Potential and Limitations of Intervertebral Disc Endogenous Repair

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Potential and Limitations of Intervertebral Disc Endogenous Repair

Zhen Li, Marianna Peroglio, Mauro Alini and Sibylle Grad*

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Abstract: Intervertebral disc (IVD) disorders, especially degenerative disc disease, reduce the quality of life, and are short of effective therapy. A new direction for treatment of chronic tissue and organ diseases is to promote regeneration by harnessing endogenous repair mechanisms. In this review, we discuss the potential of endogenous repair in the IVD, the recent findings on endogenous IVD progenitor cells, and stem cell niches involved in IVD endogenous repair. We also highlight the factors which may restrict IVD self-healing. Ultimately, advanced therapeutic attempts to boost endogenous repair in the IVD are discussed, including bioactive factor delivery, gene therapy, activation of endogenous IVD progenitor cells and chemokine mediated stem cell homing.

Keywords: Annulus fibrosus, endogenous repair, intervertebral disc, nucleus pulposus, progenitor cells, stem cell niche.

1. INTRODUCTION

Endogenous repair exists in the regeneration process of many organs, such as liver, gut, skin, kidney, muscle, nervous system, heart, and bone [1, 2]. In general, upon tissue damage or degeneration, paracrine factors are released to recruit native stem cells and activate local progenitor cells. Within a tissue-specific niche which contains the supportive factors, tissue self-repair is then initiated and promoted.

The ability of endogenous repair is tissue specific. Some organs such as liver, gut and bone have a high self-repair capability due to abundant access to native progenitor cells and an inductive niche composed of supportive factors [1, 2]; while other organs and tissues, such as articular cartilage, possess a minimal self-repair capability due to insufficient number and activity of native progenitor cells [3]. The intervertebral disc (IVD) is a flexible joint composed of the central nucleus pulposus (NP), the surrounding annulus fibrosus (AF), and the cartilaginous endplates which serve as a connection to the vertebral bone [4]. Similar to articular cartilage, the IVD is an avascular joint with low inherent cell density and limited nutrition supply [5, 6], high degeneration rate and low endogenous repair capability. As reported recently, low back pain caused mainly by IVD degeneration has become the leading cause of disability worldwide [7]. Therefore, it is of critical importance to understand the potential and limitations of IVD endogenous repair. Improved knowledge about IVD repair mechanisms would facilitate the development of new therapies for disc diseases and ultimately low back pain.

2. EVIDENCE OF ENDOGENOUS REPAIR IN THE IVD

Evidence of endogenous repair exists from many in vivo studies which investigated animal models for IVD degeneration and regeneration. Melrose et al. created annular incisions in ovine lumbar discs to induce degenerative events [8, 9]. The outer AF showed progressive degeneration at 3 to 12 months post-surgery, followed by self-healing re-organization at 26 months post-surgery [8]. In another study using the same model, increased matrix gene expression (collagen type I, collagen type II, and aggrecan) was noticed in NP and AF tissue at 3 months post-surgery, which indicated an attempted repair response during the disc degeneration process [9]. Benz et al. reported complete healing of nucleotomized discs at 6 months post-surgery, based on histological, biochemical and gene expression assessment compared with non-injured control discs [10]. Zhang et al. compared different types of lesions for generating an IVD degeneration model. AF lesions in goat IVDs were created with no. 10 blade, no. 15 blade and 4.5 mm drill. At 2 months post-surgery, no. 10 blade and no. 15 blade defects did not lead to any histological difference compared with uninjured controls [11]. The above studies indicate that sheep and goat have an endogenous repair capability upon acute disc injury. As the ovine model shares many similarities with human discs, in terms of cell type [12], matrix composition [13], and biomechanical properties [14, 15], human IVDs may also maintain a similar self-repair attempt after acute traumatic injury. However, this condition is very different from chronic disc degeneration, which is predominant in most cases in human patients. It is likely that endogenous repair is very limited in the latter condition due to less active cells and less supportive niches.

Hutton et al. and Ganey et al. have established an IVD degeneration model and investigated repair of damaged IVDs in a canine model [16-18]. Neither long-term compressive loading [16] nor nutrition blocking [17] did induce disc degeneration, which indicated that canine IVDs may be resistant to these degenerative approaches. In a partial nucleotomy model, the disc tissue surrounding the defect showed an increase in gene expression of aggrecan, collagen type I and collagen type II, and protein level of collagen type II from 6....
to 12 months post-surgery, which suggested an endogenous repair capability at long-term after acute injury. Mizrahi et al. induced disc degeneration in mini-pigs [19]; they found that degenerated NP cells showed higher proliferation rate and colony forming unit rate in 2D culture compared with healthy NP cells, which indicates a regenerative attempt in degenerated NP tissue. However, it is known that certain canine breeds and porcine IVDs contain residual notochordal cells [20, 21], which are not present in adult human IVDs. The endogenous repair mechanism of non-chondrodystrophic canine IVDs would therefore be quite different from that of human IVDs.

