Studying Bone Metastasis in Culture

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Introduction

Breast cancer and several other cancers have a predilection to metastasize to the skeleton [1]. It is estimated that as many as 30 percent of women treated for breast cancer will have a recurrence, and that about 70 percent of these will develop bone metastases. Metastasis to the bone often is associated with significant morbidity related to bone loss, in the case of breast cancer, or bone gain, in the case of prostate cancer. There is also bone pain, hypercalcemia, fractures and spinal cord compression. There is evidence that metastases can remain dormant in the bone for many years, even decades, and then begin to grow.

Bone metastasis is difficult to study because early-stage colonization is obscured by the material nature of the bone. Once metastasis has grown to the point of symptoms of bone pain, it is usually not curable [2]. The inaccessibility of the bone makes it difficult to detect bone metastasis but also to study the processes of colonization and dormancy in the bone. Animal models are useful but not suitable for detailed mechanistic studies. Standard tissue culture reduces the complexity and permits one to grow bone cells. However, there are limits to the time of culture. Furthermore, every medium change with cell culture leads to a change of microenvironment and loss of growth factors and cytokines.

We have adapted a specific type of culture system, a bioreactor [3] based on the principle of simultaneous growth and dialysis pioneered by Rose [4] (Figure 1). We began with a simple system of osteoblasts (bone forming cells) with the plan of increasing the complexity of the system to include bone degrading cells (osteoclasts) and metastatic cancer cells.

Growth and Differentiation of Osteoblasts in the Bioreactor

The bioreactor (Figure 1) consists of two compartments, a cell growth space (5 ml) separated from a medium reservoir (30 ml) by dialysis membrane. The upper and lower membranes are composed of Surlyn® which is liquid impermeable but gas permeable. The cells are seeded on the lower membrane and cultured in a standard humidified, 37°C incubator. Ports permit access to both upper and lower chambers. Due to the nature of the component parts, it is necessary to sterilize the assembled bioreactors by irradiation.

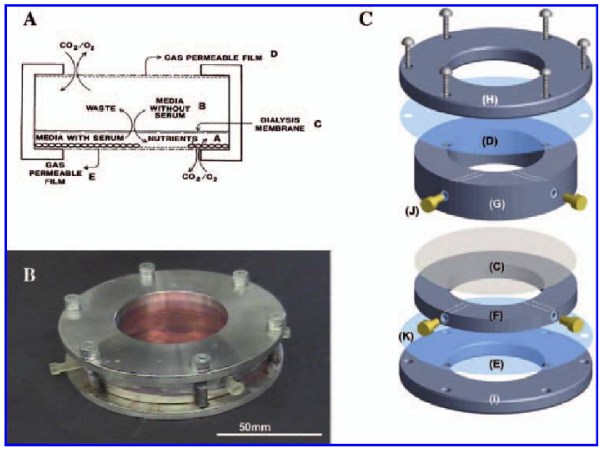

FIGURE 1: Compartmentalized bioreactor design. A, cross-sectional diagram indicating the separation of the cell growth space (A) from the basal medium reservoir (B) by a dialysis membrane (C). Cells are grown on a gas permeable but liquid impermeable film (E). The whole device is brought together in a liquid-tight fashion using screws. Liquid access is through Luer taper ports [5].

We followed the maturation of MC3T3-E1 murine osteoblasts over time from a few weeks to nearly a year [6]. The osteoblasts grew, differentiated, produced a matrix and then became osteocyte-like cells (Figure 2).

Interaction of Metastatic Breast Cancer Cells into an Osteoblast Matrix

We introduced human metastatic breast cancer cells, MDA-MB-231, into bioreactors in which osteoblasts had grown for at least 2 months and had produced a rich matrix (Figure 3). Within 3 days of culture, the cancer cells attached, aligned "single file" and formed invadopodia through the matrix. Interestingly, the osteoblasts also aligned parallel with the cancer cells. These behaviors are characteristic of metastasizing cells seen in human bone pathology. In contrast, when a metastasis-suppressed isologous line, MDA-MB-

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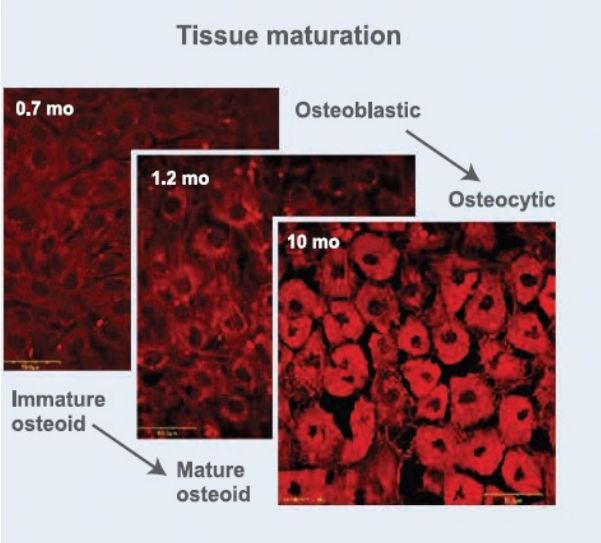

FIGURE 2: Growth and differentiation of osteoblasts in the bioreactor over time. MC3T3-E1 cells grew into multiple layers and differentiated, producing an extracellular matrix over time. By 10 months the cells exhibited properties of osteocytes [6].

