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Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases

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Abstract

Adipocytes are important but underappreciated components of bone marrow microenvironment, and their numbers greatly increase with age, obesity, and associated metabolic pathologies. Age and obesity are also significant risk factors for development of metastatic prostate cancer.

Adipocytes are metabolically active cells that secrete adipokines, growth factors, and inflammatory mediators; influence behavior and function of neighboring cells; and have a potential to disturb local milieu and dysregulate normal bone homeostasis. Increased marrow adiposity has been linked to bone marrow inflammation and osteoporosis of the bone, but its effects on growth and progression of prostate tumors that have metastasized to the skeleton are currently not known. This review focuses on fat-bone relationship in a context of normal bone homeostasis and metastatic tumor growth in bone. We discuss effects of marrow fat cells on bone metabolism, hematopoiesis, and inflammation. Special attention is given to CCL2- and COX-2-driven pathways and their potential as therapeutic targets for bone metastatic disease.

Keywords

Prostate cancer; Bone metastasis; Adipocytes; Inflammation; COX-2; CCL2

1 Introduction

Bone is a major component of the system that regulates energy metabolism [1, 2]. It is also a major site of metastasis from prostate cancer [3]. Bone metastases occur in 75–80 % of prostate cancer patients and have devastating consequences including bone fractures, pain, hypercalcaemia, and spinal cord compression [4, 5]. Age, obesity, and associated metabolic conditions are considered significant risk factors for aggressive prostate cancer (PCa) [6–15]. Almost 50 % of men with metastatic (M1) PCa are age 75 or older [14]. Independently of age, obesity increases the risk of developing high-grade PCa [16–19], having biochemical recurrence and disease progression after radical prostatectomy [20–23] and radiation treatment [24, 25], as well as increased rate of metastasis and PCa-specific death [26–28]. Notably, risk of developing metastatic disease appears to be 2–3-fold higher in obese and overweight compared to normal-weight men receiving the same treatment [29]. The mechanisms behind obesity-induced changes in the bone microenvironment and their impact on metastatic processes are not well understood.

Adiposity-driven chronic inflammation and oxidative stress are already known risk factors in many cancers including prostate [30–32]. They are also extremely likely to impact both the predisposition and the ability of the tumor to grow and thrive in the skeleton. Increased marrow adiposity is a known culprit in accelerated bone resorption and osteoporosis. Notably, the preferred sites for prostate tumor growth in the bone are the axial skeleton and long bone metaphyses, sites known to be under active remodeling and to have increased marrow cellularity [33]. The increased metastatic potential has been observed in response to experimental treatment with calciotropic hormone, androgen ablation, or cyclophosphamide [34–36]. An adipocyte-rich metabolically active red bone marrow appears to be particularly attractive to metastatic cells colonizing the bone [37, 38]. When enriched in metabolically active fat, the bone marrow niche has a potential to accelerate these events and become even more conducive to tumor growth and survival.

2 Bone-fat relationship: impact of marrow adiposity on bone homeostasis

2.1 Age-induced effects on bone

It is becoming recognized that the relationship between bone and fat formation is reciprocal [39, 40]. Bone marrow adipocytes and osteoblasts share common progenitor cells, known as bone marrow mesenchymal stromal cells (MSCs) [41, 42]. Their lineage commitment appears to be dynamically regulated by the presence of adipogenic (e.g., PPAR γ 2) vs. osteogenic (e.g., Runx2, Cbfa1) factors in the bone microenvironment [39, 43]. Adipocyte numbers in the bone marrow strongly correlate with age [39, 43–45]. Bone marrow of newborn infants is primarily hematopoietic and contains no fat, whereas 70 % of appendicular skeleton of adults is occupied by adipocytes [43, 45]. Parallel to an increase in fat content, bone acquisition is reduced during growth, and the osteoblast numbers and bone mass decrease [1, 43, 46]. Adipocyte-derived fatty acids accelerate osteoclast differentiation and prolong osteoclast survival [47], while inhibiting differentiation of MSCs to osteoblasts [48]. This is in line with observations from mouse models of aging, where increased adiposity and associated enhancement of PPAR γ 2 expression, as well as reduced Cbfa1

levels, correlate with decreased bone mass [49, 50]. In addition to changes in MSC fate, aging bone is associated with increases in osteoclast differentiation, process driven by PPAR γ and pro-osteoclastic cytokines: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κ B ligand (RANKL) [43, 44, 51, 52]. Together, these age-related changes in adipo-, osteoblasto-, and osteoclastogenesis pathways have been linked to low bone mineral density (BMD) and osteoporosis [43, 50].