3. EVIDENCE OF PROGENITOR CELLS IN THE IVD

One of the key factors in endogenous repair is the native progenitor cells. Research from the last decade has shown increasing evidence of progenitor cells present in the IVDs. An overview over progenitor cell populations isolated and characterized from whole disc, and NP and AF tissues is provided in Tables 1-3. Risbud et al. first identified that NP cells and AF cells from human degenerative disc expressed surface markers that are characteristic of stem cells, including CD90, CD73, p75 low affinity nerve growth factor receptor (p75NTR), CD105, CD166, CD63, CD49a, CD133. Furthermore, these cells could differentiate into osteogenic, adipogenic and chondrogenic lineages [22]. Henriksson et al. confirmed that a small percentage of primary cells isolated from degenerative human disc expressed stem cell markers CD105 and CD166, as well as other stem cell markers, including Notch1, Delta4 and Jagged1 [23]. Feng et al. confirmed these findings in adolescent human scoliotic AF cells. They further identified neuronal stem cell marker expression in these cells and found that these cells could differentiate into neuronal cells and endothelial cells [24]. A subsequent study from the same group suggested that the AF cells are also capable of differentiating into chondrocytes and osteoblasts under appropriate stimuli in vivo [25]. Blanco et al. identified stem cells from degenerated human NP tissue and compared them with bone marrow derived mesenchymal stem cells (MSCs) from the same patient. They found that the NP derived stem cells were similar to bone marrow MSCs, in terms of cell morphology, surface marker expression, and multilineage differentiation potential, with the exception that NP derived stem cells were not able to differentiate into adipocytes [26]. In another study with similar experimental set-up, Liu et al. identified stem cells derived from degenerated human disc endplate cartilage tissue and compared them with bone marrow MSCs from the same patient. The endplate cartilage derived stem cells also shared most similarities with bone marrow MSCs in terms of stem cell characteristics [27]. A recent pioneering study from Sakai et al. identified a tyrosine kinase endothelial receptor (Tie2) and disialoganglioside 2 (GD2) double positive cell population as NP progenitor cells [28]. These multipotent cells form spheroid colonies in 2D culture and express aggrecan and collagen type II. The Tie2 positive, GD2 negative cells were identified as precursor cells of Tie2 and GD2 double positive cells. The proportion of Tie2 positive NP cells decreased with aging and disc degeneration in human disc samples, which may be a major cause of age related disc degeneration. This study published in Nature Communications initiated a series of publications on disc progenitor cells. Erwin et al. isolated progenitor cells from canine NP tissue [29]. These cells expressed Sox2, Oct3/4, Nanog, CD133, Nestin and neural cell adhesion molecule, and were able to differentiate into chondrogenic, adipogenic, and neuronal lineages. Yassen et al. found that rabbit NP and AF cells expressed proliferation and progenitor cell markers, including proliferating cell nuclear antigen (PCNA), C-kit, CD166, Notch1, and Jagged1 [30]. They also compared young and mature rabbit discs and found that the proportion of cells expressing these markers decreased with age. Mizrahi et al. isolated NP cells from healthy and induced degenerated porcine discs [19]. NP cells from healthy and degenerated discs both expressed stem cell surface markers, such as CD29, CD90 and CD44. Degenerated NP cells showed higher proliferation rate, colony forming unit rate, and superior osteogenic and adipogenic differentiation capability; while healthy NP cells were superior for chondrogenic differentiation and proteoglycan production. Characterization of Rhesus monkey IVD cells confirmed stem cell surface marker expression, clonogenicity, and multipotency of both NP and AF cells [31]. Compared with bone marrow MSCs from the same animal, most of the stem cell surface markers showed similar expression profiles. Brisby et al. identified cells expressing stem cell markers in human degenerated disc [32]. Pellet culture of these cells within conditioned media from bone marrow MSCs enhanced the expression of stem cell markers, while pellet culture of these cells within conditioned media from healthy disc cells showed beneficial effects.

