**GDF-5 Supplementation Enhances hMSC Chondrogenesis**

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**Introduction** Deficient secretion of bioactive growth/differentiation factor 5 (GDF-5) contributes to the induction of arthritis, as naturally GDF-5 secretion acts to maintain cartilage matrix stability. As arthritic cartilage fails to self-repair, treatment of joints with mesenchymal stem cells (MSCs) may contribute to the slowing of disease progression and/or repair of fibrillated cartilage. The aim of this project was to assess the effects of MSC treatment with GDF-5 in vitro, focusing on the stimulation of cartilage-like matrix (ECM) deposition. These data support the combination of GDF-5 and hMSCs as a clinical therapeutic for arthritic cartilage.

**Materials and Methods** Bone marrow (BM) was harvested from the iliac crest of healthy, consenting donors. MSCs were isolated from the BM by direct plating using traditional methods. MSCs were expanded and induced towards chondrogenesis by pellet culture in incomplete chondrogenic medium (ICM) supplemented with 10ng/ml TGF-beta3 (CCM) and treated with 100, 150 or 200 ng/ml GDF-5 for 21 days. Glycosaminoglycan (GAG) content was determined histologically by Safranin O staining, and quantitatively by DMMB assay. DNA content was determined by Pico Green analysis. For immunohistological (IHC) analysis, paraffin embedded samples were re-hydrated, blocked in 2.5% BSA and incubated with anti-collagen II or X antibodies. After incubation with a secondary antibody, samples were developed with DAB.

**Results** MSCs, when pelleted and cultured in CCM, undergo chondrogenesis including the deposition of a collagen and GAG containing ECM. As assessed by Safranin-O staining and DMMB analysis (Fig 1), pellets treated with GDF-5 contained greater amounts of GAG as compared to CCM controls, suggesting enhanced chondrogenic differentiation. Consistent enhancement was observed in pellets treated with 150ng/ml GDF-5. Although treatment with GDF-5 resulted in enhanced GAG deposition, IHC analysis for collagen type II illustrated the presence of similar quantities of collagen type II in pellets treated with both CCM and 150ng/ml GDF-5 (Fig 2). Collagen type X expression, a marker of hypertrophy, was eliminated in GDF-5 treated pellets when expression was observed in CCM cultured pellets (Fig 2).

**Discussion** Treatment of MSC chondrogenic pellets with 150ng/ml GDF-5 resulted specifically in significant enhancement of GAG deposition, but no obvious change in collagen II secretion. The presence of GDF-5, however, eliminated collagen X from the ECM, an indication of undesirable hypertrophy. Therefore, the co-administration of GDF-5 with MSCs as a therapeutic for arthritis may result in enhanced chondrogenesis as well as the prevention of cellular hypertrophy.