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Improving the Osteogenic Behavior of Human Mesenchymal Stromal Cells

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INTRODUCTION: Bone marrow contains mesenchymal stromal cells (MSCs), which can undergo osteoblastic differentiation when cultured under the appropriate conditions. The utilization of MSCs to generate osteoblasts presents an attractive opportunity for addressing orthopaedic problems that require substantial bone formation. To achieve this goal, it is first necessary to develop protocols that optimize MSC osteogenesis. Recombinant BMP-2 and BMP-7 are in clinical use for bone healing, but MSCs of human origin are only moderately responsive to these proteins, and deposit limited amounts of mineral *in vitro* in response to them (1). Recent reports indicate that other BMPs may be more important for hMSC osteogenesis (2). Another promising strategy is pre-treatment of these cells with histone deacetylase inhibitors (HDIs) (3). Here, we investigated the effects of BMP-6, BMP-9and HDIs on the osteogenic behavior of hMSCs in monolayer culture.

METHODS: Human bone marrow was obtained from patients undergoing primary hip arthroplasty, and the mononuclear cell fraction was cultured on tissue culture plastic in order to isolate and expand the number of MSCs. Second-passage cells were plated at a density of 10^4 cells/well in 24-well plates containing basal medium (low-glucose DMEM with 10% fetal bovine serum, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 50 µg/ml ascorbic acid, and 10 mM β-glycerol phosphate). After 24h, rhBMP-2 (100 ng/ml), rhBMP-6 (100 ng/ml) or rhBMP-9 (100 ng/ml) were added to the cultures..Additional hMSCs were cultured under similar conditions to test the effects of pre-treatment with two clinically-used HDIs: valproic acid (VPA) and trychostatin A (TSA). After 24h in basal medium, hMSCs were pretreated for 4 days with increasing concentrations of VPA (1, 3 or 5 mM) and TSA (100, 500 or 1000 nM). After 4 days osteoblastic differentiation was induced by supplementing the medium with dexamethasone (10^{-7}M); untreated hMSCs were used as controls. Alkaline phosphatase (ALP) activity (normalized by DNA content) was analyzed at day 10 as a marker of early osteoblastic differentiation. Alizarin red staining and calcium assay were performed at day 21 as indicators of mineral deposition.

RESULTS: Treatment of monolayer cultures of hMSCs with rhBMP-6 and -9 resulted in moderately better mineral deposition than treatment with rhBMP-2 (*Figure 1*). Furthermore, pre-treatment with VPA or TSA prior to the initiation of differentiation also increased mineral deposition in a dose-dependent manner.

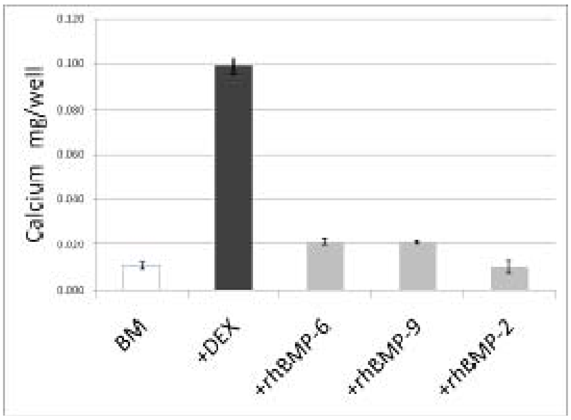

Fig. 1: Calcium deposition in response to rhBMP-6, -9, -2.

DISCUSSION & CONCLUSIONS: The data suggest that BMPs -2 does not effectively induce osteogenesis in isolated hMSCs cultured as monolayers. BMP-6 and –9 moderately enhance the osteogenic differentiation of hMSCs but they are far less potent than dexamethasone in this regard. These findings are intruiging, given the popularity of using recombinant BMPs in the clinic. Pre-treatment of hMSCs with clinically available HDIs (VPA and TSA) also enhanced osteogenic differentiation, which implies that HDIs provide an additional strategy for enhancing bone engineering.

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