Table 1. Characterization of progenitor cells from disc tissue.

<table>
<thead>
<tr>
<th>Species and status of tissue</th>
<th>Surface markers detected (+)</th>
<th>Surface markers undetected (-)</th>
<th>Surface marker detection methods</th>
<th>Multilineage differentiation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human degenerated disc NP/AF tissue and primary cells [23]</td>
<td>Notch1, Delta4, Jagged1, CD117, Stro-1, Ki67</td>
<td>-</td>
<td>immunohistochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Human degenerated disc NP/AF tissue and primary cells [21]</td>
<td>Notch1, Stro-1, CD117, CD105, CD166</td>
<td>CD45</td>
<td>flow cytometry</td>
<td>-</td>
</tr>
<tr>
<td>Human degenerated disc cartilage endplate tissue, plastic adherent [27]</td>
<td>CD73, CD90, CD44, Stro-1, CD105, CD166, CD133</td>
<td>CD14, CD34, CD19, CD45, HLA-DR</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Human degenerated disc tissue [32]</td>
<td>Oct3/4, Stro-1, CD105, CD90, Notch1</td>
<td>-</td>
<td>qPCR, immunohistochemistry</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Characterization of progenitor cells from NP tissue.

<table>
<thead>
<tr>
<th>Species and status of cells/tissue</th>
<th>Surface markers detected (+)</th>
<th>Surface markers undetected (-)</th>
<th>Surface marker detection methods</th>
<th>Multilineage differentiation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human degenerated NP cells, plastic adherent [22]</td>
<td>CD90, CD73, p75NTR, CD105, CD166, CD63, CD49a, CD133</td>
<td>CD34</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Rat healthy NP cells, 2D expanded [22]</td>
<td>CD90, CD29, CD44</td>
<td>CD45</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic</td>
</tr>
<tr>
<td>Rabbit healthy NP tissue [23]</td>
<td>Delta4, Jagged1, Stro-1</td>
<td>Notch1, CD117, Ki67</td>
<td>immunohistochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Rat healthy NP tissue [21]</td>
<td>Notch1, Delta4, Jagged1, CD117</td>
<td>-</td>
<td>immunohistochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Minipig healthy NP tissue [21]</td>
<td>Notch1, Delta4, jagged1, CD117, Ki67</td>
<td>Stro-1</td>
<td>immunohistochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Human degenerated NP cells, plastic adherent [26]</td>
<td>CD90, CD73, CD105, CD166, CD106</td>
<td>CD24, CD45, CD34, CD14, CD19, HLA-DR</td>
<td>flow cytometry</td>
<td>osteogenic, chondrogenic</td>
</tr>
<tr>
<td>Human degenerated and mouse NP cells, spheroid colony forming Tic2+ GD2+ [28]</td>
<td>CD271, Flt1, CD44, CD49f, CD56, CD73, CD90, CD105, CD166</td>
<td>CD45, CD11c, CD14, CD31, CD34, CD144, UE-1, vWF, CD146</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Nonchondrodystrophic canine NP cells, colony forming [29]</td>
<td>Sox2, Oct3/4, Nanog, CD133, Nestin, neural cell adhesion molecule</td>
<td>Protein 0, Brachyury</td>
<td>qPCR</td>
<td>adipogenic, chondrogenic, neurogenic</td>
</tr>
<tr>
<td>Rabbit healthy NP tissue, and primary cells [30]</td>
<td>PCNA, C-kit, CD166, Notch1, Jagged1</td>
<td>-</td>
<td>qPCR, western blot</td>
<td>-</td>
</tr>
<tr>
<td>Porcine healthy NP, primary cells [19]</td>
<td>CD29, CD90, CD44</td>
<td>-</td>
<td>flow cytometry, immunohistochemistry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
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<td>Porcine degenerated NP, primary cells [19]</td>
<td>CD29, CD90, CD44</td>
<td>-</td>
<td>flow cytometry, immunohistochemistry</td>
<td>osteogenic, adipogenic</td>
</tr>
<tr>
<td>Rhesus monkey healthy NP cells, plastic adherent [31]</td>
<td>Notch1, CD44, CD166, Ki-67, CD9, CD146, CD106, HLA-DR, CD29</td>
<td>CD34, CD45, CD144, CD117, CD271, CD90, FLK-1</td>
<td>flow cytometry, qPCR</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Human NP cells, plastic adherent [33]</td>
<td>CD105, CD44, CD29</td>
<td>CD34, CD45, CD14, HLA-DR, CD24</td>
<td>Flow cytometry</td>
<td>osteogenic, chondrogenic</td>
</tr>
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</table>

