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The Molecule Mechanisms of Bone Metastasis in Breast Cancer

Guang-Xian Zhong, Zhao-Yang Wu, Xia Zhu and Jian-Hua Lin*

Department of Orthopaedics, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350004, P.R.China

Abstract

Breast cancer is the second most common cancer diagnosed worldwide, affecting approximately one in eight women during their lifetime. Strategies targeting the primary tumour have markedly improved, but systemic treatments to prevent metastasis are less effective. Breast cancer frequently spreads to the skeleton and causes destructive osteolytic bone metastases. Breast cancers express chemokine receptors, integrins, cadherins, and bone-resorbing and bone-forming factors that contribute to the successful and preferential spread of tumor from breast to bone. Once breast cancer cells arrest in bone, bone is a storehouse of a variety of cytokines and growth factors and thus provides an extremely fertile environment for the cells to grow. This article reviews the specific molecule mechanism of the changes of breast cancer cells, bone marrow microenvironment and the interaction between cancer cell and microenvironment in breast cancer.

Keywords: Breast cancer; Bone metastasis; Molecular mechanisms

Introduction

Breast cancer is now the most frequently diagnosed malignant tumor and the second leading cause of cancer-related deaths among women worldwide [1,2]. More than 90% of breast cancer patients was died from the metastases at distant site rather than the primary tumor. Strategies targeting the primary tumor such as early diagnosis by screening, improved surgical techniques and implementation of adjuvant therapies have markedly improved the survival of primary breast cancer, however, the systemic treatments to prevent metastasis are less effective [3]. Bone is the most common distant site to which the breast cancer metastasizes, and the percentage of bone metastasis reached about 80%-90% [4]. A serious complications caused by the bone metastasis of breast cancer such as bone destruction and associated bone pain, fracture, hypercalcemia, and paralysis due to spinal cord compression were occurred and were incurable. Thus, it is very important to understand the molecular mechanisms of bone metastasis of breast cancer and then find the effective targeted drug therapy.

Although the molecular mechanism of bone metastasis of breast cancer is of the highest importance, it has not yet been fully cleared. More than a century ago, Stephen Paget proposed the "seed and soil" theory, that the tumor cells was the seeds, and the bone microenvironment was the soil, the microenvironment of bone tissue may serve as fertile soil on which the cancer cells may grow [5]. This notion has been widely accepted since then and remains the basic principle in the field of cancer metastasis even to the present time. The "Homing hypothesis" suggested that the bone metastasis of breast cancer was due to the tumor cells specifically to bone settlers and homing, the specific types of cancer cells was acted on different organs by chemokines. Although the two hypotheses mentioned above have been accepted, there were still some other factors which influenced the bone metastasis. The process of bone metastasis of breast cancer was complex and was interrelated with many cytokines, growth factors and molecular signaling pathways.

In this review, we elucidated the specific molecule mechanism of the changes of breast cancer cells, bone marrow microenvironment and the interaction between cancer cell and microenvironment which developed in recent years.

The Migration, Adhesion and Invasion of Breast Cancer Cell

The mechanism of migration

The chemokines is a low molecular weight cytokine secreted by different types of cells. It could selectively attract and activate different leukocyte subpopulations and are key mediators of a variety of patho-physiological states, including hematopoiesis, inflammation, infection, allergy, atherosclerosis, reperfusion injury, as well as malignant tumors [6-8]. Chemokines bind and activate a number of specific or promiscuous, G-protein-coupled seven-transmembrane receptors. Chemokines and their receptors promote cell directional migration by the rearrangement of cytoskeletal protein and the adhesion of endothelial cells, which play important role in the targeted migration process of tumor cells to bone marrow [9].

Recent researches suggested that the interaction between the Stromal cell-derived factor-1(SDF1/CXCL12) and its receptor (CXCR4) played important role in the migration and adhesion of tumor cells to the endothelial cells of bone marrow [10-12].

Compared to the normal breast epithelium cells, the mRNA levels of CXCL12/SDF-1 of primary breast cancer cells and metastatic breast cancer cells were overexpressed. The CXCL12 could promote the pseudopodia formation of MDA-MB-231, and subsequently induced chemotactic and invasive responses. In vivo, neutralizing the interactions of CXCL12/CXCR4 significantly impairs metastasis of breast cancer cells to bone marrow [13]. Zhang et al. [14] also demonstrated the similar effect of CXCR4 by overexpressing and knockdown the CXCR4 gene in breast cancer cell.

