

Skeletal Tropism of Bone Metastasis in Breast Cancer



Nitin Rao and Daotai Nie*

Department of Medical Microbiology, Southern Illinois University School of Medicine and Simmons Cancer Institute, USA

Submission: February 21, 2017; **Published:** March 27, 2017

***Corresponding author:** Daotai Nie, Department of Medical Microbiology, Southern Illinois University School of Medicine, PO Box 19626, Springfield, IL 62794, USA, Tel: 217-545-9702; Fax: 217-545-3227; Email: dnie@siu.edu

Review

Breast cancer is the most commonly diagnosed cancer in women, with approximately 182,000 women diagnosed annually in the United States-representing 26% of all cancer incidence in women. It carries the lifetime mortality risk of 3.4% in women. The defining feature of “malignancy” in breast cancer is its ability to metastasize or travel to distant sites to form secondary cancers. Breast cancer also demonstrates exquisite preference to metastasize to bone, followed by liver, lung and brain a process known as organ tropism. The molecular factors implicated in the skeletal tropism of breast cancer will be reviewed.

General Principles of Metastasis

The malignancy of cancer is hallmarked by its ability to develop metastatic disease, which accounts for ~90% of cancer-related mortality. Metastasis is the process by which a primary tumor leaves its original site and migrates to distant sites through direct extension, hematogenous spread, or lymphatic spread. This “invasion-metastasis cascade” involves the following seven steps [1].

A. In situ carcinoma invades into the basement membrane by inducing epithelial-mesenchymal transition (EMT) and remodeling the extracellular matrix. EMT represents a switch from an epithelial cell gene program to a mesenchymal gene expression program, which promotes an invasive phenotype. This involves releasing complex molecular signals that result in replacement of E-cadherin by N-cadherin expression leading to pliable scaffolding between epithelial cells, Rho-dependent motility and loss of cancer cell polarity, weak cytokeratin expression, recruitment of tumor-associated macrophages, and degradation of extracellular matrix with proteases (matrix metalloproteinases).

B. This allows intravasation into blood or lymphatic “highways”, often through conduits of recruited microvessels driven by secreted Vascular Endothelial Growth Factor

C (VEGF-C). In breast cancer, lymphatics that drain the mammary gland first collect neoplastic cells in the most proximal lymph node-the sentinel node.

C. Neoplastic cells then circulate through these vessels to reach distant sites. To progress onto the next step, circulating neoplastic cells must survive the austere conditions that systemic circulation imposes.

D. These masses then undergo extravasation into distant sites, and each type of cancer demonstrates organotropism-favoring some organs over others. Breast cancer particularly exits not only into bones and lungs, but also brain and liver. This is mediated by factors including chemokines and Transforming Growth Factor-Beta (TGF-B).

E. The cancer cells then form dormant micrometastasis (<2mm diameter) that are typically below traditional detection thresholds.

F. Some of these micro metastases become activated given a suitable microenvironment through a reversal of EMT-Mesenchymal-epithelial transition (MET)- and resemble the primary tumor. Factors such as epigenetic regulation and histone modification (H3K4-methylation and H3K9-methylation) had been implicated in this process.

G. The cluster of cells that thrive under these environments then form a colony- leading to a macro metastasis (>2mm diameter), which is integral to a clinically significant metastatic disease. The adaptive processes involving colonization is specific to each cancer cell and the site of colonization. Hence, breast cancer cells colonize the bone marrow via different cellular signaling than prostate cancer, and these signaling pathways are distinct from those involving colonization of the lungs.

This is not a comprehensive description of principles driving metastasis, but serves as a general outline of this inefficient

process.

