

Review Article



Application of Biomarkers for the Prediction and Diagnosis of Bone Metastasis in Breast Cancer

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Conflict of Interest

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ABSTRACT

The most common metastatic site of breast cancer is the bone. Metastatic bone disease can alter the integrity of the bone and cause serious complications, thereby greatly reducing health-related quality of life and leading to high medical costs. Although diagnostic methods and treatments for bone metastases (BM) are improving, some patients with early breast cancer who are at high risk of BM are not diagnosed early enough, leading to delayed intervention. Moreover, whole-body scintigraphy cannot easily distinguish BM from non-malignant bone diseases. To circumvent these issues, specific gene and protein biomarkers are being investigated for their potential to predict, diagnose, and evaluate breast cancer prognosis. In this review, we summarized the current biomarkers associated with BM in breast cancer and their role in clinical applications to assist in the diagnosis and treatment of BM in the future.

Keywords: Biomarkers; Bone neoplasms; Breast neoplasms; Prognosis

INTRODUCTION

Breast cancer is one of the most common and most fatal neoplastic diseases affecting women worldwide [1]. In 2017, there were a total of 252,710 newly diagnosed breast cancer cases reported in the United States alone [2]. Although the cure rate for breast cancer has been rising in Western countries, it remains the leading cause of cancer-related death in women [3]. The most common site of metastasis is the bone, which has been shown to occur in 50%–70% of the patients with breast cancer [4]. Due to the increased osteoclast activity and bone destruction, tumor-associated bones can cause skeleton-related events, which have been reported to significantly reduce health-related quality of life and result in high medical costs [5]. Bone metastases (BM) can often result in bone pain, spontaneous fractures, systemic hypercalcemia-induced metabolic consequences, and paraplegia (if the metastatic sites are present in the vertebrae) [6,7]. To date, whole-body scintigraphy is considered the gold standard for determining, quantifying, and describing the location and extent of BM; however, this method lacks specificity though it offers high sensitivity [8]. Furthermore, the rapid diagnosis of BM is impaired by the similarities between BM and non-malignant bone diseases in terms of imaging results. In addition, with the passage of time and combined with the action of anti-reabsorption drugs, the radiological appearance of BM may spontaneously

change, making follow-up more difficult [9]. Therefore, a highly sensitive and specific method that could also monitor the effect of bisphosphonate treatment on bone metabolism is needed for the clinical diagnosis of BM.

Currently, cancer biomarkers encompass key parameters in all aspects of cancer staging and recurrence. In particular, the analysis of biomarkers related to BM could markedly advance—and provide a reference value—for the diagnosis of BM [10,11]. These biomarkers have been extensively studied in breast cancer-associated BM. In this review, we summarize disease-specific biomarkers that could be used to predict and diagnose the occurrence and development of BM, or to evaluate their antitumor efficacy in breast cancer BM (Table 1).

BONE METASTASIS-ASSOCIATED BIOMARKERS IN BREAST CANCER

Proteins

Hormone receptors

Growing evidence suggests that different subtypes of breast cancer manifest unique patterns of metastasis and clinical outcomes [12-14]. Depending on the hormone receptor (HR) and human epidermal growth factor 2 (HER2) status, the metastasis pattern and prognosis may also differ, with the bone tissue being reported as the optimal metastasis site for HR-positive tumors [15,16]. For instance, compared with estrogen receptor (ER)-negative tumors,

Table 1. Biomarkers associated with BM in breast cancer

Biomarkers	Prediction	Diagnosis	Prognostic	Predicting treatment efficacy	References
Proteins					
HR	√				[17,18]
NTX		√	√	√	[21-23,25]
CTX	√	√			[8,24,71]
CAPG and GIPCI	√		√	√	[28]
OPG		√			[33]
RANKL		√			[33,34]
BAP		√	√	√	[8,22,23,25,38]
TRACP-5b		√	√	√	[23,38,40,41]
ICTP		√		√	[38,71]
DOCK4	√				[44,45]
FGF23			√		[48]
PTHrP(12-48)		√			[51]
Genes					
IL-1B	√				[52,72,73]
MAF	√				[54]
15-gene signature		√			[58]
Kang's signature		√			[55,58]
ZNF217	√				[61]
miRNAs					
miR-214-3p		√			[67]
miR-218	√				[69]
miR-126		√	√		[70]
miR-206			√		[70]
miR-335		√	√		[70]

