INTRODUCTION: Decorin, one of the small proteoglycans (PGs), is present in the extracellular matrix of articular cartilage. Decorin was shown to be involved in collagen fibrillogenesis and in interaction with matrix molecules, e.g., fibronectin and growth factors. TGF-ß plays important roles in cartilage growth, maintenance and repair. Since decorin can bind to TGF-ß, it may regulate the effect of TGF-ß on chondrocytes. We previously reported [1] that the expression of decorin is increased in osteoarthritic (OA) cartilage and hypothesized that increased levels of decorin might negatively affect the function of TGF-ß by forming a complex with the growth factor, thereby keeping it from binding to its cell surface receptor. This may cause the abrogation of the effect of TGF-ß on chondrocytes, including the synthesis of the major matrix molecules, and may lead to a shift in the biological processes that are necessary for the maintenance of the homeostasis in the cartilage matrix. Therefore, it is important to determine the combined effect of decorin and TGF-ß. Thus, the goal of this study was to elucidate the effect of TGF-ß on chondrocytes, the combination of these two factors on chondrocytes during matrix assembly. For this purpose, human articular chondrocytes were cultured in alginate beads to maintain phenotype stability; and to determine the changes at both message and protein levels of several matrix components triggered by TGF-ß and/or decorin.

MATERIALS AND METHODS: Human ankle joints with no known joint disease were received from nine donors (55 - 70 years old) through the Regional Organ Bank of Illinois (with informed consent of the family), and used with the permission of the Internal Review Board.

CULTURE OF HUMAN CHONDROCYTES IN ALGINATE BEADS: Chondrocytes from full-depth human ankle cartilage samples were released by pronase/collagenase digestion and resuspended in 1.2% alginate solution at a density of 4x10⁴ cells/ml. The beads were polymerized, the chondrocytes in the beads were cultured in DMEM/F12 medium with ITSC supplementation in the absence or presence of 5ng/ml rhTGFß, 5 μg/ml rhdecorin, or the mixture of these two factors (tissues from donors for each experiment in triplicates), for 16 days with daily changes of medium. After the culture period, beads were dissolved in citrate buffer and, by centrifugation, two components were separated: the cells with their cell-associated matrix and the further removed matrix (FRM) containing matrix proteins synthesized and deposited by chondrocytes. DNA content of the cells in each bead was determined by PicoGreen DNA assay (Molecular Probes) and experimental data were normalized to DNA content. Proteoglycan content in the beads and the medium was determined by the DMB assay [3]. Protein content determination: Beads were reconstituted in 2.4% alginate in the presence of barium chloride and components of the FRM were extracted from the insoluble beads with 4M guanidine-HCl in the presence of protease inhibitors. Purified polyclonal or monoclonal antibodies to biglycan, decorin, fibromodulin, link protein and type II collagen were bound to Magnabind IgG beads (Pierce) and used to immunoprecipitate the matrix proteins. Western blot hybridization: Immunoprecipitated matrix components were separated by SDS-PAGE followed by transfer to nitrocellulose membranes, and probed with the appropriate antibodies. Signals were detected with the enhanced chemiluminescence assay (Amersham) and band densities were quantified. mRNA levels of the cells: Total RNA was isolated from chondrocytes released from the alginate beads. Semi-quantitative reverse transcription-polymerase chain reactions (RT/PCR) were used to determine the amount of mRNA of the matrix proteins in the cultured cells normalized to that of GAPDH.

DISCUSSION: Articular chondrocytes cultured in alginate beads produce, de novo, a matrix rich in PGs. Our studies showed that most of the matrix proteins expressed by chondrocytes were incorporated into the newly-built matrix and were detectable at both message and protein levels within the beads. However, collagen type II and decorin were unable to be incorporated into the matrix during 16 days of culture. After the chondrocytes were cultured for 90 days, both decorin and type II collagen were readily detectable in the alginate beads. This result provides further evidence that collagen type II is the major binding partner of decorin in articular cartilage. TGF-ß up-regulated the synthesis of the matrix components to various extents. Aggrecan and link protein, being binding partners, were up-regulated to the same magnitude. Biglycan and decorin were moderately up-regulated by TGF-ß, but fibromodulin increased greatly at both message and protein levels. When TGF-ß was pre-incubated with decorin, the positive effect of TGF-ß on the synthesis of major matrix components was diminished while it remained unchanged for the small PGs. It was due to the fact that decorin itself down-regulated the expression levels for aggrecan, link protein and collagen type II; however, it did not affect that of the small PGs.

In summary, the proposed mechanism for the combined action of TGF-ß and decorin is as follows: decorin binds to TGF-ß, and the decorin-bound TGF-ß is still able to regulate the metabolism of chondrocytes. Decorin and TGF-ß express their effects on chondrocytes independent of each other. Therefore, decorin can counteract the effect of TGF-ß, not through binding to it and thereby preventing TGF-ß from binding to its receptors, but by expressing opposite effects on chondrocytes. Thus, a well-regulated interplay between TGF-ß and the small PGs is important for proper cartilage formation.

REFERENCES:

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