A Type I Collagen Matrikine Fragment is Involved in Breast Cancer Cells Metastasis to Bone and Promoting Osteolysis

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**Introduction:**

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. This relationship was first described by Paget in 1889 and the term “seed and soil” was coined to explain this preferential metastasis of breast tumors to bone [1]. The osteolysis that occurs in patients is mediated by factors that come from the tumor cells. The tumor cells thrive and expand in the bone microenvironment and provide the osteoblasts and osteoclasts with factors that promote osteolysis. The mechanisms of osteolysis and targeting of bone by the breast cancer cells are still unknown. 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. Recent studies of breakdown fragments of extracellular matrix (ECM) proteins have shown novel biological activity of the fragments [2]. Recently we have identified a type I collagen degradation fragment that can be generated either MMPs or Cathepsin K cleavage of type I collagen. This matrikine has been shown to induce the expression and production of MMPs and promote the formation of osteoclasts in vitro [3]. These observations have let us to hypothesize that type I collagen matrikine fragment, CB4II, may have several functions in breast tumor metastasis to bone and osteolysis. Bone is an active remodeling tissue and accounts for the major of type I collagen turnover in the body. 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 inflammatory chemokines and cytokines such as PTHrP, receptor activator of nuclear factor kappa B (RANK) and Runx2 that stimulate osteoclasts formation. These events result in increase osteolysis due to increase protease production and osteoclasts formation. 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 aim of the study was to investigate the production of this matrikine fragment by cathepsin K in an in vivo mouse model of breast tumor metastasis.

**Materials and Methods:**

Mouse metastasis model: Human fetal tibiae fragments were implanted subcutaneously into either control or tumor-bearing immunodeficient Rag-1 -/- or Rag-1 -/-, CatK -/- mice (N=3 each). Four weeks later, 0.5x10^6 MDA-MB-231 cells were injected through the mouse skin directly into the marrow of previously implanted bone, as described [4]. Four weeks following injection of tumor cells, the mice were sacrificed and the bone implants harvested and extracted for Western blot and immunohistochemical analysis. Western Blot analysis: Human tibiae fragments were homogenized in RIPA extraction buffer. Protein concentration was determined by BCA. Equal amounts of protein was loaded and separated by SDS-PAGE. Nylon membranes were probed with anti-CB4II rabbit antibody. Immunohistochemistry: Mice tibiae fragments were fixed in formalin and decalcified for immunohistochemistry. Serial sections were stained with anti-CB4II rabbit antibody and staining was visualized by florescence microscopy.

**Results:**

Western blotting of control human tibiae fragments tibiae (3 different mice) implanted in Rag-1 -/- mice revealed the presence of CB4II containing type I collagen matrikine fragments from normal bone turnover while MDA-MB-231 tumor-bearing tibiae show increased amounts of CB4II containing type I collagen fragments (Fig 1A) whereas in (Fig 1B) both control and MDA-MB-231 tumor-bearing tibiae implanted in Rag-1 -/- mice had a marked decrease in CB4II containing type I collagen matrikine fragments as compared to Rag1 -/- mice. These results indicate that cathepsin K plays an important role in generating CB4II type I collagen fragments and CatK -/- mice have decreased tumor-mediated osteolysis due to decrease in generation of CB4II matrikine.

The generation of this matrikine was also seen at osteolytic sites by immunohistochemical analysis of the human tibiae injected with MDA-MB-231 cells, and its generation was reduced in Cathepsin K null mice. These observations have let us to hypothesize that type I collagen fragment, CB4II, may have several functions in breast tumor metastasis to bone and osteolysis. Bone is an active remodeling tissue and accounts for the major of type I collagen turnover in the body. 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 inflammatory chemokines and cytokines such as PTHrP, receptor activator of nuclear factor kappa B (RANK) and Runx2 that stimulate osteoclasts formation. CB4II can induce transcription factors such as NF-kb in breast tumor cells which in turn stimulate osteoclasts formation. Recently NF-kb has been shown to induce OMP-CSF and promote osteolytic bone metastasis and CB4II may play a role in this process. These events result in increased osteolysis due to increased protease production and osteoclasts formation. 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. A schematic of the mechanism of CB4II in tumor mediated bone osteolysis is shown in Fig. 2.

**Discussion:**

In this study, we have investigated the role of Cathepsin K in the generation of type I collagen matrikine CB4II. We have shown for the first time that Cathepsin K contributes to the generation of the CB4II matrikine during breast tumor mediated osteolysis. Also the generation of this matrikine is significantly reduced in Cathepsin K null mice. These observations have let us to hypothesize that type I collagen fragment, CB4II, may have several functions in breast tumor metastasis to bone and osteolysis. Bone is an active remodeling tissue and accounts for the major of type I collagen turnover in the body. 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 inflammatory chemokines and cytokines such as PTHrP, receptor activator of nuclear factor kappa B (RANK) and Runx2 that stimulate osteoclasts formation. CB4II can induce transcription factors such as NF-kb in breast tumor cells which in turn stimulate osteoclasts formation. Recently NF-kb has been shown to induce OMP-CSF and promote osteolytic bone metastasis and CB4II may play a role in this process. These events result in increased osteolysis due to increased protease production and osteoclasts formation. 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. A schematic of the mechanism of CB4II in tumor mediated bone osteolysis is shown in Fig. 2.

**Fig. 1. Western blot analysis of human tibiae implanted in Rag-1 -/- and Rag-1 -/- Cat K -/- mice.**

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**Fig. 2. Feedback of CB4II containing type I collagen fragments in promoting Osteolysis.**

Recently it has been shown that women on bisphosphonate treatments prior to the development of breast tumors have a low incidence of bone metastasis [5]. This observation could be the result of bisphosphonate inhibition of bone remodeling and therefore inhibit the production of matrikine CB4II in bone. Studies studying the CB4II matrikine in human breast tumor patients with bisphosphonate treatment would give us insight into this mechanism and may be a target for breast tumor mediated bone metastasis.

**References:**