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INVITED REVIEW

Circulating biomarkers for diagnosis and therapeutic monitoring in bone metastasis

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Abstract

Bone is a frequent site of metastasis for multiple types of solid tumors in organs such as prostate, breast, lung, etc., accounting for significant morbidities and mortalities of afflicted patients. One of the major problems of bone metastasis is lack of biomarkers for early diagnosis and for monitoring therapeutic responses. Medical imaging modalities such as computerized tomography, magnetic resonance imaging, and radioactive isotope-based bone scans are currently standard clinical practices, yet these imaging techniques are limited to detect early lesions or to accurately monitor the metastatic disease progression during standard and/or experimental therapies. Accordingly, development of novel blood biomarkers rationalizes extensive basic research and clinical development. This review article covers the up-to-date information on protein- and cell-based biomarkers of bone metastasis that are currently used in the clinical practices and also are under development.

Keywords Bone metastases · Circulating biomarkers · Bone turnover markers · Bone microenvironment · Bone metastatic cancers

Introduction

Bone metastasis is a multi-step process involving detachment from the primary tumor, intravasation, survival in the bloodstream, extravasation in the bone microenvironment, dormancy, and subsequent outgrowth and colonization in the unique microenvironment comprising hard tissue (i.e., calcified matrices) and soft tissue (i.e., bone marrow). Nearly all types of solid cancers metastasize to bone, but several types of cancer, most notably breast and prostate cancers, preferentially develop bone metastasis. From the clinical aspect, bone metastasis causes specific morbidities known as skeletal-related events (SREs) including pathologic bone fracture, spinal cord compression, hypercalcemia, and bone pain [1, 2]. Accordingly, bone metastasis remains a major cause of mortality of afflicted patients.

The matrix of bone tissue is densely calcified yet the internal hollow is filled with well-vascularized soft tissue (bone marrow) [3]. Disseminating metastatic cancer cells first locate adjacently to the endosteal surface, and interact with various types of bone and marrow cells to form micro-metastatic colonies termed as metastatic niche [4, 5]. Meanwhile, osteoblasts first become activated and produce diverse cytokines and growth factors essential to activate osteolysis and proliferation of cancer cells [6]. However, these micro-metastatic lesions are undetectable using current imaging technologies.

Current standard diagnostic approach for bone metastasis use imaging modalities, including whole-body bone scintigraphy (WBS) and magnetic resonance imaging (MRI). Since WBS is highly sensitive for detecting both osteolytic and osteoblastic lesions [7, 8], WBS is commonly utilized

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to screen patients for bone metastasis. On the other hand, flare phenomenon, an inadvertently increased uptake despite favorable treatment responses, often results in misdiagnoses and misguided changes in treatment plans [9]. Whole-body MRI is the most sensitive method for detection of cellular changes in bone, but MRI is unsuitable for routine follow-up examinations or screening large cohorts. Given these limitations of WBS and MRI, there is a substantial clinical demand for practically feasible and sensitive diagnostic modalities to sensitively detect micro-metastatic bone lesions.

Over the past decades, many studies have been conducted to develop alternatives to invasive biopsy for diagnosing cancer and monitoring treatment response. Classically, measurement of serum or urine protein biomarkers has been used, and more recently, liquid biopsy techniques have received much attention. Liquid biopsies involve isolating tumor-derived entities such as circulating tumor cells, circulating tumor DNA, and tumor extracellular vesicles, that are present in the body fluids of cancer patients [10]. Regarding bone metastasis, especially due to the characteristics of hard tissue, collecting and processing the biopsy specimen are difficult in cancer clinics, and thus there remains a strong clinical unmet need for non-invasive diagnostic tests. Here, we will review the protein- and cell-based biomarkers as diagnostics for bone metastasis.

Protein-based biomarkers of bone metastasis

Bone turnover markers (BTMs)

Bone metastasis alters normal bone homeostasis, creating a pro-tumorigenic bone marrow microenvironment, i.e., so-called vicious cycle of bone metastasis. At this time, activated osteoclasts and osteoblasts propel bone turnover. Therefore, numerous investigators first focused on BTMs as potential biomarkers of bone metastasis in the past few decades. BTMs can be broadly categorized into bone resorption and bone formation markers. Bone resorption markers include by-products of type-I collagen (the major type of collagen in bone matrix) degradation or osteoclastic enzymes/cytokines, whereas bone formation markers include mainly by-products of osteogenesis or osteoblastic enzymes released by active osteoblasts (Table 1).

