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Matricellular proteins as regulators of cancer metastasis to bone

Timothy N. Trotter¹ and Yang Yang^{1,2}

¹Department of Pathology, University of Alabama at Birmingham, Birmingham, AL

²Comprehensive Cancer Center and the Center for Metabolic Bone Disease, University of Alabama at Birmingham, Birmingham, AL

Abstract

Metastasis is the major cause of the death in cancer patients, and a frequent site of metastasis for many cancers is the bone marrow. Therefore, understanding the mechanisms underlying the metastatic process is necessary for future prevention and treatment. The tumor microenvironment is now known to play a role in the metastatic cascade, both at the primary tumor and in metastatic sites, and includes both cellular and non-cellular components. The extracellular matrix (ECM) provides structural support and signaling cues to cells. One particular group of molecules associated with the ECM, known as matricellular proteins, modulate multiple aspects of tumor biology, including growth, migration, invasion, angiogenesis and metastasis. These proteins are also important for normal function in the bone by regulating bone formation and bone resorption. Recent studies have described a link between some of these proteins and metastasis of various tumors to the bone. The aim of this review is to summarize what is currently known about matricellular protein influence on bone metastasis. Particular attention to the contribution of both tumor cells and non-malignant cells in the bone has been given.

Keywords

Matricellular proteins; ECM; Bone microenvironment; Bone matrix; bone metastasis

Introduction

Metastasis is a complicated, multi-step process that remains a major challenge in the treatment of cancer. Indeed, roughly 90% of cancer patient mortality is due to metastasis [1]. Though the impact of metastasis on patient outcome is clear, this process as a whole remains poorly understood. An early theory describing the mechanism of metastasis, known as the “seed and soil” hypothesis, was proposed by Stephen Paget in 1889 [2]. Paget theorized that cancer cells (seed) need a compatible microenvironment (soil) to survive and form a distant

Corresponding author: Yang Yang M.D., Ph.D. Department of Pathology, University of Alabama at Birmingham, WTI 320A, 1824 6th Avenue South, Birmingham, Alabama 35294 Telephone (205) 996-6228, Fax (205) 975-6615; ; Email: yangyang@uab.edu.

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metastasis. This concept, that the microenvironment supports tumor growth and metastasis at local and distant sites, has become the focus of intense study over the past two decades and has changed the way we think about tumor biology.

One of the most common sites of metastasis is the bone marrow. Post-mortem analysis revealed that roughly 70% of patients with breast or prostate cancer had bone metastases [3]. This figure is relatively high in thyroid, lung and renal cancer as well, with an incidence of approximately 35–40% in each case [3]. In addition, multiple myeloma, a hematologic malignancy of B cells, predominantly localizes to the bone marrow and progresses from primary bone sites to new bone sites [4]. High frequencies of bone metastasis in these cancers can be viewed somewhat as a result of high blood flow to the bone marrow [5]. However, many studies demonstrate that the bone microenvironment plays a critical role in attracting metastatic tumor cells and supporting subsequent growth in bone [6]. The bone marrow microenvironment is a complex system composed of a number of cell types, such as osteoblasts and osteoclasts. In addition is a non-cellular component that includes soluble signaling mediators, such as hormones and cytokines. Finally, the extracellular matrix (ECM) is a meshwork of secreted proteins that provides both structural support and biochemical cues for cells in the bone marrow. It is comprised primarily of type I collagen, but also contains other structural proteins such as fibronectin [7]. However, a group of molecules exist in the ECM that do not serve a structural function. Rather, these proteins fine-tune cell-ECM interactions and cellular functions [8]. Accumulating evidence has shown that these so called “matricellular” proteins are often dysregulated in cancer, both by cancer cells and by host cells (covered in more detail in [9] and [10]). In this review, we will introduce matricellular proteins in the bone ECM and summarize what is known about their involvement in cancer metastasis to bone.

Small leucine-rich proteoglycans (SLRPs)

SLRPs are a family of 18 relatively low molecular weight proteins (~36–42 kDa). SLRPs are characterized in part by a central domain containing a variable number of tandem leucine-rich repeats (LRRs) with the conserved motif LXXLxLXXNxL; where L represents leucine but can be substituted with isoleucine, valine or other hydrophobic amino acids, and X can be any amino acid [11]. The majority of these proteins contain glycosaminoglycan chains (GAGs) that are differentially processed during aging or development. They are involved in ECM assembly, hydration, and cytokine binding in interstitial connective tissue, such as bone and tendon, as well as other tissues [12]. In addition, SLRPs can affect signaling through membrane receptors, such as receptor tyrosine kinases (RTK) and toll-like receptors (TLR). They are involved in a wide variety of biological processes, particularly inflammation, bone morphogenesis and neural development [11–13].