2.2 Obesity and bone

Effects of age on bone metabolism and function are further complicated by obesity and associated metabolic pathologies. Traditionally, obesity was thought to be protective against dysregulated bone metabolism and osteoporosis, mainly because of positive impact of body weight on bone formation [53, 54]. This has been attributed specifically to mechanical loading-driven proliferation of osteoblasts and osteocytes through Wnt/ β -catenin pathway [53, 55]. However, the fact that bone marrow fat localizes to the trabecular area, the site of active bone remodeling, indicates its involvement in bone degradation [1]. Indeed, emerging evidence is increasingly suggesting that expanded adipose tissue mass positively correlates with low BMD [53], although the link between adiposity and increased risk of osteopenia and bone fractures appears to be age-, site-, and sex-specific [53, 56]. In men specifically, the risk of osteoporotic fractures positively correlates with percent body fat, waist circumference, and waist-to-hip ratio [56–58]. Notably, men also have larger amounts of bone marrow fat than age-matched women [1, 59, 60], an observation that further supports the link between marrow adiposity and accelerated bone remodeling. Additional evidence of negative impacts of obesity on bone health comes from diet-induced obesity mouse models. Increased marrow adiposity in obese mice is accompanied by decreases in trabecular bone volume and overall reduced BMD [61–63]. These structural changes are paralleled by increases in osteoclast activity, upregulation of bone-degrading proteases such as cathepsin K, and escalated levels of proinflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor α , (TNF- α) [53, 61–65]. Unfortunately, due to lack of controlled studies on effects of obesity on bone health in humans, the relationship between adiposity and bone turnover remains complex and controversial.

2.3 Marrow adipocytes and bone metabolism: tale of two adipokines

Bone marrow fat, known as yellow adipose tissue (YAT), is proposed to have a mixed white (WAT) and brown fat (BAT) characteristics [39, 66], or potentially even form a distinct class with unique properties [67]. The BAT-like phenotype appears to be involved in providing energy for hematopoietic and mesenchymal compartments [1]. In fact, it has been hypothesized that bone marrow fat can act as a localized energy reservoir and turn on *de novo* osteogenesis in emergency situations [1, 68]. Its WAT-like functions involve clearing and storing circulating triglycerides and regulating fatty acid metabolism [1, 69, 70]. This suggests that fat cell involvement in regulating events in the bone microenvironment is dynamic and complex.

For a long time, adipocytes have been considered as passive occupants of bone marrow niche or cells filling the spaces after trabecular bone loss [39, 43, 71]. In fact, adipogenesis was suggested to be a default pathway for MSCs that were not able to differentiate into

osteoblasts or chondrocytes [43], or a support system in a form of heat for hematopoietic cell development [1, 68]. However, there is a recently growing understanding that bone marrow fat is not inert; it serves as an insulin-sensitive endocrine tissue that affects bone mass, energy expenditure, and insulin metabolism [72, 73]. Marrow adipocytes secrete hormones, cytokines, and fatty acids that have profound effects on metabolism and function of other neighboring cells in the bone microenvironment [43, 45, 61, 63, 64, 74]. Fat cells, including those within bone marrow space, are a significant source of leptin and adiponectin, the adipokines whose receptors are expressed by osteoclasts and osteoblasts [1]. Both of these hormones have been shown to regulate processes in the bone.

Action of leptin on the bone appears to have both positive and negative consequences and is not fully understood [1, 53]. Circulating leptin levels increase in obesity [53, 75], but their correlation with bone mass and fracture risk in humans is not conclusive [1, 76], possibly due to leptin resistance [77]. A positive link has been demonstrated between serum leptin levels and BMD, especially in women, yet a number of other studies suggested no correlation [78]. Several *in vitro* studies demonstrated positive effects of this peptide hormone on osteoblast proliferation and suppression of osteoblast-dependent osteoclast recruitment [79–81]. In mice, a majority of studies indicated that leptin has a negative influence on bone metabolism and function stemming from its ability to enhance the sympathetic output to bone from the hypothalamus [77]. Yet, a number of other studies reported increased bone formation rate, higher mineral content and mineral density, and reduced number and size of bone marrow adipocytes that appear to be a result of peripheral effects of leptin on bone [1, 77, 82, 83]. In line with these results, ob/ob and db/db mice, both of which are leptin receptor-deficient, exhibit reduced bone mass coupled with significant increase in the number and size of adipocytes in the femoral marrow, suggesting anabolic effects of adipocyte-derived leptin on bone [1,78].

Adiponectin (ACRP30) is a peptide hormone with pivotal roles in glucose metabolism and energy homeostasis [84]. It circulates at much higher concentrations than other adipocyte-derived factors, and its levels are clearly inversely proportional to body mass index (BMI) and visceral adiposity [84, 85]. Its structure is surprisingly similar to that of TNF- α , a cytokine with dynamic roles in regulation of energy metabolism and insulin sensitivity [86]. This similarity may be the potential mechanism behind adiponectin's ability to mitigate the negative effects of TNF- α on insulin signaling [87]. Despite having clearly defined roles in glucose metabolism, adiponectin's effects on bone, similarly to those of leptin, are controversial and a subject on ongoing debate [1]. Based on a number of clinical studies, circulating levels of this hormone negatively correlate with BMD, particularly in older adults [1, 88], although a positive association between ACRP30 levels and fracture risk is only apparent in older men, and not older women, indicating potential effects of sex hormones in this process [1, 76]. Adding to the complexity of adiponectin's effects on bone are the observations from animal studies showing only transient or no effects on bone mass, and the parallel *in vitro* results demonstrating inhibitory effects of this adipokine on osteoclastogenesis and promoting effects on osteoblastogenesis [1, 86, 88–90]. In line with *in vitro* findings, the bone phenotype of adiponectin-deficient mice exhibits age-dependent

increase in trabecula volume and number, suggesting that adiponectin is indeed a likely contributor to the link between fat and bone mass [88, 90] that calls for further exploration.

