THE RESPONSE OF HUMAN MESENCHYML STEM CELLS TO MECHANICAL STRAINS CHANGES AS A FUNCTION OF DIFFERENTIATION STATE

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Introduction: Osteoblasts differentiate from bone marrow borne pluripotent mesenchymal precursors, or stem cells (MSCs). Mechanical forces applied to bones are important signals that direct the balance between bone formation and resorption. The cues that stimulate MSCs to leave the precursor cell pool in vivo and to begin to differentiate into osteoblasts, and ultimately osteocytes, remain unknown, but the process must be modulated at some point by mechanical forces. To meet the need for new cells, the precursors, and/or their developmental descendants, must mitotically expand as well as progress along the differentiation pathway. We therefore hypothesized that mechanical cues might affect either proliferation or differentiation, or both, and that the effect of mechanical signals may enhance or restrict the progression of the cell through specific steps of the lineage. In this study, we examined the effect of mechanical loading by substrate deformation on MSC proliferation and differentiation at two stages of the osteoblast lineage.

Methods: Human MSCs (hMSCs) derived from bone marrow aspirates were expanded in culture. Using a vacuum driven cell loading device which deforms the bottom plates of standard cell culture dishes, undifferentiated hMSCs were loaded in tension / compression for up to 3000 cycles daily at 1 Hz, at peak strains of ±1200 µstrain. Cells were harvested and counted on days 1,2,4,6 and 8 of loading. Initial plating density was 100,000 cells per 60mm dish. We also examined the effect of 4 days of loading on MSCs after an 8 day pretreatment in medium supplemented with 10-7 M dexamethasone, 50 µM ascorbate-2-phosphate, as assayed by this mechanically induced mitotic expansion of the undifferentiated MSCs showed no statistically significant increase in cell number in either the loaded cells or the unloaded cells. (not shown) There was a 1.5 to 2 fold larger increase in cell number, alkaline phosphatase activity and calcium incorporation were determined as outcome variables in these studies; statistical evaluation was by analysis of variance.

Results: In undifferentiated hMSCs, loaded for 3600 cycles daily, (Fig. 1) there was a 1.5 to 2 fold larger increase in cell number 24 hours after treatment in loaded cells compared to unloaded controls. This trend continued on days 2-4. Unless the cells were passaged at this point, proliferation then ceased and cell number plateaued at 2M cells per 60mm dish. In contrast, cells that were pretreated with dexamethasone and ascorbate for 8 days prior to loading showed no statistically significant increase in cell number in either the loaded cells or the unloaded cells. (not shown) This mechanically induced mitotic expansion of the undifferentiated MSCs had no effect on their subsequent ability to differentiate when challenged with 10-7 M dexamethasone and 50 µM ascorbate-2-phosphate, as assayed by alkaline phosphatase expression (Fig. 2) and capacity to mineralize extracellular matrix. There was no difference whether the cells were loaded for 5, 15, 30 or 60 minutes. In cells pretreated with dexamethasone and ascorbate, on the other hand, there was a delayed but marked (to 25% of control) and sustained suppression of alkaline phosphatase expression. (Fig. 3) and inhibited mineralization of the extracellular matrix in the loaded (3600 cycles) compared to the unloaded cells.

Discussion: Possible models for the enhancement of osteoblast differentiation by mechanical loads include that (1) mechanical signals stimulate the proliferation of undifferentiated stem cells, leading to an increased pool of cells from which osteogenic cells can then differentiate and/or (2) that mechanical signals enhance the differentiation along the osteogenic lineage of cells in a preexisting pool of precursor cells. Our results to date are consistent with the first model, in that undifferentiated MSCs proliferate vigorously in response to mechanical stimulation, without any detrimental effect on their ability to differentiate when challenged with dexamethasone and ascorbate. The sustained downregulation of early and late differentiation markers when the cells were loaded at a later stage in the lineage, (consistent with the transitory osteoblast stage), is suggestive of a partial de-differentiation. However, the mitotic response seen in the undifferentiated cells remains absent, suggesting that this de-differentiation is incomplete. Further studies of this response, including detailed profiles of stage specific differentiation markers will be necessary to fully characterize this response.

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Fig. 1 Growth curves of loaded and un-loaded undifferentiated hMSCs

Fig. 2 Alkaline phosphatase activity as a function of time in OS medium. Cells were loaded daily for 6 days for the durations indicated, then harvested and tested for ALP activity. A representative sample is shown, trends were identical in cells from 4-12 donors.

Fig. 3 Alkaline phosphatase activity as a function of time. Cells were transferred to OS medium at 0 days and were harvested for 1 hour daily from days 0-10.