Osteoarthritis-derived Chondrocytes Are Promising Source Of Cartilage-tissue Engineering As the Multipotent progenitors

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Introduction: As the natural healing capacity of damaged articular cartilage is poor, joint surface injuries are a prime target for regenerative medicine. Although in young patients, autologous chondrocyte implantation (ACI) can be applied to repair cartilage defects, elderly patients with osteoarthritis (OA) are lacking an appropriate long-lasting treatment alternative, partly since it is not known if chondrocytes from OA patients have the same chondrogenic differentiation potential as chondrocytes from donors not affected by OA. Recently it is reported that multipotent progenitor cells are present in adult human articular cartilage and that their frequency is increased in OA cartilage. Our hypothesis was therefore, that OA chondrocytes (OACs) could be used as the cell source for cartilage tissue engineering. We studied this hypothesis in OACs by using immunocytochemical, molecular biology, and histological techniques.

Methods: Human bone marrow and OA cartilage was harvested from patients (n=24) undergoing knee joint replacement for OA. The acquisition of human bone marrow and cartilage was approved by both Institutional Review Boards. Human non-OA cartilage was harvested from patients who underwent bipolar hemiarthroplasty for femoral neck fracture and arthroscopic Bankart repair for recurrent shoulder dislocation (n=7). These freshly isolated cells defined as primary (P0) cells were plated in tissue culture flasks. The term passage number was defined according to the number of times chondrocytes were trypsinized and replated in monolayer culture (described as P1 to P3). Cell surface expression of MSC markers was analyzed by flow cytometry. For osteogenic and adipogenic differentiation, OACs and MSCs were cultured to subconfluent levels; osteogenic and adipogenic medium was then added for 21 days. For chondrogenic differentiation and re-differentiation, 2 x 10^5 cells were centrifuged for 5 min at 500g in a 15-mL polypropylene tube. The pellet was then treated with chondrogenic medium supplemented with 10ng/mL TGF-β3 for 21 days. Gene expression was evaluated by RT-PCR and cell surface expression of MSC markers were analyzed by flow cytometry. Histological and immunohistochemical staining was performed to evaluate each differentiation ability. In addition we assessed expression of Micro-RNA in OACs during re-differentiation. All data were presented as mean ± standard deviation (SD). Statistical significance was determined using Mann-Whitney U-test. P values less than 0.05 were considered significant.

Results: Flow cytometry analyses showed that the cell surface marker profile of OACs has a resemblance to that of MSCs (Fig.1A). The percentage of MSC marker expression of OACs was high (70%~92%) at P0 (Fig.1B). OACs and MSCs differentiated into chondrogenic, adipogenic, and osteogenic lineages. Osteogenic calcium deposition was induced under the osteogenic condition (Fig. 2A). Adipogenic induction was indicated by the accumulation of lipid vacuoles (Fig.2B). Chondrogenic-differentiated pellets were stained with Safranin O and Collagen II. The intensity of safranin O-stained proteoglycans was higher in OACs pellets than in MSCs (Fig. 2C). The mRNA expression of Sox9, Col2a1, COMP, and Aggrecan in OACs were higher during re-differentiation by pellet culture compared with uninduced cells (Fig.2D). Expression of MMP13 and ADAMTS5 in OACs were higher than non-OACs at P0 (Fig.3A). However, expression of these genes in OACs were decreased to the same level as non-OACs during re-differentiation (Fig.3B). The expression of miR-140 and miR-27b in OACs during re-differentiation were higher than...
Single-immunolabeling of OACs and MSCs. (A) The cell surface expression of CD29, CD44, CD73, CD90, CD105, CD45 and HLA-DR antigens were analyzed individually by flow cytometry of P2 or P3 cells (non-OA n=3, OAC n=8, MSC n=5). (B) Positive marker expression of OACs (n=4) were analyzed primary chondrocytes (P0) and up to 2nd
Differentiation potentials of OACs and MSCs. (A) Osteogenic
differentiation. Mineralization in treated cells was revealed by Alizarin Red S staining. (B) Adipogenic differentiation. Lipid vacuoles were staining with Oil Red O. (C) Chondrogenic differentiation. Cell pellet were stained with Safranin O and Collagen II. (D) Each mRNA expression were increased during re-differentiation by pellet culture comparing with that of the monolayer culture on 14 days (OAC n=8, MSC n=5).
catabolic genes in non-OACs (n = 4) and OACs (n=4) at P0. (A) The mRNA expression of MMP13 and ADAMT55 in OACs were higher than non-OA chondrocytes. (B) The mRNA expression of MMP13 and ADAMT55 in OACs (n =4) were decreased to the same level as non-OACs (n = 4) during re-differentiation by pellet culture. (C) The expression of miR-27b and miR-140 were increased during re-differentiation by pellet culture (n = 4).

Discussion: The expressions of MSC markers such as CD29, CD44, CD73, CD90, and CD105 in OACs after monolayer culture were up-regulated. Moreover, OACs could be differentiated into adipogenic, osteogenic, and chondrogenic lineages. Suggesting that OACs will meet the minimal criteria for hMSCs as proposed by the International Society for Cellular Therapy. In particular, it seemed that OACs showed higher chondrogenic potentials than MSCs in this study. Immunohistochemistry confirmed that OACs were capable of producing Collagen II. In addition, the gene expression of catabolic markers such as MMP13 and ADAMT55 up-regulated in OACs in monolayer culture were decreased to the levels of healthy cartilages after re-differentiation by pellet culture. MiR-140 is recently proved to play an important role in the pathogenesis of OA by a mechanism that could at least partly be involved in the regulation of ADAMT55. On the other hand, miR-27b regulates the expression of MMP-13 in OA chondrocytes. Normal human articular cartilage expresses miR-140 and miR-27b, and their expression are significantly reduced in OA tissue. Our results demonstrated that the expression of miR-140 and miR-27b were up-regulated, and the expression of ADAMT55 and MMP13 were down-regulated in OACs during re-differentiation by pellet culture. Our findings suggest that OACs have regenerative potentials (to normal chondrocytes rather than MSCs) and are promising cell-source of cartilage-tissue engineering as multipotent progenitors.

Significance: In clinical practice, there is no appropriate therapies other than implant arthroplasty for patients with progressive OA. The results of the present study suggest that OACs might be promising source of cartilage-tissue engineering as multipotent progenitors.

Acknowledgments:


ORS 2014 Annual Meeting
Poster No: 1285