The Potential of Umbilical Cord Derived Mesenchymal Stem Cells in Intervertebral Disc Repair

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- Congress Abstract

Introduction Nucleus pulposus (NP) is the center and major compartment of intervertebral disc (IVD). Mesenchymal stem cells (MSCs) are a type of stem cell source under intensive investigation for their potential to regenerate NP. MSCs have been identified from various sources with different characteristics. There are indications that fetal or close to fetal tissue sources contain cells with relatively undifferentiated phenotype with respect to MSCs from adult sources. Moreover, evidences have shown that umbilical cord-derived MSCs (CMSCs) may have better chondrogenic differentiation potential than bone marrow-derived MSCs (BMSCs).[1]

We hypothesize CMSCs might be a suitable stem cell source for NP regeneration. The aim of this research is to analyze the paracrine effect of MSCs on NP cells, and compare the effect of BMSCs and CMSCs in an attempt to identify a better MSC source for future clinical application.

Materials and Methods Human BMSCs, CMSCs, and degenerated NP cells (three batches each) were isolated and characterized from patients undergoing spinal fusion and patients at caesarean delivery, respectively, after IRB approval was acquired. Conditioned media (CM) was collected after 48 hours exposure to MSC monolayer. Cell proliferation and cytotoxicity were assessed by MTT assay after 1, 3, and 7 days in MSC-CM. Proteoglycan content of NP cells in both types of MSC-CM were measured by DMMB assay after 14 days in culture. Gene expression of degeneration-related molecules of NP cells in MSC-CM, including *CDH2, CD55, FBLN1, Sox9, KRT19, KRT18*, and *MGP*, were determined by real-time RT-PCR. Protein expression of KRT19 in degenerated NP cells before and after MSC-CM treatment was examined by immunocytochemistry and confocal microscopy. All results were normalized to the control group in which the NP cells were cultured in basal medium.

Results Human BMSCs and CMSCs that we isolated satisfied the minimum criteria of MSCs; that is, they were CD73(+), CD105(+), CD146(+), CD14(−), CD45(−), C34(−), and had tri-lineage differentiation potency. The overall metabolic activities of NP cells measured by MTT

reading were significantly enhanced in MSC-CM than that in control basal medium, especially in CMSC-CM. This is accompanied by a slight increase in proteoglycan production. We demonstrated that MMP12, MGP, and KRT19 are the major differential expressed genes between scoliotic and degenerated human NP cells. We found that MGP and MMP12 were significantly downregulated, while KRT19 expression was significantly upregulated, in NP cells treated by MSC-CM, especially CMSC-CM. The increased KRT19 expression in NP cells was also confirmed at protein level by confocal microscopy.

Conclusion This is the first comparative study that discussed how different sources of MSCs affect the biological activities of cultured NP cells through paracrine effect. MGP[2] and KRT19[3] has recently been reported to be associated with IVD degeneration. In this study, MSC-CM effectively upregulated KRT19 while downregulated MMP12 and MGP, suggesting that MSC-CM may promote a recovery of NP cell phenotype. In line with this finding is that MSC-CM treatment also enhanced overall cell metabolic activities, reduced apoptosis, and enhanced proteoglycan production of NP cells in culture. In all aspects tested, CMSC-CM showed stronger effect than BMSC-CM, suggesting that CMSC is a superior source of MSCs for future clinical application for IVD regeneration.

Disclosure of Interest