Bisphosphonate Effects on Breast Cancer Colonization of Three-Dimensional Osteoblastic Tissue

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Statement of Purpose: Breast cancer is the second most commonly diagnosed cancer in women in the United States, accounting for nearly 27% of all female cancers in 2009.[1] Breast cancer frequently metastasizes to bone, with bone metastases occurring in approximately 70% of patients with advanced disease.[2] Breast cancer uncouples normal bone remodeling, resulting in increased bone degradation and the release of factors from the bone matrix that support tumor growth. Current therapies target this "vicious cycle" between tumor cells and the skeleton.[3] Bisphosphonates are a family of drugs that bind avidly to mineralized bone where they are internalized by osteoclasts and signal osteoclast destruction, resulting in reduced bone degradation.[4] While the effects of bisphosphonates on osteoclasts are well understood, effects on osteoblasts vary among studies. The purpose of this study was to investigate the effects of zoledronic acid, a nitrogen-containing bisphosphonate, on breast cancer colonization of three-dimensional (3D) osteoblastic tissue *in vitro.*

Methods: Mineralized 3D osteoblastic tissue was grown from murine calvarial pre-osteoblasts (MC3T3-E1) in a specialized bioreactor based on the principle of simultaneous-growth-and-dialysis.[5] The bioreactor is a compartmentalized cell-culture system with a 5 mL cell growth space separated by dialysis membrane from a 30 mL medium reservoir. Osteoblastic bone tissue grown in the bioreactor for up to one year recapitulated bone development including visually-apparent mineral deposits and phenotypic maturation of osteoblasts into osteocytes.[6] This 3D bone model provides a unique test system in which cancer cell interactions with osteoblastic tissue at controlled phenotypic maturities can be microscopically monitored in real time. Using this system, human metastatic breast cancer cells (MDA-MB-231^{GFP}) were co-cultured for 6 days with osteoblastic tissue in the actively mineralizing phase (90 days of continuous culture). A single dose of zoledronic acid (at 0.50 μM and 0.05 μM) was added to the growth chamber of the bioreactors 3 days after co-culture was initiated. Osteoblastic tissue was stained with Cell Tracker Orange or AlexaFluor 568, and cultures were monitored daily using confocal microscopy.

Results: Without added zoledronic acid, breast cancer (BC) cells were observed to attach, penetrate, and colonize osteoblastic (OB) tissue in a continuous process that ultimately marshaled osteoblasts into linear files similar to that observed in authentic pathological tissue (Figure 1A). A single dose of zoledronic acid delayed cancer-cell penetration and colony formation (Figure 1B), with osteoblasts retaining the characteristic cuboidal shape observed in controls for the first 2 days of co-culture. Thereafter, cancer-cell colonization progressed to the filing stage.

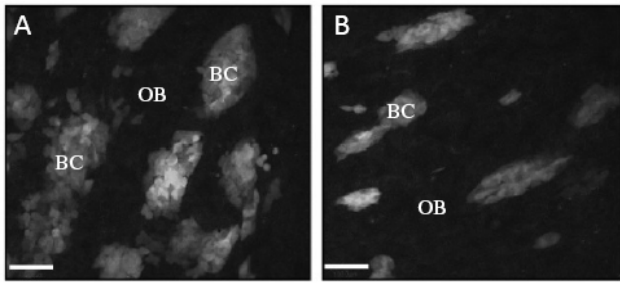

Figure 1.

Conclusions: Concentrations of zoledronic acid that minimally affect osteoblast function are capable of delaying breast cancer progression to bone. However, it is unclear whether zoledronic acid treatment of osteoblasts challenged with cancer cells in the bioreactor resulted in a true delay of breast cancer progression or occurred due to diffusion of zoledronic acid from the growth chamber to the medium reservoir. To address this concern, future work will ensure that zoledronic acid is added to both bioreactor compartments. This study has shown that the bioreactor is a useful device for the study of drug effects on the early stages of breast cancer cell interactions with bone tissue.

References:

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