Influence of Growth Factors (BMP-2, BMP-7) on Human Rotator Cuff Tendon Cells

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Study aim:
Surgical repair of rotator cuff (RC) tears is prone to high retear rates as a consequence of incomplete tendon-bone regeneration. Many biomechanical approaches have improved time-zero mechanical stability, however, recent techniques may even deteriorate tissue regeneration due to mechanical tissue strangulation. In order to explore biological aspects of tendon-bone healing, it was the aim of the present study to first establish and characterize a primary human rotator tendon cell (tenocyte) culture. Subsequently, the influence of growth factors BMP-2 and -7 on tenocyte cell activity, matrix production and -expression was determined. Since their effect on bone cells is well described, a beneficial effect of these factors on tenocytes would be promising to improve RC-repair.

Materials and Methods:
After written informed consent, tissue samples were obtained from patients undergoing arthroscopic RC-repair (M. supraspinatus, Long head biceps tendon). After enzymatic cell isolation cells were cultivated (DMEM/HAM’S F12, 10% FCS, 1% Pen/Step), and characterized by Real-time PCR-analysis of several markers (COL I-III, Aggrecan, Decorin, Biglycan, Osteocalcin, Scleraxis) to rule out metaplasia towards chondroid or osteogenic cells. Subsequently, cells were incubated with BMP-2 (100, 500, 1000 ng/ml) and BMP-7 (100, 500, 1000, 2000 ng/ml), alone as well as in combination (100, 500, 1000 ng/ml BMP-2 + 1000 ng/ml BMP-7). At day 0, 3 und 6, cell activity was assessed (Alamar Blue Assay). Collagen Type I production (COL-I, ELISA) was determined from supernatant at day 6 to quantify production of extracellular tendon matrix (ECM). Expression of several tendon-, bone- and cartilage-related markers (including COL-I-III, Osteocalcin, Scleraxis) to rule out metaplasia (including COL-I-III, Osteocalcin, Scleraxis) was evaluated by Real-Time PCR at day 6.

Statistics: ANOVA with Bonferroni or Dunnett-Test.

Results:
The characterization of the harvested cells revealed that the primary tendon cell cultures show an expression profile that differed significantly from chondrocytes in terms of lower Aggrecan- and COL-II expression and from osteoblasts in terms of lower Osteocalcin-expression (Fig. 1).

The application of the two different growth factors to the cells showed dose-dependent effects. Application of BMP-2 significantly increased COL-I production of the tenocytes (Fig. 2 a). Cell activity was decreased after application of a higher BMP-2 dosage over time compared to non-treated control. A dose-dependent, significant increase of COL-I production and expression (Fig. 2 b) as well as significantly increased cell activity were observed for BMP-7 incubated cells when compared to non-treated controls. However, combination of both factors resulted in decreased parameters compared to BMP-7 application alone. Expression of Scleraxis and Collagen Type III in the tenocytes was slightly increased by application of BMP-2 and BMP-7. The investigation of the Collagen Type II and Osteocalcin expression showed no tendon cell differentiation towards a chondrogenic or osteoblastic phenotype.

Discussion:
First, the present approach represents a precise characterization of a primary human rotator cuff tenocyte culture. Both BMP-2 and -7 have well-described effects on differentiation and proliferation of osteoblasts and are currently used in clinical applications. Furthermore, both substances promote tendon graft integration into bone tunnels in animals[1-3]. BMP-7 stimulates ligament and tendon cell proliferation as well as expression of several markers in cell cultures of rodent and bovine origin[4,5]. The present in vitro study describes further effects on human tendon cell biology for the first time. Both tendon and bone tissue potentially need to be addressed to improve regeneration of the rotator cuff. In particular, stimulation of tenocyte activity as well as increased production and expression of COL-I (the main component of tendon-ECM) may improve the integration between tendon and bony footprint.

The current data suggest further investigations especially of local application of BMP-7 to the site of RC tendon-bone repair in order to biologically stimulate their solid reintegration.

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References:
1. Rodeo et al. AJSM, 1999
2. Mihelic et al. AJSM, 2004
3. Ma et al. AJSM, 2007