The stability of cartilage phenotype in human bone marrow stromal cells and chondrocytes

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Introduction:

To repair cartilage lesions, modern cell-based treatments focus on the employment of various cell types like Bone Marrow Stromal Cells (BMSCs) and chondrocytes. To be successful on the long term, stability of the cell phenotype in the generated cartilage is required. Recent studies show that besides BMSCs, chondrocytes are also multipotent. In this study we analyze the stability of the chondrogenic phenotype of BMSCs, and primary and dedifferentiated chondrocytes. To induce a chondrogenic phenotype the 3 cell types are pre-cultured in chondrogenic medium. To test the phenotypic stability of these cells an assay was used where chondrogenic medium was switched to adipogenic or control medium. Finally, we evaluate and compare the phenotype of the different cell types by RT-PCR and histology.

Materials and Methods:

Human BMSCs were expanded for 3 passages. Primary chondrocytes from human knee joints were used immediately, or dedifferentiated by expansion for 2 passages in monolayer. The 3 cell types were pre-cultured for 10 days on chondrogenic medium, both in 2-d monolayer and 3-d alginate beads. For the following ten days cells remained on chondrogenic medium or switched to adipogenic or control medium (no chondrogenic factors). At day 20, histology and RT-PCR analysis were used to evaluate the effects of the medium switch of the cells.

Results:

Collagen 2 (COL2) expression confirmed that chondrogenesis was induced on chondrogenic medium in all cell types after 10 days and even more obviously after 20 days. When chondrogenic medium was switched to adipogenic, a tempering of chondrogenic phenotype (COL2) in all cell types was observed. Furthermore, primary and dedifferentiated chondrocytes showed decreased expression of the hypertrophic marker collagen 10 in the adipogenic medium, while BMSCs did not. Furthermore the switch to adipogenic medium gave elevated aP2 expression in all 3 cell types. This occurred most clearly in the BMSCs, which was confirmed by histology, as BMSCs were able to create lipid vacuoles when switched to adipogenic medium, while primary and dedifferentiated chondrocytes were not.
Discussion and Conclusions:

These results indicate that the chondrogenic phenotype is not stable after 10 days of chondrogenic culture. BMSCs seem less stable than primary and dedifferentiated chondrocytes. Future plans include creating a more stable cartilage phenotype in BMSCs, via switching experiments with a longer chondrogenic pre-culture time.