***Corresponding author:** Dr. Jian-Hua Lin, Department of Orthopaedics, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350004, PR China, Tel: +86 59187981029; E-mail: jianhual@126.com

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The mechanism of adhesion

Integrins: Integrins are heterodimeric transmembrane glycoproteins which are responsible for a variety of cell-cell and cell-extracellular matrix (ECM) adhesion and cell migration [15]. In cancer metastasis, integrins have been shown to mediate cancer cell attachment to vascular endothelial cells and to matrix proteins such as laminin and fibronectin underlying endothelium, which is an initial step in tumor colonization [16]. The osteoclasts express high levels of integrin av β3 which play important role in the osteolytic bone metastasis mediated by osteoclasts [17]. avβ3 is a receptor for osteopontin, fibronectin, and vitronectin, these ECM proteins are important bone matrix proteins, and avβ3 has been identified as a critical integrin in skeletal metastasis of breast cancer [18]. In breast cancer, it is necessary for tumor cell clolnization to bone by binding avβ3 to osteopontin [19]. The bone metastatic cancer cells have a higher expression of avβ3 than the primary tumor [20], which promoting adherence to the bone matrix by binding osteopontin expressed by bone stromal cells [19]. This overexpression of avβ3 in the tumor cells leads to increased tumor cell adhesion, migration and invasion to bone as well as enhanced osteoclasts recruitment within the bone microenvironment [21].

Cadherins: Epithelial cadherin (E-cadherin) is a 120 kDa cell surface glycoprotein involved in calcium-dependent, epithelial cell specific, cell adhesion. E-cadherin has homophilic properties in cell-cell adhesion and thus may cause homotypic cell aggregation, which is likely to be important in controlling embryogenesis and morphogenesis [22]. Recently, evidence has accumulated that E-cadherin also plays a key role in cancer invasion and metastasis [23]. E-cadherin expression was increased in populations of MCF-7 breast cancer cells with reduced invasiveness, whereas relatively low levels of E-cadherin were detected in the highly invasive human breast cancer cells MDA-MB-231[24,25].

The mechanism of invasion

Matrix metalloproteinases: The matrix metalloproteinases (MMPs) are a family of zinc dependent proteases that play a major role in assisting tumor cells during metastasis by proteolytic degradation of structural components of extracellular matrix [26]. Numerous studies demonstrated that the inhibition of MMPs by synthetic and natural inhibitors resulted in a corresponding inhibition of cell invasion. Conversely, up-regulation of MMPs by inducers, transcriptional enhancement, or transgene constructs usually led to enhanced tumor cell invasion, monitored *in vivo* by histology, or in model systems by appearance of tumor cells in distant sites and *in vitro* by Matrigel or ECM invasion [27].

Bone Sialoprotein (BSP): The BSP is a sialic acid-rich, phosphorylated glycoprotein, which is a major non-collagenous extracellular protein of mineralized tissues such as bone, dentin, cementum, and calcified cartilage [28]. BSP is synthesized by osteoblasts, osteoclasts, osteocytes and chondrocytes [29] and has an apparent molecular weight of 60-80 kDa due to extensive post-translati onal modifications including N- and O-linked glycosylation, serine and threonine phosphorylation, tyrosine sulfation, and sialylation[30]. It contains an Arg-Gly-Asp (RGD) sequence, which is a common recognition site for integrins such as alpha-v-beta3 and alpha-v-beta5 [31]. The interaction of BSP and the integrin alpha-v-beta3, which is up-regulated on activated endothelial cells, promotes cell attachment and plays a fundamental role in allowing BSP to engage with endothelial cells, osteoclasts and tumor cells [29]. BSP was found in primary malignancies such as breast [32], prostate [33] and thyroid cancers [34]. Specifically, the BSP-mediated interaction between tumor cells and bone tissue was suspected to participate in the pathogenesis of bone metastasis [29]. It is assumed that the secretion of BSP from tumor cells provides a selective advantage for their survival via binding to alpha-v-beta3 integrin and factor H, which protects them from complement- mediated lysis [35]. Kovacheva et al. [36] reported that the decrease BSP levels by a combination of the tetracycline-controlled transcription activation system ("Tet-Off system") and RNA interference could resulted in significant anti-proliferative, anti-migratory and anti-clonogenic effects in breast cancer cell MDA-MB-231.