2.4 Bone marrow adipocytes and regulation of hematopoietic niche

Numerous studies have linked chronic inflammation with visceral adiposity [91–93]; however, little is known on how bone marrow fat influences inflammatory processes in the skeleton. Bone marrow adipogenesis and osteogenesis are processes tightly linked to hematopoiesis, although their exact effects on hematopoietic stem cell (HSC) niche are not clearly delineated. Human bone marrow adipocytes have been reported to support differentiation of CD34⁺ of HSCs into myeloid and lymphoid pathways [94]. Accordingly, myelopoiesis was shown to positively correlate with increased adipogenesis and reduced osteoblastogenesis in SAMP6 mouse model of aging [46]. An enhancement in hematopoietic and lymphopoietic bone marrow cell populations was also demonstrated in diet-induced obese mice in correlation with increased marrow adiposity [74]. At the same time, lipid-filled adipocytes in the bone marrow have been linked to repression of growth and differentiation of HSCs [95, 96] and have been considered as the negative regulators of hematopoietic niche [1, 97]. This suppressive activity has been primarily attributed to the reduced production of granulocyte colony-stimulating factor (GM-CSF) and granulocyte stimulating factor (G-CSF) and increased secretion of neuropilin and lipocalin-2 [96, 98, 99]. Interestingly, while inhibiting HSC progenitor cells, adipocytes appear to positively affect the primitive HSCs via secretion of adiponectin and TNF- α [100, 101], a phenomenon proposed to play a role in preserving hematopoietic stem cell pool while preventing progenitor expansion [96]. Indeed, aging in humans and mice, a process associated with increased marrow adiposity [39, 43–45], induces myeloid-biased differentiation in HSCs [102], while promoting overall decrease in marrow cellularity [103]. Collectively, these studies underline the complex nature of bone marrow microenvironment and suggest that the hematopoietic environment in the marrow is governed by the dynamic relationship between adipocyte and osteoblast pathways.

Myeloid cells are the major cell type in undifferentiated bone marrow, which give rise to monocytes, macrophages, and granulocytes [36, 104]. Important contributors to their expansion in the bone marrow are proinflammatory, myelogenic cytokines such as interleukin 6 (IL-6) [36, 105]. Indeed, IL-6 is one of the bone marrow-derived inflammatory genes whose expression is highly upregulated, along with IL-1 and TNF- α in mice fed high-fat diet [63]. All three of these cytokines are highly present in adipose tissue and have been associated with obesity, adipose tissue dysfunction, and metabolic dysregulation [106–108]. They are also known mediators of osteoclastogenesis and bone resorption, predominantly through the regulation of the RANKL/RANK/ osteoprotegrin (OPG) pathway [53, 109]. Blocking TNF- α or IL-1 activity in ovariectomized mice attenuates osteoclast formation and prevents subsequent osteolysis of the bone [110], and neutralizing IL-6 reduces IL-1-driven bone degradation [111]. It has been documented that patients with periodontitis-, pancreatitis-, inflammatory bowel disease-, and rheumatoid arthritis-driven chronic inflammation exhibit accelerated bone resorption and bone loss [53]. Increased circulating levels of IL-6, TNF- α , and C-reactive protein (CRP) have been shown to positively correlate

with hip fracture risk in elderly men and women [112], results further underlining the link between proinflammatory events and dysregulated bone remodeling.

2.5 Adiposity and bone marrow inflammation: the role of CCL2/COX-2 axis

One of the key myelogenic molecules in the bone marrow is a C-motif chemokine ligand 2 (CCL2, MCP1) [36, 105], a low molecular weight monomeric polypeptide known largely for its role in obesity-induced infiltration of adipose tissue by inflammatory cells via its interaction with CCR2 receptor [113–116]. CCL2 overexpression is a major culprit of chronic inflammation in adipose tissue and a contributor to insulin resistance [117, 118]. Accordingly, CCR2 deficiency in a mouse model of diet-induced obesity attenuates the macrophage accumulation and significantly reduces inflammation and systemic insulin resistance [116]. Improved *in vivo* insulin sensitivity and reduced macrophage content are also observed in mice with preexisting obesity after short-term pharmacologic antagonism of CCR2 [116]. Within bone marrow microenvironment, CCL2 is expressed by several cell types and is an important player in regulating skeletal homeostasis [115]. In fact, CCL2 is one of the most strongly expressed genes in osteoporotic bone [119]. This cytokine significantly increases the number of bone-resorbing osteoclasts when coupled with RANKL [120]. CCL2 is also secreted by osteoblasts that have been stimulated with inflammatory factors [121]. Interestingly, osteoblast-derived CCL2 appears to be involved in monocyte recruitment [115, 122], and in line with these functions, CCL2 deficiency in mice results in higher bone mass [123].

