Regulatory Gene Expression in the Chondrogenesis of Adult Mesenchymal Stem Cells

INTRODUCTION:
While the use of mesenchymal stem cells (MSCs) for the repair of focal cartilage damage have shown to be beneficial, the complex mechanisms that regulate the process of chondrogenesis (cartilage cell formation) from transformed MSCs remains largely unexplained. Furthermore, although it is believed that the control of chondrogenic transformation is related to the regulation of gene expression, the exact changes have not been well established. To better understand these changes, a study was conducted to determine the alterations in the genes expressed during chondrogenesis by measuring and comparing the global gene expression of undifferentiated MSCs to biochemically induced chondrogenic differentiated MSCs (C-MSC) in vitro.

METHODS:
Two ml of bone marrow (BM) aspirates from femur or tibia were harvested from ten patients (n=10) undergoing various types of surgery. Mononuclear cells were isolated from human BM aspirate using Ficoll–Paque PREMIUM method. Cell culture was performed using protocol previously established. Cells were characterized and identified using flow cytometry and reverse transcriptase-polymerase chain reaction (RT-PCR) methods. MSCs were cultured in alginate scaffolds using chondrogenic medium containing transforming growth factor-β3 (TGF-β3) and fibroblast growth factor-basic (BFGF) to promote chondrogenic transformation. C-MSCs were examined and compared to human chondrocytes to confirm chondrogenic differentiation. These examinations include (1) histological and immunohistochemical analysis, (2) gene expression analysis by RT-PCR (3) 1,9-dimethylmethylene blue (DMMB) assay: GAG expressions. Gene expression analyses on conventional cartilage markers (aggrecan/ACAN; cartilage oligomeric matrix protein/COMP) were examined using real time PCR (qPCR) at different time points. 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. SPSS statistical software package (ver.13) was used for all statistical analysis. This study was conducted following the approval from the Medical Ethics Committee board in University of Malaya (reference number: 369.19).

RESULTS:
MSC characteristics of the isolated cells were confirmed and described elsewhere. Chondrogenic differentiated MSCs demonstrated phenotypic expression similar to chondrocyte cultures. Histological examinations were positive for type II collagen and safranin-O, and negative for type I collagen. MSCs cultures also expressed significant amount of GAG concentrations comparable to that in human chondrocytes but were significantly higher than undifferentiated MSCs (ANOVA: p<0.05). In contrast, expression of ACAN was significantly reduced (Figure 1).

CONCLUSION:
This study demonstrated significant changes in the genes expressed during chondrogenesis, reflecting a shift in the regulation of specific genes, which ultimately results in the alteration of two important pathways during the process of chondrogenesis (i.e. ID and NOTCH pathways). These changes involved more than 400 genes of which the roles of certain genes (e.g. SLC7AC) have not been established in previous literature. Furthermore, we have demonstrated that these changes appeared to be dynamic in their temporal response suggesting the need to further understand the global changes in gene expression during the course of chondrogenic transformation, as opposed to a single time point determination.

REFERENCES:
1. Chong et al. 2008. Identification of human bone marrow derived mesenchymal stem cells (MSCs) for studies of chondrogenic differentiation. Abstract of the 13th Biological Sciences Graduate Congress, Singapore: annexure

Microarray analysis revealed a wide range of differences in gene expression between MSCs and C-MSCs, denoting a major change in cell characteristics and expression (Figure 2). Further analysis using the GeneSpring software revealed significant alterations in genes involved mainly in the two following pathways, both of which have been reported to be associated with chondrogenesis: 1) ID pathway (i.e. interferon gamma-inducible protein 16 (IF16); inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3); SMAD family member 3 (SMAD3)), and 2) NOTCH pathway (i.e. ADAM metalloproteinase domain 17 (ADAM17); nuclear factor of kappa light polypeptide gene enhancer in B-cell, alpha (NFκB1/α); SMAD3. Genes which were up-regulated included MMP-13, IBSP, COL10A1, PRG4, APOD, whilst other genes including CPA4, EFEMP1, PDE5A, STC2, RFT34 were amongst those found to be down-regulated.

Figure 2: Using the LIMMA software package from Biconductor, the differences in the number of genes expressed in MSC (n=5) versus MSC-driven chondrocytes (n=5) were estimated. Log-ratio > 1 denotes an up-regulation, while < 1 demonstrates a down-regulation of gene expression.

Figure 1: The relative gene expression of ACAN and COMP by MSC-driven chondrocytes seeded in alginate scaffolds at different time points.