Table 1 Circulating protein-based biomarkers of bone metastasis

Circulating protein biomarker	Body fluid	Type of cancer	References
Bone turnover markers (BTMs)			
Bone resorption-related			
N-telopeptide of type-I collagen (NTX)	Urine, serum	BC, PC, LC, solid tumors	[12–17]
C-telopeptide of type-I collagen (CTX)	Urine, serum	BC, PC, LC	[17–20, 28]
Cross-linked carboxy-terminal telopeptide of type-I collagen (ICTP)	Serum	BC, PC, LC	[18, 20–22]
Tartrate-resistant acid phosphatase (TRACP)	Serum	BC, PC	[17, 22–25]
Receptor activator of nuclear factor κB-ligand/osteoprotegerin (RANKL/OPG)	Serum	BC, PC	[17, 26]
Bone formation-related			
Pro-collagen-type I N-terminal propeptide (P1NP)	Serum	BC, PC, ovarian cancer	[17, 18, 21–23, 27]
Pro-collagen-type I C-terminal propeptide (P1CP)	Serum	BC, PC	[16, 28]
Alkaline phosphatase (ALP)	Serum	PC, solid tumors	[14–16, 23, 29–31]
Osteocalcin (OCN)	Serum	PC, LC	[32, 34, 35]
Protein biomarkers			
Sclerostin (SOST)	Serum	BC, PC	[37, 38]
Dickopf-1 (DKK1)	Serum	BC, NSCLC	[39–41]
Osteopontin (OPN)	Serum	PC,RC	[42, 43]
Parathyroid hormone-related protein (PTHrP)	Serum	BC, LC	[44, 45]
Prostate-specific antigen (PSA) Serum level with a Gleason score (PSA ≥ 20 ng/mL, a Gleason score ≥ 8)	Serum	PC	[46, 47]
Prolactin (PRL)	Serum	BC	[48]
CCL20	Serum	BC, RC	[49, 50]
Stress-induced phosphoprotein-1 (STIP1)	Not tested	RC	[51]
Peroxiredoxin-4 (PRDX4) and L-plastin (LPC1)	Not tested	BC, PC, RC (TCGA data)	[52]

BC breast cancer; *PC* prostate cancer; *LC* lung cancer; *RC* renal cancer; *NSCLC* non-small cell lung cancer

Among bone resorption-related BTMs, urinary N-terminal cross-linked telopeptide of type-I collagen (NTX) is the most reported indicator for the risk of SRE and death in patients with bone metastasis and for monitoring response to anti-resorptive bisphosphonate [11–17]. Similar to NTX, C-telopeptide of type-I collagen (CTX), cross-linked carboxy-terminal telopeptide of type-I collagen (ICTP), and pyridinoline/deoxypyridinoline (PYD/DPD) bridging collagen molecules have showed positive correlations with bone metastasis [16–22]. Multiple reports have reported that cancer patients with elevated levels of these BTMs in serum or urine are at a high risk for bone-specific recurrence, but not for other (soft tissue) metastases [18]. In addition to collagen degradation by-products, tartrate-resistant acid phosphatase (TRAP), an enzyme of osteoclasts, is highly detected in the serum of bone metastasis patients, and the receptor activator of nuclear factor κB ligand and osteoprotegerin ratio (RANKL/OPG), an index of osteoclastogenesis-related cytokines used to measure degree of bone resorption, were also significantly detected in the serum of breast cancer patients with bone metastasis, showing clinically applicable sensitivity and specificity [17, 22–26].

Regarding bone formation-related BTMs, there are many reports that peptides cleaved from pro-collagen such as pro-collagen type 1 N- and C- terminal propeptide (P1NP and P1CP) can be used as biomarkers for bone metastasis in breast and prostate cancer [16–18, 21–23, 27, 28]. P1NP is considered an useful diagnostic and prognostic factor for bone metastasis, supported by correlation of high serum P1NP levels with shorter time to the development of bone metastases and lower overall survival in patients with stage I–III breast cancer [27]. Alkaline phosphatase (ALP), an enzyme and differentiation marker of osteoblasts, is a representative BTM that has been shown to associate with bone metastasis in prostate cancer and solid tumors [14–16, 23, 29–31]. Although serum levels of osteocalcin (OCN), another marker of bone formation, have been suggested as a marker for bone metastasis, the fact that OCN reflects the response to treatment through its hormonal effects and inconsistent results in the non-small cell lung cancer (NSCLC) patients with bone metastasis raise questions about accuracy [32–35]. Rather than serum OCN levels, OCN-positive circulating osteoblastic cells as a cell-based biomarker showed a potential to be used as a biomarker for bone metastasis and will be discussed later in this article [36].