Also important to skeletal metabolism and function is cyclooxygenase-2 (COX-2), an inducible enzyme responsible for the increased prostaglandin levels [124, 125]. COX-2 deficiency does not appear to have significant effects on overall skeletal phenotype, but its activity is essential for fracture healing [126, 127]. Notably, COX-2 overexpression in the bone has been tied to prostaglandin E₂ (PGE₂)-driven effects on skeletal metabolism [124]. Initially found to stimulate osteoclastogenesis and bone resorption, PGE₂ also has been shown to enhance bone formation [124]. Studies utilizing bone marrow cells from COX-2-deficient mice or treated with selective COX-2 inhibitors demonstrated that COX-2-induced endogenous PGs enhance pro-osteoclastogenic effects of several factors, such as IL-1, IL-6, IL-11, IL-17, TNF- α , PTH, vitamin D₃, and BMP-2 [124]. PGE₂ appears to have indirect effects on bone resorption either via upregulation of RANKL or by inhibition of its decoy receptor osteoprotegerin (OPG) expression in osteoblastic cells [124]. In general, the PGE₂ effects on bone appear to be time and context-dependent: continuous PGE₂ administration induces bone loss in rats, whereas an increase is observed with intermittent administration [128].

It is noteworthy that during inflammatory events, high expression of COX-2 is often coupled with CCL2 upregulation [129–131]. Specifically, its major metabolite PGE₂ is known to induce differentiation of immunoregulatory cells [125, 132], and importantly, COX-2 inhibitors appear to prevent CCL2 production by activated macrophages [133, 134]. Under normal physiological conditions, COX-2 expression in macrophages is low and increases in response to proinflammatory stimuli [135]. In fact, both the COX-2 expression and the PGE₂ release by macrophages have been shown to be stimulated by CCL2 and to be

important for macrophage migration [136–138]. Interestingly, studies from our laboratory demonstrate that bone marrow macrophage invasiveness through collagen I matrix increases with age (Fig. 1(a, b)) and is highly stimulated by bone marrow adipocyte-derived factors (Fig. 1(a, c)). Notably, age and obesity are also potent inducers of COX-2 and CCL2 levels in murine bone marrow cells (Fig. 1(d, e)), a finding suggesting that the CCL2/COX-2 axis may be playing a functional role in obesity-driven inflammatory events in bone.

3 Marrow adiposity and bone metastases from prostate cancer

3.1 Effect of adipocytes on prostate cancer cells

Numerous studies have suggested that the biological behavior of tumor cells is affected by adipocyte-supplied factors [139–144]. Adipocytes and associated inflammatory cells secrete adipokines and cytokines, which are known contributors to tumor development and progression [144, 145]. This is particularly true for cancers that grow in adipocyte enriched microenvironments or have a predisposition to metastasize to fat-rich sites (e.g., breast, gastric, and ovarian cancers) [144]. Adipocytes are capable of inducing repolarization of vimentin, downregulating E-cadherin, and promoting tumor cell invasiveness [143]. The presence of adipocytes in the tumor microenvironment leads to an abundance of lipids, which are critical for signaling, cellular trafficking, and migration, particularly when environmental glucose stores are low [146]. Transformed cells have the ability to utilize and store lipids including glycosphingolipids, sphingomyelin, and cholesterol to gain growth advantage in comparison to cells of normal epithelium [147]. Lipids appear to be particularly important to prostatic tumor progression. Prostate tumor cells depend on fatty acid synthesis, have capabilities to upregulate lipogenic enzymes, and utilize both *de novo* and dietary lipids to sustain growth and proliferation (reviewed in [148, 149]). Interestingly, the favored pathway utilized by prostate cancer cells to generate energy and sustain survival appears to be β -oxidation [144, 150]. Activation of this pathway has been suggested as a mechanism supporting tumor cell viability during conditions of high stress and especially in response to oxidative radical-enriched environments [146, 148].

In line with the evident importance of lipid metabolism to prostate tumor growth, there appears to be a positive association between adipocyte presence, especially within the visceral and periprostatic adipose tissues, and prostate cancer progression and poor prognosis [144]. Adipose tissue adipocytes have been shown to stimulate proliferation, differentiation, and invasive potential *in vitro* [140, 142]. Clinical studies have linked obesity with prostate cancer aggressiveness, biochemical recurrence, and incidence of metastases [7, 19, 20, 23, 28, 29]. Interestingly, metastases from prostate cancer most commonly occur in adipocyte-rich metabolically active red bone marrow [37, 38]. Tumor cells appear to be attracted to marrow adipocytes, and the interaction between the two cell types results in translocation of adipocyte-stored lipids to the metastatic tumor cells [38, 142], resulting in their increased motility [37]. In addition, data from our laboratory suggest that uptake of bone marrow adipocyte-derived fatty acids and upregulation of lipid chaperone fatty acid-binding protein 4 (FABP4) and IL-1 β in tumor cells drives their progression in the bone metastatic site [152]. However, despite increasing evidence linking skeletal metastases and death from prostate cancer with age and obesity, conditions which

are implicated in marrow adiposity [8, 14, 19, 21, 29], the mechanisms of marrow fat cell involvement in metastatic growth in bone are still not clearly understood and warrant further studies.

