Dissecting out multiple mechanisms of breast cancer-induced bone destruction: Molecular Evidences

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INTRODUCTION:
The skeletal system, comprising of more than 200 bones, is the largest reservoir for advanced metastatic breast cancer. Breast cancer has a well-recognized predilection for skeletal metastasis. Osteolytic metastatic breast cancer causes pain, fracture, spinal cord compression, and hypercalcemia of malignancy. Surgical treatment of pathological fractures further pose complications not limited to infection, bleeding, thrombosis, embolism, and death. Use of bisphosphonates have been shown to reduce skeletal events but fail to consistently prevent fractures. Denosumab treatment share the same shortcomings in addition to having a high cost. Not only that, both bisphosphonates and denosumab are associated with avascular necrosis and atypical fractures. The need for more effective management of skeletal events and cancer control in advanced osteolytic metastatic breast cancer largely remains unmet. Our study aims to identify new therapeutic targets, verify pathophysiologic mechanism, and translate our findings into therapeutic applications. We have identified MAPK (pERK1/2) as an important mediator of cancer-induced bone destruction and cancer growth with promising potential in kinase-targeted cancer therapy.

MATERIALS AND METHODS:
1. Identification of new therapeutic targets: In order to identify a key pathway leading aggressive bone destruction, 5 different types of well-established human breast cancer cells were implanted into nude mice tibia and breast. The use of T-B-cell deficient mice allowed for a unique experimental platform to examine the interaction between breast cancer cells and bone cells. At 4 weeks, we measured the tumor size and bone destruction using radiographs and microCT. We then compared the phosphorylated kinases between the least and most osteolytic breast cancer cells and confirmed the expression of candidate kinases using immunoblotting.
2. Mechanisms and Pathophysiology: Downstream pro-osteoclastogenic and anti-osteogenic proteins were defined using RT-PCR array and immunoblotting. We also examined if kinase inhibitors could enhance cancer cell death by using colony forming assay in the co-culture condition with osteoblasts.
3. Human Pathological Specimens: Breast cancer cells from human pathology specimens (N=12) were collected from pathological fracture sites and screened for the presence of pERK1/2 (Data not shown).
4. Therapeutic Translation: We experimented whether a kinase inhibitor could indeed prevent cancer growth and bone destruction in MDA231-bearing mice (N=3).

RESULTS:
MDA-MB-231 cells demonstrated the most aggressive growth in the tibia in comparison to other well-established breast cancer cells such as MCF-7, MDA-MB-157, and HCC1806 cells (p<0.01). Despite high mechanical stiffness of the bone compartment, breast cancer cells grew more aggressively in bone than in the orthotopic breast regions (Figure 1A). Protein kinase array showed that high levels of MAKP (pERK1/2) distinguishes MDA-MB-231 cells from MCF-7. Confirmative immunoblotting revealed a high correlation between pERK1/2 expression and bone destruction (Figure 1C). Loss-of-function experiments using a clinical MEK1-pERK1/2 inhibitor confirmed regulation of RANKL/OPG ratio that is known to enhance osteoclastogenesis by osteoblasts (Figure 1E).

DISCUSSION AND CONCLUSIONS:
Our results suggest that pERK1/2 is a therapeutic target that mediates cancer growth and bone destruction. Kinase-targeting therapies using FDA-approved clinical-grade inhibitors have great potential to enhance clinical outcomes of patients with advanced osteolytic metastatic breast cancer by increasing pro-apoptotic proteins, decreasing anti-apoptotic proteins and pro-osteoclastogenic cytokines, and reducing pathological fractures with associated pain and disability. Our study introduces a new concept of kinase-targeted therapies for cancer treatment in general.

SIGNIFICANCE:
Our data suggest that treatment of osteolytic cancer with kinase-targeted agents may have beneficial clinical outcomes.

Figure 1. A, higher pERK1/2-expressing MDA-MB-231 or MCF10B121 show more aggressive bone destruction and larger cancer mass than MCF with lower pERK1/2 (N=12, all P<0.05). B, Gene expression level related to cancer growth in tibia whereas AKT, p38, NTRK and PIK3 are not C. Breast cancer cells produce RANKL that is not correlated with pERK1/2. D, the activated breast cancer cells induced changes of osteoclasts via the activation to osteoclastogenic cytokine. E, the ratio of osteoclasts increased by conditioned media of MDA-MB-231 cells and MEK inhibitor reduced RANKL/OPG ratio.

Figure 2. MDA-MB-231 cells induce osteoclasts and osteoblasts via osteoclastic activity. A, The MDA-MB-231 cells induces proliferation of osteoblasts via osteoinductive effect. B, MDA-MB-231 cells induces osteoclastic activity. C, MDA-MB-231 cells induce osteoclasts via the expression of RANKL in the media.