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Review Article

RANK/RANKL/OPG: The Axis of Breast Cancer Bone Metastasis Evil?

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Abstract

Breast cancer bone metastasis remains the leading cause of death in individuals with advanced breast cancer of patients presenting with metastasis, ~ 70% is osseous. Generally, breast cancer bone metastasis is osteolytic, resulting in breakdown of the bone matrix. Treatment options are limited, and once breast cancer has metastasized to bone, it is incurable. The RANK/RANKL/OPG triad plays a critical role in breast cancer bone metastasis. Not only do the interactions of this triad facilitate and regulate normal bone turnover, it is also central to many signaling pathways in the metastatic bone microenvironment. Radiating from the RANK/RANKL/OPG axis, like spokes in a wheel, are factors such as TGF-beta, PTHrP, M-CSF, CCN2 and other cellular signaling pathways involved in the progression of breast cancer bone metastasis. Crosstalk between breast cancer cells and the bone microenvironment results in a vicious cycle of metastatic tumor promotion, progression and osteolysis. Furthermore, mounting evidence suggests that the RANK/RANKL/OPG axis also plays an important role in the development of breast carcinogenesis. However, the relationship between their differential expression in normal mammary epithelium and breast tumors is understudied and conflicting data from the literature muddies the role of each player in this triad in progression towards breast cancer bone metastasis. Thus, this review discusses the current status of RANK/RANKL/OPG expression and their involvement in breast cancer progression and metastasis and highlights the need to further understand the role of the triad.

ABBREVIATIONS

BCBM: Breast Cancer Bone Metastasis; Casr: Calcium-Sensing Receptor; CCN2: CCN Family Protein 2; CREB: cAMP Response Element-Binding Protein; EMT: Epithelial To Mesenchymal Transition; ER: Estrogen Receptor; ERK: Extracellular Signal-Regulated Kinase; IL-6: Interleukin-6; IL-8: Interleukin 8; JNK: C-Jun N-Terminal Kinase; M-CSF: Macrophage Colony-Stimulating Factor; MMP: Matrix Metalloproteinase; Nfatc1: Nuclear Factor of Activated T-Cells; OPG: Osteoprotegerin; PKA: Protein Kinase A; PKC: Protein Kinase C; PR: Progesterone Receptor; PTHrP: Parathyroid Hormone-Related Protein; RANK: Receptor Activator of Nuclear Factor Kappa-β; RANKL: Receptor Activator of Nuclear Factor Kappa-β Ligand; RR_LO: RANK/RANKL/OPG; STAT5: Signal Transducer And Activator Of Transcription 5; TGF-β: Transforming Growth Factor-β; TNF: Tumor Necrosis Factor; TRAF6: TNF Receptor Associated Factor 6; TRAIL: TNF-Related Apoptosis-Inducing Ligand; VEGF: Vascular Endothelial Growth Factor

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in American women [1] and the second leading cause of cancer-related deaths [2]. There are multiple risk factors associated with breast cancer, but the main two are gender and age [3]. Other risk factors include genetic predisposition, breast density, endogenous hormone levels, number of pregnancies, and use of oral contraceptives, obesity, and tobacco use and alcohol consumption [4]. Each year, over 200,000 new cases of invasive breast cancer and 60,000 new cases of in situ breast cancer are diagnosed in the United States [1]. Approximately 1 in 8 women in the United States will develop invasive breast cancer at least once during their life and 1 in 36 women will still die as a result of the disease [1], causing over 40,000 deaths.

Metastasis is the main cause of breast cancer-associated death [5] as localized disease is not considered fatal. Bone is the most common site to which breast cancer metastasizes. About 70% of breast cancers that metastasize spread to the bone [6].

Other sites for breast cancer metastases include lungs, liver and brain [5,7]. Five-year life expectancy decreases from about 95% for individuals with non-metastatic breast cancer to less than 25% for patients with metastatic breast cancer [8]. Once breast cancer has spread to the bone, it is deemed incurable [6]. Some of the current treatment options for bone metastases include radiotherapy and radiopharmaceuticals, orthopedic surgery, bisphosphonates, endocrine and cytotoxic treatments [9], most of which have drawbacks due to unfavorable side effects. Thus, understanding the underlying mechanisms in the pathogenesis and particularly the protein interactions governing the etiology of breast cancer-derived bone metastasis is critical for effectively preventing and treating the disease through the development of more targeted treatments, resulting in increased survivorship and quality of life. One such network may involve the Receptor Activator of Nuclear Factor Kappa-B (RANK)/ Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)/Osteoprotegerin (OPG) (RR_LO) axis, yet relatively little is known about expression, interactions and mechanisms of action of this signaling pathway in breast cancer metastasizing to the bone. Here, we review the current understanding of the RR_LO triad in development of breast cancer and bone metastases and discuss the need for further examination of this understudied area that may yield a critical clue as to why bone is preferentially targeted by breast cancer metastasis.