Decorin is the most well studied SLRP in bone metastasis. This protein is found in normal connective tissues, including the bone, where it binds to and cross-links collagen fibrils [14]. Decorin is also found in tumor ECM and is mainly attributed to surrounding, non-malignant stromal cells (tumor cells express little decorin) [15–17]. However, decorin expression is decreased in stromal cells of many tumors compared to normal tissues and inversely correlates with prognosis of breast and lung carcinomas [16, 18, 19]. Declined decorin in the

bone marrow matrix is also associated with the clinical progression of multiple myeloma [20]. Interestingly, decorin acts as a tumor suppressor in a number of cancers, including breast, prostate, ovarian and colon [16, 21–23]. This function is partially due to the ability of decorin to bind to and inhibit function of RTKs such as epidermal growth factor receptor (EGFR), insulin-like growth factor receptor I (IGF-IR) and hepatocyte growth factor receptor (c-Met) [24–26]. For example, recombinant decorin reduces hypoxia inducible factor-1 α (HIF-1 α) and vascular endothelial growth factor A (VEGF-A) in MDA-MB-231 breast cancer cells through inhibition of c-Met, as well as induces anti-angiogenic molecules thrombospondin-1 and metalloproteinase inhibitor 3 (TIMP3) in these cells [27]. Subcutaneous injection of a Matrigel plug containing MDA-MB-231 cells, hepatocyte growth factor (HGF) and decorin results in decreased angiogenesis compared to a plug containing cells and HGF alone [27]. In addition, recombinant human decorin decreases adhesion of breast and colorectal cancer cell lines, whereas conditioned media from breast cancer cells reduces expression of decorin in fibroblasts *in vitro* [28].

With regards to the bone marrow, decorin expression is decreased in mesenchymal stem cells (MSCs) and osteoblasts from myeloma patients compared to healthy individuals [29]. Decorin expression is further decreased in these cells from myeloma patients with osteolytic disease compared to non-osteolytic patients. Osteoblasts express decorin at higher levels than MSCs [30]. Decorin expression by osteoblasts also suppresses myeloma cell survival, even when cultured with osteoclasts, and inhibits tube formation by HUVEC cells as well as differentiation of osteoclasts *in vitro* [30]. Myeloma cells co-cultured with osteoblasts decrease decorin expression in osteoblasts, but antibody-mediated neutralization of myeloma cell-derived macrophage inflammatory protein-1 α (MIP-1 α) inhibits this effect [31]. In addition, knock-in of decorin in osteotropic MDA-MB-231 breast cancer cells results in decreased bone metastasis along with diminished osteoclast numbers [32]. Moreover, systemic delivery of Ad.dcn, an oncolytic adenovirus carrying human recombinant decorin, inhibits skeletal metastasis of PC-3 prostate cancer cells and decreases osteoclast numbers and tartrate-resistant acid phosphatase 5b (TRAP5b) levels *in vivo* [33]. Thus decorin is not only involved at the primary tumor site, but is also active in the bone where it protects against bone metastasis and destruction.

Currently, very little is known about other SLRPs in the process of bone metastasis. Bone marrow stromal cell-derived biglycan may have similar anti-bone metastatic functions as decorin. Biglycan in an ECM scaffold produced by mammary mesenchyme cells can “normalize” breast cancer cells *in vitro* [34]. In addition, decreased biglycan is found in paired bone metastases compared to primary tumors in breast cancer patients [35]. Biglycan expression is increased in mouse osteoblastic cells by a secreted isoform of ErbB3 from prostate cancer cells, although the effects of this change are currently unknown [36]. Asporin is upregulated in bone marrow stromal cells in a bone xenograft model of prostate cancer [37]. Although asporin is poorly understood it is linked to multiple bone-related diseases, warranting further investigation in bone metastasis and cancer-induced bone disease [38]. Lumican expression in breast stromal cells correlates with higher tumor grades, lower patient age, and decreased estrogen receptor levels in breast tumors, indicating bad prognosis for these patients. However, decreased lumican levels in cancer cells are associated with advanced cancer and bone metastasis [35, 39–41]. Lumican decreases

invasion and metastasis in some contexts, therefore it is interesting to speculate that this differential expression pattern in tumor versus stroma represents an attempt by surrounding cells to contain expanding neoplastic cells [42–44].

