Umbilical Cord Derived Mesenchymal Stem Cells Impose Better Paracrine Effect Than Bone Marrow Mesenchyma Stem Cells in Nucleus Pulposus Regeneration

INTRODUCTION

Intervertebral disc (IVD) degeneration accounts for most cases of low back pain, which usually starts from the inner nucleus pulposus (NP) area. Increasing investigation into NP regeneration highlights mesenchymal stem cells (MSC) as a potential stem cell source to repopulate the damaged and dehydrated NP. Despite the majority of researches investigate the role of MSC derived from bone marrow, MSC has been identified from various sources with different characteristics. MSC from bone marrow may not be the idea cell source for NP repair, as they are demonstrated to have a tendency of spontaneous osteogenesis [1]. There are indications that fetal or close to fetal tissue sources contain cells with relatively undifferentiated phenotype with respect to MSC from adult sources. Moreover, evidences have shown that umbilical cord (C)-MSC may have better chondrogenic differentiation potential than bone marrow (B)-MSC [2]. We hypothesize CMSC might be a more suitable stem cell source than BMSC for NP regeneration. The aim of this research is to analyse the paracrine effect of MSC on NP cells, and compare the effect of MSC from human bone marrow and human umbilical cord in an attempt to identify a better MSC source for future clinical application.

MATERIALS AND METHODS

Three batches of BMSC and CMSC, and three batches of NP were isolated and characterized from patients undergoing spinal fusion and patients at caesarean delivery respectively, after IRB approval were acquired. Conditioned media was collected after 48hr exposure to confluent MSC monolayer. Cell proliferation and cytotoxicity were assessed by MTT assay after 1, 3 & 7 days in MSC-CM. Proteoglycan content of NP cells in both types of MSC-CM were measured by DMMB assay after 7 & 14 days in culture. Gene expression of degeneration related molecules of NP cells in MSC-CM, including Cadherin-2, CD55, FBLN1, Sox9, KRT19, KRT18, MGP, were determined by realtime RT-PCR. All results were normalized to the control group in which the NP cells were cultured in basal medium.

RESULTS

BMSC and CMSC both possessed spindle-shaped cell morphology in culture. Flow cytometry analysis showed similar expression profile, including negative for CD14, CD34 and CD45, and positive for CD73, CD90 and CD105. They both demonstrated tripotency and can be induced into adipocytes, osteoblasts and chondrocytes (Figure 1).

MTT reading of NP cells in MSC-CM were significantly enhanced than that in control basal medium, especially in CMSC-CM (Figure 2). It is also noticed that cells in conditioned media adopted a more spreaded morphology.

DISCUSSION

We attempted to search for a better source of MSC for IVD regeneration, as the typical source of MSC from bone marrow has spontaneous osteogenesis tendency, which increases the risk of calcification of IVD in the long term. In our study, MSC-CM exerted anti apoptosis/proliferation stimulation effect on NP cells cultured in alginat beads, and effectively upregulated KRT19 while downregulating MGP, stimulating the NP towards a younger phenotype. In all aspects tested, CMSC showed improved regeneration effect in promoting NP cell bioactivities and restore NP cell gene expression than BMSC, which confirms CMSC as a promising source of MSC for future clinical application for NP regeneration.

NEW SIGNIFICANCE

This is the first study revealing how the soluble factors secreted by MSC affect the biological activities of cultured NP cells. Moreover, the effect of MSC stimulation on the gene expression of MGP, MMP12 and KRT19, which are very recently reported to be NP markers related with degeneration, is studied here for the first time. Furthermore, this is also the first study searching for an alternative, and possibly better source of MSC other than MSC from bone marrow for IVD regeneration, revealing an exciting field of MSC application.

REFERENCE