Breast Cancer Cells Metastasize to Bone through E-Selectin Positive Vascular Gateways

Trevor Price¹, Monika Burness³, Ayelet Sivan², Renee Cheng¹, John L. Mangnani¹, Dorothy A. Sipkins¹

¹Division of Hematological Malignancies and Cellular Therapy, Dept. of Medicine, Duke University, Durham, NC
²University of Chicago, Chicago, IL
³University of Michigan, Ann Arbor, MI
⁴GlaxoSmithKline, Inc., Gaithersburg, MD

Introduction

The Bone marrow (BM) has been suggested to serve as a protective environment for disseminated breast cancer cells (BCCs). The precise molecular signals that circulating BCCs use to identify and adhere to BM vasculature are unknown, yet these interactions are essential for the development of bone metastases. To address this question, we used in vivo fluorescence microscopy to track the interactions of estrogen receptor (ER)+ and ER- BCC lines with bone in real time in an intracardiac injection xenograft mouse model. Our data show that circulating tumor cells enter the bone through unique vascular niches where endothelial cell expression of the adhesive molecule E-selectin and of the chemokine SDF-1 are both upregulated. These vascular niches are the same sinusoidal capillary beds that serve as the site of entry for peripherally circulating hematopoietic stem and progenitor cells (HSPCs) returning to the BM. Similar to HSPCs, BCCs aberrantly express the major cell surface molecules known to regulate hematopoietic cell entry and exit through the BM, including CXCR4 (SDF-1 receptor) and multiple E-selectin ligands. While neither CXCR4 blockade nor general chemokine signaling inhibition in BCCs prevented BM dissemination, E-selectin blockade by the specific glycomimetic antagonist GMI-1271 decreased BCC entry in the BM by approximately three fold at 2 and 24 hours after treatment. Moreover, in studies comparing FACS isolated stem and non-stem MCF-7 BCCs, GMI-1271 inhibition of E-selectin specifically targeted CD44+CD24 breast cancer stem cell homing. Non-stem CD44+CD24+ BCCs demonstrated significantly less BM homing compared to stem BCCs, and their BM trafficking was less E-selectin dependent. These data reveal for the first time a molecular mediator of bone metastases at the point-of-entry for BCCs in the BM. In addition, the ability of BCCs to hijack HSPC trafficking pathways to metastasize to bone correlates with E-selectin ligated expression and may be a specific characteristic of the BCC stem cell subpopulation.

Method

A

(DID
- E-Selectin antagonist
- BM
- HSFPCs
- MCF-7 (ER+)
- MDA-MB-231 (ER-)

Human Bone Cancer Cells:
- MCT-7 (ER+)
- MDA-MB-231 (ER-)

(B) - Intravitreal Confocal Microscopy of calvarial bone Marrow: In-vivo confocal microscope imaging of the calvarium of the mouse is used to achieve single-cell resolution of BCC bone marrow homing. This method allows for measurement of the relative number, localisation and micro-environmental interactions of bone marrow homed BCCs.

Results

Fig 1: BCCs home to sinusoidal vascular regions of the bone marrow with upregulated expression of SDF-1 and E-selectin. Female SCID mice injected with 4 × 10⁶ conjugated antibodies against (A) E-selectin and (B) SDF-1 post 1C engraftment of ISD labeled BCCs. Cells home to sinusoidal capillary beds rich with upregulated expression of adhesive molecules E-selectin and SDF-1.

Discussion

This work identifies E-selectin, as opposed to SDF-1, as an important microenvironmental signal mediating the homing of circulating metastatic breast cancer cells to the bone marrow. A novel E-selectin antagonist (GMI-1271) was shown to significantly inhibit the number cells homing to E-selectin positive sinusoidal vasculature. Importantly, BCCs expressing a stem cell immunophenotype (MCF-7 – CD44+/CD24+) were seen to preferentially home to E-selectin positive regions and antagonism using GMI-1271 also significantly inhibited stem cell homing. For the first time this data reveals E-selectin as an important microenvironmental component mediating metastatic entry of BCC stem cells into the bone marrow and the activity of E-selectin antagonist GMI-1271 as an inhibitor of this process.