Although BTMs are robust markers for bone metastasis, BTMs are not cancer type specific but are a general marker of bone metabolism, and the usefulness of BTMs is limited by cancer patients' characteristics that can affect levels of BTMs, such as age, sex, underlying kidney and/or liver diseases, and hormonal therapy [11]. Indeed, most of the studies compared cancer patients with or without bone metastasis with healthy subjects, not with the benign metabolic bone disease or fracture patients who can have comparable changes of BTMs. Further study is needed to validate it. Furthermore, due to its wide range of sensitivity and specificity values, the use of BTM as a sole marker cannot yet allow the replacement of imaging techniques (sensitivity and specificity for each biomarker are summarized in Supplementary Table 1). However, with the advantage of liquid biopsy diagnosis that multiple biomarkers can be measured at once, it may be possible to overcome sensitivity and specificity problems with a combination of biomarkers and to make a personalized diagnosis.

Additional protein-based biomarkers

Several studies have indicated that Wnt signaling is closely related to bone metastasis. Therefore, proteins secreted by cancer cells that alter the activity of Wnt signaling are involved in bone metastasis. Sclerostin (SOST), a Wnt inhibitor, is highly secreted by metastatic breast and prostate cancer and promotes osteolysis by inhibiting osteoblast differentiation [37, 38]. A Wnt-antagonist dickkopf-1 (DKK1) is increased in bone metastatic breast cancer and prompts osteoblast apoptosis [39, 40]. Serum levels of DKK1 have also been identified elevated in NSCLC patients with bone metastasis [41].

High plasma osteopontin (OPN) level was associated with distant metastases and low survival rates in renal cell carcinoma (RCC) patients, and OPN alone or in combination with BTMs showed significant differences secondary to presence or absence of bone metastases or survival rates [42, 43]. In a retrospective study of serum levels of parathyroid hormone-related peptide (PTHrP) in hypercalcemic lung cancer patients, high PTHrP was found to associate with increased bone metastasis incidence and decreased median survival [44]. Furthermore, in breast cancer, PTHrP (amino acids 12–48) levels were significantly increased in the plasma of patients with bone metastasis than in patients without bone metastasis, and the clinical measurement of PTHrP (12–48) in combination with NTX improved the detection of bone metastasis [45]. Prostate-specific antigen (PSA) as a bone metastasis marker of prostate cancer is controversial. However, there is an opinion that bone scans should be considered because patients with high PSA ($\geq$20 ng/mL), locally advanced disease, or a Gleason score of 8 or higher have a higher risk of bone metastasis [46]. Although PSA alone cannot be used as a marker for bone metastasis in prostate cancer, PSA may be useful in combination with other markers [47].

Additional circulating effectors of bone metastasis have been investigated, including prolactin. High expression of the prolactin receptor (PRLR) in primary breast cancer cells was found to correlate with a shorter time to relapse,

including relapse in bone, which results from increased osteoclast differentiation followed by osteolysis and secretion of the growth factors [48]. Serum CCL20 levels were significantly increased in renal cancer patients with bone metastasis [49]. Lee et al. demonstrated that metastasis-free survival and overall survival were decreased in breast cancer patients with high CCL20 expression, and administration of anti-CCL20 antibodies inhibited osteolytic breast cancer bone metastasis in mice [50]. Stress-induced phosphoprotein-1 (STIP1) was highly expressed both intracellularly and extracellularly in bone metastatic tissue samples from RCC patients and was upregulated in bone-seeking cells. STIP1 promoted the proliferation and migration/invasion of RCC tumor cells, while secreted STIP1 increased the differentiation and activation of osteoclasts along with increased cathepsin K production [51]. In addition, Tiedemann et al. showed that L-plastin is released through exosomes in human breast cancer cell lines, and suggested that L-plastin and peroxiredoxin-4 (PRDX4) secreted from breast cancer cells, as mediators of osteoclastogenesis, can contribute to bone colonization in a number of osteotropic cancers such as breast cancer, prostate cancer, and kidney cancer [52]. The above-mentioned circulating protein-based biomarkers are summarized in Table 1.

