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Effect of transforming growth factor- β on up/down regulation of integrin- β 1 in primary chondrocyte culture

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INTRODUCTION: Regeneration of a damaged or non-functioning tissue requires adhesion of cells to their extracellular matrix (ECM). Thus the investigation of the level of synthesised cell adhesion molecules (CAMs) in cell culture systems play major roles in cell and tissue engineering. Adhesion of chondrocyte to a collagen type-II rich matrix, is dependent on cell adhesion molecules (CAMs) and integrins and cells adhere to ECM through integrins.

METHODS: Monolayer-expanded primary chondrocyte cells derived from fourth passage were used in this work. The cells were isolated from knee joint of neonate Sprague-Dawley rat, and the protocol has been described in detail elsewhere [1]. Eight 22 mm² glass coverslips were sterilised with eight petri dishes labelled as Control, TGF- β 1, TGF- β 2, TGF- β 3, TGF- β (1+2), TGF- β (1+3), TGF- β (2+3), and TGF- β (1+2+3).

Chondrocyte cells were cultured, expanded in monolayer culture system and 8×10^6 cells were resuspended in 40 ml DMEM media supplemented with 10% FCS. Five ml of cell suspensions were subjected to seeding on each coverslip. Five ml of cell suspension was also seeded on coverslips labelled as control. Remaining cell suspensions were aliquoted in 5 millilitres and supplemented with TGF- β 1, TGF- β 2, TGF- β 3, TGF- β (1+2), TGF- β (1+3), TGF- β (2+3), and TGF- β (1+2+3). All cell cultures were incubated at 37°C for 24 hours. After 24 hours, cells were fixed by 1% formaldehyde and immunocytochemically stained for integrin β 1 (CD29).

RESULTS: The isolation and purification of cartilage cell (chondrocyte) and cultivation of this cell in planar culture system showed that cells with low density produced fibroblast like morphology and synthesised collagen type-I instead of collagen type-II. Monolayer culture of the chondrocyte resulted in dedifferentiation of cells and production of stress fibres. This characteristic was prevented by high density and 3D multilayer chondrocyte culture. TGF- β 2, TGF- β 3, and manipulated TGF- β (2+3) exhibited similar synthesis of integrin- β 1

(CD29) to control (Figure 1A-B), but TGF- β 1, TGF- β (1+2), TGF- β (1+3), and TGF- β (1+2+3) decreased the expression of integrin- β (Figure 1C-D). This is likely due to gene expression level of TGF- β and the chondrogenic transcription factors Sox-9, c-fos, or c-jun seen to be necessary for chondrogenesis (remains unchanged), and on the other hand, to high expression of β 1 integrin, which plays major roles in cell-matrix interactions in chondrocytes.

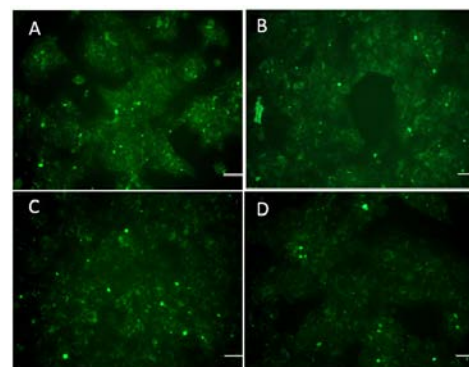


Fig. 1: Immunofluorescence micrographs of chondrocytes stained for integrin- β 1 subunit: A) control; B) TGF- β 3; C) TGF- β 1 and D) TGF- β (1+2); (Scale bar = 50 μ m).

DISCUSSION & CONCLUSIONS: The weak presence of integrin β 1 revealed that additions of such transforming growth factor- β and their manipulated forms down-regulated integrin β 1 leading possibly to the down regulation of cartilage formation.

Results also suggested that TGF- β (1+2) (Figure 1D) could be utilized in fabrication of biodegradable scaffolds for 3D chondrocyte culture, due to its ability to induce cell proliferation.

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