3.2 Regulation of tumor growth in bone by adipocyte-derived leptin and adiponectin

Clinical and epidemiological data provide conflicting reports on the role of leptin in prostate carcinogenesis [152], although positive associations have been found with more advanced, hormone-refractory disease [153]. *In vitro* studies suggest that this adipokine exerts mitogenic effects in prostate cancer cells via modulation of MAPK pathway [154], and its activity in tumor cells appears to be dependent on hormonal status and to require JNK activation [155]. In the bone microenvironment, effects of adipocyte-derived leptin on tumor growth have been suggested to be indirect and occur via leptin-mediated stimulation of bone resorption [156, 157]. In addition, leptin expression appears to be tied to COX-2/prostaglandin pathways. Specifically, PGE₂ has been shown to induce leptin expression and regulate lipolysis in murine and human adipose tissue [158, 159]. It is likely that COX-2 activity will also affect leptin signaling in the bone, with potential downstream effects on metastatic tumor cells.

Although not completely defined, the association of adiponectin with prostate cancer risk and progression appears to be better understood. Circulating levels of adiponectin are inversely correlated with obesity as well as prostate cancer incidence and aggressiveness [160, 161]. Based on limited *in vitro* studies, adiponectin is a potent inhibitor of prostate cancer cell growth and survival through downregulation of STAT3 signaling [162] and activation of AMPK pathway [161]. Adiponectin receptors are differentially expressed in prostate cancer cell lines and tissues, and their interactions with this adipokine vary depending on the androgen status [163, 164]. In addition, low adiponectin levels in combination with increased leptin secretion have been suggested to regulate prostate cancer progression via modulation of p53 and bcl-2 expression [165]. There have been no studies to date examining the direct role of adiponectin in growth and survival of tumor cells in bone. Similarly to leptin, adiponectin is likely to modulate tumor cell behavior at least in part via its known effects on bone cells and inhibitory effects on adipogenesis [166]. In addition, its reported role(s) in activation of COX-2-prostaglandin pathway and modulation of hematopoiesis [166, 167] suggest involvement in modulation of bone microenvironment with likely implications for affecting tumor growth. Further studies are needed to determine whether a direct interaction of this potent adipokine with receptors on metastatic tumor cells has any impact on their growth and survival in bone.

3.3 COX-2 in bone tumor microenvironment

COX-2 overexpression and aberrant signaling have been implicated in disease progression and decreased survival in several malignancies including breast, prostate, colon, bladder, and lung cancers [168]. COX-2/PGE₂ signaling is hijacked in carcinogenesis, predominantly to promote chronic inflammation and immunosuppression associated with tumor evasion of the immune system [169]. Studies have shown that COX-2/PGE₂ overexpression in breast tumor cells results in recruitment of regulatory T cells (Tregs) to the primary tumor site and the apoptosis of anti-tumorigenic CD8⁺ T cells [170]. Eventually, tumor cells progress and

metastasize to distant sites, including the bone, and the overutilization of COX-2 by tumor cells may be a selective advantage to metastasis and colonization in the skeletal sites [171]. In the context of bone metastatic disease, COX-2 and PGE₂ overexpressions are the major culprits in tumor-associated bone degradation [172, 173]. Increased COX-2 levels in breast cancer cells were shown to promote tumor colonization, osteoclastogenesis, and development of lytic lesions in a mouse model of bone metastasis [174]. In primary osteosarcomas, COX-2 inhibition suppresses proliferation of COX-2-overexpressing UTOS cells and decreased PGE₂ levels [175]. In metastatic melanomas, blocking of PGE₂ receptor with EP4 antagonist was shown to attenuate osteolysis of the bone [176], a process that was mimicked by use of selective COX-2 inhibitor celecoxib [177]. These effects were mediated by TNF- α , a potent stimulator of tumor proliferation and adhesion, and by COX-2-dependent VEGF overexpression [177].

Several studies have linked COX-2 expression and activity with prostate tumor progression and metastasis. Selective COX-2 inhibition was shown to suppress PC3 tumor growth *in vivo* via induction of tumor cell apoptosis and decreased angiogenesis due to VEGF downregulation [178]. One of the key factors implicated in COX-2 production by the tumor cells is tumor milieu-derived transforming growth factor β (TGF- β) [179]. PC3 cells treated with exogenous TGF- β exhibit elevated COX-2 expression and increased migration and invasion, events appearing to be linked to the activation of PI3K/Akt/mTor pathway by TGF- β and PGE₂ [179]. TGF- β -driven regulation of COX-2 expression and activity is particularly relevant to progression of metastatic tumors in bone. Indeed, TGF- β is known to stimulate bone resorption via production of pro-osteoclastogenic prostaglandins [180].