The Chronology of Breast Cancer Bone Metastasis (BCBM)

Breast cancer cells have an arsenal of tools to facilitate escape from the confines of the primary tumor microenvironment. These include restructuring the extracellular matrix [10,11] and secreting proteases [11] and angiogenic factors [12], allowing the cancer cells to invade into circulation and become metastatic. Metastatic breast cells then release cytokines and growth factors [13-16] that promote adhesion in osseous tissue and subsequent proliferation [17]. While osseous breast cancer metastasis can occur in any bone, it most often occurs in bones proximal to the body midline, especially the spine [18]. Initially, circulating breast cancer cells preferentially adhere to the bone marrow endothelium [19].

Under normal circumstances, bone matrix is constantly fluctuating to maintain homeostasis through osteoblast - osteoclast activity. Osteoblasts are derived from mesenchymal cells that synthesize collagen fibers [20]. Once surrounded by newly deposited bone matrix, the osteoblasts differentiate into osteocytes. Osteoclasts are derived from a hematopoietic cell lineage [21]. Through release of proteolytic enzymes and lowering the extracellular pH, osteoclasts are able to resorb the bone matrix by dissolving hydroxyapatite crystals, subsequently releasing calcium and phosphate [22].

Once breast cancer cells have invaded the bone marrow, the balance between osteoblast and osteoclast activity is disrupted and the interaction between the dynamic bone environment and breast cancer cells facilitates the development of bone metastasis. Bone metastasis has been shown to induce higher rates of osteoclast activity [23,24]. Moreover, osteoblast differentiation is inhibited and apoptosis is induced [24]. This altered osteoclast - osteoblast dynamism is facilitated by the secretion of growth factors and cytokines produced by breast cancer cells such as parathyroid hormone-related protein (PTHrP) [25], interleukin-6 (IL-6) [26], interleukin-8 (IL-8) [27], macrophage colony-stimulating factor (M-CSF) [28], matrix metalloproteinase's (MMPs) [29-32], and tumor necrosis factor (TNF) [33]. Secretion of these proteins plays an important role in facilitating metastatic behavior by directly or indirectly influencing osteoblasts and osteoclasts as well as their precursors. This in turn leads to higher osteoclast differentiation which leads to an increase in the secretion of growth factors and cytokines such as transforming growth factor-β (TGF-β) by osteoclasts which stimulates further breast cancer growth [34]. This results in a recursive loop that facilitates further metastasis and the pathogenic deterioration of bone integrity.

Molecular Mechanisms of BCBM: The Role of RR_LO

The molecular mechanisms surrounding BCBM are very complex. It remains unclear how the RR_LO triad, a signaling pathway most commonly known for its role in normal bone remodeling, regulates BCBM. RANK is a type I transmembrane protein and a member of the TNF receptor family [35,36]. RANKL is a type II transmembrane protein that belongs to the TNF super family [36]. OPG is a glycoprotein and decoy receptor to RANKL. It is also a member of the TNF receptor super family [37,38]. OPG, RANK and RANKL are widely expressed in many tissues including skin, smooth muscles, respiratory, gastrointestinal, and lymphoid tissues [38-41]. Interestingly, RR_LO also has an observed role in mammary gland development [42], which may help elucidate its possible importance in BCBM and as potential biomarkers and prognostic factors should be changed to as a potential biomarker and a prognostic factor.

The Role of RR_LO Interactions in Mammary Gland Development

Since the role of RR_LO interactions is well known in bone development (See [43] for review), it will only be discussed in this review in context of possible links to BCBM. The importance of the relationship of RR_LO in mammary gland development is much less understood and will be discussed below.