Small integrin-binding ligand N-linked glycoproteins (SIBLINGs)

SIBLINGs consist of 5 members: osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). This group has relatively little sequence homology and is poorly conserved between species, but all share an Arg-Gly-Asp (RGD) motif that is important for cell attachment and signaling [45]. SIBLINGs have a flexible structure that likely helps facilitate binding to a wide variety of partners, including matrix metalloproteinases (MMP), integrins and collagen [45, 46]. Many SIBLING functions are determined by various levels of post-translational modification, such as proteolytic processing or glycosylation [47]. SIBLINGs are especially important for bone formation and maintenance, particularly in mineralization [48]. However, these proteins are also expressed in other tissues under certain conditions. Moreover, accumulating evidence supports the role of this family in multiple stages of cancer progression, including proliferation, invasion, and metastasis [49].

OPN was originally identified with bone sialoprotein as a major component of the mineralized bone matrix [50]. OPN has since been found in multiple normal tissues, including numerous epithelial cell types, activated leukocytes, kidney tubule cells, arterial endothelial and smooth muscle cells, cells of the inner ear, and sites of wound healing [51]. In the bone, OPN is produced throughout the osteoblast lineage from pre-osteoblasts to osteocytes, as well as by bone resorbing osteoclasts [52, 53]. OPN is critical for anchoring osteoclasts to the bone surface via the integrin $\alpha_v\beta_3$ during bone remodeling [54, 55]. Experiments using an OPN knockout mouse model give further evidence that OPN is necessary for this process. While adult bones appear relatively normal, OPN knockout mice are resistant to bone loss due to ovariectomy, decreased mechanical stress or continuous parathyroid hormone treatment [56, 57].

OPN expression by tumor cells has been proposed as a predictive marker for bone metastasis [58–60]. Interestingly, specific OPN polymorphisms were found to correlate with poor survival and bone metastasis of lung cancer patients [61]. OPN was also identified as a part of the “bone-metastasis signature” of breast cancer cells that metastasize to bone, and induced expression of OPN in combination with interleukin 11 (IL-11) in OPN-negative breast cancer cells promotes bone metastasis [62]. These findings indicate that OPN in cancer cells alone is a critical factor for bone metastasis. The bone marrow microenvironment is also an important source of OPN for tumors. OPN deficient mice show decreased growth in and metastasis to bone of melanoma cells, and OPN-deficient mice form increased trabecular bone in the presence of intra-tibial melanoma tumors compared to wild-type mice [63, 64]. OPN may also be involved in early bone colonization of cancer cells through binding $\alpha_v\beta_3$ integrin on cancer cell surfaces and initiating angiogenesis within the bone marrow [65, 66]. In addition, the ability of OPN to facilitate bone destruction by promoting osteoclast function would allow release of bound growth factors from the bone

matrix, and OPN itself is involved in signaling from growth factors such as transforming growth factor β (TGF- β), HGF and basic fibroblast growth factor (bFGF) [67–69]. OPN also promotes migration of multiple tumor types [70, 71]. In one study, OPN from bone marrow derived cells enhanced migration of MCF-7 breast cancer cells in a Transwell co-culture system [71]. Bone marrow cells in overt myeloma patients produce more OPN than myeloma precursor disease [72]. In addition, myeloma cells express and bind to OPN [72, 73]. Plasma OPN levels in myeloma patients positively correlate with disease progression, bone destruction and angiogenesis [74]. OPN is normally an important component of the hematopoietic stem cell (HSC) niche in the bone where it regulates HSC pool size and localization [75]. Acute lymphoblastic leukemia (ALL) cells were recently reported to bind OPN near osteoblasts where the ALL cells became dormant and resistant to chemotherapy [76]. This effect can be blocked by OPN neutralization. Considering OPN directly interacts with the cancer stem cell marker CD44 and regulates stem cells in the bone, OPN may support takeover of the HSC niche by cancer stem cells [77, 78]. However, this does not appear to have been directly addressed yet.

