Discovery of Clinically Actionable Genes in Breast Cancer Bone Metastases

Rebecca J. Watters, PhD
Research Assistant Professor, Department of Orthopaedic Surgery
University of Pittsburgh

Background: Currently, it is estimated that ~150,000 individuals with metastatic breast cancer are living in the US, but actual numbers are not known given that these data are not well collected. These estimates may be greater since ~3.1 million Americans are living with breast cancer and ~30% of those patients will develop metastatic disease. There are data to support that bone metastases occur in 65-80% of stage IV patients, specifically those with estrogen receptor (ER) positive tumors, representing a significant proportion of breast cancer patients. According to the "seed and soil" hypothesis, the bone microenvironment provides a rich climate for growth due to its mineralized extracellular matrix containing abundant growth factors and ionized calcium that support cancer cell growth. Bone metastases result in increased morbidity and pain due to bone fractures and nerve compression. The current standard of care involves treatment with bisphosphonates to target osteoclast driven resorption, and surgery for pathological fractures. Unfortunately, these measures cannot prevent new bone metastases from developing. In addition, hormonal therapy targeting ER will inhibit tumor growth, but can also lead to accelerated bone loss or osteopenia, resulting in an increased number of fractures with many patients developing endocrine therapy resistance after 5 years of treatment. In addition to obvious physical side effects, treatment for and living with metastatic disease can also cause undesirable mood disorders, chronic fatigue syndrome, and chronic pain that significantly reduce the quality of life for these patients. **There is an obvious unmet need for new therapeutic targets to treat breast cancer related bone metastasis.**

Approach: The goal of this proposal is to identify and validate both novel and specific therapeutics to target breast cancer bone metastasis. Previous studies from our lab utilized sets of primary ER-positive breast tumors and patient-matched bone metastases from retrospective clinical studies, with RNA-sequencing to define gene expression changes in breast cancer cells following metastatic colonization in the bone and after years of endocrine therapy. Analysis for expression gains and losses in clinically actionable genes were examined. Interestingly, expression gains in genes like EPHA3, PTPRD, and PDGFRA were absent in *de novo* bone metastasis cases, yet highly recurrent in long-term endocrine-deprived cases that had developed resistance to therapy. These genes have been associated with growth and progression of cancer cells. _Therefore, we hypothesize that targeting these clinical actionable, treatment-driven gains in breast cancer recurrences may provide a novel approach to targeting bone metastases in patients that have developed endocrine resistance._ To address this hypothesis, we will validate if EPHA3, PTPRD, and/or PDGFRA are highly expressed in an independent cohort of over 40 bone metastases collected from a prospective study of breast cancer patients, which is complete with clinical information including treatment regimens. Samples include bone metastases that are *de novo* and those that have developed after the removal of the primary tumor and during endocrine treatment. In addition, we will target expression of these genes in ER positive and long-term estrogen deprived/resistant cell lines via both CRISPR genome editing and approved pharmacological inhibitors for potential expedited translation to the clinic.

Impact: Effective treatments are available for targeting the primary tumor, however we are lacking knowledge regarding the process of how and why patients develop bone metastases. The need to specifically address the underlying mechanisms of metastasis are apparent since stage IV breast cancer therapy shifts from curative to palliative, as it is a rare exception, based on current science, that a patient with distant disease can be cured of breast cancer. **The impact of our study is significant as we aim to identify novel therapeutics to treat existing breast cancer related bone metastasis, and to detect other clinical actionable genes that can be targeted to overcome treatment resistance and unnecessary side effects.** Successful completion of our proposed study to identify novel therapeutics could result in the reduction of individuals living with metastatic bone disease. This would result in a significant decrease of deaths, since these patients account for the majority of ~40,000 individuals per year in the US that die due to breast cancer.