RANKL-RANK signaling has been shown to affect the expansion of mammary stem cells during normal mammary gland development [44,45]. Additionally, RANK and RANKL play an important role in development of the lactating mammary gland during pregnancy. Using a RANK/RANKL mouse knock-out model, Fata et al., showed that lobuloalveolar development was impaired during pregnancy. This impairment was linked to a deficiency in the differentiation, proliferation, and survival of mammary epithelial cells [46]. In addition, RANKL expression in mammary cells is mainly regulated by progesterone and prolactin expression [47-50]. RANKL appears to be the main paracrine effector of progesterone for inducing mammary cell proliferation and expansion, and the resulting mammary gland development [44, 45,47,49,51-54]. Progesterone induces the proliferation of mammary epithelial cells through the progesterone receptor (PR) via a paracrine signaling mechanism mediated from PR-positive to neighboring PR-negative cells [55]. RANKL was shown to bind to the PR in a hormone-dependent manner [55]. This occurs

when progesterone recruits signal transducer and activator of transcription 5 (STAT5) to complex with RANKL upon PR binding [55] (Figure 1).

RANKL and RANK both appear to have roles in mammary gland development, mammary epithelial and stem cell proliferation, differentiation and survival. In contrast, the role and mechanism of action of OPG remains unclear. This is in part, due to the paucity of studies investigating its function in normal breast epithelium. Based on its function as a decoy receptor to RANKL and its regulation of RANKL and RANK signaling [56,57], the involvement of OPG in regulating mammary gland development is also highly probable. Furthermore, OPG was observed in mammary epithelial cells [42]. Similarly, another study found the presence of OPG in human milk samples with levels of OPG more than 1000-fold greater than in normal human serum [58]. Since OPG is significantly raised in human milk samples postpartum, it suggests that not only RANKL-RANK signaling but also OPG might play a role in breast development during lactation. In contrast, using OPG knock-out mice, Mizuno et al, did not observe any abnormalities in the mouse mammary gland development, suggesting that OPG does not play a direct role in mammary gland development [59]. It is also possible that OPG might be inversely correlated with mammary epithelial cell proliferation [60].

RR_LO in Breast Cancer

Increasing evidence suggests a vital role of the RR_LO axis in tumor formation, progression and metastasis. Many studies show RANKL contributes directly to breast cancer formation and metastasis [53,54,61,62]. A major cancer-associated pathway regulated by RANKL-RANK binding is the NF-κB pathway. This pathway is activated in breast cancer cells that form tumor spheres (spheres formed by NF-κB activation in epithelial to mesenchymal transition (EMT), up regulation of interleukin-1B and interleukin-6 as well as cancer stem cells) [63]. A study by Palafox et al., found that RANKL lead to cancer progression and metastasis in mammary epithelial cells by inducing EMT and stemness [64]. Furthermore, several studies have also shown that an inhibition of RANKL or PR-associated pathways suppresses mammary tumor formation in mice [45,53,54,65].

However, when evaluating RANK, RANKL, and OPG expression at a transcriptional level, inconsistencies between studies and cell lines become apparent. For example, many breast cancer cell lines analyzed have been found to lack RANKL on the mRNA level with the exception of HCC70, while OPG and RANK seem to be expressed by most breast cancer cell lines [42,66-69]. Several studies have also shown a lack of RANKL expression in primary and metastatic breast tumors, suggesting a possible down regulation in RANKL expression during the transformation from normal mammary epithelium to breast cancer. However, studies by Nicolin et al., found that MCF-7 and MDA-MB-231 breast cancer cells express RANKL protein, despite other studies finding a lack of RANKL mRNA expression [69-71]. Another study analyzed breast tumor tissues of patients and found that RANKL was expressed in 78.4% of patients and was associated with lower cell proliferation and improved skeletal disease-free survival [72].

While results from these studies do not paint a clear picture of the landscape for RANKL expression, even less is known about RANK expression. RANK is expressed in breast cancer cells lines such as MDA-MB-231, MCF-7, Hs578T, and ZR75-1 [73,74]. Santini et al., found that lower RANK mRNA expression in primary breast cancer specimens is associated with a longer overall survival [75].