BSP is present in mineralized tissues such as bone, dentin, cementum and calcified cartilage and is a significant component of the bone ECM where it comprises up to 8% of all non-collagenous proteins [48]. BSP binds hydroxyapatite through three polyglutamic acid domains where it provides nucleation points for crystal formation [79]. BSP is not only expressed in bone forming cells such as osteoblasts, osteocytes and chondrocytes, but is also expressed in bone resorbing osteoclasts. Therefore BSP acts to regulate both bone formation and bone destruction, illustrated by mixed phenotypes seen in genetic animal models [80]. BSP null mice display delayed bone mineralization and decreased bone formation rates but increased trabecular bone volume and decreased osteoclasts [81]. At the same time, transgenic mice overexpressing BSP display mild dwarfism, lower bone mineral density and lower trabecular bone compared to wild-type mice [82]. BSP transgenic mice also show increases in osteoclastic activity and decreases in osteoblast populations, leading to an uncoupling of bone formation and resorption [82]. Therefore, finely-tuned BSP levels seems to be necessary for a proper balance between bone formation and resorption. Changes in BSP levels are also observed in cancer, evaluated both by serum levels and directly in tumors. Indeed, studies in multiple myeloma, breast, prostate, renal, thyroid, and lung cancers have shown elevated levels of BSP that often correlate with disease progression and bone metastasis [83–88]. Serum BSP may also serve as a prognostic index for some cancers, such as breast cancer metastasis to bone and bone destruction in myeloma patients [85, 88].

Multiple studies indicate that BSP is not simply a prognostic marker in cancer, but has direct roles in this process. MDA-MB-231 breast cancer cells transfected with human BSP display increased invasion *in vitro* and an enhanced primary tumor growth rate after being injected into mammary fat pads of nude mice [89]. Intra-cardiac injection of these BSP-expressing cells results in increased growth at metastatic sites, although the incidence of metastasis was the same between groups. In a similar study, MDA-MB-231 breast cancer cells transfected with human BSP, with or without antisense BSP cDNA, show a dose response in bone metastasis after intra-cardiac injection into nude mice [90]. Cells containing human BSP result in the most osteolytic bone metastases, followed by control cells, while antisense BSP cDNA containing cells result in the least bone metastasis. In addition, BSP in the bone

microenvironment contributes to cancer cell affinity for bone. BSP binding of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins on cancer cells promotes proliferation, adhesion, and migration, possibly through activation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) pathways [91–93]. BSP also forms a complex on the cell surface of BM stromal cells with MMP-2 and $\alpha_v\beta_3$ that facilitates migration through matrices, possibly representing a mechanism that enhances tumor cell migration and invasion [46]. Osteoclasts are also an important source of BSP in bone metastasis. One study found that osteoclast specific overexpression of BSP results in increased bone metastasis of 4T1 mouse breast cancer cells with greater osteolytic lesion areas and increased osteoclast numbers compared to wild type mice [94]. Therefore, BSP may help drive the “viscous cycle” of bone resorption and release of latent factors from the bone matrix. Accordingly, inhibiting BSP in breast cancer cells via a tet-regulated miRNA results in decreased migration, proliferation, and colony formation *in vitro* and reduced bone metastasis *in vivo* [95]. Treatment with an anti-BSP antibody reduces osteolytic lesion size and enhances bone formation in nude rats inoculated with MDA-MB-231GFP cells [96]. Finally, *in vivo* nanoparticle delivery of an antisense oligonucleotide to BSP decreases both bone metastasis incidence and size [97].

DMP1 is highly produced by osteocytes in bone and odontoblasts in teeth and is important for proper mineralization in these tissues [98, 99]. DMP1 expression is reported to be increased in numerous tumor types, including breast, colon, lung, prostate and thyroid carcinomas [100–102]. Tumor cell-derived DMP1 was found to increase invasion of colon cancer cells independent of BSP or OPN *in vitro* by forming complexes on the surface of tumor cells with MMP-9 and $\alpha_v\beta_3$ integrin, $\alpha_v\beta_5$ integrin, and/or CD44 [103]. However the effect of DMP1 in cancer appears cell type specific; a study in breast cancer shows that tumor cells expressing low levels of DMP1 are more likely to metastasize to bone, and inhibition of DMP1 *in vitro* promoted migratory capacity of non-invasive MCF-7 cells [104].

Similar to OPN and BSP, DMP1 in the bone microenvironment may support homing of cancer cells to bone through integrin mediated activation of focal adhesion kinase and subsequent activation of MAPK pathways, although this will need to be directly tested [105]. DMP1 could also be involved in the determination of osteolytic versus osteoblastic metastatic lesions by regulating of osteoblast function. One study showed that conditioned medium from an osteolytic prostate cancer cell line significantly reduced DMP1 expression in osteoblasts compared to conditioned medium from an osteoblastic prostate cancer cell line [106]. In addition, DMP1 may play a negative role in angiogenesis as DMP1 inhibits VEGF-mediated tube formation of HUVEC cells *in vitro* and overexpression of DMP1 reduces angiogenesis in a glioma model [107]. Overall, although it stands to reason that DMP1 aids in certain bone metastasis, as other SIBLING family members OPN and BSP do, it may play an inhibitory role in some types of cancer, such as breast cancer. More *in vivo* experiments are needed to clarify DMP1’s role in bone metastases of vary type of cancers.

