Title: The Relationship between Human Preadipocytes and Mesenchymal Stem Cells

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Recently, there have been many reports found in the literature describing the uses and characteristics of mesenchymal stem cells. Mesenchymal stem cells are undifferentiated fibroblast like pluripotential cells that can form bone, cartilage, muscle and adipose tissue when exposed to various differentiating media in vitro.[1] Currently, they are isolated from adult human bone marrow or fetal tissue. Human preadipocytes are fibroblast-like undifferentiated cells found within adipose tissue. These cells can be expanded in vitro without differentiation and under proper culture conditions accumulate lipid and develop an adipose-like morphology.[2] We hypothesize that preadipocytes have similar multilineage differentiation potential as human mesenchymal stem cells when exposed to appropriate conditions. In addition, adipose tissue may be a source for the harvest of mesenchymal stem cells.

Method: Adipose tissue harvested from a TRAM procedure was processed to isolate the preadipocyte component. The isolation of preadipocytes was performed by the protocol described by Rodbell.[3]

The tissue was subjected to a collagenase digestion buffer, filtered through a 210μm-mesh sieve, and the stromal vascular fraction (pellet) was treated with an erythrocyte-lysing buffer. Once isolated the preadipocytes were plated at a concentration of $1x10^4$. These cells were expanded in culture for 4 population doublings until $9x10^6$ cells were available for experimentation.

A portion of the cells were plated at a density of $5x10^3$ per cm^2 and exposed to culture conditions known to support osteogenic differentiation of human mesenchymal stem cells isolated from bone marrow. The osteogenic differentiating media consisted of DMEM, 10% fetal calf serum, 0.1μm dexamethazone, 50μm L-ascorbic acid 2-phosphate, and 10mM beta-glycerophosphate.

The control group was exposed to culture conditions known to promote lipid accumulation and adipose differentiation. This media was made up of DMEM/Ham's F12, 10% fetal calf serum and antibiotics.

The culture media was changed every 48-72 hours. After 25 days in culture the cells were stained for adipose differentiation by Oil-Red-O and deposition of calcium phosphate by the method of Von Kossa. In addition the cells were evaluated for alkaline phosphatase activity using photo spectrometric analysis as well as direct staining in vitro of the cells. The control for the alkaline phosphatase activity was a human osteoblastic cell line.

Results: After 14 days in culture the cells exposed to osteogenic media showed microscopic signs of bony deposition and by 25 days bone nodules were grossly visible in the culture dish.

The cells subjected to the adipogenic differentiating media were stained with Oil-Red-O which stains lipid red. There were lipid droplets accumulating intracellularly and coalescing to form larger lipid vacuoles. These were absent in the cells exposed to the osteogenic differentiating media.

Staining by the method of Von Kossa revealed numerous areas of calcium phosphate deposition in the wells exposed to the osteogenic differentiating media. These areas correlated with the bone nodules seen grossly. These darkly stained regions, seen in all views within the well, represent extracellular bony matrix and calcium phosphate deposition (Figure 1).

In the control cultures, no bone formation was detected but numerous cells showed lipid accumulation consistent with mature adipocytes (Figure 2).

The cells exposed to the osteogenic differentiating media had greater expression of alkaline phosphatase activity when compared to the cells exposed to the adipogenic differentiating media but less than the control osteoblasts. This difference was statistically significant. When the two cell populations where stained for alkaline phosphatase activity it was grossly evident that the cells exposed to the osteogenic media expressed alkaline phosphatase and the cells exposed to the adipogenic media did not (Figure 3).

Conclusion: We have demonstrated that human preadipocytes can undergo osteogenic differentiation and deposit an extracellular bony matrix consistent with calcium phosphate deposition under proper culture conditions. They also express alkaline phosphatase activity after thirty days exposure to an osteogenic differentiating media, which is a marker for osteogenic differentiation.

These findings raise the possibility that human preadipocytes are in fact mesenchymal stem cells. Since mesenchymal stem cells offer wide therapeutic potential in areas of tissue engineering and cellular therapy, preadipocytes from fat normally discarded after surgical procedures could also be used for these purposes.