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Contribute to Breast Cancer-Associated Osteolytic Lesions

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14. ABSTRACT Bone metastasis is a common event during breast cancer progression. Matrix metalloproteinases (MMPs) are often overexpressed in breast cancer and play an important role in tumor progression. Metastatic breast cancer is typically osteolytic and we hypothesize that specific stromal and tumor MMPs contribute to the growth and the development of osteolytic lesions. To address the role of individual stromal MMPs in vivo, we used an intratibial model that recapitulates breast tumor induced osteolysis. We demonstrated that stromal MMP-2 is required for mammary tumor growth in bone by contributing to the proliferation and the survival of the tumor. Developing our understanding of the roles of specific MMPs in breast induced-bone osteolysis will hopefully open the way for new therapeutics.					
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Introduction

In the United States, this year, 80% of the women who will succumb to breast cancer, will present with evidence of bone metastasis at the time of their death[1]. The process of breast to bone metastasis illustrates the “seed and soil theory” which explains that certain tumors spread to specific organs depending on the complexity of the interactions between the tumor cells (seed) and their environment (soil)[2]. The skeleton is a common site for metastasis in many cancers such as breast, lung or prostate cancer. Breast to bone cancer metastases are typically osteolytic and induce bone destruction[3].

The MMPs are a family of enzymes that degrade the extracellular matrix and a variety of signaling molecules and cell surface receptors such as osteopontin, $\text{TNF-}\alpha$, $\text{TGF-}\beta$ and RANKL[4-6]. Thus, MMPs, by cleaving and/or solubilizing these functional factors, can modify the communication between tumor cells and the host microenvironment[7]. In the normal bone stroma, a number of MMPs have been detected, in osteoclasts, the cells responsible for bone resorption, such as MMP-2, -3, -9, -13 and -14[8]. Our understanding of the contribution of specific MMPs to pathological conditions such as breast cancer induced osteolysis is limited. The use of broad spectrum MMP inhibitors can decrease and even prevent breast tumor induced osteolysis in animal models[9-11]. However the contribution of specific MMPs to the observed tumor growth and osteolysis remains to be determined. In the current project, we will investigate the role of specific MMPs in mammary tumor growth induced osteolysis and determine the molecular mechanisms through which specific MMPs contribute to this process.