BM = bone metastases; HR = hormone receptor; NTX = N-terminal cross-linked telopeptide of type I collagen; CTX = C-terminal cross-linked telopeptide of type I collagen; CAPG = macrophage-capping protein; GIPCI = PDZ domain-containing protein GIPCI; OPG = osteoprotegerin; RANKL = receptor activator of nuclear factor κB ligand; BAP = bone alkaline phosphatase; TRACP-5b = tartrate-resistant acid phosphatase type 5b; ICTP = cross-linked carboxy-terminal telopeptide of type I collagen; DOCK4 = dedicator of cytokinesis 4; FGF23 = fibroblast growth factor 23; PTHrP = parathyroid hormone-related protein; IL-1B = interleukin-1B; MAF = musculo-aponeurotic fibrosarcoma oncogene homolog; ZNF217 = zinc finger protein 217; miRNA = microRNA.

ER-positive tumors relapse later; however, they are associated with a higher rate of bone recurrence [17]. Moreover, a retrospective analysis of 263 patients with primary invasive metastatic breast cancer, which evaluated the expression of ER, progesterone receptor, epidermal growth factor receptor, HER2, Ki-67, as well as other markers revealed that patients with ER+/HER2-/Ki-67^{hi} had a higher probability of BM [17]. A related study reported similar results in 490 patients with breast cancer, with BM occurring in up to 72% of the HR-positive patients [18].

N-terminal cross-linked telopeptide of type I collagen and C-terminal cross-linked telopeptide of type I collagen

The N-terminal cross-linked telopeptide of type I collagen (NTX) and C-terminal cross-linked telopeptide of type I collagen (CTX) become cross-linked and are released during bone resorption [19]. Antibodies against the alpha-2 chain are used to detect NTX, which are readily detected in serum or urine; however, NTX urine results must be adjusted for dilution, which might increase the measured variability. In contrast, urinary measurements of CTX are reportedly less accurate at concentrations below 200 µg/L, therefore, serum or plasma samples are often used [20].

Several studies have suggested that NTX and CTX might serve as sensitive indicators of breast cancer-induced BM. For instance, the levels of NTX in a small cohort of 19 patients with BM were significantly higher than those in patients with breast cancer without BM (n = 65), or with other bone pathologies other than metastases (n = 22) [21]. Furthermore, Coleman et al. [22] found that in bisphosphonate-treated patients with BM (n = 1,824), the risk of skeletal complications and disease progression in patients with high and moderate levels of NTX was 2-fold higher than that in patients with low levels of NTX. In addition, NTX could be used as an indicator for monitoring the treatment response of breast cancer patients with BM. Chung et al. [23] found that in patients with breast cancer BM who responded to antitumor therapy, the level of NTX decreased significantly after treatment, whereas it increased in non-responders. Additionally, CTX was found to predict—and enable the diagnosis of—breast cancer BM. Zulauf et al. [8] tested the serum CTX level in breast cancer patients without (n = 28) and with BM (n = 50), and found that the level of CTX in patients with BM was significantly higher than that in patients without BM. They postulated that CTX levels could be used as a tool to detect and exclude BM [8]. Similarly, in a large phase III clinical trial (AZURE trial), Brown et al. [24] measured the levels of CTX in 872 patients with early-stage breast cancer and found that high CTX levels were prognostic for BM recurrence. Lastly, although CTX and NTX levels were not found to be predictive for the response to zoledronate in adjuvant therapy, they are expected to be indicators of drug efficacy for BM treatment [24,25].

