

## SOX9 TRANSDUCTION AND TGF $\beta$ -3 TREATMENT OF LATE PASSAGE HUMAN ARTICULAR CHONDROCYTES IN PELLET CULTURE POTENTIATES CARTILAGE MATRIX FORMATION

SR Tew\*, Y Li\*\*, LM Tweats\*, T Katopodi\*, RE Hawkins\*\* and TE Hardingham\*

UK Centre for Tissue Engineering, \*Stopford Building, University of Manchester, Manchester, UK;

\*\*Paterson Institute for Cancer Research, Christie Hospital, Manchester, UK.

**INTRODUCTION:** The expansion of articular chondrocytes for tissue engineering purposes leads to loss of chondrocytic function that can be difficult to regain. We have transduced SOX9, a transcription factor crucial for the induction and regulation of the chondrocyte phenotype<sup>1</sup>, into monolayer expanded human articular chondrocytes using a retroviral vector<sup>2</sup>. The cells were grown as pellet cultures in the presence of TGF $\beta$ -3 and IGF-1 and the expression of marker genes and extracellular matrix (ECM) production was compared with that of control cultures.

**METHODS:** Human articular chondrocytes (HAC), from tissue obtained following total knee arthroplasties, were grown as monolayers and between passages 2-5 cell growth was accelerated by stimulation with growth factors. During this time the cells were transduced with a retrovirus bicistronically expressing human SOX9 and green fluorescent protein. After transduction, the cells were >90% positive. Control cells were transduced with a retrovirus containing GFP only. Subsequently, the cells were further expanded in monolayer to passages 7-10 and then grown as pellet cultures for 14 days in medium containing 10ng/ml TGF $\beta$ -3 and/or 100ng/ml IGF-1. Glycosaminoglycan (GAG) accumulation was measured using the dimethylmethylene blue assay on papain-digested pellets. Pellets were fixed in 4% formaldehyde and embedded in paraffin wax for histological analysis. 5 $\mu$ m sections were cut and stained with 0.1% safranin-O. Total RNA was prepared from pellet cultures using Tri Reagent. cDNA was reverse transcribed and then amplified by PCR with an MJ Opticon 2 using a SYBR Green Core Kit (Eurogentec) with collagen I, II and SOX9 specific primers. Relative expression levels were normalised using GAPDH. Protein extracts were prepared by grinding the pellets in RIPA buffer. They were run on 4-12% Nu-PAGE gels and western blots were carried out using antibodies to collagen I and II and GAPDH.

**RESULTS:** SOX9 transduction led to larger, heavier pellets after 14 days in culture. The size of the pellets was further increased by the addition of

TGF $\beta$ -3 and IGF-1 to the medium during culture. These growth factor treated SOX9 transduced cultures displayed elevated levels of GAG retention in the pellets as assayed using DMB. Histologically, SOX9 transduced chondrocytes treated with TGF $\beta$ -3 and IGF-1 displayed strong safranin-O stained ECM populated by rounded cells. Control pellets under all conditions did not display cartilage matrix morphology and neither did SOX9 transduced cells cultured without growth factors or with IGF-1 alone. Treatment of transduced cells with TGF $\beta$ -3 and IGF-1 led to maximal collagen II gene expression 2 times higher than in growth factor free SOX9 transduced cultures and nearly 1700 times higher than growth factor free control cultures. Collagen I was upregulated in all pellet cultures when TGF $\beta$ -3 was present and did not seem to be affected by SOX9 expression levels. Western blotting of protein extracts of growth factor treated pellets showed a large increase in collagen II accumulation in the pellets formed from SOX9 transduced cells when compared to control cell pellets. Collagen I was found at similar levels in both transduced and non-transduced cell pellets.

**DISCUSSION:** Passaged HAC show major loss of expression of matrix genes. We have shown that continuous expression of SOX9 via a retrovirus improves collagen II expression in monolayer culture of late passage HAC and also enhances their ability to respond to 3-dimensional cultures<sup>2</sup>. We have used these cells to produce a maximal chondrogenic system by stimulating them in pellet cultures with TGF $\beta$ -3 and IGF-1. In these cultures, synthesis of ECM genes and the retention of ECM proteins around the cells is significantly upregulated. Furthermore, under these conditions, the cells show a typically rounded chondrocyte-like morphology not seen in any of our other pellet cultures. It would appear that the synthesis of appropriate cartilage matrix components and their localized retention is dependant on SOX9 expression and is an important factor in further influencing the morphology and phenotype of monolayer expanded articular chondrocytes.