Osteonectin: Osteonectin is a 32-46 kDa glycoprotein which was originally discovered in bone by its ability to bind to type I collagen. It was characterized by three domains including acidic domain (AA 1-52), the follistatin-like domain (AA 53-137) and extracellular Ca^{2+} binding domain (AA 138-286), each domain has its specific function [37]. The report by Campo McKnight et al. [38] suggested that the osteonectin stimulated breast cancer cell motility and enhanced chemoattraction of breast cancer cells toward vitronectin. Jacob et al. [39] reported that in the breast cancer cell line MDA-MB-231, osteonectin could induce an increase in MMP-2 activity, whereas in the non-bone-metastasizing HT-1080 and B16-F10 cells, osteonectin could not induce any change in MMP activity. These results demonstrate that osteonectin is a specific inducer of collagenase activity in those cancer cells that preferentially metastasize to the bone.

Changes in the Bone Marrow Microenvironment

Bone is a unique tissue which consists of two physically and biologically distinctive structures. The outer structure of bone consists of hard mineralized matrix in which cellular and metabolic activities are relatively low. The inner structure of bone consists of bone marrow. The bone marrow is multicellular, which makes it one of the most metabolically active tissues in the body. It includes hematopoietic stem cells, stromal cells, immune cells such as macrophages and lymphocytes, and unidentified cells. All cells in the bone marrow except for the hematopoietic stem cells produce a number of growth factors and cytokines which mediate cell-to-cell interactions in autocrine, paracrine, and/or juxtacrine fashion. The bone microenvironment is comprised of a mineralized extracellular matrix and specific cell types that are under the control of local and systemic factors [40]. Tumor cells in breast cancer produce adhesive molecules that bind them to marrow stromal cells and bone matrix, and then directly or indirectly induce the formation of osteoclasts. In turn, bone resorption by osteoclasts releases growth factors from the bone matrix that stimulate tumor growth and bone destruction [41].

Osteoclasts

Osteoclasts arise from precursor cells in the monocyte-macrophage lineage that differentiate into inactive osteoclasts [42]. Activated osteoclasts are terminally differentiated myeloid cells that resorb bone and eventually undergo apoptosis. The bone microenvironment plays a critical role in the formation of osteoclasts through the production of macrophage colonystimulating factor and receptor activator of nuclear factork B (RANK) ligand (RANKL) by stromal cells or osteoblasts [43,44]. Research had demonstrated that the process of bone destruction itself was responsible for stimulating the growth of breast cancer cells in bone, and may even increase the production of bone-destroying mediators by the breast cancer cells [24]. Osteoclasts resorb bone by secreting proteases that dissolve the matrix and producing acid that releases bone mineral into the extracellular space under the ruffled border of the plasma membrane of osteoclasts, which faces bone and is

the resorbing organelle of the cell [45]. The adherence of osteoclasts to the bone surface is critical for the bone resorptive process, since agents that interfere with osteoclast attachment block bone resorption [46].

Osteoblasts

Osteoblasts are specialized bone-forming cells that express parathyroid hormone (PTH) receptors and have several important roles in bone remodeling: expression of osteoclastogenic factors, production of bone matrix proteins, and bone mineralization [47]. Osteoblastic cells comprise a diverse population of cells that include immature osteoblast lineage cells and differentiating and mature matrix-producing osteoblasts. Osteoblasts eventually become osteocytes. Bone morphometric proteins are critical factors that stimulate the growth and differentiation of osteoblasts many factors can enhance the growth and differentiation of osteoblasts [48], including platelet-derived growth factor, fibroblast growth factor, and transforming growth factor β [49].

Insulin-like growth factors (IGFs)

The insulin-like growth factor (IGF) signaling system plays a critical role in the growth and development of many tissues and regulates overall growth, particularly prenatal growth. The IGF system has also been implicated in various pathophysiological conditions, and is thought to play a particularly prominent role in tumorigenesis. The IGF system is comprised of the IGF ligands (IGF-I and IGF-II), cell surface receptors that mediate the biological effects of the IGFs, including the IGF-I receptor (IGF-IR), the IGF-II receptor (IGF-IIR), and the insulin receptor (IR), as well as a family of IGF-binding proteins (IGFBPs) [50-52]. Amplification of the IGF-IR locus at band 15q26 has been reported in a small number of breast cancer and melanoma cases [53]. During tumorigenesis, overexpression of the IGF-IR is presumed to increase the cellular responsiveness to the IGFs, in terms of proliferation and inhibition of apoptosis. It has been suggested that the IGF-IR itself can function as an oncogene, based upon the phenotype of fibroblasts overexpressing the IGF-IR [54]. However, the relevance of this system to human cancer in general is unclear. Studies of IGF-IR expression in breast and prostate that employed immunohistochemistry or matched cell lines corresponding to normal and tumor tissue revealed that normal epithelium and early-stage tumors both express abundant levels of the IGF-IR, and that IGF-IR expression is significantly reduced in advanced, metastatic cancer [55,56]. Nevertheless, the report by Hellawell et al. [57] challenged this view, reporting that IGF-IR expression was decreased in certain metastatic prostate cancer samples, as compared to benign or carcinoma tissue.

