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14. ABSTRACT Bone metastasis is one of the major causes of morbidity and mortality in breast cancer (BC) patients. However, only a few human BC cell lines can efficiently metastasize to bone whereas most BC cell lines cannot. Recently, it was shown that systemic administration of the conditioned medium by a melanoma cell line redirected the metastatic dissemination of a weakly metastatic lung carcinoma cell line to the organ sites that were metastasized by the melanoma cell due to the formation of metastasis-permissive niches by bone marrow-derived cells (BMDCs) in these organ sites. The current project was proposed to test the hypothesis that factors secreted by metastasis-competent BC cells may condition bone marrow for the successful homing and skeletal remodeling of circulating BC cells. Athymic nude mice were treated with media conditioned by metastasis-competent BC cells and then inoculated with nonmetastatic BC cells in arterial circulation. The treatment induced limited bone metastasis. Bone histology analysis is currently being performed.					
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Introduction

Bone metastasis is one of the major causes of morbidity and mortality in breast cancer (BC) patients. While several molecular pathways have been shown or implicated to promote bone metastasis of human BC cells, how metastatic BC cells specifically take residence in skeletal tissue remains unknown.

It is widely believed that bone provides an optimal environment for BC cells to adhere and proliferate, which is the “seed-and-soil” hypothesis. However, only a few human BC cell lines can readily metastasize to bone when inoculated in the arterial circulation in mice. For example, human BC MDA-MB-231 cells cause noticeable osteolytic bone metastasis in 4 weeks whereas it takes about 6 months for human BC ZR-75-1 cells to cause significant osteoblastic bone metastasis when the cells were injected in the left ventricle of female nude mice (1). Human BC MDA-MB-435 cell and its variant metastasize to bone in 4-5 weeks in the same animal model system, but caused very limited osteolysis (2). Thus, the modulation of the “soil” by different “seeds” may determine the efficacy of the “seeding” as well as the subsequent remodeling of the “soil”.

Recently, Kaplan et al. (3) showed that systemic administration of the conditioned medium by a melanoma cell line redirected the metastatic dissemination of a weakly metastatic lung carcinoma cell line to the organ sites that were metastasized by the melanoma cell, not by the lung carcinoma cell. This was due to the formation of metastasis-permissive niches by bone marrow-derived cells (BMDCs) in these organ sites after the treatment with the melanoma cell-conditioned medium. The BMDCs then attracted the lung carcinoma cells to these organ sites. Thus, this new concept suggests that tumor cell-secreted factors can initiate site-specific construction of metastasis-permissive niches before the arrival of metastatic cells. On the basis of these observations, we hypothesized that factor(s) secreted by metastasis-competent BC cells may condition bone marrow for the successful homing and skeletal remodeling of circulating BC cells. Our initial study indicated that the media conditioned by metastasis-competent BC cells had limited effect on bone metastasis of metastasis-incompetent cells. We are currently analyzing mouse bone tissues and investigating whether the presence of orthotopic mammary tumors formed by metastasis-competent BC cells will enhance bone metastasis of circulating BC cells that do not metastasize to bone by themselves.

Body

Experimental design

Five-week-old athymic nude female nude mice were injected i.p. daily with 300 μ l conditioned medium (CM) from metastasis-competent human breast cancer MDA-MB-231 or MDA-MB-435-F-L cells for 5 days prior to the intracardiac injection of metastasis-incompetent human breast cancer ZR-75-1-GFP cells. ZR-75-1 cells were stably transfected with a green fluorescent protein (GFP) expression plasmid containing puromycin-resistant gene. After the intracardiac injection, CM (300 μ l per mouse) was injected every alternate day for more than 3 months. Control animals (n=10) were injected with 300 μ l serum-free medium. Bone metastasis was monitored by green fluorescence imaging for the detection of the GFP-labeled ZR-75-1 cell-formed tumors and periodic X-ray radiography.

Experimental results

Skeletal metastasis was not detected by whole mouse green fluorescence imaging after 6 and 12 weeks and by X-ray imaging after 6 weeks. In order to determine whether intracardiacally injected ZR-75-1 cells homed to the bones, bone marrow of tibiae and femora of some mice was flushed out using the cell culture medium and a syringe attached to an 18 gauge needle after 12 weeks of the experiment to isolate puromycin resistant ZR-75-1-GFP cells. However, we failed to detect any bone marrow derived ZR-75-1 cells. Injection of CM was continued once a week for four more weeks and then stopped.

The experiment was terminated after 24 weeks. Green fluorescence imaging and X-ray imaging were performed. One animal out of a total of eight in the control group developed spine and Lung metastasis. One animal in the MDA-MB-435-F-L CM treated group developed metastasis in femur/tibia (Fig. 1). Another two animal in the same group developed spine and lung metastases. Thus, three out of eight mice treated with CM from MDA-MB-435-F-L developed metastases to bone suggesting that the CM might have promoted the metastatic potential of ZR-75-1 cells. All femur/tibia bones were formalin fixed and decalcified for future histomorphometric analysis. On the other hand, the mice that were treated with CM from MDA-MB-231 cells did not develop metastasis.

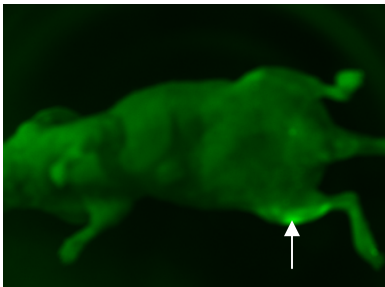


Fig. 1. Development of bone metastasis by intracardiacally injected ZR-75-1-GFP cells in a mouse treated with CM from MDA-MB-435-F-L cells as detected with green fluorescence imaging. The arrow indicates the GFP-expression tumor in the femur/tibia.

Ongoing research

Due to a delay in finding personnel to carry out the project, we have not completed the proposed research and have requested a one-year no cost extension. We are currently processing the femur/tibia bone with ZR-75-1 cell-formed tumor to determine whether the tumor caused osteolysis, which is often observed in breast cancer patients.

In light of the limited promoting effect of the CM from MDA-MB-435-F-L and MDA-MB-231 cells on the metastasis of ZR-75-1 cells, we will determine whether growing orthotopic tumors formed by MDA-MB-435-F-L or MDA-MB-231 cells will promote bone metastasis of intracardiacally injected ZR-75-1 cells. If this is the case, it is likely that factors produced by the MDA cells may be labile and/or not produced in vitro. Future studies will be focused on the identification of the factors produced by the MDA cells that promote the metastasis of ZR-75-1 cells in vivo.

KEY RESEARCH ACCOMPLISHMENTS

1. Generated GFP-expressing, puromycin-resistant stable ZR-75-1 cells.
2. Completed study to determine whether systemic treatment with conditioned media from MDA-MB-231 or MDA-MB-435-F-L cells can promote bone metastatic potential of ZR-75-1 cells.

REPORTABLE OUTCOMES

Cell line: GFP-expressing, puromycin-resistant ZR-75-1 cells

CONCLUSIONS

Systemic treatment in female nude mice with the medium conditioned by MDA-MB-435-F-L cells showed moderate effect in promoting bone metastasis of ZR-75-1 cells. However, the medium conditioned by MDA-MB-231 cells did not affect the metastatic potential of ZR-75-1 cells. Our observations suggest that the production of metastasis-promoting factors by metastasis-competent breast cancer cells may require the interaction between the cancer cells and stromal cells in an in vivo environment. Our ongoing research will address this hypothesis.

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