Two major theories exist regarding why cancer metastases exhibit tropism. Paget postulated in 1889 that cancer cells are “seeds” and thrive when they have found their proper “soil”. This alludes to microenvironments of a tissue possessing a hospitable stroma, receptor density, and growth factors favoring colonization. However, this “seed and soil” theory does not adequately explain why contralateral metastases are rare- there is a 98% probability that contra lateral breast involvement is due to a second, isolated, primary tumor [1]. James Ewing in 1928 offered an alternate theory, proposing metastasis occurs through purely mechanical routes and anatomical distribution of vessels [2]. For example, colon cancer most commonly metastasizes to liver because it is the first major organ to be exposed to blood arriving from the portal vein and cancer cells become entrapped in its microvessels. However, this doesn't explain why blood draining from the breast to the heart and spleen rarely lead to metastasis in these organs. Hence, there appears to be a host stroma-cancer microenvironment interaction.

Osteotropism in Breast Cancer

Bone homeostasis is under systemic and local control, integrating endocrine, paracrine, neural, and mechanical stimuli [2]. Locally, it is closely regulated by growth factors exchanged between osteoclasts and osteoblasts [1]. Receptor activator of NFB Ligand (RANKL) is produced by osteoblasts and is maintained on its cell membrane. RANK receptor is found on the surface of osteoclast precursors, and RANKL-RANK receptor binding is a trophic signal of osteoclasts that stimulates its maturation. Osteoblasts also produce an inhibitory decoy receptor called osteoprotegerin (OPG), which can bind RANKL and regulate osteoclast activation by RANK receptor. Therefore, osteoclast activation is determined by the dynamic balance between RANKL and OPG-resulting in constant skeletal turnover that is integral to mechanical adaptation. Osteoblasts also contain receptor for parathyroid hormone (an endocrine regulator), and norepinephrine (regulator released by sympathetic nerves), which physiologically stimulate the release of RANKL [3]. Breast cancer cells commandeer these mechanisms to promote a hospitable microenvironment for colonization. The earliest and most common site of distant metastasis of breast cancer is bone [4]. Cite that 50% of individuals with newly diagnosed advanced breast cancer have bone metastasis, followed by liver (30% of individuals), lung (26%), and brain (7%).

Approximately 70% of women die with bone metastases, and this is the site of the highest tumor burden. Breast cancer classically causes osteolytic lesions, but osteoblastic lesions are found in 25% of the cases [1]. Breast cancer cells migrate to the highly vascular bone microenvironment, interact with the bone stroma and secrete Parathyroid Hormone-related Peptide (PTHrP), driving osteoblast-induced osteoclastic resorption

by upregulating RANKL: OPG ratio. Osteoclastic resorption of bone has been found to be a key factor in promoting a favorable microenvironment due to its release of growth factors, chemokines, and cytokines from the bone matrix that favor cancer cell colonization. These include platelet-derived growth factor (PDGF), bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), insulin-like growth factor-1 (IGF-1), and Transforming Growth Factor (TGF) and their downstream effector pathways. These factors stimulate cancer proliferation, and production of more PTHrP-creating a vicious cycle of a positive feedback loop [4]. Antibodies against PTHrP have been shown to suppress osteolytic lesions in mice [1,5]. Bussard et. al. (2008) previously commented on how the release of these growth factors is accelerated breast cancer metastasis, but posed the question why most cancers such as colorectal adenocarcinoma are transient in the bone marrow but do not present as clinically as metastatic disease of the bone. They suggested that there are bone-specific interactions with breast cancer cells that “home” them to the bone rather than another site such as the adrenals [4,5].

