted BM-MSCs did not survive well and died in 4 days after injection [121], a large proportion of BM-MSCs may die or undergo apoptosis after transplantation into the degenerated IVD.

BM-MSCs for IVD regeneration in vivo

Although pioneer in vitro studies with BM-MSCs have mainly focused on their survival in the microenvironment of IVD and differentiation toward disc-like cells, the data on the therapeutic property of BM-MSCs for degenerative IVD support their potential clinical application. Research groups around the world have investigated BM-MSC transplantation in an attempt to reverse IVD degeneration in preclinical studies based on the utilization for BM-MSC's pleiotropic functions: poor immunogenicity, differentiation into disc-like cells, and preserving the structure and function of IVD. Research for in vivo trial design is proved in the Table. For example, in a mouse model of IVD degeneration, it was observed that BM-MSCs arrested the degeneration based on both chondrogenic differentiation and enhancing proliferation of notochordal cells or NP progenitors [24]. Similarly, site-dependent differentiation of BM-MSCs helping to decrease the degeneration of IVD was also concluded in other research [119,122–124]. Apart from these, BM-MSC injection or combined with specific biomaterial into degenerative IVD probably resulted in an improvement of the ECM and disc height [116,119,125–129], as well as effectively preserving the annular structure [42,122]. Importantly, recent study has provided additional insight that autologous BM-MSCs can regenerate the NP and maintain perfusion and permeability of the vertebral EP and subchondral bone that highlight another potential function of BM-MSCs for IVD degeneration [130]. Despite these encouraging reports, there is no study to reveal whether BM-MSCs will improve the biomechanical and kinematical properties of the whole IVD after in vivo administration. But it is noted that all the previous in vivo studies are consistent with the possibility of using BM-MSCs for clinical purposes.

Clinical application and future perspectives

On the basis of their capacity to rescue the degenerating IVD in animal studies, autologous marrow mesenchymal cells from ilium have been used for the first time to treat degeneration in 67- and 70-year-old female patients with LBP and leg pain; collagen sponge containing marrow mesenchymal cells was transplanted into the degenerated IVD percutaneously; radiograph, computed tomography, and

Table
Transplantation of BM-MSCs for IVD regeneration

Year	Species	Target organ	Cell source and number	Tracing method	Scaffolds	Mechanism of BM-MSC effect	Reference
2003	Rabbit	L2–L3 to L4–L5	Autologus, 4×10^4/40 μL	Ad-lacZ	Atelocollagen gel	MSCs preserved annular structure and differentiated into IVD cells	[122]
2004	Rat	Coccygeal disc	Allogenic, 5×10^5/50 μL	CM-DiI	15% hyaluronan gel	MSCs proliferated for more than 3 wk and increased the disc height	[126]
2005	Rabbit	L2–L3 to L5–L6	Allogenic, 1×10^5/20 μL	Ad-lacZ	No	MSCs survived 6 mo and increased the amount of PGs	[120]
2005	Rabbit	L1–L2/L3–L4/L4–L5	Autologus, 2×10^4/20 μL	GFP	Atelocollagen gel	MSCs differentiated into NP-like cells and increased PGs content	[123]
2006	Rabbit	L2–L3 to L4–L5	Autologus, 4×10^4/40 μL	Ad-lacZ	Atelocollagen gel	MSCs helped to restore the disc height and T_2w signal intensity	[127]
2008	Rabbit	L2–L3 to L4–L5	Allogenic, 1×10^5/15 μL	Transgenic Laz marker	No	MSCs survived for more than 24 wk and located in the AF with an AF-like morphology	[42]
2009	Mouse	Caudal 4–5	Allogenic, 1×10^3/1 μL	GFP	No	MSCs underwent chondrogenic differentiation and stimulated the endogenous notochordal cells	[24]
2009	Rat	Coccygeal 3–4	Human, 1.5×10^4/15 μL	Xenogenic	No	MSCs survived for 2 wk and increased the heights and signal intensity of IVD	[125]
2009	Porcine	L1–L2/L3–L4/L4–L5	Human, 5×10^5	Xenogenic	Puramatrix hydrogel	MSCs survived up to 6 mo and differentiated toward disc-like cells	[119]
2009	Rat	Coccygeal disc	Human, 5×10^4/5 μL	Cell Tracker Orange	No	MSCs differentiated into chondrocytic phenotype after 3-wk transplantation	[124]
2010	Rabbit	L3–L4 to L5–L6	Allogenic, 2×10^6/40 μL	No	Pure fibrinous gelatin	MSCs slowed the rate of DHI decrease	[128]
2011	Porcine	L2–L3 to L5–L6	Autologus, 1.25×10^5/200 μL	Quantum dots	Hydrogel	MSCs regenerated the NP and protected the function of vertebral EP	[130]