Given the important and intimate interactions between

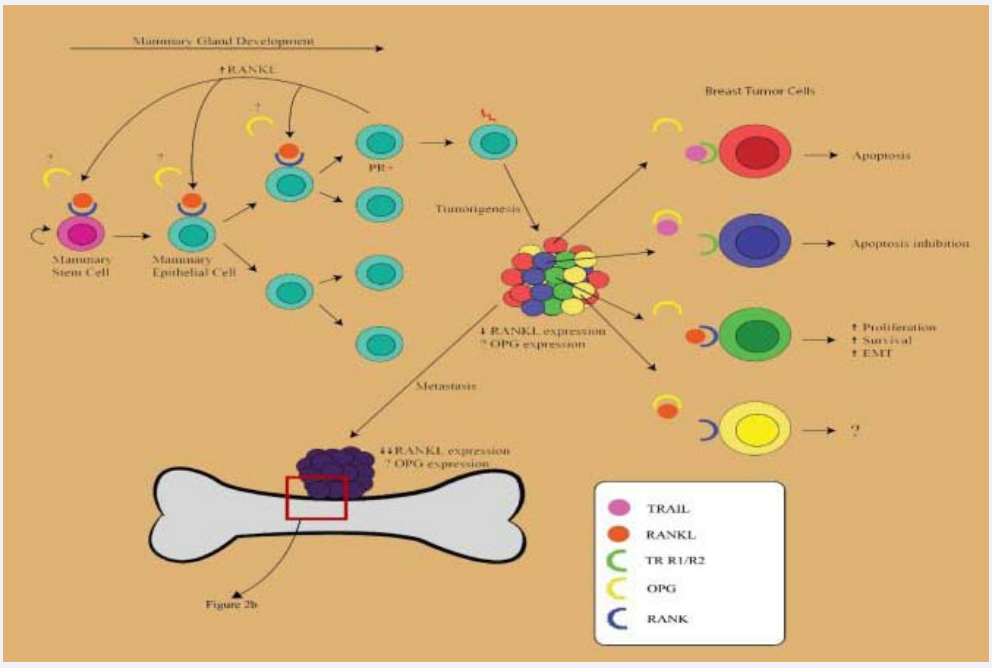

Figure 1 An overview of the role of the RANK/RANKL/OPG axis in mammary gland development, tumorigenesis, and metastasis.

the RR_LO triad members, surprisingly little attention has been given to OPG in comparison to RANKL. One study reported that OPG was expressed in only 45.9% of breast tumor samples and its expression was associated with a smaller tumor size, node negativity, and lower cell proliferation [72]. Another study found 55% of primary breast tumors sampled expressed OPG, while it was highly expressed in mammary epithelium with columnar alteration. It was also expressed in endothelial cells but was absent in non-malignant lobules and ducts as well as myoepithelial cells [76]. This study further demonstrated that OPG expression is positively correlated with ER/PR status [76]. Another study found that increased OPG mRNA levels analyzed in primary breast cancer specimens correlate with longer overall survival [75]. A recent study investigated BRCA mutations and their correlation with OPG serum levels. Results showed that women post menopause with BRCA1/2 mutations have lower OPG serum levels than those without the mutation. Interestingly, OPG levels were inversely correlated with mammary epithelial proliferation [60]. However, since only serum levels were analyzed, it cannot be accurately predicted if we see the same correlation with OPG levels within the mammary tissue.

The RR_LO Axis in Normal Bone Remodeling

RANK is expressed on precursors of osteoclasts. Binding of its ligand, RANKL, initiates the differentiation of osteoclast precursors into mature osteoclasts through the activation of the NF-κB pathways [36,39,77]. This results in the upregulation of Nuclear Factor of Activated T-cells (NFATc1), which is the master transcriptional regulator for osteoclast differentiation [78]. OPG is also expressed by osteoblasts. Once bound to RANKL, OPG inhibits RANK/RANKL interactions [36] and down regulates osteoclast differentiation [36,79] (Figure 2a). Thus, a larger RANKL/OPG ratio expressed by osteoblasts is an indicator of an increased rate of osteoclastogenesis. A study by Nelson et al. showed that OPG binds to RANKL with an affinity that is approximately 500 fold higher than the binding affinity of RANK to RANKL [35].