Effect on proteoglycan production, demonstrating the differential response of the cells to changes in the environment. Guan et al. isolated disc progenitor cells by plastic adherence of NP cells from human discs, and confirmed stem cell marker expression and multipotency of these cells [33]. After culturing these cells with transforming growth factor beta 1 (TGFB1), the disc progenitor cells obtained a mature disc cell phenotype, as indicated by markedly increased CD24 expression. A recent study on rabbit AF cells confirmed existence of progenitor cells in AF tissue by stem cell surface marker detection, colony forming analysis, and multipotent differentiation assessment [34].

In summary, the above studies clearly indicate that progenitor cells exist in the IVD. In general, surface markers expressed in stem cells (CD105, CD90, Notch1, and CD166) have been detected in disc progenitor cells, whereas surface markers for hematopoietic and endothelial lineages (CD34, CD45, and CD117) have been absent. It was also shown that the number of disc cells expressing progenitor cell markers decreases with age and/or degeneration [28, 30]. Most of the studies isolated disc progenitor cells by plastic adherence and/or colony forming. Only Sakai's group successfully identified specific surface markers for NP progenitor cells, namely Tie2 and GD2 [28]. Compared with bone marrow MSCs from the same donor, NP progenitor cells showed lower flow cytometry mean fluorescence intensity on CD105, CD166 and CD106 [26], and cartilage endplate progenitor cells possessed lower CD105 and CD166 positive proportions [27]. Progenitor cells in degenerated discs show a regenerative attempt as indicated by higher proliferation rate and higher colony forming unit rate compared with progenitor cells in healthy discs. However the progenitor cells in degenerated discs also lose their chondrogenic differentiation capability compared with the cells from healthy discs [19]. The above study was conducted in vitro, while the function of disc progenitor cells upon damage and/or degeneration in situ is still unknown. Therefore, further research exploring the properties of disc progenitor cells and their potential for endogenous repair is needed.
Table 3. Characterization of progenitor cells from AF tissue.

<table>
<thead>
<tr>
<th>Species and status of tissue</th>
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<tr>
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<td>CD90, CD73, p75NTR, CD105, CD166, CD63, CD49a, CD133</td>
<td>CD34</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Human scoliosis AF cells, plastic adherent [24]</td>
<td>CD29, CD49e, CD51, CD73, CD90, CD105, CD166, CD24, Stro-1, nesin, neuron-specific enolase</td>
<td>CD31, CD34, CD45, CD106, CD117, CD133</td>
<td>flow cytometry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Rabbit healthy AF tissue [23]</td>
<td>Notch1, Delta4, Jagged1, CD117, Stro-1, Ki-67</td>
<td>-</td>
<td>immuno-histochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Rat healthy AF tissue [23]</td>
<td>Notch1, Delta4, CD117</td>
<td>Jagged1</td>
<td>immuno-histochemistry</td>
<td>-</td>
</tr>
<tr>
<td>Minipig healthy AF tissue [23]</td>
<td>Notch1, Delta4, Jagged1, CD117, Stro-1, Ki-67</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Rabbit healthy AF tissue, and primary cells [30]</td>
<td>PCNA, CD166, C-kit, Jagged1, Notch1</td>
<td>-</td>
<td>immuno-histochemistry, qPCR, western blot</td>
<td>-</td>
</tr>
<tr>
<td>Rhesus monkey healthy AF cells, plastic adherent [31]</td>
<td>Notch1, CD44, CD166, Ki-67, CD9, CD146, HLA-DR, CD90, FLK-1</td>
<td>CD106, CD29, CD34, CD45, CD144, CD117, CD271</td>
<td>flow cytometry, qPCR</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
<tr>
<td>Rabbit healthy AF cells, colony forming [34]</td>
<td>CD29, CD44, CD166, Oct4, Nucleostemin, SSEA-4</td>
<td>CD4, CD8, CD14</td>
<td>qPCR, immuno-histochemistry</td>
<td>osteogenic, adipogenic, chondrogenic</td>
</tr>
</tbody>
</table>

4. STEM CELL NICHES INVOLVED IN IVD ENDOGENOUS REPAIR

In addition to the progenitor cells inside the IVD, stem cell niches surrounding IVDs, and within the blood and bone marrow system, also play pivotal roles in the endogenous repair of IVDs. Endogenous stem cells from these origins function to maintain physiological homeostasis and may be activated and recruited during tissue repair.

