

ERK1/2 activation is essential for aggressive growth and bone destruction in osteolytic metastatic breast cancers: Clinical and translational study

Jung Ho Back, Paco Kang, Saqib Nizami, Danielle Stamer*, Jocelyn Compton**,
Do Yu Soung, Lee Song, Francis Y. Lee

Robert Carroll and Jane Chace Carroll Laboratories, Department of Orthopaedic Surgery, Columbia University
Medical Center, New York, New York, U.S.A.

* Yale University, New Haven, CT** University of Iowa, Iowa City

Introduction: The skeletal compartment is the largest reservoir for advanced metastatic breast cancers. Metastatic breast cancers have an unusual predilection for the skeletal milieu. Osteolytic metastatic breast cancers cause pain, fractures, spinal cord compression, and hypercalcemia of malignancy. Surgical treatments of pathological fractures are associated with infection, bleeding, thrombosis, embolism, and death. Bisphosphonates certainly reduce skeletal events but do not consistently prevent fractures. Denosumab shares these issues in addition to prohibitive expense. Both bisphosphonates and denosumab are associated with avascular necrosis and atypical fractures. Therefore, the need for more effective managements of advanced osteolytic metastatic breast cancers with respect to skeletal events and cancer control remains unmet. Our study covers the identification of new therapeutic targets, verification of mechanistic importance, and therapeutic translation. We identified pERK1/2 as an important mediator of cancer-induced bone destruction and cancer growth.

Materials and Methods:

1. Identification of new therapeutic targets: To define a key pathway leading to aggressive bone destruction, we implanted 5 different types of well-established human breast cancer cells into the nude mouse tibiae and breast regions. The use of T-/B-cell deficient mice allowed for a unique experimental platform to examine the interactions 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 phosphorylated kinases between the least and most osteolytic breast cancer cells (MCF7 vs MDA-MB-231) and confirmed the expression of candidate kinases using immunoblotting.
2. Mechanisms and Pathophysiology: We next defined downstream pro-osteoclastogenic and anti-osteogenic proteins using RT-PCR array and immunoblotting. We also examined whether kinase inhibition alone or in combination with doxorubicin enhances cancer cell death using MTT cell survival assay and flow cytometry.
3. Human Pathological Specimens: We examined whether breast cancer cells in human pathology specimens (N=12) from the pathological fracture sites show the presence of pERK1/2.
4. Therapeutic Translation: We examined whether a kinase inhibitor indeed prevents the cancer growth and bone destruction in MDA-MB-231-bearing mice (N=5).

Results: MDA-MB-231 cells grew the most aggressively in the tibia in comparison to other well-established breast cancer cells such as MCF-7, MDA-MB-157, or HCC1806 cells ($p < 0.01$). Despite high mechanical stiffness of the bone compartment, breast cancer cells grew more aggressively in the bone than in the orthotopic breast regions. Protein kinase array screening examination showed that MAKP (pERK1/2) distinguishes MDA-MB-231 cells from MCF-7. Confirmative immunoblotting revealed a high correlation between pERK1/2 expression and bone destruction. Loss-of-function experiments using a clinical MEK1-pERK1/2 inhibitor confirmed regulation of RANKL, sclerostin, MCSF1, and other cytokines that are known to enhance osteoclastogenesis. Metastatic breast cancer cells in pathologic specimens from patients with pathological fractures showed pERK1/2 expression. MEK1-pERK1/2 inhibitors induced death of breast cancer cells by increasing Bim (cell death/pro-apoptotic protein) and decreasing Bcl-xL (cell survival/anti-apoptotic protein). A MEK1-pERK1/2 inhibitor dramatically reduced cancer growth and bone destruction in breast cancer-bearing mice.

Discussion & Conclusions: Our results suggest that pERK1/2 is a therapeutic target that mediates cancer growth and bone destruction. Kinase-targeting adjuvant therapies using FDA-approved clinical-grade inhibitors may enhance clinical outcomes of patients with advanced osteolytic metastatic breast cancers 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 combined conventional cytotoxic and targeted therapies for cancers in general.

Keywords: Targeted therapy, Metastatic Breast Cancers, Osteoclasts, Pathological Fractures





