Analysis of TGF-β3 and BFGF Induced Chondrogenic Differentiation In Human Bone Marrow-Derived Mesenchymal Stem Cells

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INTRODUCTION: Due to its ability to differentiate into different mesenchymal cell lineages, it has been suggested that for the repair of focal cartilage damage, the use of mesenchymal stem cells (MSCs) as a therapeutic agent may be beneficial. However, the complex mechanisms that regulate the process of chondrogenesis (cartilage cell forming) from MSCs remain largely unexplained. A study was thus conducted to better understand the extracellular matrix and the regulation of genes expression during chondrogenic differentiation of MSCs.

METHODS: Two ml of bone marrow (BM) aspirates from femur or tibia were harvested from patients (n=6) undergoing orthopaedic procedures. Mononuclear cells were isolated from human BM aspirates using Ficoll–Paque PREMIUM method. Cells were characterized and identified using flowcytometry. MSCs were cultured in alginate scaffolds using chondrogenic medium containing transforming growth factor-β3 (TGF-β3) and fibroblast growth factor-basic (BFGF) to promote chondorgenic transformation. Chondrogenic-MSCs (C-MSCs) were examined and histologically compared using Safranin O staining to that of human chondrocytes as a means to determine chondrogenic transformation. Microarray gene chips (Affymetrix Gene 1.0 ST Array: 28,869 Well–Annotated Genes) were used to compare the genes expressed in MSCs and C-MSCs cultures. The data attained were analyzed using Agilent GeneSpring analysis platform and R language in combination with Bioconductor packages.

RESULTS: MSC characteristics of the isolated cells were confirmed and described elsewhere[1,2]. The formation of proteoglycan extracellular matrix for the chondrogenic-MSCs were observed in Haematoxylin-Fast Green-Safranin O staining, which had similar positive expression of proteoglycan extracellular matrix staining as that of human articular chondrocytes. However, these depositions were not observed in non-treated MSCs that were cultured as monolayers using basal growth medium.

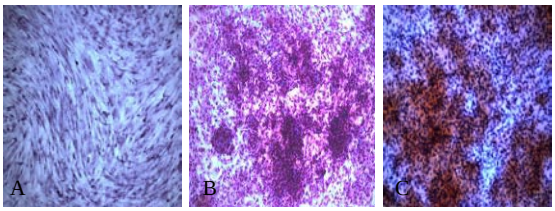

Fig. 1. The proteoglycan was stained orange to red. The cell nuclei were stained black, while the background was stained blue green. A: MSCs. B: CMSCs. C: Clonetics® Normal Human Articular Chondrocytes

Table 1. Summary of the differences in gene regulation between MSC and C-MSC. Log ratio=+1 denotes 1 fold increase. (Significance: P<0.05)

Correlation: *p*-val < 0.05		**Correlation: *p*-val < 0.001**	
Log-ratio > 1	*Log-ratio* < -1	*Log-ratio* > 1	*Log-ratio* < -1
260	240	223	187

Microarray analysis revealed a wide range of differences in genes expressed in MSCs and C-MSCs, denoting a major change in gene regulation.

DISCUSSION & CONCLUSIONS: Significant changes in the expression of more than 400 genes occur dynamically during the course of chondrogenic transformation of MSCs. This dynamic response should be considered when interpreting the overall changes in the genetic expression during chondrogenesis.

REFERENCES: [1] Chong et al. Abstract of the 13th Biological Sciences Graduate Congress, Singapore:2008 annexure. [2]Mansor et al. European Cell and Materials 2008;16(2):64