The role of prostaglandins in tumor-induced osteolysis of the bone is further evident from studies in mice injected intratibially with PC3 cells that have been transfected with a decoy fragment of PGE₂ [181]. Suppression of PGE₂ pathway resulted in reduced tumor growth, decreased osteolysis, and increased tumor cell death, in parallel with attenuated expression of COX-2 and other pro-inflammatory factors (e.g., IL-1 β and IL-6) [181]. Interestingly, in a recent elegant study by Liu et al., COX-2 expression in tumor-associated MSCs was shown to be induced by tumor cell-derived IL-1 β , a process that promoted skeletal growth and progression of prostate PC3-ML tumors and was inhibited by IL-1 β inhibitor, anakinra [182]. This is in line with previous reports suggesting that in addition to tumor cells themselves, cells of the tumor-associated stroma are key contributors to increased COX-2 expression [183, 184]. In fact, tumors that arise from IL-1 β -producing carcinoma cells appear to induce COX-2/mPGES1/PGDH/PGE₂ response in MSCs to support own growth and progression [182, 184].

3.4 CCL2/CCR2 axis and metastatic tumor growth in bone

Growing evidence suggests critical involvement of CCL2/CCR2 axis in modulating the bone microenvironment to support tumor colonization and growth [115]. An important study utilizing bone metastasis tissues from rapid autopsy program at University of Michigan has demonstrated that patients with prostate tumor metastases to the spinal cord have increased levels of CCL2 in the skeletal lesions compared to soft tissue tumors [185]. The same group also revealed that endothelial cells are one of the major sources of bone marrow-derived

CCL2 involved in macrophage/monocyte recruitment [185, 186]. In addition to their expression in endothelial cells, CCL2 and its receptor CCR2 have been localized to other cell types in the bone microenvironment, including the metastatic prostate cancer cells [186, 187]. This localization to various components within the marrow space appears to promote an autocrine/paracrine signaling that aids in tumor growth and survival [115]. For instance, recent studies from our laboratory have shown that bone marrow macrophages contribute to increased CCL2 levels in the bone marrow in response to prostate tumor challenge [188]. We have provided evidence for the existence of paracrine signaling between macrophage- and tumor cell-derived CCL2/CCR2 axes, which supports previous reports on prolonged survival and abolished metastasis in response to simultaneous blockade of tumor- and macrophage-derived CCL2 in mice bearing prostate and breast tumors [186, 189, 190].

The pivotal role for CCL2 in bone metastasis has been largely attributed to its effects on osteoclast differentiation and function [115]. Metastatic prostate cancer cells appear to secrete much higher levels of CCL2 compared to primary tumor cells [191]. Tumor cell-derived CCL2 promotes osteoclast differentiation [191, 192] that can be attenuated by CCL2 neutralization [193]. Accordingly, prostate cancer cells that overexpress CCL2 show higher incidence of tumor metastasis and tumor-induced osteolysis of the bone [193]. In line with these findings, targeting CCL2 expression in tumor cells with shRNA leads to reduced bone destruction and osteoclast presence in the tumor [194]. On the other hand, expression of CCL2 by bone-building osteoblasts may also be important to tumor progression in bone, particularly in a context of development of blastic lesions, a common occurrence in prostate cancer [115]. Under normal conditions, CCL2 expression by osteoblasts is low, and its upregulation is stimulated by inflammatory factors and associated with recruitment of monocytes [115]. It is currently not known if osteoblast-derived CCL2 has any direct effects on tumor cells in bone. However, given the fact that the osteoclast-osteoblast pathways are tightly coupled in the bone microenvironment and both are key regulators of bone homeostasis, the activity of CCL2 is likely to be a determinant of how either pathway impacts prostate tumor progression and survival in the marrow niche.

There is no doubt that CCL2 is emerging as a key contributor to site-specific metastasis from prostate and several other cancers [115]; yet the pathways associated with tumor addiction to CCL2 are only beginning to be uncovered. A recent study reported that N-cadherin expression is increased with tumor grade and directly regulates tumor CCL2 production through PI3K/Akt signaling and tumor neovascularization [195]. Additional studies uncovered a possible role for CCL2 in pro-tumorigenic effects of cyclophosphamide, a DNA alkylating chemotherapy drug [36]. Specifically, cyclophosphamide-treated tumor-bearing mice grew larger tumors, displayed significant vascular destruction, and promoted prostate tumor seeding and metastasis in bone, effects that were abrogated with CCL2 inhibition [36]. This suggests that CCL2 signaling may be a critical factor behind chemotherapy-induced bone remodeling and consequent successful tumor adaptation in bone.

3.5 COX-2 and CCL2 pathways: common link between marrow adiposity and bone metastasis?

Clear, positive links have been established between obesity and CCL2 expression [114, 116, 118]. Similar associations have been made between COX-2 and obesity-induced inflammation and insulin resistance [196]. In addition, both factors have been strongly implicated in prostate tumor progression and metastasis [115, 124, 125, 197]. The activities and functions of CCL2 and COX-2 appear to be closely connected, by the virtue of the fact that both factors are often overexpressed together [129–131], and inhibition of one appears to affect expression of the other [133, 134, 198].

