Effect of Co-culture with Chondrocytes on Adipose Tissue-derived Mesenchymal Stem Cells

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Introduction
Although bone marrow provides the most universal source of MSC, adipose tissue also offers an abundant and easily accessible pool of MSCs. Adipose tissue-derived mesenchymal stem cells (ATMSCs) obtained from liposapirates have the multi-lineage potential to differentiate into adipogenic, chondrogenic, myogenic, and osteogenic cells. However, several studies indicated that ATMSCs have lower chondrogenic potential compared with MSCs from bone marrow. The aim of this study was to test the hypothesis that co-culture of ATMSCs with chondrocyte or culturing ATMSCs under conditioned medium from chondrocytes might promote chondrogenesis from ATMSCs.

Materials and Methods
Chondrocytes isolated from unaffected cartilage of four patients (mean age: 76 years, range: 62-86) who underwent total knee arthroplasty from arthritis. The ATMSCs were isolated from 4 patients (mean age: 45 years, range: 29-66) undergoing elective liposuction. To examine cell-cell interactions in co-culture, in-vitro pellet cultures were carried out by mixing 1.25 x 10^5 ATMSCs (P3) and 1.25 x 10^5 chondrocytes (P3) in the same pellet, and compared with the pellets from 2.5 x 10^5 ATMSCs or from 2.5 x 10^5 chondrocytes. These were cultured in chondrogenic medium that did not contain growth factors. In order to effect of conditioned media from chondrocytes culture on ATMSCs, 2.5 x 10^5 ATMSCs were cultured in soups (soup: conditioned medium from chondrocytes culture). After the 21st day of in-vitro culture, the pellets were harvested for DNA quantification, analysis of the glycosaminoglycan content, reverse transcription, and real-time PCR for collagen type I (COL1A1), collagen type II (COL2A1), collagen type X (COL10A1), and Sox9. Safranin-O staining also was carried out to assess the proteoglycan production.

Results
Prior to co-culture, ATMSCs were labeled with TSR50 (TER50: cell membrane labeling kit). After three weeks, immunofluorescence showed ATMSC- chondrocyte interaction in pellet. The pellets were digested after 3 weeks of culture, and the DNA contents and GAG were measured (Fig. 1, 2). GAG contents increased from ATMSCs either by co-culturing with chondrocytes (10.7%, p<0.05) or by culturing under conditioned medium from chondrocytes culture (9.6%, p<0.05). Real-time PCR analysis showed that COL2A1 slightly increased by 20% when ATMSCs were treated with conditioned medium from chondrocytes culture. COL1A1 gene expression was stationary and COL10A1 gene expression slight increased in the conditioned medium (Fig 3). Although SOX-9 and COL2A1 gene expression was several times greater in the ATMSCs-chondrocytes co-culture than in ATMSCs only, these gene expressions were still several times lower than those in chondrocytes only. Interestingly, COL1A1 and COL10A1 gene expressions were greater in ATMSCs-chondrocytes co-culture than in ATMSCs only, let alone than in chondrocytes (Fig.3). The results from Safranin-O staining generally paralleled those from GAG analyses: greater metachromatic staining when treated with conditioned media or in ATMSCs-chondrocytes co-culture (Fig 4).

Discussion and Conclusions
Conditioned media from chondrocyte culture has moderate enhancing effect on the chondrogenesis from ATMSCs. Co-culture of ATMSCs with chondrocytes does not seem to further promote chondrogenesis from ATMSCs.

References

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Disclosures
Include commercial conflict of interest disclosure information here, or a statement that authors have nothing to disclose.