Macrophage-capping protein and PDZ domain-containing protein GIPC1

Macrophage-capping protein (CAPG) is a calcium-sensitive, actin-bound protein that contributes to the regulation of cytoplasmic and nuclear structure; it has been reported to modulate cell migration and invasion [26]. The PDZ domain-containing protein, GIPC1 is localized in the cytoplasm and peripheral membrane, where it acts as an adaptor protein that connects receptor interactions to intracellular signaling pathways, including cell cycle regulation, because of which, its expression has been associated with certain cancers [27]. The expression of these 2 biomarkers was clinically validated by immunohistochemistry in tumor tissues from patients enrolled in the AZURE trial. Patients with high expression of both proteins (CAPG^{hi}/GIPC1^{hi}) not only had a higher risk of BM, but also benefitted more from adjuvant zoledronate treatment against the first BM relative to the control group [28].

These results highlighted the importance of the CAPG/GIPC1 biomarkers in the prediction of BM as well as in the selection of patients for adjunctive bisphosphonate therapy [29].

Osteoprotegerin and receptor activator of nuclear factor κ B ligand

The protein receptor activator of nuclear factor κ B (RANK) and its ligand (RANKL) play a key role in the development and maintenance of osteoclastic activity [30]. Osteoprotegerin (OPG) is a decoy of RANKL and serves as a natural osteoclast activity regulator by inhibiting the binding of RANK to RANKL [31]. However, BM results in an imbalance in these processes by interfering with the RANK/RANKL/OPG pathways through the downregulation of OPG or upregulation of RANKL [32]. Accordingly, RANKL and OPG levels, and the RANKL/OPG ratio have been reported to exhibit high specificity and sensitivity in the diagnosis of breast cancer with BM. Upon comparing the serum OPG and RANKL levels in patients with breast cancer with and without BM, Elfar et al. [33] found that the serum OPG levels in breast cancer patients with BM were significantly reduced, whereas RANKL levels were significantly increased. In addition, the RANKL/OPG ratio was significantly increased in BM patients, with an appropriate sensitivity (73%) and specificity (72%) [33]. Furthermore, Shaker and Helmy [34] found that RANKL exhibited a better diagnostic ability to detect BM, and that the combination of RANKL with HER2 resulted in a higher discriminatory detection of BM (compared to RANKL alone). Thus, both serum OPG and RANKL are promising biomarkers for the diagnosis of BM. RANKL also serves as a target in the treatment of BM. Specifically, denosumab, the first complete human immunoglobulin G2 monoclonal antibody against RANKL, is currently being used in the clinical treatment of bone-related cancer pathologies [35]. However, denosumab had no effect on survival in patients with BM, suggesting that inhibition of osteoclast resorption alone may not be sufficient to inhibit tumor proliferation [36].

Bone alkaline phosphatase

Bone alkaline phosphatase (BAP) is a tetramer protein located in the plasma membrane of osteogenic cells. It enters the bloodstream in the form of a dimeric protein through the activity of phosphatidylinositol glycanase or via membrane vesicles. As a specific marker of osteoblasts, the serum BAP level has been shown to reflect the metabolic status of osteoblasts [37]. Two related studies confirmed that the serum levels of BAP in patients with BM from breast cancer were significantly higher than those in breast cancer patients without BM [8,23]. In addition, changes in the serum BAP levels could reflect the efficacy of bisphosphonate treatment for BM [38].

Tartrate-resistant acid phosphatase type 5b

Tartrate-resistant acid phosphatase type 5b (TRACP-5b) is secreted primarily by activated osteoclasts [39]. Lumachi et al. [40] found that TRACP-5b was the most accurate marker for the diagnosis of BM in postmenopausal patients with luminal-type invasive ductal carcinoma, and that it was more efficacious when combined with BAP and the amino-terminal propeptide of type I collagen. Similarly, Chung et al. [23] also found that the activity of serum TRACP-5b was higher in patients with BM, and that TRACP-5b was superior to NTX with respect to antitumor treatment response. Like BAP, the changes in the activity of serum TRACP-5b were associated with the treatment efficacy of bisphosphonates in the context of BM [38]. Furthermore, patients with BM—exhibiting a higher activity of TRACP-5b—had a significantly shorter overall survival (OS) time [41].

