Endogenous Cell Homing for Intervertebral Disk Regeneration

Sibylle Grad, PhD
Marianna Peroglio, PhD
Zhen Li, PhD
Mauro Alini, PhD

Intervertebral disk (IVD) degeneration is characterized by a gradual loss of cellular function and related breakdown of extracellular matrix. This process leads to a decrease in the mechanical stability of the spine and activation of nociceptive receptors that trigger painful back and neck symptoms.[1] Implantation of mesenchymal stem cells (MSCs) has been shown to counteract the degenerative processes in animal models of disk degeneration and in some initial clinical studies.[2] The regenerative activity of MSCs when injected into the disk may partially rely on their potential to differentiate toward an IVD-like phenotype upon exposure to the appropriate microenvironment. In addition, therapeutic MSCs also release trophic factors that may stimulate the metabolism of disk cells and suppress inflammatory reactions.[3,4] However, in spite of these promising perspectives, clinical application of MSCs has certain limitations. Harvesting of autologous bone marrow for isolation of MSCs is an invasive procedure, and the cell processing can be complex and costly. Potential adverse events may occur, such as cell leaking and osteophyte formation.[5,6]

Recruitment or homing of endogenous progenitor cells is being investigated as an alternative concept. As a reaction to cell or tissue damage, cells will release various signaling molecules, aiming to facilitate tissue repair. Certain chemokines efficiently attract progenitor cells from their niches within surrounding tissues, thereby enabling cell-mediated healing.[7] Progenitor cell recruitment has primarily been investigated in the context of wound healing, myocardial infarction, and bone repair.[8-10] For IVD regeneration, cell homing appears challenging, given that the disk is an avascular tissue and that circulating or bone marrow–derived stem cells need to migrate over longer distances to reach the inner anulus fibrosus or the nucleus pulposus tissue.

Using a whole-organ culture model of induced IVD degeneration, we have shown that human bone marrow–derived MSCs (hBMSCs) were capable of migrating through the end plate of a bovine coccygeal disk. While a low basal level of migration was observed in disks cultured under simulated physiologic conditions, the number of recruited cells was significantly enhanced by induced degenerative settings.[11] Specifically, a degenerative state was achieved by culturing the disks under limited nutrition, exposing disks to high-frequency dynamic compressive loading and causing mechanical damage by annular needle puncture.[12] Furthermore, the expression of certain chemokine receptors was significantly upregulated in MSCs cultured with degenerative disk–conditioned medium, confirming the responsiveness of the cells to the degenerative disk environment.[13] The feasibility of MSC recruitment in the disk in vivo was recently evaluated on a mouse tail model of induced disk degeneration.[14] However, only a limited number of bone marrow cells were recruited in the disk degeneration group, which is probably due to its avascular nature.

From the AO Research Institute Davos, Davos, Switzerland.

None of the following authors or any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article: Dr. Grad, Dr. Peroglio, Dr. Li, and Dr. Alini.

J Am Acad Orthop Surg 2015;23: 264-266

http://dx.doi.org/10.5435/JAAOS-D-15-00096

The finding that MSCs could migrate through the end plate of the IVD was further explored by injecting stromal cell–derived factor 1 (SDF-1, also known as CXCL12), into the cavity of a partially nucleotomized disk in organ culture.[15] The chemoattractant was delivered using a hyaluronan-based thermoreversible injectable hydrogel, which had previously been shown to support disk cell growth, matrix production, and MSC differentiation toward the disk-like phenotype.[16,17] Fluorescent-labeled hBMSCs were applied onto the end plate and incubated for 48 hours. Microscopic evaluation revealed a significantly enhanced migration of cells into the disks that had been treated with SDF-1 containing hydrogel compared with disks treated with the hydrogel alone or with SDF-1 in saline. Interestingly, recruited MSCs were identified in both the anulus fibrosus and nucleus pulposus tissues, indicating migration through both extracellular matrix structures.[15]

These results indicate that recruitment of MSCs toward the center of a damaged disk is feasible and that MSC migration can be enhanced by a chemoattractant delivery system consisting of a suitable hydrogel and a potent chemokine. Yet, tissue-specific chemoattractants may be released by a damaged or degenerative disk. To identify the factors released by the disk cells in response to tissue damage, the proteomic profile of the conditioned medium of a whole disk maintained under induced degenerative settings was analyzed.[13] Proteomic analysis revealed CCL5/RANTES (chemokine [C-C motif] ligand 5/regulated on activation, normal T cell–expressed and secreted) and CXCL6 (chemokine [C-X-C motif] ligand 6) as two main chemotactic factors secreted by the degenerative disk. Interestingly, depletion of CCL5/RANTES from degenerative disk–derived medium markedly reduced its chemoattractive activity toward MSCs, suggesting that CCL5/RANTES plays a major role in the recruitment of regenerative cells in the disk. Presence of CCL5/RANTES and its receptors was also confirmed in histologic sections of bovine and human degenerative disks and was associated with painful disk diseases.[13,18,19]

In conclusion, there is clear evidence that homing of endogenous regenerative progenitor cells can occur in the disk and can be advanced by chemoattractant delivery systems. Further research is necessary to identify the most effective chemotactic factor and uncover potential side effects. Finally, recent studies have described the presence of progenitor cells at different locations of the healthy and degenerative IVD.[20] Mobilization, augmentation, and activation of these endogenous progenitor cell populations represent attractive targets for future regenerative strategies.[21]