Transforming growth factorβ (TGFβ)

The TGF-β family includes a large number of structurally, and functionally, related proteins. The members of the TGF-β family act as multifunctional regulators of a wide range of biological processes such as morphogenesis, embryonic development, adult stem cell differentiation, immune regulation, wound healing, inflammation and cancer. The first member of the family, TGF-β1, was discovered in 1983 because of its ability to stimulate the growth of cultured rat fibroblasts in soft agar [57]. In patients with metastatic breast cancer, PTHRP expression levels are increased in bone metastases compared with the primary tumor [58]. Yin et al. [59] showed that TGF-β in the bone microenvironment induced tumoural PTHRP production that resulted in enhanced bone resorption. The TGF-β in bone could also modulate many other pro-metastatic and osteolytic factors.

Bone morphogenetic protein (BMP)

The bone morphogenetic proteins (BMPs), members of the TGF-β family, are expressed in a variety of human carcinoma cell lines, their roles in tumor progression have not been fully clarified. Katsuno et al. [60] reported that BMP-2 promoted the motility and invasiveness of the MDA-231-D cells in vitro. Moreover, expression of dominant-negative receptors for BMPs in the MDA-231-D cells inhibited invasiveness in vitro and bone metastasis in the xenograft model. These results suggest that BMPs promote invasion and bone metastasis of breast cancer.

Fibroblast growth factors (FGFs)

FGFs are a family of 23 growth factors that signal through tyrosine kinase receptors (FGFR1-4) and are involved in bone growth and development [61,62]. Mutations in FGF receptors have been associated with many skeletal disorders including achondroplasia (a form of skeletal dwarfism), Apert syndrome, Beare-Stevenson cutis gyrata, Crouzon syndrome, Pfeiffer syndrome, non-syndromic craniosynostosis, osteoglophonic dysplasia, and Muenke syndrome [62]. FGFs are also involved in tumor growth and angiogenesis [63]. Breast cancers express both FGFs and FGFRs [63]. Breast tumors have higher expression of FGFR-1 than normal breast epithelium [63]. Overexpression of FGF-1 and FGF-4 in MCF-7 breast cancer cells increased tumor growth, blood flow rate, and lung metastases in vivo [64]. FGFs may also affect the invasive ability of breast cancer cells by stimulating secretion of matrix metalloproteinases. FGF-2 treatment of MCF-7 cells stimulated MMP-9 secretion in vitro [65].

Parathyroid hormone-related protein (PTHrP)

The calciotropic hormone PTH is an endocrine remodeling signal generated to maintain calcium homeostasis. PTH is secreted by the parathyroid glands in response to reduced serum calcium and acts peripherally on kidneys and bone and indirectly on the intestine to maintain serum calcium homeostasis. In the bone microenvironment, PTH activates a seventransmembrane G-protein-coupled receptor, the PTH receptor on the surface of osteoblastic cells [66]. Binding of PTH to its receptor activates protein kinase A, protein kinase C, and calcium intracellular signaling pathways in these cells [67] and induces a wave of transcriptional responses that produce/modulate secretion of molecules that recruit osteoclast precursors, induce osteoclast differentiation and activation, and establish bone resorption.

Breast cancer cells express calcium-sensing receptors, CaSR [68]. Activation of CaSR by free calcium increases PTHrP expression by breast cancer cells [68]. Therefore, bone-stored calcium may contribute to the increased levels of PTHrP found in breast cancer cells that have metastasized to the bone compared to the primary tumor and other sites of metastases [69-71]. Calcium may therefore be an important component within the bone microenvironment contributing to breast cancer bone metastasis.

