Microarray study of chondrocyte secreted factors inducing osteogenic differentiation of bone marrow stromal cells

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Statement of Purpose: In this work we established osteogenic differentiation of bovine bone marrow stromal cells (BMSCs) induced by coculture with bovine chondrocytes encapsulated in three dimensional alginate bead matrices. In particular we aim to identify factors secreted by chondrocytes that induce BMSCs to differentiate to osteoblasts. The study was designed to first identify the genes significantly over-expressed by chondrocytes that were in coculture with BMSCs, and then follow up with subsequent protein studies to understand the time profile of induction underlying this intercellular signaling. A microarray analysis was performed on the chondrocytes to identify the factors differentially expressed using the Affymetrix GeneChip Bovine Genome Array platform. Genes that showed significant upregulation across the two points of study belonged to the broad categories of secreted proteins and binding proteins. During all these studies alkaline phosphatase (ALP) levels were assayed in BMSCs to establish differentiation of BMSCs to osteoblasts. Calcium deposition was observed in the BMSC monolayers. Results indicate that chondrocytes encapsulated in a three dimensional matrix could effectively induce osteogenic differentiation of BMSCs via soluble factors secreted into the culture medium.

Methods: Bovine bone marrow was harvested by asceptically cutting through the metatarsal bone at mid-shaft and drawing out the marrow fluid into a syringe with media. The marrow was plated in cell culture flasks and passaged when confluent. Articular cartilage was harvested from the metatarsal-phalangeal joints and the isolated cells were directly encapsulated into alginate beads and stabilized overnight in Dulbecco's modified Eagle's medium(DMEM) + 10% FBS. For the study, separate biological triplicates were maintained for each of the groups : coculture, control, osteogenic control and conditioned media. All groups contained BMSCs in monolayer in 6 well plates. The coculture group contained membrane bound inserts which contained the alginate beads in which the chondrocytes were encapsulated. The osteogenic control group contained BMSCs cultured in α-MEM with an osteogenic supplement of 10nM dexamethasone, ascorbic acid, and sodium β-glycerolphosphate. The conditioned media group was supplemented with secreted media from an isolated well plate containing chondrocytes in alginate beads. The 21 day study was done over 5 time points (1,4,8,14 and 21). At each time point ALP and DNA was assayed using standard lab procedures. Microarray analysis was carried out using RNA isolated from encapsulated chondrocytes at days 4 and 14. All gene expression values were log transformed normalized to an absolute control that was served by RNA that was isolated immediately from harvested chondrocytes. Significantly expressed genes in each time point were selected as those genes containing a 4-fold or higher up / down regulation in the coculture group as compared to the control group or atleast a 4 fold change between the two time points.

Results: Our studies indicated that chondrocytes encapsulated in three dimensional matrices can be used to induce osteogenic differentiation of BMSCs via soluble factors secreted into the medium. ALP being an early marker of osteogenesis, showed a significant peak in the coculture and osteogenic control groups by Day8. Microarray results from the chondrocytes showed that approx. 60% of the genes in the genome array were detected at all time points. The number of genes that showed greater than 4-fold expression on Day 4, on Day 14 and between Days 4 and 14 were 245, 105 and 109 respectively. Most genes in these subsets code for secretory or membrane binding proteins. Meaningful targets will be identified and validated for elucidation of the signal transduction pathway involved in the process.

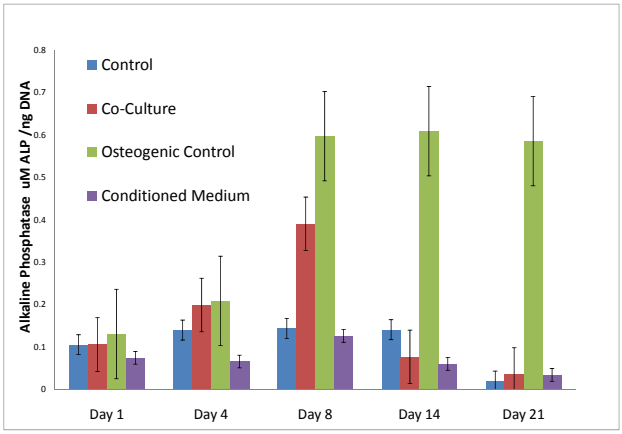

Figure: Alkaline Phosphatase levels (in uM ALP / ng DNA) normalized to the proliferation in each group. The coculture group shows a transient response in ALP levels as against the constant expression of the osteogenic control group. (all values reported as Mean±SD, n=3)

Conclusions: Our results demonstrated osteogenic differentiation in the coculture group across the early time points. The transient behavior of ALP expression of the coculture group as against the constant trend of the osteogenic control and conditioned media groups suggests an interaction between the two cell types exists that allows for this expression pattern, in close accordance to results furnished by previously published results in other coculture systems[1]. The microarray analysis reveals that the categories of the genes that show severe differential expression support the hypothesis of an active regulation that exists between the two cell types allowing for transient mutual changes in phenotype and function[2] that is similar to a typical process of endochondral ossification.

References: 1) Thompson AD. Tissue Eng Part A. 2009:15(5):1181-1190, 2) Henrotin YE.Osteoarthr Cartilage 2005:13:988-997