References

1. Ito K, Creemers L: Mechanisms of intervertebral disk degeneration/injury and pain: A review. *Global Spine J* 2013;3(3):145-152.
2. Yim RL, Lee JT, Bow CH, et al: A systematic review of the safety and efficacy of mesenchymal stem cells for disc degeneration: Insights and future directions for regenerative therapeutics. *Stem Cells Dev* 2014;23(21):2553-2567.
3. Wang YT, Wu XT, Wang F: Regeneration potential and mechanism of bone marrow mesenchymal stem cell transplantation for treating intervertebral disc degeneration. *J Orthop Sci* 2010;15(6):707-719.
4. Murphy MB, Moncivais K, Caplan AI: Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med* 2013;45:e54.
5. Vadalà G, Sowa G, Hubert M, Gilbertson LG, Denaro V, Kang JD: Mesenchymal stem cells injection in degenerated intervertebral disc: Cell leakage may induce osteophyte formation. *J Tissue Eng Regen Med* 2012;6(5):348-355.
6. Omlor GW, Bertram H, Kleinschmidt K, et al: Methods to monitor distribution and metabolic activity of mesenchymal stem cells following in vivo injection into nucleotomized porcine intervertebral discs. *Eur Spine J* 2010;19(4):601-612.
7. Vanden Berg-Foels WS: In situ tissue regeneration: Chemoattractants for endogenous stem cell recruitment. *Tissue Eng Part B Rev* 2014;20(1):28-39.
8. Liu X, Liao X, Luo E, Chen W, Bao C, Xu HH: Mesenchymal stem cells systemically injected into femoral marrow of dogs home to mandibular defects to enhance new bone formation. *Tissue Eng Part A* 2014;20(3-4):883-892.
9. Martins-Green M, Petreaca M, Wang L: Chemokines and their receptors are key players in the orchestra that regulates wound healing. *Adv Wound Care (New Rochelle)* 2013;2(7):327-347.
10. Dong F, Harvey J, Finan A, Weber K, Agarwal U, Penn MS: Myocardial CXCR4 expression is required for mesenchymal stem cell mediated repair following acute myocardial infarction. *Circulation* 2012;126(3):314-324.
11. Illien-Jünger S, Pattappa G, Peroglio M, et al: Homing of mesenchymal stem cells in induced degenerative intervertebral discs in a whole organ culture system. *Spine (Phila Pa 1976)* 2012;37(22):1865-1873.
12. Illien-Jünger S, Gantenbein-Ritter B, Grad S, et al: The combined effects of limited nutrition and high-frequency loading on intervertebral discs with endplates. *Spine (Phila Pa 1976)* 2010;35(19):1744-1752.
13. Pattappa G, Peroglio M, Sakai D, et al: CCL5/RANTES is a key chemoattractant released by degenerative intervertebral discs in organ culture. *Eur Cell Mater* 2014;27:124-136, discussion 136.
14. Sakai D, Nishimura K, Tanaka M, et al: Migration of bone marrow-derived cells for endogenous repair in a new tail-looping disc degeneration model in the mouse: A pilot study. *Spine J* 2014.
15. Pereira CL, Gonçalves RM, Peroglio M, et al: The effect of hyaluronan-based delivery of stromal cell-derived factor-1 on the recruitment of MSCs in degenerating intervertebral discs. *Biomaterials* 2014;35(28):8144-8153.
16. Peroglio M, Grad S, Mortisen D, et al: Injectable thermoreversible hyaluronan-based hydrogels for nucleus pulposus cell encapsulation. *Eur Spine J* 2012;21(suppl 6):S839-S849.
17. Peroglio M, Eglin D, Benneker LM, Alini M, Grad S: Thermoreversible hyaluronan-based hydrogel supports in vitro and ex vivo disc-like differentiation of human mesenchymal stem cells. *Spine J* 2013;13(11):1627-1639.
18. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Norton HJ, Hanley EN Jr: Production and expression of RANTES

(CCL5) by human disc cells and modulation by IL-1-β and TNF-α in 3D culture. *Exp Mol Pathol* 2014;96(2):133-138.

19. Kepler CK, Markova DZ, Dibra F, et al: Expression and relationship of proinflammatory chemokine RANTES/CCL5 and cytokine IL-1β in painful human intervertebral discs. *Spine (Phila Pa 1976)* 2013;38(11):873-880.

20. Sakai D, Nakamura Y, Nakai T, et al: Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat Commun* 2012;3:1264.

21. Sakai D, Grad S: Advancing the cellular and molecular therapy for intervertebral disc disease. *Adv Drug Deliv Rev* 2014.

Errata

Case Study: AAOS Clinical Practice Guideline: Management of Hip Fractures in the Elderly

Karl C. Roberts, MD, and W. Timothy Brox, MD

(Vol 23, No 2, February 2015, pp 138-140)

On page 139, column 1, in the paragraph beginning under Management, the parenthetical reference to the AAOS Guideline, which now incorrectly recommends the use of regional anesthesia, should correctly recommend the use of general or spinal anesthesia:

(The AAOS Guideline strongly recommends the use of general or spinal anesthesia "unless a clear and compelling rational for an alternative approach is present," which is the situation in this case.)

Multiple Epiphyseal Dysplasia

Steven Anthony, DO, Richard Munk, MD, William Skakun, DO, and Michael Masini, MD

(Vol 23, No 3, March 2015, pp 164-172)

On page 164, in the author notes section in the right hand column, Dr. Skakun is noted as being affiliated with The Ohio State University. In fact, Dr. Skakun is affiliated with Ohio University.

The *Journal* regrets these errors.