Lineage-Specific Repression of Immediate Early Gene Family Transcription Factor Genes During Early Chondrogenesis in Mesenchymal Stromal Cells (MSCs)
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Introduction
The multipotent and proliferative characteristics of Mesenchymal Stromal/Stem Cells (MSCs) are ideally suited to the development of novel cell therapies. A model of chondrogenesis induced by TGFβ family agonists and high cell mass growth conditions (pellet culture) is well established for these cells, as are molecular markers of chondrogenesis such as induction of Sox family transcription factors and ECM proteins such as aggrecan. Less well established are molecular events downstream of these external factors and upstream of the emergence of these markers. We have focused on the in vitro chondrogenic potential of these cells to identify molecular targets that may advance cell therapies for damaged cartilage.

Methods
Mouse and human MSCs were treated with TGFβ agonists to induce differentiation into chondrocytes in pellet culture. RNA was harvested at 0, 16, 32 and 48 hours and analyzed by oligonucleotide microarray. Differential expression was confirmed by qRT-PCR. Adipogenic, osteogenic and chondrogenic culture conditions were standard except for permutations as indicated.

Results
Significant increases in the established chondrogenic markers Sox9 and aggrecan, measured by qRT-PCR, were used to establish a relevant time course for early chondrogenesis. Global transcript profiling by microarray was used to identify early changes in gene expression as these cells differentiate into chondrocytes in vitro. Clustering of genes by temporal profiles revealed 127 up- and 284 down-regulated transcripts within 16 hours. (Figure 1)

Figure 1: Up-regulation of Sox9, followed by AGC, indicates chondral differentiation (left). Gene clusters at these time points show many early regulated genes (right)

Differential expression for a selection of genes from each cluster was confirmed by qRT-PCR. We focused on a set of immediate early response gene family transcription factors (IEG-TFs); c-Fos, EGR-1, ATF3, ATF5, Nr4a1 and Zfp521, all of which are significantly repressed within 16 hrs of induction of differentiation. Repression was also observed in human MSCs subjected to chondrogenic differentiation conditions (figure 2).

Figure 2: repression of IEG TFs is conserved in mouse and human MSC models of chondrogenesis. Pink = human; Blue = mouse

Maximal repression of all IEG transcription factors was seen only in complete chondrogenic conditions and not seen in osteogenic or adipogenic cultures, suggesting a lineage-specific mechanism regulating repression of these transcripts. Monolayer cultures of MSC’s in complete chondrogenic media, which lacks serum, showed no repression of these transcripts over the same time course, eliminating a mechanism solely dependent on de-activation through serum response elements present in these genes’ promoters.

Figure 3: Maximal repression of IEG TFs requires complete chondrogenic conditions and is lineage specific. Blue=complete chondro, orange=adipo, pink = osteo and brown = chondro in monolayer.

Discussion
A set of IEG TFs are expressed in MSCs that show chondro-specific patterns of repression upon differentiation. Understanding these molecular events and their consequences will contribute to the successful manipulation of these cells in tissue engineering or cell therapy applications.