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PRINCIPAL INVESTIGATOR: Carol V. Gay, Ph.D.

CONTRACTING ORGANIZATION: The Pennsylvania State University
University Park, Pennsylvania
16802-7000

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Carol V. Gay, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)The Pennsylvania State University
University Park, Pennsylvania 16802-7000

E-Mail: cvg1@psu.edu

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The hFOB osteoblast cell line was cultured in both undifferentiated and differentiated states and tested for the capacity of the cell layers to occlude fluorescent-tagged dextrans of 4-, 20- and 40 kD molecular weight. We found that diffusion of all three dextrans through the undifferentiated osteoblast layer occurred at a rate of 45-70% in two hours whereas diffusion through layers of differentiated cells occurred at a rate of 10% in two hours. Osteonectin, a likely chemoattractant for breast cancer cells, was present in media from the undifferentiated cells. Undifferentiated osteoblasts are potentially a source of chemoattractant for breast cancer cells *in vivo*.

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INTRODUCTION: The objective of this study was to develop a model to determine 1) if osteoblasts secrete breast cancer chemoattractants in a unidirectional manner into bone matrix and 2) if a layer a cultured osteoblasts becomes permeable to allow diffusion of chemoattractants into the bone marrow compartment. Osteonectin is likely one of the major chemoattractants (Jacobs et al., 1999). An osteoblast cell line, hFOB, was cultured to confluence and the permeability of the layer tested with fluorescein-tagged dextrans of varying molecular weights. The effect of parathyroid hormone and conditioned media of breast cancer cells (MDA-MB-435) on enhancing permeability of the osteoblast layer was tested.

BODY: The first aim was to test layers of cultured osteoblasts for their ability to occlude molecules which have a molecular mass similar to osteonectin. We used the hFOB cell line, which has been transformed with a temperature sensitive SV40 large T antigen plasmid to allow proliferation to occur at 34° to 37° C; this cell line can be stimulated to differentiate by switching the temperature to 39° C (Harris et al, 1995). As shown in Fig. 1, we found that the undifferentiated osteoblasts, when confluent, permitted diffusion of 4-, 20-, and 40-kD FITC-dextran at a level of 45, 60 and 70%, respectively, in 2 hours. Diffusion through layers of differentiated osteoblasts was substantially less, ~ 10% in two hours. We selected the range of dextran sizes on the basis that osteonectin has a molecular mass of ~ 40 kD. The smaller dextrans were tested to provide insight into how well the confluent osteoblasts adhered to each other.

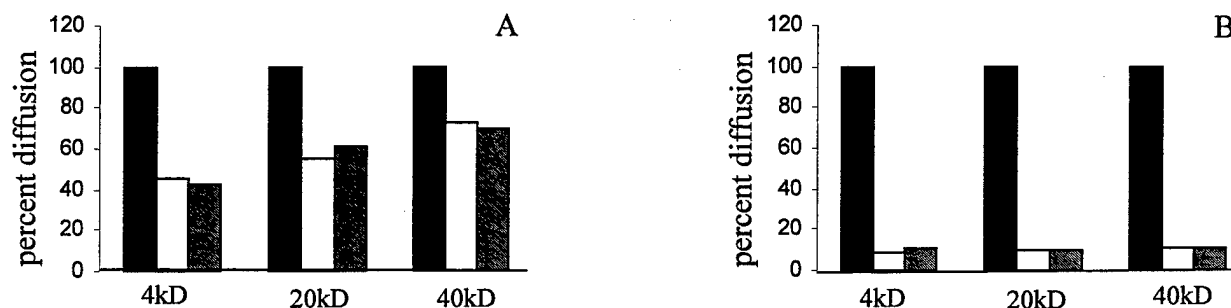


Figure 1: Diffusion of 4kD, 20kD and 40kD FITC-dextran through (A) an undifferentiated layer of confluent hFOB cells and (B) a differentiated layer of confluent hFOB cells. Black bars denote diffusion through wells without cells, white bars denote diffusion of dextran through hFOB cells, striped bars denote diffusion through hFOB cells with the addition of 10nM PTH (see Aim 2).

The second aim was to determine if permeability of the osteoblast layer could be altered by parathyroid hormone (PTH). The reason for testing PTH was based on reports in the literature that show that PTH causes osteoblasts to change shape and become detached (Fitzpatrick and Bilezikian, 1996). Both 1-34 and 1-84 PTH were tested at concentrations of 1-10nm. We also tested conditioned media from breast cancer cells (MDA-MB-435) because these cells secrete a variety of growth factors, including PTHrP. We found no changes in diffusion of FITC-dextran through a monolayer of hFOB cells in response to PTH or conditioned media. In order to assure that the hFOB cell line expresses PTH/PTHrP receptors, we immunostained with

anti-PTH/PTHrP-R using monoclonal MMS-610P (BABCO Richmond, CA) developed against opossum kidney PTH/PTHrP-R and found strong positive staining of the hFOB cells. Staining occurred in the perinuclear region occupied by Golgi as well as secretory granules and the secretory surface of the cells.

Finally, we have tested media of undifferentiated, but confluent, hFOBs and found osteonectin to be present. This is consistent with our hypothesis that osteonectin can diffuse between a leaky layer of osteoblasts. How well (or poorly) differentiated cells secrete osteonectin into media remains to be tested. However, this observation suggests that loosely connected, undifferentiated osteoblasts are a source of bone-derived osteonectin found in the bone marrow space.

KEY RESEARCH ACCOMPLISHMENTS:

- Developed a model for testing diffusion of breast cancer chemoattractants across a layer of osteoblasts.
- Showed that the model substantially occludes molecules of greater than 4 kD when the osteoblasts have differentiated, whereas undifferentiated osteoblast layers occlude poorly.
- Undifferentiated osteoblasts with poor lateral attachments secrete osteonectin and are a possible source of chemoattractant *in vivo* for breast cancer cells.

REPORTABLE OUTCOMES: The results obtained support an NIH grant submitted on the topic of "Functional Studies of Osteoblasts" and a grant submitted to the Pennsylvania Department of Health entitled "Specificity of Breast Cancer Cells for Bone". In addition, the results will provide preliminary evidence for a revised grant on Specificity of Breast Cancer Cells for Bone to be submitted to the National Cancer Institute for the February 1, 2002 deadline.

CONCLUSIONS: In this one year of support, we have learned a) how to culture osteoblasts in a manner that allows diffusion through osteoblast layers to be tested; b) that undifferentiated osteoblasts are loosely connected and that differentiated osteoblasts are tightly connected; c) undifferentiated osteoblasts secrete osteonectin into media. This latter finding indicates that, *in vivo*, undifferentiated osteoblasts could be the source of chemoattractants that lure the breast cancer cells into the bone marrow space.

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