Osteonectin (ON)

ON, also known as secreted protein, acidic, cysteine-rich (SPARC), is a calcium and collagen binding protein abundant in tissues undergoing remodeling, such as the gut mucosa,

healing wounds, and in the bone – particularly the pericellular matrix around osteoblasts and osteocytes [108]. Functionally, ON is anti-adhesive, modulates growth factor activity, and is an inhibitor of cell proliferation by inducing arrest in the G1 phase of the cell cycle [108]. ON is produced by osteoblasts in bone where it is important for proper bone matrix formation, as indicated by osteopenia seen in ON-null mice and decreased ON expression in osteogenesis imperfecta patients [109]. ON also modulates angiogenesis, particularly through interaction with VEGF-A, platelet-derived growth factor (PDGF), bFGF and TGF- β . In addition, ON is rapidly processed by proteases such as cathepsins and MMPs, with the resulting products having individual biologic activities [110]. As tumors are often described as “wounds that never heal,” it is no surprise that ON is implicated in various aspects of tumor biology. Indeed, the roles of ON in tumor progression have been intensively studied, however many of the results indicate that these functions are highly tissue and context dependent. Overexpression of ON is associated with aggressive phenotypes and poor outcomes in multiple cancers [111, 112]. Prostate cancer cell expression of ON increases in metastatic sites compared the primary tumor [113]. ON expression is also associated with decreased E-cadherin and increased N-cadherin expression in melanoma cells and is increased in less differentiated breast and prostate cancer cells, suggesting a role in epithelial-mesenchymal transition (EMT) [114, 115]. In contrast, some researchers report that ON has either no effect or antagonistic effects on tumor progression, even in these same cancer types [116, 117]. The different effects seen in different models may be the result of the tumor microenvironment as a whole, such as local concentrations of growth factors and proteolytic enzymes that interact with ON.

The function of ON in bone metastasis is equally complicated. One study shows preferential migration of PC3 prostate cancer cells to bone extracts from WT mice compare to SPARK-null mice [118]. The same study found that interaction of ON with cell-surface $\alpha_v\beta_3$ and $\alpha_v\beta_5$ upregulates VEGF expression in prostate cancer cells, potentially providing both a direct growth advantage and increased angiogenic potential for these cells in bone [119]. Prostate cancer cells are also upregulate ON in mouse osteoblastic cells. The increased ON from the osteoblastic cells enhances migration of prostate cancer cells, providing evidence that ON is involved in a feedback loop that supports tumor growth in bone in some cases [36]. Breast cancer cell migration is enhanced by recombinant ON, although ON is not found to be directly chemoattractive. However, ON is capable of enhancing migration towards vitronectin [120]. Another group found that overexpressing ON in MDA-MB-231 breast cancer cells results in decreased metastasis to multiple organ sites, including bone [121]. In addition, decreased ON expression has been suggested to be part of the gene signature of breast cancer bone metastasis [122]. Serum ON is inversely correlated with bone disease at the time of diagnosis in multiple myeloma patients, and another study reported hypermethylation of the SPARC gene leading to decreased ON expression in myeloma cells [123, 124]. Finally, intraosseous implantation of RM1 prostate cancer cells into ON knockout mice results in increased osteoclast numbers and osteolysis compared to WT mice [125]. Once again, the varying effects of ON on bone metastasis may be due to different compositions of specific microenvironments, illustrated by a study demonstrating that Cathepsin K produced by bone marrow stromal macrophages processes ON in PC3 prostate cancer bone tumors [126].