Awolaran et al. [2] presented the first systematic review of 15 proteins implicated in “homing” breast cancer cells to the bone in articles published between (2003) and August (2016). The group excluded studies exclusively carried out in vitro without in vivo testing and included only studies using bone tropic models that specifically demonstrated osteotropic effects of specific gene products. The group then categorized each molecular factor in four categories-cell proliferation & differentiation, bone mineralization & remodeling, cell adhesion, and chemokine signaling, with several markers having overlapping putative roles. In this systematic review, IL-11 was the oldest factor elucidated in 2003 by Kang et al. whose physiological role is osteoclast formation, especially when activated by TGF. Its pathological role is consistent with current literature supporting the role of IL-11 promoting angiogenesis, tumor cell invasion, and primarily osteoclastogenesis – often working in tandem with Connective tissue growth factor (CTGF), Chemokine receptor CXCR4, and Osteopontin. However, whether IL-11 independently activates osteoclasts or promotes RANKL release is unclear, and the exact mechanism of action has yet to be elucidated. Jamieson-Gladney et al. [6] investigated the chemokine receptor CX3CR1 which is physiologically expressed in natural killer cells, and respond to fractalkine (CX3CL1) to initiate migration and adhesion. The group found that CX3CR1 tissue microarray distribution increased with malignant transformation and MDA-MB-231 line expressed high levels (whereas MDA-MB-436 did not) and CX3CR1 increased homing in the presence of CX3CL1 originating from the endothelium of the bone marrow through the MAPK pathway. Furthermore, when CX3CL1-null mice were inoculated with CX3CR1 expressing MDA-MB-231 cells, the group noted over a 70% reduction in tumor cells homing to the bone compared to wide type models.

Awolaran et al. [2] noted that a similarity between the aforementioned factors is that wildtype expression of these proteins maintains normal bone homeostasis, and is integral in the osteogenic activities of healthy breast tissues in breast tissue development and lactation. Breast cancer cells commandeer these proteins in a similar mechanism to promote osteogenesis to their own benefit – to create a conducive microenvironment for capillary adhesion, extravasation, and colonization.

Elefteriou [4] analyzed the role of sympathetic nerves in acquiring skeletal metastases. He notes that chronic stress and depression are prevalent in individuals of low socioeconomic standing and is also associated with increased cancer recurrence and reduced survival in women with a history of breast cancer. The mechanism involves chronic release of catecholamines by sympathetic nerves, particularly norepinephrine (NE), and glucocorticoids by adrenal glands. Acute and chronic stress, traumatic emotional events, and depression results in prolonged sympathetic nervous system (SNS) activity and hypothalamic-pituitary and adrenal (HPA) axis activity. Osteoblasts express receptors for NE, which increases release of RANKL- leading to osteoclast recruitment and microenvironment for osteotropism.

Elefteriou cites previous studies that showed NE signals leading to SDF1/CXCL12 activation-which increased homing of breast cancer cells. His group previously used MDA-MD-231 and 4T1 breast cancer cells and mouse models of learned helplessness in parenthesis to demonstrate increased metastatic homing into bone and increases osteolytic lesions. Further studies into the SNS involvement in the metastatic process would include mouse models in which β 2-Adrenergic Receptors can be modulated with exogenous NE and β -blockers (ie. Propranolol) and monitoring homing response. However, the challenge is being able to replicate endogenous regulation of catecholamine release.

Micrometastasis

Skeletal micrometastasis in breast cancer is an important prognostic marker of risk of relapse with metastatic disease, even after radical mastectomy [1]. one study demonstrated 36% micrometastases in the bone marrow of patients with stage I, II, or III breast cancer. Within four years, 25% of marrow-positive patients died from cancer, compared to 6% who were marrow-negative, representing approximately a 4-fold increased risk of relapse or death. Another study documented 10-fold risk of death. Weinberg comments that a challenge in detecting micrometastasis is a dormancy state of these cells or clumps of cells – representing a G0-like phase for a long period without proliferating. Circulating microRNAs in peripheral blood are possible future avenues for detection of micrometastasis and show some promise in detecting tumor progression, but their significance is yet to be completely elucidated Roth et. al. (2010), investigated miR10b, miR34a, miR141, and miR155, and found that miR10b and miR34a are correlated with

occurrence of overt metastasis. However more prospective and higher-powered studies are underway to ascertain their exact prognostic implications [8]. highlighted recent studies showing aberrantly expressed vascular cell adhesion molecule-1 (VCAM-1) in escaping dormancy and forming macrometastasis. VCAM-1 is an immunoglobulin-like adhesion molecule with Ig domains expressed on endothelial cells. VCAM-1 binds with α 4 β integrins under the influence of inflammatory cytokines. Physiologically, it mediates leukocyte adhesion and extravasation. VCAM-1 clustering by Ig cross-linking or integrin binding leads to activation of Rac1 (Rho-like GTPase) that remodel tight junctions between endothelial cells to facilitate leukocyte extravasation.

