CYTOSKELETAL TENSION ENHANCES OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL CELLS

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INTRODUCTION

Cells isolated from adipose tissue possess multilineage potential, but strategies for driving the AMCs to a particular lineage have yet to be elucidated. A recent study has shown that cell density and shape regulate the differentiation of human mesenchymal stem cells (hMSCs) [1]. In hMSCs, RhoA and Rho Kinase (ROCK), which mediate tension in the actin cytoskeleton, are directly involved in density- and shape-dependent differentiation [1]. The RhoA/ROCK pathway is particularly interesting to us because it has also been implicated in the mechanotransduction of signals from the extracellular matrix to a cellular response [2,3].

To explore density-dependent differentiation of AMCs, we varied cell density and examined its effect on adipogenesis and osteogenesis. We inhibited ROCK to determine whether cytoskeletal tension is involved in the differentiation of our cells. Finally, we used microarray analysis to elucidate the mechanisms of density-dependent differentiation in AMCs and to determine whether genes that are regulated by density are also mechanosensitive.

METHODS

AMCs were harvested from inguinal fat pads of three-week-old FVB mice. After expansion, early passage (P1) cells were plated at low (2,500 cells/cm^2) and high (25,000 cells/cm^2) density. Cells were cultured in a bipotent differentiation media containing both osteogenic and adipogenic factors. At 1 week, adipogenic differentiation was determined by staining the cells with Oil Red O. Early osteogenic differentiation was assessed by staining cells for alkaline phosphatase (ALP) and by quantifying ALP activity normalized to total protein. These differentiation assays were performed both with and without the drug Y-27632, which inhibits ROCK.

RNA was harvested from cells at low and high density in bipotent differentiation media 24 hours after plating. Gene expression analysis was performed on cDNA microarrays containing nucleotide sequences for over 20,000 mouse genes.

RESULTS AND DISCUSSION

We observed greater ALP staining in our low density cells. Conversely, Oil Red O staining was more pronounced in the cells plated at high density. Oil Red O staining appeared identical with and without the addition of the ROCK inhibitor Y-27632. However, we saw a decrease in ALP staining at low density with Y-27632 (Fig 1). The quantitative ALP activity assay confirmed the staining results (data not shown). Specifically, ALP activity in cells at low density without Y-27632 was significantly higher than in all other conditions.

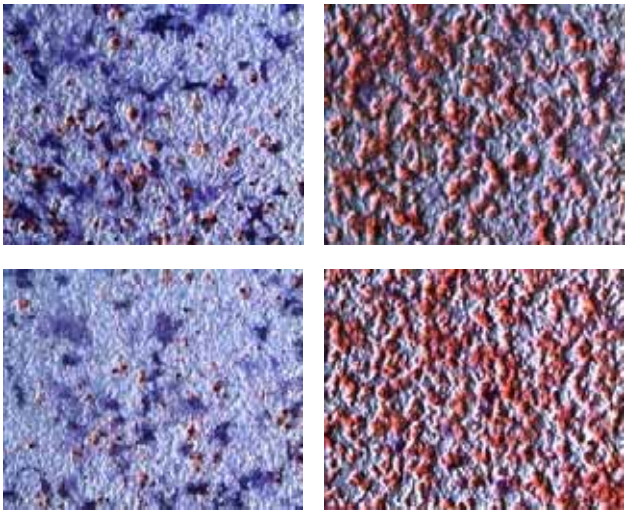

Figure 1: Microscopic (10x) view of ALP staining in blue and Oil Red O staining in red. Left: cells at low density; Right: cells at high density; Top: cells in bipotent media; Bottom: cells in bipotent media with Y-27632.

Some of the genes that are significantly upregulated in low density cells are also mechanosensitive. For example, connective tissue growth factor and thrombospondin 1 were expressed more highly in our low density cells and were upregulated with tensile strain in primary rat calvarial cells [4]. Similarly, calponin was more highly expressed in our low density cells and was upregulated with uniaxial tensile strain in rat bone marrow progenitor cells [5]. A thorough review of our microarray data is ongoing.

CONCLUSIONS

This study demonstrates that cell density influences osteogenesis and adipogenesis in AMCs and strongly suggests that increased cytoskeletal tension in the low density cells enhances osteogenesis. Our microarray data indicate that some similarities exist between genes that are sensitive to density-mediated changes in cytoskeletal tension and mechanosensitive genes. In the future, we plan to explore whether the mechanism of cytoskeletal tension is similar in density-dependent osteogeneisis and in mechanotransduction of AMCs.

REFERENCES

1. McBeath et al *Dev Cell* 2004. 2. Ridley *J Cell Biol* 2000. 3. Chiquet et al *Matrix Biology* 2003. 4. Segev et al *Bone* 2004. 5. Hamilton et al *Tissue Eng* 2004.

ACKNOWLEDGEMENTS

This study was funded by NIH RO1 DE14526.