Studies on placing CCL2 and COX pathways as potential links between adiposity and prostate cancer are just beginning to emerge. Increased CCL2 and CCR2 expressions were recently implicated in accelerated tumor progression in prostate tumor-bearing mice on high-fat diet (HFD) [199]. In addition, we have shown recently that inflammatory events during prostate tumor progression in bone involve both the CCL2/CCR2 and COX-2 axes [188]. Interestingly, both the CCL2 and COX-2 pathways appear to be also highly upregulated in prostate bone tumors from mice with diet-induced obesity (Fig. 2). HFD is a known inducer of bone marrow adiposity [61, 63, 64, 152]. We utilized our established xenograft intratibial model of prostate bone tumor growth [188], which we have exposed to low-fat diet (LFD) vs. HFD diets [152] and assessed host (murine)- and tumor (human)-derived CCL2 and COX-2 expression. Our data show that host CCL2 and COX-2 are upregulated in tumor-bearing tibiae of mice receiving normal (LFD) diet (Fig. 2(a)), a result in agreement with our previous findings [188]. Notably, levels of host-derived CCL2 and COX-2 are highly escalated in mice on HFD (Fig. 2(a)) in parallel with accelerated tumor progression in bone [152]. Levels of human tumor-derived CCL2 are also highly increased in bone tumors (Fig. 2(b)) but not subcutaneous tumors (Fig. 2(c)) from HFD mice, suggesting specific functional involvement of CCL2/CCR2 axis in adiposity-driven bone tumor growth. Human tumor-derived COX-2 levels are low under both LFD and HFD conditions (data not shown) suggesting that the majority of COX-2 activity in the bone tumor microenvironment is host-supplied. Collectively, these results underline the involvement of CCL2 and COX-2 pathways in growth and progression of prostate tumors in bone and suggest that these pathways may be functionally tied to adiposity-driven metastatic events in the skeleton.

3.6 COX-2 and CCL2: therapeutic implications

Bone marrow niche is a complex and dynamic environment where multiple cell types, factors, and events shape the development and progression of metastatic disease. Inflammatory pathways, which under normal physiological conditions are involved in the maintenance and protection of normal bone homeostasis, become “hijacked” by the tumor to promote its growth and survival. COX2 and CCL2 are such examples of inflammatory factors that are important to normal bone function, but when present in overabundance under conditions of high adiposity and inflammation, have a potential to modulate the microenvironment to support aggressive tumor behavior.

Due to strong evidence of CCL2 involvement in prostate cancer cell proliferation, migration, and invasion, as well as tumor growth in bone [115, 186, 187, 191], this chemokine is becoming a potential target for therapy in men with metastatic disease. Specifically, two humanized monoclonal antibodies have reached phase II clinical trials: Carlumab (CNTO 888), targeting CCL2 and MLN1202 against its receptor CCR2 (Table 1). Carlumab showed promise in preclinical studies both as a single agent slowing prostate tumor growth and reducing macrophage infiltration in mice, and in combination with docetaxel where it induced tumor regression and reduced tumor burden [200]. Unfortunately, a phase II study with Carlumab in metastatic prostate cancer patients with castrate-resistant disease who failed prior docetaxel treatment did not reach clinical objective [200]. Less than sufficient suppression of the target was suggested as a potential reason for the lack of therapeutic benefit. The outcomes of phase II trial with MLN1202 are currently not known.

There is also mounting evidence based on preclinical and epidemiological studies that COX-2 inhibition may have therapeutic benefits for prostate cancer patients [201–206]. Several phase II and III clinical trials have been initiated to evaluate the efficacy of Celecoxib in advanced prostate cancer (Table 1). Aspirin, a nonspecific NSAID, is also being examined in combination therapies for localized as well as metastatic disease [207, 208]. NSAIDs have been previously demonstrated to inhibit tumorigenesis through proapoptotic, anti-tumorigenic, and immune-modulating mechanisms [209, 210]. They have been shown to inhibit constitutive and RANKL-induced NF κ B activation in osteoclasts [211]. One of the suggested mechanisms of aspirin-mediated effects on prostate tumor cells is modulation of prostaglandin receptor subtype EP3 [212].

It appears that inhibiting CCL2/CCR2 and COX-2 pathways may offer several benefits, particularly in a context of blocking adiposity-induced inflammation and maintaining homeostasis in the bone microenvironment. More studies are needed, however, to fully elucidate the mechanisms and benefits of targeting these axes in bone metastatic disease.

4 Conclusions

At present, metastatic prostate cancer remains an incurable disease, and treatments that are available to patients with skeletal lesions are mainly palliative [213]. Targeting the prostate tumors that have colonized the bone has proven extremely challenging due to complexity and dynamic nature of the bone marrow metastatic niche. The contribution of marrow adipose tissue to metastatic events in the bone is understudied and not well understood. Although considerable research has been dedicated to examining effects of marrow adiposity on bone health, especially in the context of osteoporosis [39, 45, 214, 215], little is known about the role of marrow fat cells in tumor cell adaptation and growth in the skeleton. Clearly, adipocytes have a potential to be active modulators of bone tumor microenvironment via direct interactions with neighboring cells as well as through secretion of adipokines and inflammatory mediators ([141–144] and Fig. 3). Understanding how they influence prostate tumor metabolism, behavior, and mechanisms of adaptation and survival in the bone marrow niche may reveal unique therapeutic targets and treatment opportunities for metastatic disease.

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Fig. 1.