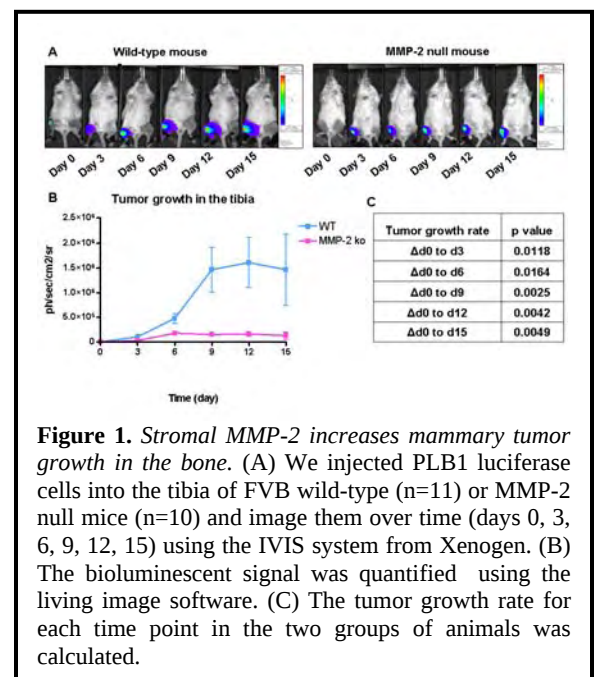
Project

Accomplishment

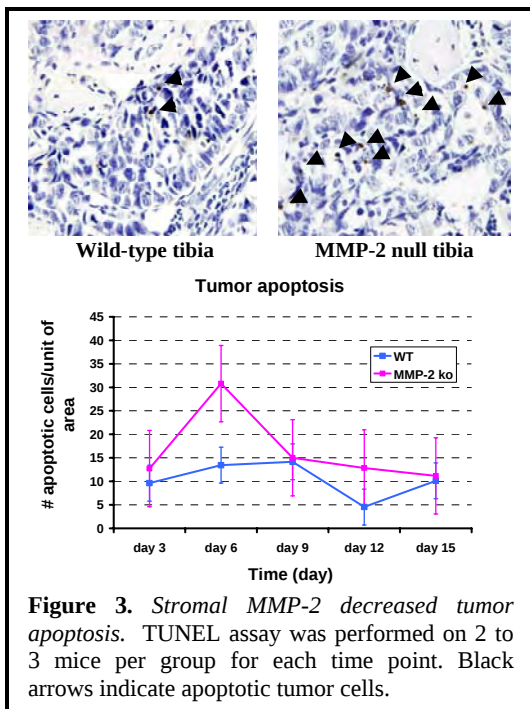
Task 1. Determination of the contribution of stromal MMP-2, -3, -7 and -9 to mammary tumor growth and osteolysis in the *in vivo* tumor:bone microenvironment.

- Intra-tibial injection of luciferase tagged PLB1 (forms primary tumors, induces bone destruction and forms lung metastases) cells studies in MMP deficient (MMP-2, -3, -7 and -9) and wild-type FVB mice (months 1-24): familiarization with real time imaging modalities such as Xenogen's IVIS system for monitoring luciferase activity (months 1-6).
- Generation of *in situ* hybridization protocols for MMPs in bone tissue (months 7-30).
- Histological analysis of wild-type and MMP deficient mice intra-tibially injected with PLB1 cells (months 3-30) and immunohistochemistry and cytochemistry for the localization of tumor cells, bone cells and immune cells in sections from intra-tibially implanted tumors.
- Collation of the data and publications of the results (months 27- 36).

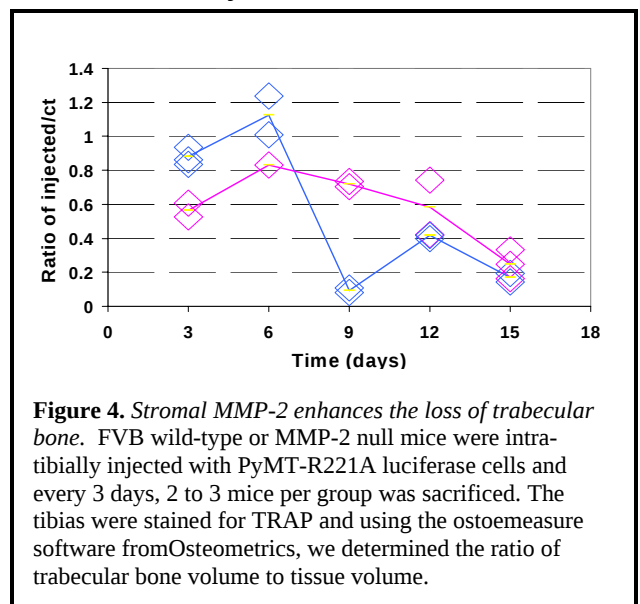
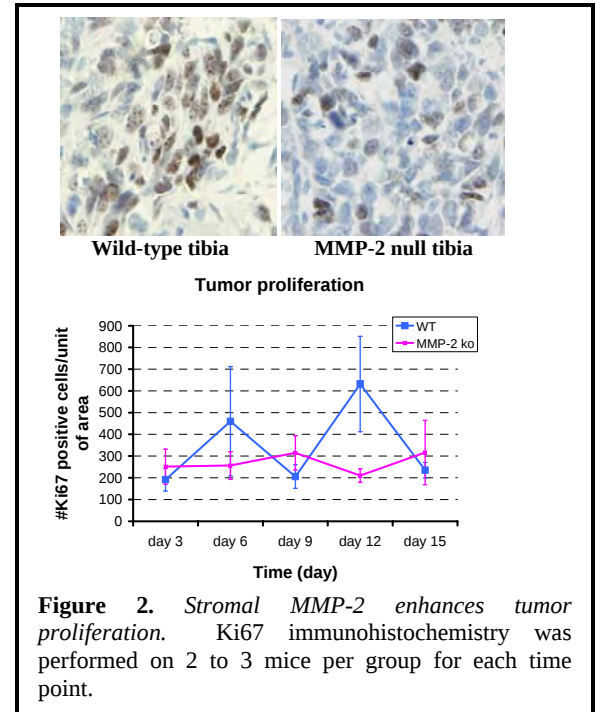
Our initial studies have focused on MMP-2 and MMP-9. To assess if stromal MMP-2 affects mammary tumor growth in the bone, we injected the PLB1 cells into the tibias of wild-type (n=11) or MMP-2 deficient (n=10) FVB mice. This cell line has been isolated from the polyoma middle T antigen (PyMT) FVB mice. These mice develop spontaneous mammary tumors that recapitulate the pathology of human breast cancer[12]. Using this cell line allows for the use of fully immune competent FVB mice representing a more accurate human scenario. After injection of the PLB1 cells tagged



