drogenic medium supplemented with TGF-beta and BMP-2. OA synovium-derived conditioned medium was obtained by culturing synovium from OA patients during 24 hours in chondrogenic medium.

Conditioned medium was added to hMSC undergoing chondrogenesis. Chondrogenesis was examined by determining mRNA levels of chondrogenic markers COL2A1 (collagen type II) and ACAN (aggrecan), as well as safranin O proteoglycan staining on histological sections.Microarray analysis (Affymetrix) was performed on day 4 and conditioned medium was added 24 hours prior to sampling. Microarray data were analyzed using Ariadne pathway software. Three chondrogenic pellets were pooled to obtain 8 array samples in total; 3 Non catabolic control (NCC) samples without conditioned medium and 4 samples treated with conditioned medium of 4 different OA patients (OA). Additionally 1 sample was treated with conditioned medium derived from a patient exhibiting no clear OA symptoms (AnOA).

Results: Chondrogenesis was induced in the hMSC strongly at 2 weeks under the described anabolic conditions (NCC). Conditioned media of the 4 different OA patients (OA) inhibited chondrogenesis of hMSC. The AnOA conditioned medium exerted a slight anabolic effect on chondrogenesis.

Biological triplicate NCC displayed a stronger Pearson's correlation (0.992 - 0.993) than the 4 OA samples (0.989 - 0.990). The NCC and OA showed the least resemblance (0.983 - 0.990).The AnOA resembled the NCC (0.989 - 0.991) more closely than the OA (0.985 - 0.991), which was confirmed by hierarchical clustering.

A set of regulated genes was determined, defined by at least a 2 fold change (FC) from NCC as well as AnOA in 3 or 4 OA samples. This list included 60 genes that were upregulated (HIGH in OA) and 20 genes downregulated (high in NCC and AnOA). The top regulated genes included CXCL6 (21.7 FC), CXCL5 (19.9 FC), SLC7A2 (7.6 FC), CXCL1 (7.5 FC), PI15 (7.4 FC), CRYGS (7.4 FC Down), AREG (6.2 FC), SAA1 (6.1 FC), OMD (5.4 FC Down), MMP3 (5.2 FC) and MMP13 (5.1 FC).

Pathway enrichment analysis was performed on the set of regulated genes, their regulators (126 genes) and targets (80 genes) as determined automatically by Ariadne Pathway software (p=0.01, 6 or more genes enriched). Pathways identified in this manner included ELK-SRF (33 hits), STAT (30 hits), CREB (29 hits), AP-1 (25 hits) and NF-kB (16 hits).

Conclusions: The AnOA condition exhibited a slight stimulatory effect on chondrogenesis. This sample appeared a suitable control in addition to NCC as confirmed by PCA and hierarchical clustering analysis. This indicates that conditioned medium has no deleterious effects on chondrogenesis. We detected the ELK-SRF, STAT, CREB, AP-1 and NF-kB pathways to be activated by OA conditioned medium. Modulating these pathways may improve chondrogenesis in conditions such as in OA.

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DEXAMETHASONE INHIBITS GELATINASE ACTIVITY DURING IN VITRO OSTEOGENESIS OF HUMAN BONE MARROW STROMAL CELLS

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Purpose: Microfracture of the subchondral bone can lead to articular cartilage regeneration through mechanisms critically tied to the repair of damaged trabecular bone. Better understanding of how bone marrow stromal cells (BMSCs) repair damaged subchondral bone could lead to the development of novel cartilage repair strategies. The purpose of this study was to further elucidate the mechanisms of BMSC osteogenesis using an in vitro cell culture assay. When cultured in vitro, BMSCs require dexamethasone (Dex) or other osteogenic inducers to undergo osteoblast differentiation. We previously observed that human BMSCs cultured without Dex in media with high levels of fetal bovine serum (FBS, 16%) express low levels of alkaline phosphatase, and easily detach from the culture dishes after several weeks of culture. We noted that BMSCs express gelatinases MMP-2 and MMP-9 and that Dex can inhibit gelatinase activity in different cell types. As cell attachment was recently shown to promote osteogenesis, we tested the hypothesis that BMSC cell attachment, collagen fibril assembly, and osteogenic differentiation is coupled to Dex-dependent inhibition of gelatinases.

Methods: Confluent BMSCs (Tulane Center for Genomics, LA) from 4 distinct human donors were cultured in complete culture media (CCM), mineralizing media (MM), or osteogenic media (OSM). CCM consisted of α-MEM, 16% FBS, and 100 U/mL penicillin/streptomycin. MM was composed of CCM with disodium β-glycerol phosphate, L-ascorbic acid-2-phosphate, and cyclodextrin. OSM contained MM plus 10 nM or 100 nM Dex. The state of cell differentiation was studied by Western blot or ELISA for stem cell (CD105) and late osteoblast markers (osteonectin and osteocalcin), and by in situ staining for alkaline phosphatase. The production of a calcified matrix was evaluated by Alizarin Red staining. Immunohistochemistry for collagen type I was used to analyze collagen fibril organization. The release of total MMP-2 (pro- and active) to the conditioned media was analyzed by ELISA, and gelatinase activity was studied by zymography.

Results: Human BMSCs after 3 cell passages consisted in partly differentiated mesenchymal cells mixed with a precursor cell population that released total MMP-2 (D0, Fig. 1A) and activate gelatinases to the media. After 2 weeks of culture, Dex suppressed an increase in total MMP-2 release ($p<0.0001$, Fig. 1A) and inhibited all gelatinase activity. In cultures without Dex, BMSC monolayers frequently detached from the dish and formed nodules with an average 9 mg of wet mass compared to 1.3 mg wet mass in cultures with Dex ($p<0.0001$). These nodules consisted in unique structures of invaginated layers of collagen type I populated with cells that formed a more cohesive network of collagen I fibrils with Dex (Fig. 1B). Dexamethasone stimulated osteoblastic differentiation of adherent BMSC monolayers after 3 weeks of culture, as assessed by an increase in alkaline phosphatase, osteocalcin, and matrix calcification.

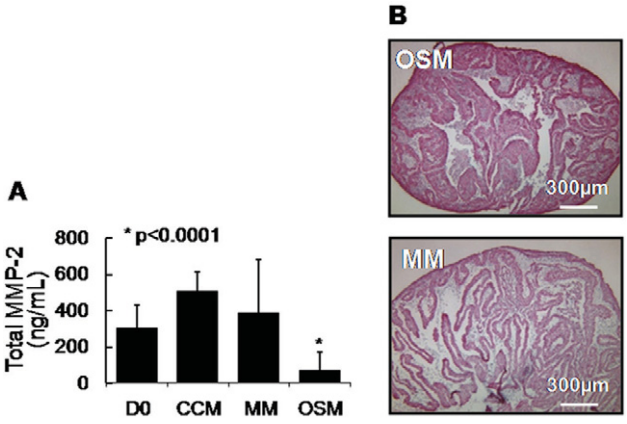

Figure 1. A. Total MMP-2 release to BMSC conditioned media at day 14 was inhibited by Dex (D0 = Day 0). B. Tissue nodules immunostained for collagen type I (red). Original mag. 5×.

Conclusions: Our results provide new evidence that Dex induces osteogenic differentiation of human BMSCs in vitro partly by inhibiting gelatinase activity which results in increased cell attachment and differentiation.