A protein recently identified to interact with the RR_LO triad, and possibly have a role in BCBM, is the CCN family protein 2 (CCN2). CCN2 is expressed in mesenchymally- derived cells including chondrocytes and osteoblasts. One study found that CCN2 bound to RANK in pre-osteoclastic RAW264.7 cells, inducing signaling pathways known to be RANK-induced such as NF-κB, p38 and Jun amino-terminal kinases (JNK) pathways. However, CCN2 did not impact RANKL-RANK binding and interactions [80]. Furthermore, CCN2 was also shown to bind to OPG, with a binding affinity close to that of OPG and RANKL. More intriguing was the finding that OPG inhibited the binding of CCN2 to RANK, suggesting that OPG might inhibit osteoclastogenesis not only by inhibiting RANKL to RANK binding, but also by suppressing CCN2 to RANK binding. Furthermore, the authors reported that CCN2 is an inhibitor of OPG's negative regulation of osteoclastogenesis, although CCN2 does not directly inhibit RANKL to OPG binding. Therefore the authors suggest that the binding site of OPG and CCN2 is distinct from that for OPG and RANKL [80] (Figure 2a).

RR_LO in Breast Cancer Bone Metastasis

Currently, the precise mechanism of action of RR_LO in promoting metastasis remains unclear. MDA-MB-231 cells that expressed RANK have shown to have a higher metastatic growth rate than MDA-MB-231 cells that were RANK-negative [81]. A study by Santini et al., showed that RANK was positively correlated with the development of bone metastases and significantly higher expressed in estrogen receptor (ER) negative tumors [82]. Trinkaus et al., found that RANK was not expressed in normal breast tissue or primary breast cancer tissue of breast cancer patients, whereas 75% of patient samples expressed RANK in tumor cells at lymph nodes. However, the sample size was small (4 patients). They further showed that 50% of breast tumor cells at the metastatic bone site expressed RANK (sample size = 20) [83]. Another study found RANK to be expressed in 74.1% of patient breast tumor samples and was associated with a poor disease-free survival rate [72]. In contrast, Bhatia et al. showed no change in RANK expression among non-neoplastic breast tissue, non-metastatic infiltrating ductal carcinoma, metastatic infiltrating ductal carcinoma, and breast cancer bony metastases tissue [84]. To date, it remains unclear from *in vivo* studies [66,72,74,85] whether RANK should be considered a prognostic biomarker for BCBM.

RANKL was shown to induce markers for metastasis such as matrix metalloproteinase-1(MMP1) and vascular endothelial growth factor (VEGF) in MDA-MB-231 breast [73,85]. Several studies suggest that RANKL promotes breast cancer metastasis by directly affecting cancer stem cells or tumor cells that express RANK [62,86,87]. Protein expression from patient samples of normal breast tissue, primary and secondary (bone) metastasized breast tumors presents inconsistencies in expression levels. RANKL expression was shown to be down regulated from normal breast tissue samples to primary breast cancer tissue to breast cancer at the site of bone metastasis in two studies [76,84]. Bhatia et al., showed that RANKL expression was found in 90% of non-neoplastic breast tissue, 62% of non-metastatic infiltrating ductal carcinoma, 31% of metastatic infiltrating ductal carcinoma, and 2% of breast cancer bone metastases [84]. In contrast, another study found that all patient samples (n=4) expressed RANKL in the secondary breast tumor at the site of osseous metastasis [88]. Yet another *in vitro* study demonstrated that when the RANKL-expressing HCC70 breast cancer cell line was co-cultured with human osteoblasts, it decreased their RANKL expression [68].

The functional advantage of a possible decreased expression of RANKL in the primary and secondary tumor remains to be elucidated. One possibility for RANKL down regulation may be associated with the binding affinity of OPG to another ligand, TNF-related apoptosis-inducing ligand (TRAIL), a ligand that binds to receptors on cells to facilitate apoptosis [89,90]. Interestingly, OPG has about equal binding affinities for both RANKL and TRAIL [91]. Some studies have proposed that the decreased RANKL expression in breast tumors and secondary tumors might be due to selective down regulation of RANKL by breast cancer cells, thus facilitating greater interactions between OPG and TRAIL, blocking TRAIL from binding to its receptor on breast cancer cells and consequently inhibiting apoptosis [76]. Holen et al., showed that OPG-expressing breast cancer cells efficiently inhibited TRAIL-induced apoptosis [92]. In contrast, Weichhaus et al., did not find an effect on TRAIL-mediated apoptosis upon OPG knockdown and suggested that there is another, as yet