Cross-linked carboxy-terminal telopeptide of type I collagen

Cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) is generated by matrix

metalloproteinases to form mature bone matrix in the process of bone dissolution [42]. Changes in serum ICTP levels can reflect the degradation rate of type I collagen and the degree of pathological damage to the bone matrix [42]. Indeed, the serum ICTP levels were significantly higher in patients with BM than those in patients without bone involvement, and levels of this marker were significantly decreased after successful treatment [38,43].

Other proteins

Deducator of cytokinesis 4 (DOCK4) is a potential predictive biomarker for BM in breast cancer. Westbrook et al. [44] detected the expression of DOCK4 in 345 breast tumor specimens and found that it was closely associated with the histological tumor type and tumor aggressiveness. Later in the AZURE study, they examined the expression of DOCK4 in tissues from 689 patients with breast cancer and found that higher levels of DOCK4 were associated with an increased risk of manifesting the first event of skeletal recurrence, with the marker being effective in predicting the first distant recurrence involving only the bone. In addition, DOCK4 might serve as a predictive biomarker for the prevention of bone metastasis by zoledronate [44,45].

Fibroblast growth factor 23 (FGF23) is predominantly expressed in osteocytes, the most abundant cells in the bone [46]. Briefly, FGF23 binds to the FGF receptor—a protein expressed on the surface of target cells—that has also been reported to play a role in tumor development and progression [47]. A study found that BM patients who exhibited a lower serum baseline FGF23 level before receiving bone-targeted agents had longer OS than those with higher baseline FGF23 levels [48].

Parathyroid hormone-related protein (PTHrP) is produced by many tumors, including breast tumors [49]. In metastatic breast cancers, tumor-derived PTHrP is one of the well-studied mediators of osteolysis [50]. A study on the plasma biomarkers for breast cancer with BM demonstrated that the levels of a unique 12–48 amino acid peptide fragment of PTHrP—designated as PTHrP(12-48)—were significantly increased in the plasma of patients with BM compared to those in patients without BM. They also found that a combination of plasma PTHrP(12-48) and serum NTX improved the accuracy and specificity of BM diagnosis [51].

In conclusion, we can clearly note that in patients with breast cancer, NTX, BAP, and TRACP-5b serve as protein biomarkers for BM. Importantly, these proteins are not only relevant for the diagnosis or prognosis prediction of patients with BM, but also in the evaluation and application of bisphosphonates in the treatment of BM. Moreover, these proteins, such as NTX, BAP, TRACP-5b, and ICTP, have demonstrated correlations with various therapeutic effects. Additionally, CAPG, GIPC1, CTX, and ICTP were reportedly correlated with breast cancer BM. All of these protein biomarkers are detectable in the serum or plasma of the patients, samples that are readily accessible via minimally invasive procedures.

Genes

Interleukin-1B

Both interleukin (IL)-1A and IL-1B might play a role in tumor formation and metastasis, with IL-1B being found to be associated with poor prognosis in breast cancer. Notably, IL-1B is a pro-inflammatory cytokine, the expression of which has been identified as a potential biomarker within primary tumors for predicting an increased risk of BM in patients with breast cancer [52]. The breast cancer cell line (MDA-IV), which has an increased capacity for metastasis in bone, exhibits a higher expression of IL-1B. Furthermore, Nutter et al.

[52] reported on the analysis of 150 patients with breast cancer whose primary breast tumors express IL-1B, and showed that IL-1B expression was significantly correlated with the occurrence of BM. Similarly, when IL-1 receptor signaling was blocked by the receptor antagonist Anakinra, the number of mice with BM derived from breast cancer was reduced compared with a placebo (40% vs. 90%) [53].

Musculo-aponeurotic fibrosarcoma oncogene homolog

The 16q23 locus, encodes the v-maf avian musculo-aponeurotic fibrosarcoma oncogene homolog (MAF) transcription factor. It regulates the expression of several genes related to breast cancer BM, and is present in osteotropic breast cancer cell lines as well as in paraffin-embedded primary breast cancer tissues. Pavlovic et al. [54] detected gain of 16q23 in paraffin-embedded breast cancer tissues by FISH and found that 16q23 gain-positive tumors were associated with a higher cumulative incidence rate of BM at any time. They then verified that patients with high expression of MAF messenger RNA (mRNA) or protein had a greater incidence of BM [54].