BM-MSCs, bone marrow–derived mesenchymal stem cells; IVD, intervertebral disc; GFP, green fluorescent protein; Ad-lacZ, adenovirus vector expressing beta-galactosidase; CM-DiI, chloromethylbenzamidodialkylcarbocyanine; PGs, proteoglycans; EP, end plate; NP, nucleus pulposus; AF, annulus fibrosus; DHI, disc height index.

magnetic resonance imaging (T1 weighted and T2 weighted) were used to assess the performance. At 2-year posttransplantation, the pain was alleviated, the vacuum phenomenon in the IVD space was improved, and the moisture contents and lumbar disc stability were increased in both patients whereas disc height gain was not found [131]. More recently, similar clinical setting was performed by Orozco et al. [132] in 10 patients (average age 37±7 year old, 4 men and 6 women) with LBP; $10\pm5\times10^6$ autologous BM-MSCs at passage 3 were injected in the NP area; clinical evaluation for back pain, disability, and quality of life and T2 weighted magnetic resonance image scanning were performed. After BM-MSC treatment of 1 year, pain relief and improvement of disability and water content in the disc were exhibited. Although disc height was not recovered and control groups were not strictly designed in these two studies, these Phase 1 clinical trials suggested that MSC-based therapy was safe and comparably effective in reducing back pain. Definitely, several underlying questions are left. Do these improvements persist in a long-term follow-up? Do BM-MSCs survive in the degenerated disc with such a harsh microenvironment? Do BM-MSCs secrete growth factors or differentiate into disc-like cells to regenerate the disc? Does BM-MSC therapy improve the biomechanical parameters of the spinal segments? It is currently impossible to answer all the questions, but the researchers have pioneered the clinical utilization of MSCs for IVD regeneration with encouraging results.

According to the results of all the experimental and clinical trials, MSCs can help to salvage the mildly degenerative disc effectively; however, the effects should not be the same in the very advanced degeneration. Second, what the literature suggests is that MSCs ''may'' increase matrix, maintain disc heights, etc., and clinical trials suggest that they can improve symptoms and reduce disability; the biggest question is how to link these two findings. The outcome parameters in the clinical trials are not sufficient to support that MSCs can or will regenerate a degenerated disc. A lot of research will still need to be done before one can draw any conclusion on the clinical usefulness. The clinical implication is not only whether it works but also how does it work and when will it not work. Third, as the nutrition transport regulator of IVD, the EP plays an essential role for disc degeneration and regeneration; the potential cross talk between the transplanted MSCs and the EP, especially the cartilaginous EP, may open a new dimension to investigate the regenerative mechanisms. Last but not the least, the issue of crucial importance concerns the safety of BM-MSCs. Indeed, BM-MSCs are currently in clinical trials with some success for bone and cartilage repair and considered safe so far but their tumorigenic risk and ectopic ossification/calcification are illustrated [133–136]; there is no direct investigation regarding the safety of BM-MSC injection for disc regeneration beyond the ectopic osteophyte formation because of BM-MSC leakage [137]. Future clinical trials with

BM-MSCs for the therapy of disc degeneration should be the ideal clinical setting in which to harvest convincing information, and if so, provide a safer and more effective route for therapeutic development.

Conclusions

In this decade, there has been a dramatic improvement in the understanding of potential BM-MSC–based clinical application for IVD regeneration. Despite this great interest in the use of BM-MSCs, survival rate posttransplantation, interaction with complicated microenvironment, and detail functions for IVD regeneration are not fully understood. The use of MSCs for IVD degeneration is still at the stage of preclinical and Phase 1 studies. In addition, the safety of MSC transplantation should also be closely studied. Only comprehensive understanding of all the previous fields can transfer MSC transplantation from bench to bedside successfully.

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