Here, we have addressed circulating protein markers identified in cancer patients and several potential targets in ongoing studies. Although many basic studies suggest new candidates for bone metastasis cancer markers, intensive follow-up studies are needed to determine whether they are clinically meaningful.

Cell-based biomarkers of bone metastasis

Circulating tumor cells (CTCs)

It has long been known that CTCs are detectable in cancer patients' bloodstream. Numerous studies have reported that CTCs are closely associated with prognosis, tumor growth, molecular subtypes, and aggressiveness. Regarding bone metastasis, multiple groups reported clinical correlation between bone metastasis and CTCs in prostate, breast, and lung cancers [53–57]. These studies used quantity (i.e., cell numbers) and specific molecular markers, such as TFF-1, RANK, and CXCR4, for the diagnostic value of CTCs. Notably, Zhu et al. collected bilateral bone marrow and blood samples from breast cancer patients without clinical bone metastasis before surgery and identified that all patients with CTC had bone micro-metastases. In contrast, there was no significant correlation between CTC and sentinel lymph node metastasis. The authors concluded that CTC serves as a predictive marker for bone micro-metastasis in breast cancer patients [58, 59]. More recently, Trapp et al. performed a prospective clinical trial (SUCCESS A trial, NCT02181101), and measured CTCs in metastatic breast cancer patients before and after chemotherapy. The authors found that patients with CTCs at both time points showed bone-only first metastatic lesions as well as multiple-site first metastatic lesions more frequently than patients without detectable CTCs [60]. The authors concluded that CTCs might serve as a liquid biopsy surveillance marker for risk stratification for further adjuvant add-on treatments. A more extensive review of CTCs was published by Iuliani et al. [61]

In contrast to significant amount of data and publications supporting the role of CTCs in cancer progression in the past two decades, as well as a few biotech companies with advanced detection technologies and U.S. Food and Drug Administration (FDA) approvals, CTCs have not yet entered oncology clinics as a standard technology. The most significant issue is that not all CTCs are the same in the disease progression. It is unclear whether CTCs enter the systemic circulation via an active biological mechanism or via shedding from the primary tumor mass or both. Extensive further investigation is required to characterize distinct molecular characteristics using multi-omics approaches [62]. One of the major hurdles for this approach is the expansion of isolated CTCs in vitro for further analysis. Microfluidic chips and biological capture technologies have been developed and are currently under optimization for CTCs [63–65].

Osteoblast-lineage cells in the circulation

In 2005, the Khosla group at Mayo Clinic first reported that osteoblast-lineage cells exist in the human blood circulation [66]. Subsequently, these circulating osteoblast-lineage cells (cOB) were further characterized to contain two distinct populations (i.e., CD34 positive and negative populations). $CD34^+$ cOB have low granularity and a small cell phenotype, whereas $CD34^-$ cOB have a larger and more granular phenotype [67]. Numerous subsequent reports from the same group and others have been published afterward, but the biological function and clinical significance of cOB have yet to be more extensively investigated. In the original discovery paper, the Khosla group demonstrated that adolescents in the active bone growth period have higher levels of cOB compared with adults. cOB also positively correlate with the pathologic changes of bone turnover in fracture, hypoparathyroidism, hereditary heterotopic ossification, or diabetes [68–72], supporting that cOB reflect changes of bone turnover and/or bone micro-architecture in either physiologic or pathologic status.

We recently performed a clinical study and demonstrated that the level of cOB, defined by $CD15^-CD34^-Ocn^+$ cells in the peripheral blood mononuclear cells (PBMCs), predicted bone metastasis progression significantly earlier than the standard image-based diagnostics (computed tomography

or bone scans). Briefly, we measured the level of cOB by flow cytometry in 92 Korean breast cancer patients and followed them up for disease progression for 18 months. The patients who had higher levels of cOB (the cutoff value was 0.069% of total $CD45^+$ PBMCs) among those who did not have clinical metastatic lesions developed de novo bone metastasis within 18 months. In addition, among those who had bone metastasis at enrollment, bone metastases in patients who had higher levels of cOB (the cutoff value was 0.045% of $CD45^+$ cells) progressed during the 18-month follow-up by bone scan images. Additional in vivo murine pre-clinical studies confirmed that cOB increased at early time points when bone micro-metastases were evident only by histology but undetectable by bioluminescence imaging. In addition, we found that cOB increased in the early phase of bone metastasis and could predict the progression of bone metastatic lesions. Taken together, the study demonstrated the clinical utility of cOB in diagnosing and monitoring bone metastasis progression in breast cancer patients [36, 73]. More recently, the Faccio group at the University of Washington in St. Louis demonstrated, using murine tumor models, that osterix (*Osx* or *Sp7*)-positive cells from the bone marrow are the origin of cancer-associated fibroblasts (CAF) in primary breast cancer tissue (the American Society for Bone and Mineral Research Annual Meeting 2022 Abstract No. 1030), and these Osx + osteoblast-lineage cells are detectable in blood samples (~ 1.5% of $CD45^+$ cells). However, the authors did not confirm the detectability of Osx^+ cells in human patient blood samples.