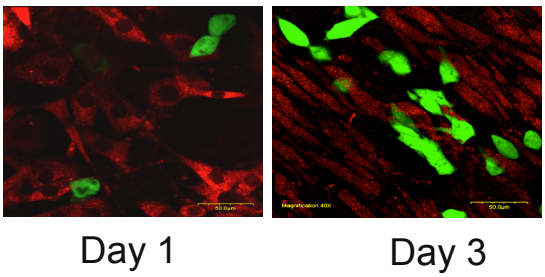

FIGURE 3: Interaction of metastatic breast cancer cells with a mature osteoid matrix in the bioreactor. MDA-MB-231 human metastatic breast cancer cells expressing green fluorescent protein were added to bioreactors containing MC3T3-E1 osteoblasts that had been grown for 5 months. The osteoblasts were stained with Cell Tracker Orange. Shown are images taken by confocal microscopy one and three days after the cancer cells were added [6].

231BRMS1, was added to the bioreactor, they barely attached and did not colonize the reactor; they behaved like dormant cells.

Dormancy in a Bioreactor

Dormancy is a clinical problem. Often many years or even decades after a woman has been treated and "cured" for breast cancer, the cancer reoccurs as bone metastases. Anecdotally, this reawakening of a dormant cell is often associated with bone trauma or fracture. Using the BRMS1 cells in the bioreactor with an osteoblast matrix, we asked if bone-remodeling cytokines could awaken the dormant cells. We found that addition of just two bone remodeling cytokines, TNFα and IL-1β, were sufficient to stimulate the dormant BRMS1 cells to grow. The activation of this signaling pathway led to production of prostaglandin PGE2. Inhibition of production of PGE2 or blocking its receptor, prevented the BRMS1 cells from entering a proliferative state [7] (Figure 4).

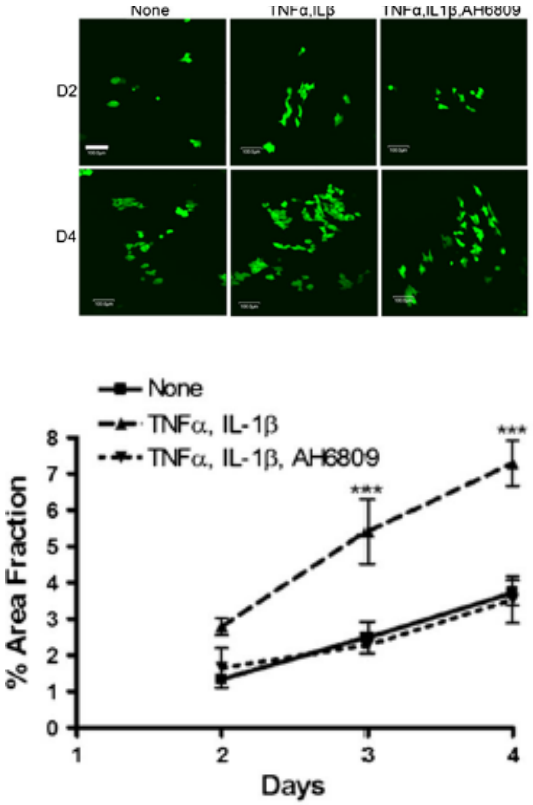

FIGURE 4: Dormancy and growth of MDA-MB-231BRMS1 cells in the bioreactor. The BRMS1 cells were introduced onto a bioreactor containing a culture of MC3T3-E1 osteoblasts, two months of age. The bone remodeling cytokines TNFα and IL-1β were added at the same time. To one set of chambers, the PGE2 antagonist AH6809 was included. Live images of the co-cultures were collected daily by confocal microscopy (Top). Image quantification was performed with Image J; statistical analysis with GraphPad Prism (bottom).

Other cellular applications

We have used the bioreactor to culture or co-culture a variety of other cell types (Figure 5). In summary, the bioreactor has proven to be a valuable and versatile tool to study the interaction of metastatic breast cancer cells with bone.

Acknowledgements

We acknowledge many undergraduates who helped prepare and assemble the bioreactors.

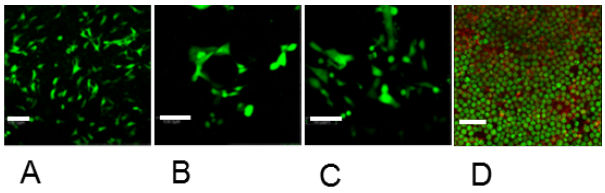

FIGURE 5: Growth and morphology of various cancer cells in co-culture with two month osteoblasts. MC3T3-E1 osteoblasts were grown in the bioreactor for two months. GFP-expressing cancer cells with a predilection to metastasize to bone were added and cultured for an additional 2 (A and B) or 7 (C and D) days. Shown are representative live images of (A) B6 mouse melanomoa, (B,C) human prostate cancer cell lines, LNCaP and PC3; (D) 5TGM-1 mouse myeloma.

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6. A. M. Mastro and E. A. Vogler, "A Three-Dimensional Osteogenic Tissue Model for the Study of Metastatic Tumor Cell Interactions with Bone," Cancer Research **69**, 4097 (2009).
7. D. M. Sosnoski, R. J. Norgard, C. D. Grove, S. J. Foster, and A. M. Mastro, "Dormancy and growth of metastatic breast cancer cells in a bone-like microenvironment," Clin. Exp. Metastasis **32**, 335-344 (2015).

Selected Publications

1. R. Dhurjati, X. Liu, C. V. Gay, A. M. Mastro, and E. A. Vogler. "Extended-term culture of bone cells in a compartmentalized bioreactor," Tissue Eng. **12**, 3045-3054 (2009).
2. A. M. Mastro and E. A. Vogler, "A Three-Dimensional Osteogenic Tissue Model for the Study of Metastatic Tumor Cell Interactions with Bone," Cancer Research **69**, 4097 (2009).
3. D. M. Sosnoski, R. J. Norgard, C. D. Grove, S. J. Foster, and A. M. Mastro, "Dormancy and growth of metastatic breast cancer cells in a bone-like microenvironment," Clin. Exp. Metastasis **32**, 335-344 (2015).