

Dormancy or growth?

A three dimensional in vitro system to study breast cancer metastases in bone

Background and Rationale

The threat of metastases or re-occurrence of metastases is a constant concern for a cancer survivor. Every headache is a brain metastasis, every joint ache is bone metastasis, every digestive upset is liver metastasis. To make matters worse, with modern technology, scientists and clinicians have found that circulating tumor cells (CTC) or bone disseminated tumor cells (DTC) are present in most survivors tested. There is no known way to selectively remove them. Does this finding mean that the individuals with these cells will suffer from recurring metastases? Not necessarily. Many tumor cells die, but others simply remain dormant. Unfortunately, some of these dormant cells can remain for years, even decades, and then awaken to become aggressively growing metastases.

What triggers this escape from dormancy? It is known that dormant cells often reside in the bone so it is reasonable to suspect that the bone microenvironment plays a key role. The bone is a rich source of cytokines and an extracellular matrix (ECM). Many bioactive molecules are produced by cells and from the ECM during the normal course of bone remodeling.

It is difficult to study metastases in the bone due to the nature of the organ. In the laboratory, most of the experimental bone metastasis models are mouse models. Their use has led to many important discoveries. Nonetheless, it is difficult to selectively manipulate individual cells and the host environment in animals. Furthermore, it is especially difficult to study dormant cells, precisely because they do not grow into large visible colonies. In order to study bone metastatic cells, we have developed a three dimensional model using a growth chamber (bioreactor) seeded with osteoblasts, the bone building cells, that differentiate and form a thick ECM that mineralizes and simulates bone. When we added metastatic human breast cancer cells (MDA-MB-231) to the osteoblast matrix culture, we found that the cancer cells behaved very similarly to their in vivo growth in bone; i.e. they penetrated the matrix, led to matrix (bone) loss, formed single cell files reminiscent of metastatic cell arrangement observed by pathologists. These behaviors are not seen in standard tissue culture. When we compared the culture of the metastasis-suppressed version of these cells, MDA-MB-231BRMS1, we saw that the BRMS1 cells remained in the culture as small groups or single cells. They did not form colonies. In previous in vivo experiments, the BRMS1 cells trafficked to and remained in bone but did not grow there. Thus, in the bioreactor, they exhibited the same characteristics as in vivo dormant cells. On rare occasion in animal experiments, the BRMS1 cells were observed to expand and colonize the bone. Why are most of these cells dormant and why did a few cells break dormancy?

Objective

Our hypothesis is that the ECM in conjunction with bone marrow cytokines is critical for both breast cancer dormancy and escape to the proliferative disease state. The in vitro bone bioreactor system will provide a superior tool to study the impact of metastatic bone microenvironment molecules on the dormancy status of breast cancer cell. The objectives are to compare metastatic, growing (MDA-MB-231 ER-), dormant (MDAMB-231BRMS1), and non-metastatic MCF-7(ER+), lines in the bone model bioreactor. The aims are 1. manipulation of the microenvironment by addition of cytokines and factors that increase during periods of accelerated bone remodeling (such as post trauma or osteoporosis) or inflammatory incidents (such as arthritis or infection); 2. simulation of bone remodeling by addition and activation of osteoclasts which will cause release cytokines and also modify the ECM. The cells will be monitored microscopically.

Potential impact of the proposed research on patients with metastatic breast cancer

This is a basic research project with a clear directive to translational outcomes. At this point chemo and adjuvant therapies are largely directed to the primary tumor but with little knowledge of effectiveness on dormant cells except indirectly in long term clinical trials. The outcomes from this study will be instrumental in development of innovative approaches to the treatment of active and dormant metastatic breast cancer. Future plans include testing for resistance to chemotherapeutic (taxanes) and adjuvant (anti-estrogens) drugs. We anticipate that specific changes in the ECM and/or cytokine profile will lead to growth of dormant cells.