Endothelin-1

Endothelin-1 has been implicated in osteoblastic metastasis from breast cancer [72]. It stimulates the formation of bone and the proliferation of osteoblasts in bone organ cultures [73], and serum endothelin-1 levels are increased in patients with osteoblastic metastasis from prostate cancer [74]. Furthermore, in an animal model of osteoblastic metastasis, treatment with a selective endothelin-1A-receptor antagonist decreased both osteoblastic metastasis and the tumor burden [72]. The antagonist had no effect on the growth of the tumor at orthotopic sites. These results suggest that blocking

osteoblast-inducing activity by tumors may decrease tumor growth and osteoblast activity and suggest that a vicious circle may also be involved in osteoblastic metastasis in which tumors induce osteoblast activity and thus the subsequent release from the osteoblasts of growth factors that increase tumor growth.

The Interaction Between Breast Cancer Cells and Bone Marrow Microenvironment

Bone is composed of a hard, mineralized bone matrix that is constantly being remodeled [75]. The osteoclast (bone resorbing cell) and the osteoblast (bone-forming cell) are the two main bone cells [75]. The formation of osteoclasts requires the macrophage-colony stimulating factor (M-CSF) produced by osteoblast and stromal cells [75]. The M-CSF stimulates precursor cells of the macrophage lineage to express the receptor RANK [75]. Then the osteoblasts and stromal cells express receptor activator of NFkappaB ligand (RANKL), which binds to RANK to stimulate osteoclast differentiation, activation, and survival [75]. Osteoclast activation is opposed by the secreted RANKL-binding protein, osteoprotegerin, also produced by osteoblasts [75]. Balanced remodeling of the skeleton occurs due to the coupled actions of the osteoclast and the osteoblast [76]. The osteoclasts resorb bone leaving a pit in which the osteoblasts (bone-forming cells) can form new bone [76].

Tumor cells in breast cancer produce factors that directly or indirectly induce the formation of osteoclasts. In turn, bone resorption by osteoclasts releases growth factors from the bone matrix that stimulate tumor growth and bone destruction [77]. This reciprocal interaction between breast-cancer cells and the bone microenvironment results in a vicious circle that increases both bone destruction and the tumor burden. Cancer cells secreted some bone-resorbing factors including parathyroid hormone-related protein (PTHrP), interleukin-6 (IL-6), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), and interleukin-11 (IL-11) [78]. Of these, PTHrP is the most studied and has been shown to play a critical role in promoting osteolytic metastases [75]. PTHrP acts through a shared receptor with parathyroid hormone (PTH) [79], the PTH/PTHrP receptor. PTHrP indirectly increases osteoclastic bone resorption by stimulating receptor activator of NFkappaB ligand (RANKL) expression on osteoblasts and bone stromal cells [80]. Mouse models of bone metastasis have been showed that the PTHrP plays an important role in breast cancer bone metastasis [81]. The osteolytic lesions produced by MDA-MB-231 cells in mouse models is reduced by inhibiting PTHrP with neutralizing antibodies [81]. In mice with an absence of hypercalcaemia or detectable circulating levels of PTHrP, neutralizing antibodies to PTHrP could block breast tumor-associated bone loss and tumor growth in bone [82]. In patients with metastatic breast cancer, PTHRP expression levels are increased in bone metastases compared with the primary tumour. A neutralizing antibody against PTHrP is currently in clinical trials to treat breast cancer bone metastasis. Interleukins 6 and 11 and VEGF also increase osteoclast formation and activity via the RANK ligand pathway, while IL-8 acts directly and indirectly on osteoclasts [83-86].

Conclusions and Future Prospects

Breast cancer is frequently associated with osteolysis. Almost all patients dying of breast cancer or with advanced breast cancer have bone metastases, and these are almost always destructive in nature. This osteolytic lesion could be contributed to the increased osteoclastic bone resorption. So far, the molecule mechanisms of the interaction between the breast cancer cell and bone marrow microenvironment have been explored by many researchers, and in animal models, some agents have also demonstrated having therapeutic effect for bone metastasis in breast cancer by inhibiting the responsible mechanisms. However, breast cancer bone metastasis is a complex process in which each stage are affecting each other, there is still no specific pharmacologic agent available for preventing or reversing bone metastasis in breast cancer patients. Thus, further efforts should be made on how to build the models of bone metastasis which can be used to incorporate all of those affecting factors mentioned above, and how can all the complex interactions in the bone microenvironment be blocked to treat breast cancer bone metastasis. With the development of animal models, molecular biology and gene chip technology, we believe that the molecule mechanism of bone metastasis will be further understood, and then the new targeting drug will be found to cure the bone metastasis in breast cancer.

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