The roles of osteoblast-lineage cells in breast and prostate cancer bone metastasis progression have long been postulated and investigated by multiple groups [6, 74–78]. For example, osteoblasts provide an endosteal niche for bone metastatic prostate and breast cancer cells during dormancy and subsequent metastatic outgrowth. In addition, osteoblast-derived cytokines and growth factors, most notably receptor activator of nuclear factor kappa-B ligand (RANKL), contribute to tumor growth, angiogenesis, and osteolysis in the metastatic bone microenvironment. In contrast, the roles of osteoblast-lineage cells in the circulation started to draw attention only recently.

Platelets, neutrophils, and other myeloid-lineage cells

Bone is a primary immune organ where majority of immune cells originate. In this sense, given that bone metastatic tumor cells interact with nearly all types of cells in the bone microenvironment, bone marrow cells—particularly mature and pre-mature immune cells—are speculated to play a role in the progression of bone metastasis. Notably, neutrophils, representing 50–70% of myeloid-lineage cells, have been shown to contribute to tumorigenesis, metastasis, and patient prognosis. The quantity of tumor-associated neutrophils (TAN) and neutrophil-to-lymphocyte ratio have been shown to correlate with poor prognosis by multiple investigators [79]. For bone metastasis, Thio et al. analyzed 1,012 patients in a retrospective cohort and found that both neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios are independently associated with survival in patients who are treated for bone metastasis [80]. In addition, Wang et al. reported that high neutrophil-to-lymphocyte ratio was associated with poor prognosis [81]. However, there are smaller clinical cohort data contradicting the correlation between neutrophils and bone metastasis [82].

In addition to neutrophils, platelets have been known for decades as an important contributing factor for metastasis. Platelet integrin, αIIbβ3, is known to involve in circulating tumor cell adhesion and invasion [83, 84]. In addition, β3 knockout mice showed 95% decrease in development of bone metastasis in an intra-cardiac injection bone metastasis mouse model [85] and also showed reduced osteolysis [86]. However, there are currently no evidence for the association between platelet counts and bone metastasis, and for diagnostic value of platelet counts in oncology clinic.

More recently, myeloid-derived suppressor cells (MDSC), immature bone marrow-derived cells in the circulation with strong suppressive activity for anti-tumoral T cells, emerge as a biomarker for bone metastasis. Park et al. demonstrated using a chemotherapy-induced bone marrow expansion mouse model that $CD11^+$ immature myeloid cells contribute to bone metastasis [87]. More recently, by analyzing more than 100 breast cancer patient blood samples, the same group demonstrated that monocytic subtype of MDSC is closely associated with bone metastasis but not with other soft tissue metastasis [88]. However, more research is required to develop MDSC as a biomarker for bone metastasis.

Conclusion

Biomarkers for early diagnosis prior to the initiation of massive osteolysis and for monitoring disease progression and/or therapeutic responses remain major clinical unmet needs in oncology practices. Diverse diagnostic markers and technologies including liquid biopsy are under extensive research and development. This review article summarized the updated information on BTM and cell-based biomarkers for bone metastasis. In conclusion, BTMs and serum protein biomarkers have been classically used, but their clinical utility has limitations in specificity and sensitivity. Recently, various biomarkers based on pathophysiological studies of bone metastasis have been proposed, but extensive further research is still required before clinical application. Meanwhile, liquid biopsy-based diagnostic techniques recently are

spotlighted and currently tried as specific diagnostic methods for bone metastatic cancer. In particular, cOB may be a strong candidate marker specifically for bone metastasis compared with CTCs and other cell-based markers. Providing rationale for further research and development.

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Declarations

Conflict of interest Sun Wook Cho is a co-founder and CEO of Cellus, Inc.

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