CCN family

The CCN family is a group of six structurally conserved matricellular proteins that plays are involved in apoptosis, ECM production, motility, proliferation and differentiation [127]. Initially named after the first three members, cysteine rich 61 (Cyr61, CCN1), connective tissue growth factor (CTGF, CCN2), and nephroblastoma overexpressed (Nov, CCN3), the family now includes the Wnt induced secreted proteins 1–3, or CCN4, CCN5 and CCN6 respectively [128]. CCN proteins all share an N-terminal secretory peptide followed by four conserved domains with sequence homologies to insulin-like growth factor binding proteins (IGFBP), von Willebrand factor type C repeat (VWC), thrombospondin type I repeat (TSR), and a carboxyl-terminal domain (CT) that contains a cysteine-knot motif [129]. Through these domains CCN proteins are capable of interacting with many other molecules, such as integrins, heparin sulfate proteoglycans, lipoprotein receptor-related proteins (LRPs), growth factors, and cytokines [130]. Among other tissues, CCN proteins are highly functional in bone where they are tightly regulated during chondrogenesis and osteogenesis and act as both positive and negative regulators of skeletal formation [130].

A number of studies have observed CCN family member dysregulation in cancer along with functional roles in tumor progression. Significant upregulation of CCN1 in a cohort of 120 breast cancer patient samples compared to 32 normal breast tissues is correlated with poor prognosis and metastasis [131]. CCN1 may also be a target for bone metastasis and related bone disease in breast cancer. Zoledronic acid (ZOL), a bisphosphosphonate that decreases bone loss and osteolytic lesion formation as well as has direct anti-tumor activity, has greater inhibitory effects on breast cancer cells expressing high levels of CCN1 compared to those with low expression. At the same time, ZOL decreases CCN3 expression in breast cancer cells through an Akt/Forkhead box O3 (FOXO3a) dependent pathway [132]. Another study found that an antibody against CCN1 upregulates MMP inhibitors TIMP1 and TIMP2, resulting in decreased migration and invasion of breast cancer cells *in vitro* and lymph node metastasis *in vivo* [133]. CCN1 is also implicated to play a role in metastasis of prostate cancer cells, although other studies indicate that CCN1 has contradictory functions in these cells by promoting both proliferation and TRAIL-induced apoptosis [134]. In myeloma, one study found that CCN1 in bone marrow mesenchymal cells is positively associated with longer time of progression from pre-malignant disease to overt myeloma and increased progression-free and overall survival of myeloma patients [135]. Interestingly, CCN1 is higher in random BM biopsies compared to focal myeloma lesions. CCN1 overexpression in myeloma cells also decreases tumor growth and osteolytic lesion formation *in vivo* [135]. Therefore, the authors of this study concluded that CCN1 may act as a protective mechanism by bone marrow cells against myeloma.

On the other hand, myeloma cells aberrantly express CCN2, and serum levels of CCN2 negatively correlates with osteolytic lesions in myeloma patients [136, 137]. In addition, CCN2 expression is upregulated in human breast cancer bone metastasis compared to a normal human epithelial cell line [138]. An earlier study identified CCN2 as part of a gene signature associated with bone metastasis but not with the metastasis to other sites of the body [62]. CCN2 is also expressed higher in overt bone metastatic breast cancer cells compared to early disseminated tumor cells in the bone marrow. These findings suggest that

CCN2 expression in cancer cells is possibly induced in bone and aids in the establishment of metastatic disease, possibly through TGF- β trapped in the bone matrix [62, 139]. Antibodies against CCN2 reduce osteolytic lesion area as well as osteoclast and endothelial cell numbers in MDA-MB-231 bone metastatic tumors [140]. The same study found that Parathyroid hormone-related protein (PTHrP) induces CCN2 gene expression in MDA-MB-231 breast cancer cells through PKA, PKC, and MAPK pathways. In addition, knocking down CCN2 in osteoclast precursor cells significantly reduces osteoclast formation *in vitro*. Finally, bone morphogenic protein-9 (BMP-9), a supporter of osteoblast differentiation, decreases bone metastasis of MDA-MB-231 cells by downregulation of CCN2 [141]. Microarray profiling of 58 breast cancer bone metastases shows CCN3 expression is increased in comparison to metastases to lung, brain and liver [142]. Overexpressing CCN3 in non-bone metastasizing 66c14 breast cancer cells does not affect primary tumor growth but enhances experimental bone metastasis. CCN3 can also decrease osteoblast differentiation while promoting osteoclast differentiation [143]. Similar results were seen in prostate cancer in which CCN3 promotes bone metastasis and tumor growth in bone [144]. CCN3 upregulates intracellular adhesion molecule-1 (ICAM-1) in prostate cancer cells and activates a signal transduction pathway that involves $\alpha_v\beta_3$ integrin, integrin-linked kinase (ILK), Akt and nuclear factor-kappa B (NF- κ B). A follow-up study found CCN3 is highly expressed in prostate cancer bone metastases and CCN3 in prostate cancer conditioned medium induces osteoclastogenesis. CCN3 also increases the RANKL/OPG ratio in osteoblasts in favor of osteoclast support [145]. These data strongly implicate CCN2 and CCN3 as bone metastasis promoting molecules in general.