with luciferase, the growth of the tumor was quantified every 3 days by a retro-orbital injection of luciferin (105ng/kg). Photoemission from the tumor was quantified with the Xenogen IVISTM imaging system (**Figure 1**). From day 3 onwards, we observed a significant difference in the tumor growth rate in the MMP-2 null mice compared to the wild-type animal ($p < 0.02$, Student's *t* Test). To investigate further the effects of stromal MMP-2 on mammary tumor growth, we sacrificed mice ($n=3$) in each group every 3 days to perform immunohistochemistry analyses. Tumor proliferation in the bone was assessed by Ki67 staining, MMP-2 deficient mice showed a decreased number of proliferative tumor cells at day 6 and 12 after injection compared to the wild-type mice (**Figure 2**). We also performed a terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphatase nick end labeling (TUNEL) assay (**Figure 3**). An increased number of apoptotic tumor cells was observed in the MMP-2 deficient mice at day 6 and day 12 after injection compared to the wild-type mice. These results indicate that stromal MMP-2 contributes to tumor growth and tumor survival in the bone. We have also confirmed that this MMP-2 effect is not confined to the PLB1 cells by injecting another clonal mammary cell line derived from the MMTV-



PyMT FVB mice. To determine the role of stromal MMP-2 in mammary tumor induced osteolysis *in vivo*, we initially focused on osteoclasts and performed tartrate-resistance acid phosphatase (TRAP) staining (a marker of active osteoclasts). Using the OsteomeasureTM software from Osteometrics, the volume of trabecular bone was determined. From this volume, the software calculated the ratio of trabecular bone volume to tissue volume and we normalized this ratio for each injected tibia to the control tibia (**Figure 4**). Between day 6 and day 9, a drastic loss of trabecular bone occurred in the wild-type mice compared to the MMP-2 null mice where we observed delayed trabecular bone loss. Using the OsteomeasureTM software, we also quantified the number of active osteoclasts present at the tumor-bone interface in the two groups of animals (**Figure 5**). We observed that the extensive trabecular bone loss occurring between day 6 and 9 in the wild-type mice, did not coincide with an increased number of active osteoclast compared to the MMP-2 deficient animals. We are currently increasing the number of animals in the study to reach statistical power. Task 1b will now be pursued to identify the cellular origin of MMP-2 in our tissue samples. We also investigated the contribution of stromal MMP-9 in tumor growth and bone resorption in our *in vivo* model, however we observed equivocal results. We are currently breeding more