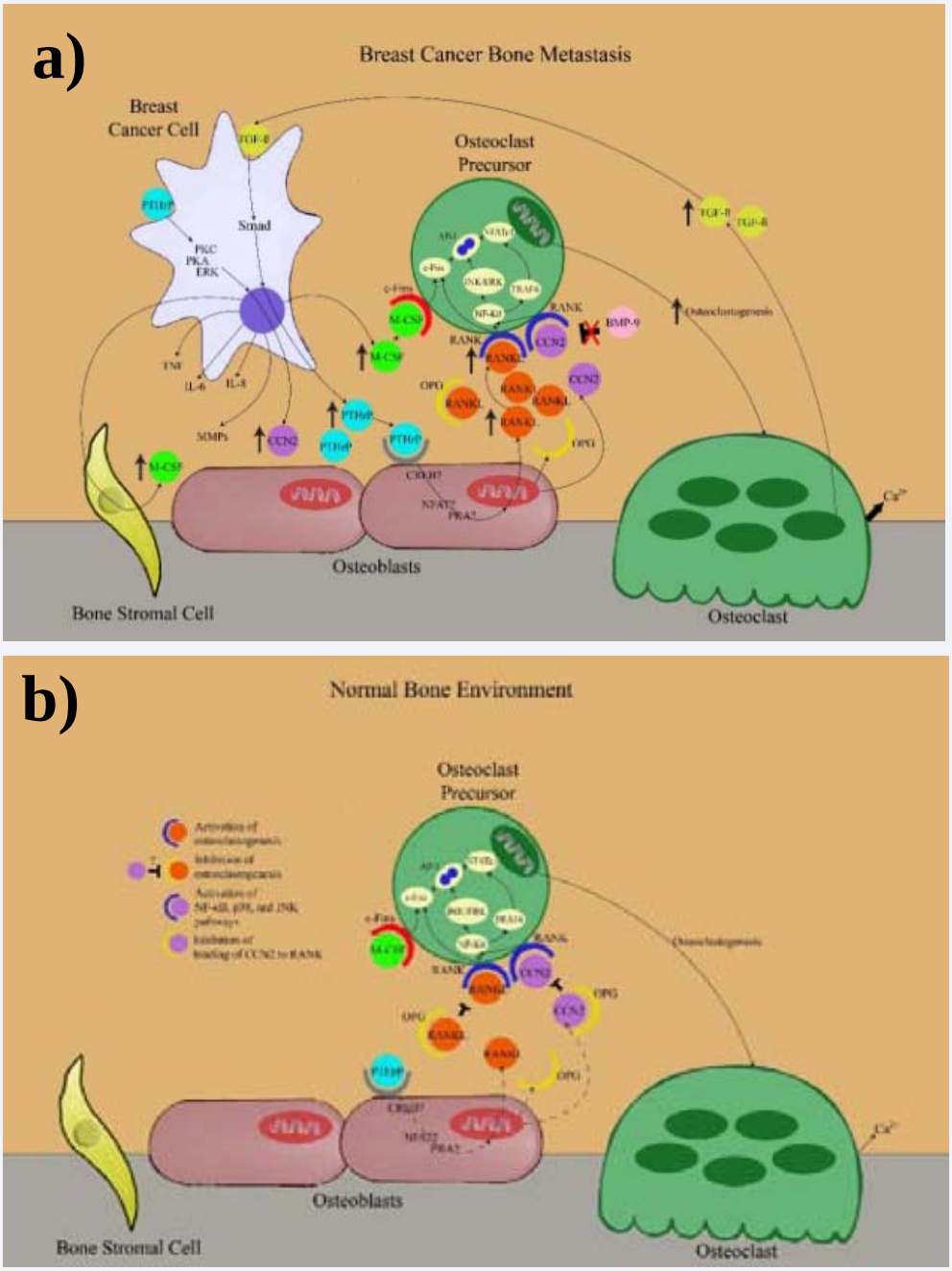

Figure 2 The RANK/RANKL/OPG axis at the core of 2a) the normal bone remodeling vs 2b) the vicious cycle of breast cancer signaling leading to osteolysis.

unknown mechanism involved in OPG's role in metastasis and possibly downregulation of RANKL [37].

Several studies also found an association between an increase in OPG and a poorer prognosis in breast cancer through increased metastasis and invasion [37,69]. Knocking down OPG expression in MDA-MB-231 significantly reduced metastasis in the chick embryo metastasis model [37]. Moreover, OPG knockdown cells showed decreased invasion through collagen, an MMP-2 substrate [37], suggesting a role for OPG in breast cancer cell migration.