Age and adiposity increase bone marrow macrophage (BMM) invasiveness and induce COX2 and CCL2 expression in the bone marrow, *a* Images of invasion filters coated with collagen I matrix. Bone marrow macrophages from 2- and 6-month-old FVB/N5 mice were plated on top of the filter (120,000 cells/filter) and allowed to invade toward control growth medium (*control*) or medium conditioned by bone marrow adipocytes (*Adipo CM*). Cells were allowed to invade for 24 h, and filters were fixed and stained with Diff-Quik dye. *b, c* Quantitation results showing numbers of invaded cells/filter $\pm$ SD. *b* Comparative analysis of invasiveness of 2- and 6-month-old BMMs under control conditions; *c* comparative analysis of 2- and 6-month-old BMMs under control and Adipo CM conditions. Three independent biological replicates with six images/filter each were analyzed, *d, e* Taqman RT-PCR analysis of CCL2 (Macrophage Chemoattractant Protein; Life Technologies probe ID: Mm00441242_m1 and COX-2 (cyclooxygenase-2; Life Technologies probe ID: Mm00478374_m1) expression in murine bone marrow in a context of age (*d*) and obesity (*e*). *d* Two months old (gray) and 6 months old (blue). Data are shown as fold increases relative to 2-month-old bone marrow, *e* LFD (green) and HFD (red) mice. Data are shown as fold increases relative to LFD mice. All data are normalized to 18S (Life Technologies probe ID: Mm03928990_m1)

Fig. 2.

Host (murine) CCL2 and COX-2 and tumor (human) CCL2 are increased with obesity in PC3 prostate bone tumors. FVB/N/N5, Rag-1^{-/-} mice caged in groups of four were started on either a LFD (10 % calories from fat; Research Diets no. D12450Bi) or a HFD (60 % calories from fat; Research Diets no. D12492i) at 5 weeks of age [152]. Mice were maintained on respective diets for 8 weeks prior to and 6 weeks following the PC3 tumor implantation into bone or subcutaneously (total of 14 weeks). *a* Taqman RT-PCR analysis of murine (host) CCL2 (Macrophage Chemoattractant Protein; Life Technologies probe ID: Mm00441242_m1) and COX-2 (cyclooxygenase 2; Life Technologies probe ID: Mm00478374_m1) in prostate bone tumors from LFD (green bars) and HFD (red bars) mice. Data were normalized to murine HPRT-1 (Life Technologies probe ID: Mm01545399_m1). *b*, *c* Taqman RT PCR analysis of human (tumor)-derived CCL2 (Life

Technologies probe ID: Hs00234140_m1) in PC3 bone tumors (*b*) and PC3 subcutaneous tumors (*c*). Data were normalized to human HPRT1 (Life Technologies probe ID: Hs02800695_m1). At least three biological replicates were analyzed for each group, and data are shown as fold changes $\pm$ SD

Fig. 3. Proposed model of adiposity-induced inflammation and its effects on progression of metastatic tumors in bone. The increased amount of marrow fat content is a consequence of age and/or obesity. Prostate cancer cells that have colonized in adipocyte-rich bone marrow niche thrive and progress via CCL2/CCR2- and COX-2-dependent pathways due to (1) increased inflammation driven by adipocyte-macrophage interactions, (2) dysregulated bone remodeling via CCL2 and COX-2 actions on osteoclasts and osteoblasts, and (3) activation of pathways downstream of CCL2/CCR2 and COX-2 axes to promote tumor growth and survival

Table 1

Clinical development of anti-CCL2/CCR2 and anti-COX-2 therapeutics

Agent/company	Mechanism of action	Preclinical findings	Clinical trials in prostate cancer
CCL2			
Carlumab (CNTO 888) Centocor, Inc. Malvern, PA	Human monoclonal antibody with high affinity and specificity for CCL2	Reduced incidence of tumor metastases, tumor burden, macrophage infiltration, and bone lesions in mice [186, 216]	- Phase I clinical trial in patients with solid tumors [217] - Phase II of metastatic prostate patients [200, 218]
MLN1202 SWOG, Portland, OR	Human monoclonal antibody against CCR2	N/A	Reached phase II clinical trial in patients with bone metastasis [219]
COX-2			
Celebrex® (Celecoxib) Pfizer Inc, Mission, KS	NSAID that selectively targets COX-2	Prostate tumor regression, apoptosis, and inhibition of angiogenesis [201, 204, 220]	- Phase II clinical trial in patients with localized prostate cancer [202] - Pilot study on Celebrex in patients with advancing or metastatic prostate cancer [203] - Phase II clinical trial of men with localized prostate cancer [221] - Phase II/III clinical trial investigating the effects of Celebrex and other antitumor therapies in patients with advanced and metastatic disease [222] - Phase II clinical trial investigating effects of Celebrex in patients with relapsed prostate cancer after radiation or prostatectomy [223]
Aspirin	NSAID that targets anti-inflammatory proteins including COX-2	Reduces tumor growth [212] and decreased prostate cancer risk [224]	- Phase III clinical trial investigation combination therapy of aspirin, dexamethasone, and diethylstilbestrol in patients with advanced or metastatic prostate cancer [208] - Clinical trial investigating the changes in COX-1 and COX-2 expression in prostate issues in patients with low grade prostate cancer [207]