Influence of Mature Adipocytes on the Function of Osteoblasts in Human Bone Marrow-Derived Mesenchymal Stem Cell Co-cultures

Wang, D; Haile, A; +Jones, LC
Johns Hopkins Orthopaedics at Good Samaritan Hospital, Baltimore, Maryland, 21239, USA
lcljones@jhmi.edu

INTRODUCTION:
The bone marrow compartment is composed of many cell types, such as osteoblasts, adipocytes, and endothelial cells in various stages of maturation. Human mesenchymal stem cells (hMSCs) derived from bone marrow are multipotent stem cells that can regenerate mesenchymal tissues such as adipose, bone or muscle. The function of bone marrow adipocytes in bone metabolism is unclear and little known about the relationship between adipocytes and osteoblasts. The increased bone marrow lipid deposition in steroid-associated osteonecrosis implies that abnormalities in fat metabolism play an important role in osteonecrosis development. The increase in lipid deposition might be explained by elevated adipogenesis of marrow mesenchymal stem cells. In order for us to understand the pathogenetic processes involved in diseases such as osteonecrosis and osteoporosis, we must understand the role of adipocytes in the normal biology of bone.

MATERIALS AND METHODS:
Human bone marrow-derived MSCs (240L) were obtained from Texas A&M Health Science Center (College Station, TX). These cells have been well characterized by their surface markers and their differentiation potential (osteoblasts, chondrocytes and adipocytes). hMSCs were cultured in alpha-minimal essential medium (α-MEM) supplemented with 10% FBS, 200 units/mL penicillin and streptomycin. The culture medium was changed every 3 days. For adipogenic induction, hMSCs cells were maintained for 6 days in adipogenic medium supplemented with 100 nM DEX (Sigma) plus 1µM Rosiglitazone (Cayman Chemical). Differentiated adipocytes were stained with oil Red O and DAPI after 12 days of induction. Confocal microscopy was performed using a Zeiss (Thornwood, NY) Axiovert 200 microscope. Western blot were also performed to determine the expression of mature adipocyte markers (A-FABP, PPAR-γ). For osteogenic induction, cells were maintained for 14 days in osteogenic medium supplemented with 10 mM β-glycerophosphate (Sigma, St Louis, MO), 10 nM Dex, and 50 µg/mL ascorbic acid (Gibco). After 14 days of differentiation, the expression of ALP and mineralization were determined by ALP and Alizarin Red S staining. ALP staining was performed by using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl (Sigma) ALP activity was also quantified by extracting protein from hMSCs. Cells were collected and sonicated in 200 µL of 10mMTris-HCl containing 0.1% Triton X-100 for 30s at 4ºC. Sonicates were centrifuged at 4ºC for 10min at 10,000 g and the supernatants were assayed for ALP activity by using an ALP assay kit and measuring at 405nm. Co-cultures were conducted using the BD Biosciences co-culture system (inserts, companion tissue culture plates). In co-culture experiments, four types of co-culture were performed: (1) undifferentiated hMSCs with undifferentiated hMSCs (UN/UN); (2) undifferentiated hMSCs with differentiated osteoblasts (UN/OB); (3) differentiated osteoblasts with undifferentiated osteoblasts (OB/UN); (4) differentiated adipocytes with differentiated osteoblasts (AD/OB). After 12 days of co-culture, cells were stained with alizarin red and quantitative analyses performed for ALP activity. All results were obtained from at least three independent experiments. Values are expressed as the mean ± standard deviation of the mean (SD). Statistical analysis was undertaken using ANOVA with the Tukey-Kramer HSD for between group comparisons. Differences between groups were considered statistically significant when P<0.05.

RESULTS:

Dexamethasone and Rosi induce differentiation of hMSCs into Adipocytic phenotype
After 12 days of treatment with 100 nM DEX and 1µM Rosi, lipid droplets were observed lipid droplets were visualized by Oil Red O staining (Fig1a) and the expression of “mature adipocytes” markers (A-FABP, PPAR-γ) was detected by Western blotting (Fig1b).

Osteoblast differentiation with hMSCs
After 12 days of treatment with osteogenic differentiation medium, osteoblast mineralization and ALP expression were demonstrated in figure 2.

Influence of Mature adipocytes on osteoblasts after co-culture
As shown in Fig 3a, osteoblast mineralization was notably inhibited by the presence of adipocytes as compared with the UN/OB and OB/OB cells. Regarding ALP activity, the UN/UN group had the least ALP activity, while the expression of ALP activity in UN/OB and OB/OB was significantly higher than AD/OB or UN/UN (p<0.05). However, there was no significant difference between UN/OB and OB/OB (Fig 3b).

DISCUSSION
The mechanisms through which human bone marrow adipocytes may influence the function of osteoblasts are not completely understood. In this study, a co-culture system was established by using human bone marrow-derived MSC with adipogenic and osteogenic induction separately. Substances released by adipocytes appear to influence the behavior of osteoblasts. Further study of the cell to cell-to-cell communication between these cell types is warranted.

REFERENCES:

Fig 1. adipogenic differentiation of hMSC with Dex and Rosi

Fig 2. osteogenic differentiation of hMSC with Alizarin red and ALP staining

Fig 3. inhibition effect of adipocytes on osteoblasts function in co-cultures