CCN4 is produced by osteoblasts and is reported to stimulate migration and VCAM-1 expression in these cells. CCN4 from osteoblasts also inhibits the expression of miR-126, a negative regulator of VCAM-1, in prostate cancer cells [146]. Another study found that CCN4 is upregulated at early stages in prostate cancer tissues and serum and anti-CCN4 antibodies reduce experimental metastasis in a prostate cancer mouse model. Interestingly, CCN4 is concentrated at the tumor-bone interface [147]. CCN4 is also associated with advanced disease in breast cancer [148]. A recent report concluded that CCN4 acts as an oncogene in breast cancer cells, however a role of CCN4 in breast cancer metastasis to bone is yet to be identified [149]. In contrast, CCN6 inhibits breast cancer metastasis by modulating BMP4 (an important protein in bone and cartilage development) suggesting a possible link between CCN6 and bone metastasis that remains to be explored [150].

Other matricellular proteins as potential targets

Other studies have highlighted additional matricellular proteins that are likely involved in bone metastasis. Hevin, a member of the SPARC family of proteins, was found to inhibit cancer cell proliferation and suppress prostate cancer metastasis to bone among other sites [151, 152]. Hevin is downregulated in a number of primary tumors, including prostate cancer, and is further downregulated in metastatic prostate cancer compared to primary tumors [153]. The anti-metastatic functions of Hevin may be mediated by de-adhesive properties, and maintaining Hevin expression in tumors and metastatic niches may prevent tumor cell colonization of these sites [154]. This may occur as a natural anti-tumor response in the bone marrow as Hevin is upregulated in bone marrow cells upon interaction with

prostate or breast cancer cells [37]. The matricellular protein periostin is also enhanced in bone marrow cells as a response to tumor cells [158]. Serum periostin is elevated in breast cancer patients presenting with bone metastasis compared to those without [155]. Periostin expression in breast cancer cells is correlated with advanced disease, the ratio of cancer stem cells, and bone metastasis in 1,086 breast cancer specimens [156]. Experiments using an anti-periostin antibody in the 4T1 breast cancer model yielded decreased primary and metastatic tumor growth, and inhibited 4T1 breast cancer cell induced differentiation of osteoclasts *in vitro* [157]. This difference in periostin and Hevin regulation illustrates well the need for a balanced network of these molecules in the bone marrow. Studies investigating the fine-tuning abilities of matricellular proteins in combination could provide great insight into the importance of different molecules at different stages of metastasis.

Tenascins are yet another group of matricellular proteins that have a variety of tumor-promoting functions, however the relative importance of these proteins in bone metastasis is not clear [158]. Tenascin-C is expressed in the HSC niche where it can bind and promote proliferation of hematopoietic cells [159, 160]. Tenascin-C is upregulated in human bone stromal cells in response to prostate cancer cells [161]. In addition, breast cancer tumors in mouse bones induce tenascin-w expression in osteoblasts. Breast cancer cell lines with high bone tropism induce higher tenascin-w expression in bone marrow stromal cells, and tenascin-w promotes breast cancer cell migration and proliferation *in vitro* [162]. These interesting findings reveal tenascins may be altered in osteoblastic cells of the HSC niche, ultimately allowing takeover by tumor cells.

Thrombospondins (TSP) are particularly known for regulation of angiogenesis [163]. Both TSP-1 and TSP-2 induce apoptosis in endothelial cells by binding CD36 on the cell surface [164, 165]. In addition, TSP-1 can inhibit VEGF signaling by directly binding VEGF [166]. All five TSP members are expressed by cells in the bone and regulate bone functions such as MSC pool size, osteoblast differentiation, mineralization and osteoclast function [167–171]. TSP proteins are also implicated in tumor growth and metastasis. Although TSP-1 and 2 reduce tumor angiogenesis, ultimately it appears that this drives enhanced migration and invasion of tumor cells that could lead to metastasis [172–174]. On the other hand, TSP-1 in a bone marrow microvasculature model induces breast cancer cell dormancy, however eventual upregulation of both TGF- β 1 and periostin in sprouting neovasculature induces metastatic growth [175]. TSP-2 was identified as a differentially regulated protein in breast cancer, being significantly downregulated in bone metastatic tumors compared to primary tumors [35]. Both TSP-1 and TSP-2 are upregulated in the bone marrow of MM patients compared to healthy donors, but another study found TSP-1 levels in bone marrow plasma positively correlates with complete or very good responders to high-dose chemotherapy [176, 177]. Thus, it appears that TSPs may have different functions in the primary tumor versus metastatic sites.