Pathologically, VCAM-1 has been shown to be highly expressed in post-dormancy breast cancer cell lines, and demonstrating osteolytic-promoting properties. Furthermore, studies have shown that antagonizing antibodies targeting either VCAM-1 or α 4 β integrin decreased relapse in mouse models. However, the caveat was that VCAM-1 appeared to have no effect on the proliferation of micrometastasis, but rather represented a positive feedback in the “vicious cycle” of osteolysis and release of growth factors that was previously alluded [8]. Aberrantly expressed VCAM-1 on breast cancer cells have been shown to bind 41 integrins on osteoclast precursors, leading to differentiation into mature osteoclasts. Future direction in treatment may revolve around blocking this interaction using drugs such as natalizumab (humanized monoclonal antibody against 4 integrin) and other small molecular inhibitors of 4 integrins to stop the micrometastases to evolve into a macrometastases.

Future Direction

The exact mechanism of osteotropism in breast cancer is yet poorly understood. While multiple studies have implicated proteins in skeletal homing of breast cancer cells, further research should be directed at isolating these markers and performing immunohistochemical and cytogenetic analysis of downstream molecular pathways. Furthermore, randomized-controlled trials should evaluate how modulating these “homing proteins” can decrease relapse into metastatic disease. Clinical trials utilizing Inhibitors of VCAM- α 4 β 1 integrin also serve as future therapeutic possibilities in decreasing breast cancer in the setting of skeletal metastasis. Lastly, unique homing properties of various breast cancer subtypes (i.e., Invasive ductal carcinoma, invasive lobular carcinoma, etc.) should be scrutinized, and their similarities and differences carefully studied.

References

1. Weinberg R (2014) *The Biology of Cancer*. (2nd edn), Garland Science, 2013, pp. 1-960.
2. Awolaran O, Brooks SA, Lavender V (2016) Breast cancer osteomimicry and its role in bone specific metastasis; an integrative, systematic review of preclinical evidence. *The Breast* (30): 156-171.
3. Coleman R (2016) Treatment of Metastatic Bone Disease and the Emerging Role of Radium-223. *Semin Nucl Med* 40(2): 99-104.

4. Elefteriou F (2016) Role of Sympathetic Nerves in The Establishment of Metastatic Breast Cancer Cells in Bone. *J Bone Oncol* 5(3): 132-134.
5. Bussard KM, Gay CV (2008) The bone microenvironment in metastasis; what is special about bone? *Cancer Metastasis Rev* 27(1): 41-55.
6. Jamieson-Gladney WL, Zhang Y, Fong AM, Olimpia M, Fatatis A, et al. (2011) The chemokine receptor CX3CR1 is directly involved in the arrest of breast cancer cells to the skeleton. *Breast Cancer Res* 13(5): R91.
7. Roth C, Rack B, Muller V, Janni W, Pantel K, et al. (2010) Circulating micro RNAs as blood-based markers for patients with primary and metastatic breast cancer. *Breast Cancer Res* 12(6): R90.
8. Chen Q, Massague J (2012) Molecular Pathways: VCAM-1 as a potential therapeutic target in metastasis. *Clinical Cancer Research* 18(20): 5520-5525.



This work is licensed under Creative Commons Attribution 4.0 License
DIO: [10.19080/IJCSMB.2017.01.555575](https://doi.org/10.19080/IJCSMB.2017.01.555575)

**Your next submission with Juniper Publishers
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats
(Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission
<https://juniperpublishers.com/online-submission.php>