Taken together, OPG is upregulated and RANKL is down regulated during tumor progression and metastasis as an increase in OPG in breast cancer cells correlates with an increase in metastasis [37,69]. Further studies need to evaluate the roles of RANKL, RANK, and OPG as regulators of bone metastasis in human breast tissue and tumors as well as potential biomarkers and therapeutic targets.

The RR_LO Triad at the Core of Many Signaling Pathways in Breast Cancer Bone Metastasis

As discussed above, OPG is significantly raised in human milk samples postpartum [58] and RANKL-RANK signaling plays a role in the mammary development during lactation. Calcium

trafficking may be an underlying link between breast and bone in regards to the triad based on the nature of mammary gland production of milk and the function of osseous tissue in calcium regulation. In the bone environment, RANKL-RANK signaling is found to trigger NFATc1, leading to osteoclast differentiation and increased bone Reabsorption and remodeling [93]. RANKL is also responsible for inducing a sustained low level calcium oscillation that promotes the nuclear import of NFATc1 by activating calcineurin-NFATc1, which is an auto regulatory feedback mechanism that enhances its own expression and activates, with the aid of c-fos, a set of genes that are vital in osteoclastogenesis [78]. Activation of NFATc1 transcription in osteoclasts requires the co-factors TNF receptor associated factor 6 (TRAF6), NF-κB and c-fos. TRAF6 is also induced by RANKL to stimulate a NF-κB pathway, which increases expression of NFATc1 by binding to NF-κB binding element in the promoter region. Thus, both the calcium-calcineurin and the TRAF6 - NF-κB pathways are utilized to activate NFATc1 activity [78].

Calcium signaling also plays an important role in the breast. Calcium-sensing receptor (CaSR), the master regulator of calcium metabolism, is expressed in mammary epithelial cells as well as breast cancer cells [94]. CaSR becomes activated during lactation and decreases levels of PTHrP in the milk and circulation, which leads to an increase of calcium in the milk [94]. During lactation there is usually an increase in bone turnover and bone resorption which frees calcium from the bone needed for lactation. It is suggested that during that time osseous RANKL is upregulated and OPG is down regulated. One study treated lactating mice with OPG and found that bone loss was reduced during lactation which, however, did not lead to a change in milk production, milk calcium levels or calcium homeostasis, unless dietary calcium was restricted [95]. Interestingly, osteoclast number remained the same but osteoblast number decreased suggesting that during lactation osteoclast activity is required for increased osteoclast numbers [95].

One longstanding paradigm is that RANKL was primarily expressed by osteoblasts [96]. However, more recent research has shown that osteocytes are the major source of RANKL in cancellous bone remodeling [97]. However, since metastatic breast cancer cells adhere to sites of bone turnover, the following discussion involves osteoblast expression of RANKL specifically.

Breast cancer cells that have migrated to the bone environment have been shown to secrete factors that increase the expression of RANKL in osteoblasts. PTHrP is known to be activated in breast cancer cells [94] and bind to receptors on osteoblasts, inducing RANKL production. Currently the exact mechanism that regulates the increased expression of RANKL in osteoblasts is not known. However, one study using mouse osteoblastic cells demonstrated that PTHrP-induced RANKL expression is dependent upon the activation of cAMP response element-binding protein (CREB) and the protein kinase A (PKA) and NFAT pathways [67]. PTHrP not only indirectly increases osteoclastogenesis, but it has also been shown to inhibit osteoblastogenesis and facilitate tumor growth of MDA-MB-231 breast cancer cells [98] (Figure 2b).

M-CSF, much like PTHrP, is released by breast cancer cells in the bone microenvironment and also up regulates RANKL expression on bone stromal cells [28]. This subsequently leads to an increase of osteoclastogenesis, resulting in osteolysis. Unlike PTHrP, M-CSF is itself a regulator of osteoclastogenesis in the normal bone environment. M-CSF, expressed by osteoblasts, binds to the c-FMS receptor on osteoclast precursors and activates the c-FOS pathway within the osteoclast precursors, up regulating NFATc1 and enabling monocytes, the precursors of osteoclasts, to fuse and form multinucleated osteoclasts [99] (Figure 2b).

