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Molecular Interaction of BMAT with Bone

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Abstract

Purpose of Review Bone marrow adipose tissue (BMAT) is a distinct adipose tissue with diverse local and systemic effects, affecting both physiological processes and pathological conditions, including hematopoiesis, bone remodeling, osteoporosis, obesity, anorexia nervosa, diabetes, and cancer. BMAT increases with age and bone loss, while the significance of this phenomenon has been neglected until recently. Bone cells and BMAT are mutually connected in terms of bone remodeling and energy metabolism. It has been suggested that high BMAT is caused by a shift in bone marrow mesenchymal stromal cell (BMSC) differentiation in favor of adipogenesis, and BMAT promotes bone loss through direct or indirect interaction with bone cells. However, it remains unclear why osteoporosis accelerates BMAT accumulation and what is the role of BMAT in bone remodeling and particularly in bone loss. The purpose of this review is to present the latest published data on the role of BMAT in physiological bone processes and during osteoporosis progression.

Recent Findings BMAT secretes numerous endocrine factors designated as adipokines as well as pro-inflammatory cytokines, which affect bone homeostasis through the regulation of osteoblast and osteoclast function. Most clinical data from osteoporotic patients demonstrate a negative relationship between BMAT and bone mass. Through technological advances in BMAT imaging, investigators are now able to quantify BMAT in humans and animal models. Pharmaceutical interventions targeting either bone loss or BMA expansion shed light in the understanding of the possible interactions between BMAT and bone cells.

Summary A neglected feature of osteoporosis progression is BMAT development. BMAT appears as a "new tissue" with unique properties, which undoubtedly plays important physiological and pathological roles, but which remains insufficiently understood.

Keywords Osteoporosis · Bone marrow adipose tissue · Bone remodeling · Animal models · Molecular mechanisms

Introduction

Bone integrity is maintained through a dynamic process, known as bone remodeling, on trabecular and cortical bones, resulting from a continuous balanced interplay between bone resorption caused by osteoclasts and bone formation employed by osteoblasts. Osteoclasts initiate the remodeling cascade by removing bone matrix and subsequently osteoblasts refill these cavities with bone matrix [1]. Disorders of bone loss, such as osteoporosis, are associated with increased rates of bone remodeling resulting in bone loss due to an overwhelming osteoclastic activity. Receptor activator of NF-κB ligand (RANKL) is a central regulator of bone remodeling by mediating osteoclast-induced bone resorption, through binding to its transmembrane receptor RANK, while it is naturally inhibited by the soluble decoy receptor osteoprotegerin (OPG) [2]. Osteoblasts are derived from bone marrow mesenchymal stromal cells (BMSC), which are multipotent and can give rise to several other distinct cell types, including adipocytes. An imbalance in the regulation of osteoblast and adipocyte differentiation is commonly observed in osteoporosis, which is associated with increased rate of bone marrow adipose tissue (BMAT) formation.

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BMAT, an adipose tissue that lies within bone marrow, has been surprisingly disregarded for many decades. In the past years, BMAT was considered as an inactive filler of the bone marrow (BM) cavity that substitutes hematopoietic cells in response to a decreasing demand for hematopoiesis. It is only in recent years that BMAT has become recognized as a specific and active fat depot, with recent advances suggesting that BMAT differs from other fat depots not only anatomically but also developmentally, functionally, and metabolically [3, 4]. Because of these unique characteristics, BMAT appears today as a "new tissue" with much potential for revealing new mechanisms on human health and disease. During the last two decades, several lines of evidence support the impact of BMAT expansion in bone, metabolic and nutritional diseases including osteoporosis, obesity, anorexia nervosa, diabetes and cancer, whereas the molecular mechanisms remain insufficiently understood [5].

BMAT Progression

In humans, BMAT is virtually absent at birth and bone cavities are mainly filled with red hematopoietic marrow, while BMAT is innately programmed to expand during life, leading to a conversion of the red marrow to fatty "yellow" marrow [6, 7]. Interestingly, a medium-aged, healthy lean adult is estimated to have BMAT that corresponds to approximately 10% of the total fat mass, and by the age of 25 BMAT occupies 50 to 70% of BM volume [8]. BMAT initially forms at terminal phalanges, and then expands towards other peripheral skeletal sites such as appendicular skeleton and eventually in the axial skeleton [9]. BMAT initiates at femur and tibiae bones at the age of 7 and continues until the age of 18. Thus, BMAT arises during normal human development, increases with age, and represents a major class of adipose tissue. In mice, BMAT begins at the distal tibia around day 7, and expands during body maturation and progression but with decreased rates compared to humans. Notably, 12-week-old C57BL/6J male mice have less than 1% BMAT volume in the tibia diaphysis, as shown by osmium tetroxide staining combined with micro-computed tomography (microCT) [10••].

Two distinct populations of marrow adipocytes have been identified, constitutive and regulated. Constitutive marrow adipocytes (cMAT) form a stable dense fat depot arising at early stages of life, occupy distal parts of the appendicular skeleton and are less responsive to stimuli [10••]. On the other hand, regulated marrow adipocytes (rMAT) are located scattered within the hematopoietic marrow of the axial skeleton and the proximal appendicular bones, and their formation and expansion occur at later phases of life in response to nutritional, hormonal, and temperature challenges [10••, 11].

