

## Integrin $\alpha 10\beta 1$ , a unique cell-surface marker for chondrogenic cells

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**INTRODUCTION:** Cartilage development and function depends on the interaction between the chondrocytes and the surrounding extra cellular matrix (ECM). Integrins, present on chondrocytes, have a central role in mediating these interactions. Our work has focused on the collagen binding integrin  $\alpha 10\beta 1$ , expressed mainly by chondrocytes<sup>1</sup>. Knockout mice lacking  $\alpha 10\beta 1$  have defect growth plate and develop a growth retardation of the long bones. The length of the hypertrophic zone is reduced and the columns of the growth plate are disorganized with rounded chondrocytes. In addition, chondrocyte proliferation is reduced and the matrix contains less collagen<sup>2</sup>. In healthy human articular cartilage  $\alpha 10\beta 1$  is expressed on the majority of the chondrocytes, however, in patients with osteoarthritis the chondrocytes loose expression of  $\alpha 10\beta 1$ , starting in the upper layer.

Integrin  $\alpha 11\beta 1$ , a collagen binding integrin mainly expressed by fibroblasts, is upregulated on cultured and dedifferentiated chondrocytes and appears to oppose the expression of  $\alpha 10\beta 1$ .

**METHODS:** Human chondrocytes were cultured in monolayer for 4 weeks and MACS sorted using a monoclonal antibody specific for  $\alpha 10$  (mAb365). mRNA expression of *COL1A*, *COL2A*, *aggrecan* and *GAPDH* was analyzed using real time PCR. C-28/I2 cells, stably transfected with  $\alpha 10$ , and wild-type C-28/I2 cells were differentiated in pellet-mass and mRNA expression of *COL2A*, *Aggrecan*, *COL1A*, *integrin  $\alpha 11$* , *MMP-13* and *GAPDH* was analyzed. Human mesenchymal stem cells (MSCs), were cultured with or without bFGF and subjected to chondrocyte differentiation in pellet-mass. On day 7, 14 and 21 mRNA expression of *GAPDH*, *COL2A*, *aggrecan*, *integrin  $\alpha 10$*  (*ITGA10*), *integrin  $\alpha 11$*  (*ITGA11*) and *SOX9* was analysed. Supernatant was analyzed for synthesis of collagen type II (CPII pro-peptide ELISA) and proteoglycan (35-S incorporation).

**RESULTS:** Integrin  $\alpha 10\beta 1$  as well as collagen II expression is down-regulated during monolayer culture and up-regulated during re-differentiation of human chondrocytes. By

sorting  $\alpha 10$  expressing cultured chondrocytes we demonstrate that high expression of integrin  $\alpha 10$  correlates with high expression of *COL2A* and *aggrecan* and low expression of *COL1A*. When we investigated the effect of growth factors on  $\alpha 10$ -expression we found that bFGF up-regulates while TGF- $\beta$  down-regulates the expression in human chondrocytes. A chondrogenic role for  $\alpha 10$  was also shown by transfecting the chondrocytic cell-line C-28/I2 with  $\alpha 10$  which resulted in elevated expression of *COL2A* and *aggrecan* and reduced expression of *COL1A*, *ITGA11* and *MMP-13* after differentiation in pellet mass.

We also found that human MSCs express integrin  $\alpha 10$  and that bFGF up-regulates the expression. Furthermore, after chondrogenic differentiation in pellet cultures, bFGF pre-treated cells, with high levels of  $\alpha 10$  have an increased mRNA expression of *COL2A*, *aggrecan* and *SOX9*, compared to un-treated cells. By analyzing the Col II pro-peptide, we could demonstrate that only the bFGF pre-treated MSCs synthesize and process collagen type II. The bFGF pre-treated MSCs also have increased proteoglycan synthesis compared to un-treated cells.

**DISCUSSION & CONCLUSIONS:** We conclude that high expression of  $\alpha 10\beta 1$  on chondrocytes correlates with high expression *COL2A* and *aggrecan* and low expression of *COL1A*. We also conclude that MSCs cultured under conditions that favours expression of  $\alpha 10$ , has an enhanced capacity to synthesize cartilage-specific molecules. Taken together our results strongly suggest that integrin  $\alpha 10$  is a unique cell surface marker for chondrocytes and MSCs with chondrogenic potential.

Our finding that over-expression of  $\alpha 10\beta 1$  in a chondrocytic cell-line resulted in increased expression of cartilage-specific markers suggests that  $\alpha 10\beta 1$  play an important role in modulating the homeostasis of cartilage.

**REFERENCES:** <sup>1</sup>L. Camper, et al. (2001) *Cell Tissue Res* **306**:107-16. <sup>2</sup>T. Bengtsson, et al. (2005) *J Cell Sci* **118**:929-36.