Henriksson et al. detected slow cycling cells with 5-bromo-2-deoxyuridine (BrdU) incorporation in the AF border to ligament zone and the perichondrium of rabbit, pig and human IVDs, and defined them as stem cell niche [23]. Using BrdU in vivo labeling technique, investigators tracked BrdU stained cells in rabbit IVDs; positively stained cells were observed in the stem cell niche adjacent to the epiphyseal plate at early time points, and later mainly in outer AF, indicating migration of cells from the stem cell niche to the outer AF of IVDs [35]. Immuno-staining revealed localization of growth and differentiation factor 5 (GDF5) in the potential stem cell niche region, along the migration route and in the outer AF. In a following study, GDF5 positive, large (> 10 μm) and small (< 10 μm) IVD cells were isolated. Migration of these cells into IVD explants without endplates was evaluated by direct co-culture. GDF5 positive and small IVD cells displayed superior migration capability, which indicates that prechondrocytic marker GDF5 signaling may be involved in the cellular migration [36].

Stem cells from the vascular system and bone marrow are the main cell sources of tissue renewal for most organs which maintain high self-repair capability [37-39]. For avascular tissues such as cartilage and IVDs, migration of stem cells from blood vessels and bone marrow to the tissue defect site is restricted by access and distance. A common procedure for articular cartilage repair is microfracture, which aims to promote access of a cartilage defect to stem cells from the subchondral bone and has shown beneficial effects on cartilage repair [40]. Unfortunately, this approach is hardly feasible for IVD repair. In a recent in vivo study, Saikai et al. established a novel tail-looping disc degeneration model in mouse [41]. They transplanted green fluorescent protein (GFP) labeled cells from whole bone marrow into the tail vein of the mice with tail-looping, and found increased bone marrow cell migration into the peripheral bone marrow and vascular canals in the endplate related to the severity of IVD degeneration, although the cell recruitment was limited due to the avascular nature of the IVD. Recent studies from our group have demonstrated that bone marrow derived MSCs are able to migrate through the cartilage endplate of organ cultured IVDs. Migration of MSCs into IVDs could be enhanced by induced degenerative conditions, including limited nutrition supply, high frequency dynamic load, and/or needle puncture [42, 43].

Proteomic assay was performed to investigate the secreted proteome of whole IVDs cultured under physiological and induced degenerative conditions. IVDs cultured under induced degenerative conditions were found to secrete higher concentrations of CCL5 compared with IVDs cultured under simulated physiological conditions. Using in vitro chemotaxis assay, CCL5 was found to be a key factor involved in recruitment of MSCs [44]. The presence of CCL5 in IVD and their relationship with disc degen-
eration has also been confirmed by other studies [45-49]. Ahn et al. detected CCL5 mRNA expression in 4 out of 23 herniated human lumbar disc specimens [46]. Kawaguchi et al. also detected CCL5 mRNA expression in human herniated and scoliotic disc tissue [47]. Li et al. screened profiles of cytokines released from organ cultured human IVDs. Eotaxin, CXCL10, CCL5, IL6, and IL8 were found to be present at high levels [45]. Kepler et al. stimulated human IVD cells with pro-inflammatory factor substance P (SP), and observed significant upregulation of CCL5 expression of AF cells [48]. Gruber et al. conducted microarray analysis of degenerated and healthy disc tissue, and found that CCL5 was significantly upregulated in more degenerated compared with healthy disc [49]. Immunostaining revealed predominant localization of CCL5 in the AF tissue, albeit some NP cells also showed positive staining. They also found that interleukin 1 beta (IL1B) and tumor necrosis factor (TNF) upregulated CCL5 expression of disc cells cultured in 3D conditions in vitro. Taken together, these studies suggest that the inflammatory chemokine CCL5 may be a primary signaling molecule involved in disc degeneration, cell recruitment, inflammation and discogenic pain [50].

5. REGENERATIVE METHODS THAT BOOST ENDOGENOUS REPAIR

Increased understanding of the repair capacities inherent to the intervertebral disc and the decrease or loss of the healing responses with aging and degeneration has led to the development of new therapies with the aim to re-activate regenerative mechanisms. The preconditions for molecular therapies to be effective are a pool of viable cells in the disc and surrounding tissues that can be mobilized and appropriate nutrition conditions to maintain their activity.

