

Defining a Calcification-Associated Stromal Niche for Biomarker-Guided Bone Metastasis Prevention in Hormone Receptor-Positive Breast Cancer

Nicole M. Cullen¹, David G. Morrison¹, Jorge A. Belgodere^{1,2,3}, Matthew E. Burow^{1,2}, Elizabeth C. Martin^{1,2}, Bridgette M. Collins-Burow^{1,2}

¹Department of Medicine, Section of Hematology and Oncology, Tulane University, New Orleans, LA 70112, USA

²Tulane Cancer Center, New Orleans, LA 70112, USA

³Department of Biological and Agricultural Engineering, Louisiana State University and Agricultural Center, Baton Rouge, LA, 70803, USA

Introduction

Bone is the most frequent site of distant recurrence in receptor-positive (ER+) breast cancer and remains a leading cause of patient morbidity and mortality. While adjuvant bisphosphonates reduce bone recurrence and improve survival in selected postmenopausal patients, understanding the biomarkers and drivers associated with breast tumor bone tropism is critical to optimizing bisphosphonate clinical use. Hydroxyapatite (HA)-containing breast microcalcifications are associated with significantly increased odds ratio of bone recurrence, suggesting that they may represent biologically active stromal niches associated with bone tropism rather than passive radiographic findings.

Methods

Thus, to model the calcified breast stromal niche, we utilized immunophenotypically validated donor-derived breast adipose stromal/stem cells (brASCs) induced *in vitro* to undergo osteogenic differentiation.

Results

We observed donor-dependent heterogeneity in brASC capacity to generate HA-rich microcalcifications by alizarin red staining. HA producing donors (HA+) and HA non-producing (HA-) donors clustered into distinct transcriptomic profiles upon RNA sequencing. Specifically, HA+ brASCs exhibited increased gene expression of C-C motif chemokine ligand 2 (*CCL2*) and C-X-C motif chemokine ligand 12 (*CXCL12*), chemokines implicated in osteolysis, breast cancer stemness, and metastatic dissemination. Compared to HA- conditioned media, HA+ conditioned media applied to ER+ cell line MCF7 significantly upregulated proliferation and gene expression of *CXCL12*, Trefoil Factor 1 (*TFF1*), and progesterone receptor (*PGR*), mediators of estrogen receptor-mediated signaling and biomarkers associated with bone tropism. Finally, we found that continuous treatment with the bisphosphonate zoledronic acid during brASC osteogenic differentiation prevented HA deposition, demonstrating the potential for bisphosphonates to directly interfere with stromal calcification in the breast.

Conclusions

Future studies will define calcification associated stromal biomarkers in archived ER+ breast tumors, determine whether calcified stromal niches promote aggressive phenotypes and bone tropism, and test whether bisphosphonates disrupt this crosstalk. This work utilizes donor-derived brASCs as an innovative platform to model the calcified breast niche and establishes a biological framework for developing stromal biomarkers to guide precision use of adjuvant bisphosphonate therapy in ER+ breast cancer.