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14. ABSTRACT
The major site of metastasis of breast cancer cells is bone. Bone metastases occur in 80% of patients with advanced disease and causes significant morbidity. The mechanisms of osteolysis and targeting of bone by the breast cancer cells are still unknown. The osteolysis that occurs in patients is mediated by factors that come from the tumor cells. The tumor cells provide a microenvironment to the osteoblasts and osteoclasts. Bone remodeling requires the degradation and turnover of type I collagen. Type I collagen is a triple helical fibrillar collagen that can be cleaved by cathepsin K found only in osteoclasts. Recently, studies of breakdown fragments of extracellular matrix (ECM) proteins have shown novel biological activity of the fragments. We believe that these fragments may be chemotactic to breast tumor cells. Once the breast tumor cells metastasize to the bone, the tumor cells secrete factors that stimulate the increase in osteolysis of bone. This leads to increase of type I collagen fragments that signal back to the tumor cells, osteoblasts and osteoclasts perpetuating this cascade. This study proposes to map the active sequences in the type I collagen breakdown fragments that can contribute to breast tumor metastasis to bone. The understanding of this pathway can lead to development of biomarkers assays to detect early bone metastases, monitor treatments in targeting bone metastases and staging of metastatic bone tumors. Prevention, early detection and treatment of bone metastasis breast tumors to bone would benefit the survival and quality of life of breast cancer patients.

15. SUBJECT TERMS
Bone, Metastasis, Collagen, Proteases

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INTRODUCTION

This study was funded to assess the ability of novel type I collagen degradation fragments that are generated by Cathepsin K cleavage found in osteoclasts to induce the upregulation of inflammatory cytokines (GM-CSF, TNF-alpha, PTHrP, IL-1, and IL-6), in breast tumor cells and osteoblasts, which stimulate osteoclast formation and increase osteolysis.

Bone is an active remodeling tissue and accounts for the major of type I collagen turnover in the body. Once the breast tumor cells metastasize to the bone, the tumor cells secrete inflammatory chemokines and cytokines such at PTHrP, receptor activator of nuclear factor kappa B (RANK) that stimulate osteoclast formation. These events result in increase osteolysis. The increase osteolysis of bone leads to increase of type I collagen fragments that signal back to the tumor cells, osteoblasts and osteoclasts perpetuating this cascade.

The major site of metastasis of breast cancer cells is bone. Bone metastases occur in 80% of patients with advanced disease and causes significant morbidity. The mechanisms of osteolysis and targeting of bone by the breast cancer cells are still unknown. The osteolysis that occurs in patients is mediated by factors that come from the tumor cells. The tumor cells provide a microenvironment to the osteoblasts and osteoclasts. Bone remodeling requires the degradation and turnover of type I collagen. Type I collagen is a triple helical fibrillar collagen that can only be cleaved by collagenases of the matrix metalloproteinase (MMP) family or by cathepsin K found only in osteoclasts. The extracellular matrix plays an important role in tissue homeostasis. It regulates cellular differentiation, migration, and proliferation. Recently it has been shown that cleavage products of ECM proteins may have novel biological properties and have been termed matrikines[1, 2].

The scope of this work includes the characterization of the ability of cathepsin K generated type I collagen cleavage fragments on breast tumor cells to produce inflammatory cytokines and proteases and to characterize the specific sequences in the type I collagen fragments that induce inflammatory cytokines and proteases.

BODY

In the first 12 months of the project, we projected that we would able to characterize and identify specific cathepsin K generated type I collagen fragments that would stimulate breast tumor cells to produce inflammatory cytokines and proteases. Below are the objectives from the original statement of work.

Tasks

Specific Aim 1: To characterize the ability of cathepsin K generated type I collagen cleavage fragments on breast tumor cells to produce inflammatory cytokines and proteases.

a) Purification of Type I matrikines:
Native rat type I collagen will be incubated with recombinant cathepsin K at 25^0C and pH 5.0 for 6h[3], cleavage products as described in Kafienah *et al*.[3], will be separated and purified by SDS-PAGE and HPLC.

b) Testing biological activity of Type I collagen fragments:
Breast tumor cell lines (MCF-10-2A, HCC1500, MDA-MB-231) will be grown *in vitro* and incubated with cathepsin k generated peptides of type I collagen.
Cells will be grown in 12 well plates in triplicate. A dose-response curve will be determined using different concentrations of matrikine fragments.

Cathepsin K, PTHrP, TNF-alpha, GM-CSF, IL-1, IL-6, RANK mRNA levels will be quantitated by Real-Time PCR (ABI -7500) and by human cytokine arrays (R&D, MN).

Cathepsin K protein will be analyzed by Western blotting with rabbit polyclonal antibodies.

Specific Aim 2: Characterize the specific sequences in the type I collagen fragments that induce inflammatory cytokines and proteases.

a) Mapping of specific matrikine sequences in purified type I collagen fragments:
To characterize the active sequences in the collagen fragments, overlapping peptides will be synthesized from bioactive fragments and then tested individually in the tumor cell lines.

b) Testing biological activity of Type I collagen matrikine fragments :
Breast tumor cell lines (MCF-10-2A, HCC1500, MDA-MB-231) will be grown *in vitro* and incubated with cathepsin k generated peptides of type I collagen.
Cells will be grown in 12 well plates in triplicate. A dose-response curve will be determined using different concentrations of matrikine fragments.

Cathepsin K, PTHrP, TNF-alpha, GM-CSF, IL-1, IL-6, RANK mRNA levels will be quantitated by Real-Time PCR (ABI -7500) and by human cytokine arrays (R&D, MN).

Cathepsin K protein will be analyzed by Western blotting with rabbit polyclonal antibodies.

The project was delayed 4 months due to hiring of a new research assistant. The research assistant was recent graduate and had to be trained in new laboratory methods. In the past 3 months we have generated Cathepsin K type I fragments that we have tested on breast tumor lines MCF-10-2A, HCC1500, MDA-MB-231. We have identify 2 regions of in the type I collagen triple helix can stimulate the tumor cells to produce inflammatory cytokines and MMPs. We are now completing the mRNA analysis and designing overlapping synthetic peptides to map the specific matrikine sequences in these 2 regions in the triple helix of type I collagen. When we receive the synthetic peptides we can map the regions and identify and test the specific matrikines on the breast tumor lines.

KEY RESEARCH ACCOMPLISHMENTS

- Identified 2 regions in type I collagen triple helix that has novel biological activity on breast tumor cells.
- Trained new college graduate in laboratory methods in matrix biology

REPORTABLE OUTCOMES

Reportable outcomes are expected for the next review.

CONCLUSIONS

There is increasing evidence that matrix molecules especially collagen and its cleavage products can act as novel matrikines that may play a role in tumor microenviroment, progression and metastasis.

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