Intra-discal administration of anabolic factors appears the most straightforward approach for boosting the cells’ metabolic activity. Proteins of the TGFbeta superfamily such as TGBF1, TGBF3, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs) have been effective in stimulating the gene expression and synthesis of collagen type II and proteoglycans in IVD cells in vitro ([51] for review). Furthermore, injection of BMP7 (osteogenic protein OP1) could restore the disc height, enhance the proteoglycan content and improve the degeneration state in rabbit disc degeneration models induced by either annular puncture or chondroitinase ABC application [52, 53]. Similarly, single direct injection of GDF5 achieved disc height increase and significant improvements in MRI and histology based disc degeneration scores in the rabbit annular puncture model [54]. GDF5 is currently investigated in phase I/II clinical trials for human disc degeneration (e.g. https://clinicaltrials.gov/ct2/show+NCT00813813?cond=%22Intervertebral+Disc+Degeneration%22&rank=51). Importantly, GDF5 and GDF6 (Cartilage Derived Morphogenetic Proteins CDMP1 and CDMP2) have been localized in human degenerative and non-degenerative disc and may thus represent physiological morphogens involved in natural IVD homeostasis [55]. Interestingly, several studies have shown that injection of the statins simvastatin and lovastatin, which are approved pharmaceuticals, could reverse the degenerative progression in rat models of disc degeneration [56-58]. The regenerative effects of simvastatin could at least partially been attributed to an up-regulation of the BMP2 signaling pathway [59].

The synthetic peptide Link-N has documented growth factor activity and may thus be a valid and cost-effective alternative. In fact, injection of Link-N into rabbit degenerate discs in vivo stimulated aggrecan expression, while suppressing MMP expression [60]; importantly, these combined anabolic and anti-catabolic effects were also evident after administration of Link-N in cell and organ cultures of human degenerative discs [61].

Mitogenic growth factors may counteract the degenerative process owing to their proliferative and anti-apoptotic effects. Insulin-like growth factor (IGF) 1, platelet-derived growth factors (PDGFs), epidermal growth factor (EGF), and fibroblast growth factor (FGF) 2 have been shown to stimulate cell proliferation and inhibit cellular apoptosis in human and animal cells in vitro ([51] for review). An attractive approach is to combine mitogenic with morphogenic growth factors to address both IVD cell growth and new matrix formation. Platelet-rich plasma (PRP) and platelet lysate (PL) preparations are autologous sources of multiple growth factors that have widely been used for orthopedic tissue repair and have recently been investigated for IVD regeneration [62]. In vitro, PRP and PL have been shown to increase cell proliferation and extracellular matrix synthesis in nucleus pulposus and annulus fibrosus cells [63, 64]; in the rabbit annular puncture model, injection of autologous PRP releasate resulted in an increase in disc height and cell count, although no effect on MRI outcome was observed [65]. Moreover, PRP was reported to suppress cytokine induced metalloproteinases and inflammatory mediators, while restoring matrix protein gene expression levels in human NP cells [66].

Given that notochordal cells, as the "original" NP cells, have known beneficial effects on the disc matrix integrity and cellular homeostasis, several studies have investigated the potential of notochordal cell derived factors for IVD regeneration ([67] for review). Recent work has indicated that factors present in notochordal cell conditioned medium have the ability to both stimulate growth and matrix production of NP cells and inhibit angiogenic reactions and vessel growth [68, 69]. Further research is essential to determine the specific factors responsible for the repair effects of notochordal cell secretome.

A critical issue with growth factor delivery is their short half-life, the requirement for high supra-physiological doses and the concomitant risk for dose-dependent adverse effects. Indeed, an acceleration of degenerative processes and increased inflammation was observed upon administration of rhBMP2 in a rabbit annular tear model [70], and annular fibrochondrocyte infiltration and inflammatory reactions occurred after repeated injection of GDF5 or IGF1 in a murine disc degeneration model [71]. Gene therapy is an attractive way to circumvent some of the disadvantages of direct protein injection and allow for a more sustained effect. Adenoviral vector mediated gene delivery of LIM mineralization protein (LMP) 1 has been shown to induce BMP2 and BMP7 expression and promote ECM synthesis by disc cells in vivo and in a rabbit model in vivo [72]. In a recent study, LMP1 was overexpressed in NP cells by lentiviral gene transfer.
Gene delivery of the transcription factor NF-
were mediated by down-regulation of MMP expression via
and anti-inflammatory effects upon TNF challenge, which
demonstrated synergistic effects in augmenting
the disc height and increasing the expression of type II collagen and proteoglycan in a rabbit IVD degeneration model [74].

