SMOOTH MUSCLE ACTIN EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES AND THEIR CONTRACTION OF A COLLAGEN-GAG MATRIX IN VITRO

*Kinner, B (A-Robert-Bosch-Foundation, Stuttgart, Germany); *Spector, M
+*Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. Department of Orthopaedic Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, 617-732-6702, Fax: 617-732-6705, BKin@rics.bwh.harvard.edu

Introduction: Cell therapies for cartilage defects, becoming increasingly important, require expansion of cell number in monolayer culture (1). During growth in two-dimensional culture chondrocytes undergo changes in their phenotype as selected genes are up- or down-regulated. Of importance is that the cells are capable of re-expressing their chondrocytic phenotype when introduced into certain environments. Recently it was shown that chondrocytes are able to express the gene for a contractile actin isoform, o-smooth muscle actin (SMA) in situ (1), and are capable of contracting a collagen matrix in vitro (2). The expression of SMA could impact the performance of transplanted chondrocytes and cell-seeded matrices employed for tissue engineering. The objective of this study was to evaluate the expression of SMA in human articular chondrocytes grown in monolayer culture and the contractile behavior of the cells when seeded into a collagen-GAG analog of extracellular matrix.

Materials and Methods: Articular cartilage obtained from 13 patients undergoing total joint replacement was used in the study. Cartilage quality was graded clinically according to Outerbridge and histologically according to Mankin. Cartilage grading Outerbridge Grade III and IV was excluded. The tissue was digested overnight at 37°C using 0.1% clostridial collagenase (type 1, ~400 U/mg). Isolated cells were grown in monolayer with DMED/F12, supplemented by 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma). Cells were used in standard conditions (37°C, 5% CO2 and 95% humidity).

3-D Cell Culture: The type I collagen-GAG copolymer sponge-like matrices were produced using a method previously described in detail (3). Briefly, type I bovine tendon collagen was blended with chondroitin-6-sulfate. The degassed and lyophilized co-precipitate was described in detail (3). Briefly, type I bovine tendon collagen was blended with chondroitin-6-sulfate. The degassed and lyophilized co-precipitate was used to produce 3-D cell culture matrices. The type I collagen-GAG matrix in vitro was used to study the growth and contractile behavior of the cells.

2-D Cell Culture: Cells were subcultured in 75 cm² flasks seeded at a density of 2*10⁴ cell/cm². Reaching confluence an aliquot of 1x10⁶ cells was used for protein extraction.

3-D Cell Culture in Collagen-GAG Matrices: The type I collagen-GAG copolymer sponge-like matrices were produced using a method previously described in detail (3). Briefly, type I bovine tendon collagen was blended with chondroitin-6-sulfate. The degassed and lyophilized co-precipitate was subsequently cross-linked for 24 hours using the dehydrothermal method. Randomly selected cell populations, either early (p2) or late passage (p6) were subsequently cross-linked for 24 hours using the dehydrothermal method. Randomly selected cell populations, either early (p2) or late passage (p6) were subsequently cross-linked for 24 hours using the dehydrothermal method. Randomly selected cell populations, either early (p2) or late passage (p6) were subsequently cross-linked for 24 hours using the dehydrothermal method. Randomly selected cell populations, either early (p2) or late passage (p6) were subsequently cross-linked for 24 hours using the dehydrothermal method.

SMA immunohistochemistry and determination of DNA content after 1, 7, 14 and 21 days.

Western Blot Analysis for SMA: Samples of 7 patients were recovered immediately after isolation for Western blot analysis. Cell extracts normalized for total protein content were resolved using SDSPAGE. As positive control 5 µg of a smooth muscle cell extract (human aorta cell) was used. The resulting gel was transferred to a PVDF membrane, incubated with the primary antibody (Sigma Chem. Co., St. Louis) and subsequently developed using a peroxidase-labeled secondary antibody and a luminol-based chemiluminescent detection system. The resulting western blots were digitized for densimetric analysis using NIH Image processing and analysis software.

Histology, Immunohistochemistry: Immunohistochemistry was performed on paraffin-embedded cartilage specimens and collagen-GAG matrices as well as on the 2-D cultures using the monoclonal anti SMA antibody and a monoclonal antibody for collagen II (II-H6B3, Developmental Studies Hybridoma Bank, Iowa, USA). Statistical Analysis: Data are reported as the mean ± S.E.M. One-factor and two-factor ANOVAs with Bonferroni post-hoc testing for multiple comparison were performed using GraphPad Prism® 3.02 Software. Statistical significance was assumed as p < 0.05.

Results: The mean age of the patients was 61 with a range of 51-74. The cartilage samples had a mean Mankin score of 4 (range, 2-8).

Expression of SMA: Immunohistochemistry revealed that 60-70% of the chondrocytes in the superficial layer were SMA-positive while 28-37% of the cells in the deep layer stained positive for this actin isoform. Immediately after cell isolation, cells from 3 of the 7 patients showed a positive result for SMA using Western blot analysis. Upon reaching confluence all samples expressed SMA. Subculturing the cells led to a significant increase in expression of SMA (up to 20-fold, p<0.0001; Fig. 1). Semi-quantitative evaluation of immunohistochemically stained slides confirmed these results. Moreover, immunohistochemistry revealed an increasing percentage of cells displaying the polymerized form of actin revealed as SMA-positive staining stress fibers. Using immunohistochemistry for collagen II, there was a marked decrease in the expression of procollagen type II, although small amounts of procollagen type II were expressed throughout the experiment up to passage 4 (Fig. 2).

Contraction Assay: The cells contracted the collagen-GAG matrices by approximately 15%. Late passage (p6) chondrocytes demonstrated significantly more cell-mediated contraction of the collagen-GAG scaffolds than the early (p2) passages (p<0.0001).

Discussion: The present study is the first report showing the increased expression of a muscle actin, SMA, in serially passaged human chondrocytes. That the SMA-containing cells have the capability to contract, was demonstrated by the cell-mediated contraction of a collagen-GAG matrix in vitro. Of importance was the demonstration that SMA-containing cells also express type II collagen and therefore retain characteristics of articular chondrocytes. Interestingly, late passage chondrocytes did not regain their chondrocytic phenotype completely when seeded to a collagen I-GAG matrix. These results may be helpful to consider when serially passaged chondrocytes are implanted into a cartilage defect. Moreover tissue engineering approaches for cartilage repair may have to identify methods to control the contractile behavior of chondrocytes.

Acknowledgments Supported by the Brigham Orthopaedic Fdn. B.K. was supported by the Robert-Bosch-Fdn.

Reference