As mentioned above, CCN2 has been shown to interact with the RR_LO triad. One study found that bone morphogentic protein 9 (BMP-9) inhibited the bone metastasis of breast cancer cells through the downregulation CCN2 [100]. Another study found that an anti-CCN2 antibody was able to inhibit the osteolytic bone metastasis that appears with CCN2 and PTHrP overproduction. They showed that PTHrP significantly upregulated CCN2 in MDA-MB-231 cells and that CCN2 expression was promoted by PTHrP via PKA-, protein kinase C (PKC)-, and extracellular signal-regulated kinase (ERK)-mediated signaling [101]. Interestingly, CCN2 is also needed for TGF-β-related signaling pathways such as Erk1/2 and Smad1 [102] (Figure 2b).

TGF-β is another important factor in this destructive BCBM loop. TGF-β is expressed by osteoblasts and osteoclasts and is upregulated during osteoclastic resorption of osteolytic bone during metastases [34]. This subsequently leads to the secretion of factors such as PTHrP, MMPs, TNF, IL-6, and IL-8 by the tumor, further driving bone destruction at the site of tumor adherence to the bone [34]. TGF-β stimulates the production PTHrP through Smad signaling in breast cancer cells, which closes the loop of this vicious cycle [103]. Furthermore, TGF-β has been shown to be involved in the epithelial-mesenchymal transition, angiogenesis and immunosuppression. Thus, TGF-β is a possible weak link in the metastasis armor to target for cancer therapy since blocking TGF-β might break the chain [104,105] (Figure 2b).

DISCUSSION & CONCLUSION

Interaction between breast cancer cells and the bone microenvironment results in a vicious cycle of bone metastasis. This vicious cycle is promoted through many factors and signaling pathways. Factors such as RANK, RANKL, OPG, TGF-β, PTHrP, M-CSF, and possibly CCN2 activate signaling pathways in breast cancer cells as well as osteoblasts and osteoclasts that lead to the resorption of the bone matrix and further stimulation of a more aggressive tumor phenotype. The RR_LO triad plays an important role in osteolytic bone metastasis progression and appears to be the center point from which many factors and signaling pathways radiate. While the function of the RR_LO axis in osteoclastogenesis is better understood, its role and expression in breast cancer remains to be elucidated. Currently, it is still unclear whether or how altered expression of RANKL and OPG in breast cancer cells directly influences BCBM. Furthermore, signaling pathways leading up to differential expression of RANKL and OPG in osteoblasts, breast cancer and mammary tissue remains largely unknown. More research is needed to understand the nature of the crosstalk between breast cancer cells and the bone microenvironment with regards to the RR_LO axis. Understanding these signaling mechanisms will ultimately lead to a more targeted therapy for breast cancer bone metastases.

PR+ mammary epithelial cells secrete RANKL. RANKL binding to RANK on mammary stem and epithelial cells leads to the expansion and proliferation of mammary epithelial cells promoting mammary gland development. The role of OPG has not yet been established. Cellular stress and mutation may cause mammary epithelial cells to undergo tumorigenesis as depicted above. When TRAIL binds to its receptor TR R1/R2, it causes the breast tumor cells to undergo apoptosis. However, TRAIL binding to OPG leads to the inhibition of apoptosis. RANKL binding to its receptor RANK on breast tumor cells causes an increase in proliferation, survival and EMT. However, RANKL expression was found to be decreased in primary breast tumor tissues. What happens when RANKL binds at higher rates to OPG needs clarification, as does its expression in primary breast tumor tissue. RANKL expression appears to further decrease as primary breast cancer cells migrate and metastasize to the bone. OPG expression of breast tumor cells at the secondary bone site needs further elucidation.

Breast cancer cells secrete certain cytokines and growth factors, such as PTHrP and M-CSF that drive osteolysis. PTHrP and M-CSF stimulate osteoblasts to increase the secretion of RANKL, which then binds at higher rates to its receptor RANK driving the differentiation of osteoclast precursors into mature osteoclasts. This leads to higher rates of bone resorption, which in turn leads to greater secretion of growth factors such as TGF-β. This stimulates breast cancer cells to further increase the production of PTHrP which continues the vicious cycle seen in breast cancer bone metastasis. PTHrP appears to influence pathways involving CREB, NFAT and PKA in osteoblasts, causing them to increase RANKL expression. CCN2, secreted by osteoblasts and chondrocytes shown in Figure (2a) might interact with the triad, as it has also been shown to bind to RANK as well as OPG. In the presence of breast cancer cells in the bone environment, osteoblasts also increasingly secrete M-CSF, which binds to the c-FMS receptor on osteoclast precursors, driving osteoclastogenesis.

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