Gene signatures

Experimental models of metastasis have elucidated sets of genes mediating site-specific metastasis of breast cancer [55-57]. The ability to form aggressive BM has been closely related to the distinctive expression profile of a defined set of genes. By comparing the transcriptional profile of parental MDA-MB-231 cells and 12 derivative subpopulations with different metastatic potentials, Kang et al. [55] found that the expression of certain genes differed significantly between cells with different capacities for BM, and that these genes (e.g., IL-11, FGF, and C-X-C chemokine receptor type 4) interacted to induce osteolytic metastasis. Another study analyzed gene expression profiling data from 157 primary tumors from patients with breast cancer with metastatic evolution and found that a novel 15-gene signature was associated with the development of BM in patients with breast cancer. This signature was found to be associated with the development of BM in both ER-positive and ER-negative tumor groups [58].

Zinc finger protein 217

Zinc finger protein 217 (ZNF217) is an oncoprotein that coordinates complex intracellular processes that control early and late stages of tumor progression, and is a major marker involved in cancer development [59]. High expression of ZNF217 mRNA in primary breast cancer is associated with poor prognosis and metastasis [60]. The mRNA levels of ZNF217 were detected in 113 human primary breast cancer tissues and it was shown that high ZNF217 mRNA expression correlated with bone metastasis. Moreover, overexpression of ZNF217 in breast cancer cells has been found to stimulate the formation of osteoclasts, thereby promoting the development of osteolytic lesions [61].

The genetic markers of breast cancer BM play a key role in predicting future BM events in patients with early-stage breast cancer. Specifically, when certain genes associated with BM are identified to be highly expressed in primary breast cancer tissues, the patient must be monitored closely for the early detection of BM, thereby allowing the early implementation of therapeutic interventions.

MicroRNAs

MicroRNAs (miRNAs) are noncoding RNAs—approximately 22 nucleotides long—that are involved in post-transcriptional regulation of gene expression, thereby coordinating a wide

range of biological processes [62,63]. In addition, miRNAs play an essential role in normal bone development and bone metastasis [64-66]. Liu et al. [67] found that miRNAs were significantly upregulated in bone specimens from breast cancer patients with osteolytic BM; meanwhile, genetic ablation of miR-214-3p in nude mice prevented the development of osteolytic BM. Furthermore, during the development of osteolytic BM, the presence of metastatic breast cancer cells can stimulate the expression of miR-214-3p in osteoclast precursor cells and promote the formation of osteoclasts. At the same time, miR-214-3p can target p53 to increase the invasiveness of breast cancer cells [68]. Specifically, Hassan et al. [69] found that miR-218, which is involved in osteoblastic differentiation, could enhance the abnormal expression of osteogenic genes, resulting in the homing and growth of cells that have metastasized to the bone. Furthermore, miR-126, miR-206, and miR-335 were downregulated in bone-homing cells and significantly inhibited the development of BM in mice once re-expressed through reverse transcriptional transduction [70].

CONCLUSION

The bone is the most common site of metastasis in breast, prostate, and lung cancers, with early-stage lesions being more difficult to detect owing to their small size as well as due to the limitations in diagnostic techniques. Diagnosis is made only in the presence of symptoms associated with BM or in the presence of an enlarged lesion; in such cases, the optimal treatment window might be missed, thus affecting patient prognosis.

Therefore, BM-related biomarkers in the serum or tumor tissues should be detected to allow for advanced prediction, early diagnosis, and prognosis of BM caused by breast cancer, thereby facilitating the early identification of high-risk patients with BM. In addition, these minimally invasive tests do not cause damage to the body of the patient the way whole-body scintigraphy does. However, the actual role of these biomarkers in the early diagnosis of BM remains unclear due to the lack of standardized or homogeneous patient studies. Furthermore, strategies for the employment of these biomarkers—or combinations—aimed at improving the accuracy of BM diagnosis remain to be developed.

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