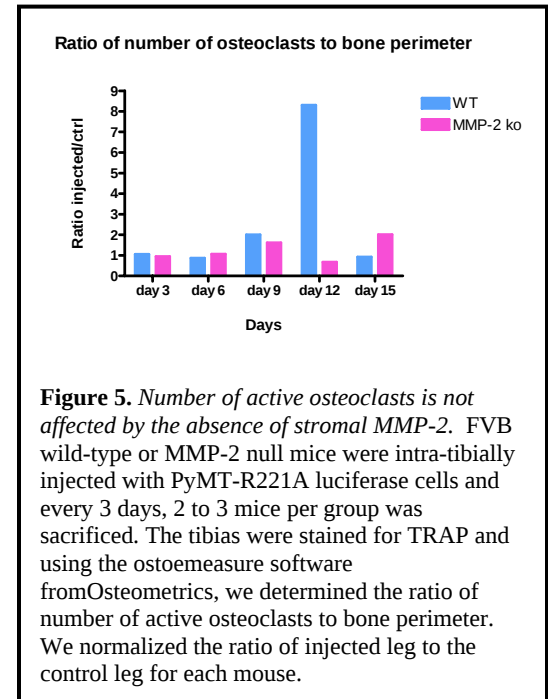
MMP-9 null mice to perform a new set of intra-tibial injections to determine the role of stromal MMP-9 in tumor growth and bone degradation.

We have now available in the laboratory MMP-3 and -7 deficient mice in the FVB background and studies will be performed as soon as sufficient mice will be available.

Task 2. *Identification of the mechanism by which specific stromal MMPs contribute to breast tumor induced osteolysis using an ex vivo approach.*

- a. Generation and characterization of the *ex vivo* calvaria model (months 1-6).
- b. Isolation of MMP deficient calvaria and histological assessment of tumor induced bone destruction. MMP deficient animals to use will be identified in Task 1, part a (months 6-32).
- c. Collation of the data and publication of results (months 34-36)

The execution of this task relies on the use of an *ex vivo* calvaria model described by Ohshima *et al*, 2003[13]. We have focused our initial effort on understanding how MMP-2 contributes to mammary tumor induced osteolysis since such a significant effect had been observed in task 1. MMP-2 deficient mice showed a difference in the amount of trabecular bone loss but not in the number of active osteoclasts, we decided to investigate the potential role of stromal MMP-2 in osteoclast function and migration. Unfortunately, the *ex vivo* calvaria model has not recapitulated the observed effects *in vivo* with respect to tumor induced osteolysis. Therefore, we are taking *in vitro* as well as *in vivo*, we will test the role of stromal MMP-2 in osteoclast migration and function. We will isolate osteoclasts from wild-type and MMP-2 deficient mice and test their ability to resorb dentin slices. Since we observed an effect of stromal MMP-2 in tumor growth and survival, we will focus on some factors in the bone that can control tumor growth



Key research accomplishment

1. Demonstration that stromal MMP-2 has a significant role in mammary tumor growth in the bone *in vivo*.
2. Stromal MMP-2 contributes to the proliferation and the survival of mammary tumor cells in the bone *in vivo*.

Reportables outcomes:

- American Society of Matrix Biology biennial meeting, October 2006, poster presentation
- 6th Annual Host -tumor Interactions and Cancer Biology joint retreat, November 2006, oral presentation, Oral talk award (1st place)
- Cancer Induced Bone Disease meeting, San Antonio, December 2006, poster presentation

Conclusions and future directions

Over the first year of our studies, we showed the significant importance of stromal MMP-2 in the growth of mammary tumor cells in bone. We demonstrated that stromal MMP-2 contributes to the growth and the survival of the tumor cell in the bone and does not affect the activation of osteoclasts. In the next two years, we

will focus on determining the molecular mechanism through which stromal MMP-2 contributes to mammary tumor growth and osteolysis.

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