Summary of matricellular protein impact on bone metastasis

Although not part of the structural component of the ECM, matricellular proteins modulate a number of cellular and biological functions that can alter the course of disease. Malignant cells in the primary tumor aberrantly express many matricellular proteins that support tumor

progression, such as OPN, BSP and ON. These changes can occur through alterations in transcription factor activation and function, such our group has shown with Runx2 in myeloma cells, or through miRNA regulation as others have reported for mir-218 [178, 179]. Regardless, these changes result in altered primary tumor growth and behavior, modulation of the tumor microenvironment, and appear to predispose some cancer cells for bone metastasis (Figure 1). At the same time, other proteins at the primary site, such as DCN, inhibit tumor progression. Downregulation of these proteins in either the malignant cells or non-malignant tumor stroma may result in an inability to contain a growing tumor. In addition, some matricellular proteins in the bone microenvironment are likely inherently attractive and supportive of certain tumors, providing a safe haven for disseminating tumor cells. Upon reaching the bone marrow microenvironment, interactions between tumor cells and bone-resident cells result in further adaptations by tumor cells as well as in an abnormal, dysfunctional stromal compartment (Figure 2). This includes increased bone resorption by osteoclasts, increased angiogenesis, altered mesenchymal stromal cell function, and in many cases defective osteoblasts. Thus, matricellular proteins not only directly support malignant cells but also mediate cross-talk that re-shapes the bone microenvironment in favor of tumor progression.

Conclusions

The ECM is an essential part of any microenvironment, including that of tumors. Mechanisms governing how certain tumors preferentially form bone metastasis remain unclear, although it is evident that manipulation of the ECM by tumor cells, local stroma, and cells in the bone marrow plays a role in this process. In this way, matricellular proteins provide interesting insight into the balancing act that controls tumor fate. There remain many unknowns in this field, such as the differing functions of various cleavage products, the importance of relative concentrations in different microenvironments, and the molecular drivers behind the changes in expression of these proteins. However, considering matricellular proteins modulate numerous components of tumor biology and the tumor microenvironment, targeting this group holds promise for attacking tumors at multiple fronts simultaneously, likely resulting in improved outcomes for cancer patients.

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Highlights

- Matricellular proteins modulate multiple aspects of tumor biology.
- Matricellular proteins are produced by tumor cells and non-malignant stromal cells.
- Matricellular proteins alter the bone microenvironment.
- Matricellular proteins contribute to cancer metastasis to bone.

Figure 1. Alterations of matricellular proteins in the ECM of primary tumors

Changes in expression of matricellular proteins at the primary tumor site are observed in many tumor types. These changes have been studied as diagnostic markers, but also affect multiple aspects of tumor biology including tumor growth, migration and invasion, angiogenesis and ultimately metastasis. Some of these proteins, such as ON, have context dependent effects that may involve different functions of cleavage products or relative abundance in the tumor tissue. Because matricellular proteins are involved in such a broad spectrum of tumor functions they may be useful targets for treatment in combination with traditional chemotherapy.

Figure 2. Matricellular proteins in the bone marrow microenvironment support tumor metastasis

Matricellular proteins mediate the interactions of tumor cells with bone-resident cells and the bone marrow microenvironment. (1) Proteins normally deposited in the bone matrix by osteoblasts and osteocytes, such as BSP and OPN, may inherently attract certain tumor cells to the bone through interactions with integrins on tumor cells. (2) Osteoblasts produce decorin that can inhibit tumor growth, however tumor cells decrease decorin in these cells and in many cases inhibit osteoblast function in general. (3) Decorin from stromal cells in the bone marrow is also reduced by tumor cells. Interaction with tumor cells alters expression of other matricellular proteins by stromal cells, such as tenascins, that may shift these cells to a more tumor-supporting phenotype. (4) Tumor cells can also induce angiogenesis through production of molecules such as OPN and CCN2. (5) Tumor cells drive osteoclast differentiation and function through a number of molecules including OPN, CCN2, CCN3 and periostin that results in release growth factors bound to the bone matrix. At the same time, osteoclasts produce matricellular molecules that reciprocally enhance tumor growth.