Several studies have applied gene therapy for induction of anti-catabolic responses. In a rabbit annular puncture model treatment of punctured discs with AAV2-5 or AAV2-TIMP1 could retard degenerative changes, as demonstrated by MRI, histological, serum marker and biomechanical analyses [75]. Injection of anti-ADAMTS5 small interference (si)RNA oligonucleotide in rabbit punctured discs led to improved MRI signals and histological scores; although no significant changes in disc height were noted [76]. Recently, microRNA-146a transfection was shown to suppress IL1 induced inflammatory and catabolic gene and protein expression in bovine disc cells [77]. The responses to siRNAs and the roles of miRNAs may be worthy of investigation for future therapeutic prospects. Nonetheless, with all gene therapy approaches using direct cell transfection in vivo, the risks of dose-dependent adverse reactions and ectopic transfection remain [78].

Few studies have addressed the concept of progenitor cell activation for IVD repair. Saraiya et al. reported that the purine analog reversine could modulate growth and morphology and induce cell plasticity of annulus fibrosus cells. Cells pretreated with reversine showed an increased response to lineage differentiation conditions along mesenchymal lineages. It was concluded that reversine may be used to generate skeletal progenitor cells for regeneration of degenerative intervertebral disc [79]. Granulocyte colony-stimulating factor (GCSF) has been shown to mobilize hematopoietic stem cells from bone marrow into the circulation and to recruit bone marrow MSCs. In an in vivo study in rats, GCSF administration to lumbar discs could not induce cell mobilization or regenerative effects in the disc. Yet, proliferating cells were found within endplates; thus effects of GCSF treatment will require further investigation [80]. Chemokine-mediated migration of MSCs towards the center of the intervertebral disc was recently investigated using an organ culture model [81]. A hyaluronan-based hydrogel release system containing stromal derived factor (SDF) 1 (CXCL12) was delivered to a partially nucleotomized bovine disc. Migration of exogenous added bone marrow derived MSCs through the endplate was significantly enhanced in discs supplemented with SDF1 containing hydrogel, compared to discs containing SDF1 or hydrogel alone. This demonstrated that cell recruitment into the disc can be accelerated by chemotactic factors, while sustained release of factors is beneficial [81].

The regulation of NP progenitor cells by Tie2-angiopoietin (Ang) 1 signaling has been shown to be disturbed during ageing and degeneration, ultimately leading to NP cell apoptosis [28]. Therefore, bioactive factors or gene therapies aiming to up-regulate the Tie2-Ang1 interaction, by increasing the number of Tie2 positive cells and/or the Ang1 availability, may represent potent targets for enhancing the endogenous repair capacity of the IVD.

### 6. LIMITATIONS OF IVD ENDOGENOUS REPAIR

Based on above mentioned studies, it is evident that the IVD possesses certain endogenous repair capability. However, in many more cases the IVD does not heal by itself upon degeneration or injury, which leads to a high social-economic burden [82]. Besides the general hallmark such as avascularity which leads to a low self-repair ability in IVD, certain other factors may explain the diversity of endogenous IVD repair potential among different patients.

#### 6.1. Aging

It is well known that the self-healing ability of human tissues decreases with aging. This also applies to the IVD. Yasen et al. noted that the protein expression of stem cell markers in rabbit IVD tissue significantly decreased with age [30]. Sakai et al. also found that the proportion of Tie2 positive NP progenitor cells in human tissue decreased markedly with age. These observations suggest that the number of inherent IVD progenitor cells decreases with age, which may lead to inferior endogenous repair in older patients.

In addition, stem cell numbers in bone marrow have been shown to decline with age [83]. Furthermore, the migration capacity of progenitor cells in the surrounding vascular and bone marrow system may also drop with age. In a recent study from our group, hyaluronan hydrogel containing SDF1 was delivered into the cavity of a nucleotomized IVD. Thereafter PKH fluorescence labeled bone marrow MSCs were applied on top of the cartilage endplate in the organ cultured IVDs. When migrated MSCs were counted after 48 h of culture, MSCs from younger donors showed significantly higher migration ability compared to MSCs from older donors [81]. This may be related to altered cell surface receptor expression, motility, and/or matrix penetrating activity of cells from older individuals.

#### 6.2. Genetic Associations

Genetic heritability is known to have an important influence on specific features of IVD degeneration, such as disc height decrease and bulging [84]. Individuals who are prone to IVD degeneration and disease are likely to possess a low IVD endogenous repair capability upon degeneration and/or injury. Genetic risk factors (genes) associated with IVD degeneration and diseases have been screened during the last 15 years [85-87]. The first gene found to be associated with IVD degeneration is vitamin D receptor [88]. A major part of the genes that have been identified to be associated with IVD degeneration and disease are related to the function and integrity of IVD extracellular matrix, including collagen type IX [89], aggrecan [90], collagen type I [91], matrix metalloproteinase 3 (MMP3) [92], cartilage intermediate layer protein (CILP) [93], and asporin [94]. Another group of genes that are involved in inflammatory pathways are also associated with IVD degeneration and disease, including *IL1* [95], *IL6* [96], tissue inhibitor of metalloproteinase 1 (*TIMP1*) and cyclooxygenase-2 (*COX2*) [97]. These genes associated with IVD degeneration are related to extracellular matrix synthesis or inflammation regulation, which are critical in the fun-
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6.3. Extent of Traumatic Lesion

In the above mentioned in vivo study [11], AF lesions were created with no. 10 blade, no. 15 blade or 4.5 mm drill for generating an IVD degeneration model. At 2 months post-surgery, no. 10 blade and no. 15 blade defects did not reveal histological changes compared with uninjured controls; while the 4.5 mm drill injury resulted in apparent extracellular matrix disturbance [11]. This indicates that IVD self-repair capability differs depending on the extent of traumatic lesion. Below a certain threshold, the IVD may be able to heal by itself. Traumatic lesion above this threshold would result in permanent degenerative effects on mechanical and biological properties of the IVD. The consequences of different types and extent of traumatic IVD lesions at organ level have been broadly studied; changes in mechanical properties of IVD motion segments, cell viability, and biosynthesis rates following injury have been comprehensively summarized in a review article by Iatridis et al. [98]. In particular, the immediate change in mechanical property is dependent on the type and extent of injuries. For example, an AF lesion next to the vertebrae may exert a stronger effect on torsional behavior compared to a lesion near the disc midplane [99]. Puncture injuries larger than 40% of disc height induced a decrease in disc axial stiffness [100]. Below this threshold size, the effect on axial stiffness was insignificant [101].

6.4. Influence of other Systemic Diseases

Systemic diseases which are associated with disc degeneration may also have an impact on the endogenous repair potential of the IVD. Obesity was suggested to be related with lumbar disc degeneration. The involvement of fat cells via altered metabolism or the release of adipokines may lead to the development of disc degeneration and low back pain [102]. The main nutrient supply to the majority of disc cells depends on the capillaries emerging from the vertebral bodies. Hence vascular diseases which cause impaired vertebral blood supply are also associated with disc degeneration, such as atherosclerosis, Gaucher disease, and sickle cell anemia [103]. In addition, osteoporosis was also found to be associated with high prevalence of lumbar disc degeneration [104]. Patients with these systemic diseases are likely to possess an inferior endogenous repair capability upon IVD degeneration and/or injury.

7. FUTURE PERSPECTIVES

Summarizing the mechanisms discussed in this article, Fig. (1) schematizes the cells and factors involved in IVD endogenous repair. There is clear evidence that progenitor cell populations exist inside the disc and in the stem cell niche close to the cartilaginous endplate and that these cells can be mobilized and induced to proliferate or acquire a differentiated phenotype. Importantly, these cells have also been recognized in human degenerative disc tissue. On the other hand, diverse molecular therapies have been considered and have shown great potential to retard or reverse degenerative processes in the IVD in vitro, in organ cultures and in preclinical models. The challenge for future preclinical and clinical research will be to translate these promising findings from different experimental models to the human situation. Safety concerns on the appropriate dosing, localization, timing and side effects exist with all exogenously applied bioactive agents. Therefore, future studies will also need to further elucidate the natural courses of healing responses to ultimately mimic these physiological processes of tissue repair in a safe and effective way. The m-

Fig. (1). Schematic illustration of intervertebral disc endogenous repair mechanisms.
lucellular therapies which aim to regenerate the IVD by activating its endogenous repair potential are best indicated at early stages of disc degeneration prior to structural changes, as the disc tissue in these patients preserves certain endogenous repair ability. Assessment of genetic predisposition for early incidence of disc degeneration, and non-invasive imaging methods to detect early degenerative changes will be needed in the future for timely diagnosis [105]. In addition, patients with discogenic pain may be considered as candidates of these therapies, as well as patients who have undergone spinal fusion or discectomy, in order to prevent further disc degeneration in the adjacent disc or nucleotomized disc respectively.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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