Volumetric BMAT analysis is performed either with invasive or non-invasive imaging methods. Invasive methods include histological analysis of bone biopsies [12], while during the last decade, non-invasive imaging approaches were developed to visualize and quantify BMAT, including magnetic resonance imaging (MRI), which is the gold standard method to estimate BMAT [13, 14] and magnetic resonance spectroscopy (MRS), which determines the fat fraction and the fat composition distinguishing saturated from non-saturated lipids [15–17]. To further study the interactions between BMAT and bone remodeling, these methods are combined with measurements of bone mineral density (BMD) and structure with dual-energy x-ray absorptiometry (DEXA) and PET-microCT [18, 19]. Lately, the introduction of osmium tetroxide staining combined with micro-CT opens new horizons in the visualization and quantification of BMAT in rodents [20••]. Based on the abovementioned imaging methods, it has been recently shown that BMAT positively correlates with BMD in 4- to 10-year-old children [21], while a clear negative correlation between BMAT and bone mass was identified during aging and osteoporosis, suggesting an interplay between BMAT and bone remodeling.

BMAT in Osteoporosis

Osteoporosis is a multifactorial metabolic disease which is characterized by low bone density, reduced bone quality, and increased risk of fractures [22]. Osteoporosis is usually underdiagnosed and undertreated because of the lack of symptoms and it is often referred as the "silent epidemic" since one in three women and one in five men above the age of 50 will experience osteoporotic fractures. Apart from bone phenotype, osteoporotic patients also exhibit high BMAT. Various clinical studies have demonstrated that osteoporotic patients have 10% higher BMAT compared to age-matched healthy subjects [23–26]. All these clinical observations support a negative correlation between BMAT and bone mass. Numerous studies applying MRI methodology showed that healthy adults display an increase of BMAT in spinal vertebrae at a rate of 7% every 10 years and a comparison between sexes revealed that BMAT is 10% higher in men than in women the period before menopause, but the ratio reverses after menopause [27, 28].

Several clinical trials studying the effectiveness of anti-osteoporosis treatments have reported effects on BMAT. Various studies demonstrate that anti-resorptive drugs effectively reduce BMAT expansion in osteoporotic women. For example, postmenopausal osteoporotic women treated with estrogen either at a long or at a short term showed a decrease in BMAT as assessed by bone biopsies or MRI [29–31]. Similar results were shown upon treatment with the parathyroid hormone teriparatide [32••]. In addition, osteoporotic women treated with the bisphosphonate risedronate showed a reduction in BMAT and improved BMD compared to the control group [33–36, 37•, 38•]. The new anti-osteoporotic drug romosozumab, an antibody that targets the anti-

anabolic protein sclerostin, efficiently improves bone mass and ameliorates BMAT [39]. Even though most intervention studies targeting improvement in bone quality manage to ameliorate BMAT, there are limited data regarding the opposite, i.e., the effect of BMAT treatment in bone mass.

The spatiotemporal pattern of BMAT formation in rodents is considered to be quite similar to humans. Through histological analysis and osmium tetroxide staining, it has been demonstrated that the BMAT accumulation in long bones substantially increases with aging. However, the percentage of BMAT in rodents is lower compared to humans and varies among different mouse strains [10••]. The negative association between high BMAT and low BMD in osteoporotic patients [40] was reproduced in ovariectomized mice [41, 42•] and other genetic osteoporotic mouse models [43••]. The use of animal models of osteoporosis can substantially improve our understanding on the pathophysiological mechanisms involved in bone resorption and BMAT expansion. Our group has recently established two distinct genetic mouse models of osteoporosis through the expression of human RANKL (huRANKL) in transgenic mice (TgRANKL). The mild osteoporosis Tg5516 model expressing huRANKL at low levels develops trabecular bone loss and adjacent increase in BMAT, while the severe osteoporosis model Tg5519 overexpressing huRANKL displays trabecular bone loss, cortical porosity, and extended BMAT throughout the BM cavity [43••]. Based on these observations, it seems that BMAT recruitment is linked with sites of active bone resorption. However, it is still unclear how BMAT and bone loss are linked during osteoporosis and which process precedes the other.

The impact of bone resorption on BMAT formation has been confirmed in various clinical and animal studies through pharmaceutical inhibition of bone resorption [29, 30, 33, 37•, 44]. However, it remains unclear whether BMAT affects bone resorption in vivo since the current data are limited [41]. Paradoxically, pharmaceutical inhibition of BMAT with a PPARγ2 antagonist bisphenol-A-diglycidyl-ether (BADGE) in normal and diabetic male mice as well as genetic studies in PPARγ knockout mice demonstrated that the loss of BMAT induced osteogenesis due to increased osteoblast activity without affecting osteoclasts and bone loss [45, 46]. Therefore, more efforts must be made in the understanding of BMAT effect on bone resorption and in osteoporosis.

Molecular Basis of BMAT Interaction with Bone Cells

First of all, BMAT could contribute as an energy supply, through lipid release, to neighboring cells like osteoblasts and hematopoietic stem cells (HSCs). The increased adipocyte numbers observed during aging and osteoporosis, may contribute to the energy maintenance of bone cells and HSCs. This hypothesis is mainly supported by in vitro experiments, where it is demonstrated that adipocytes can transfer free fatty lipids (FFAs) to hematopoietic cells through a controlled process termed lipolysis to support proliferation and survival [47, 48].

As both BM adipocytes and osteoblasts are derived from BMSCs, also known as skeletal stem cells (SSCs), it is reasonable to assume that increased BMAT formation is associated with a reciprocal suppression of osteogenesis [49–51]. Distinct sets of transcription factors are activated during the commitment of precursor cells into osteoblasts or adipocytes. Runt-related transcription factor 2 (RUNX2) and transcription factor Sp7 (Osterix) regulate osteoblast differentiation [52, 53], while CCAAT/enhancer binding proteins (C/EBP) and peroxisome proliferative activated receptors gamma (PPARγ) promote adipogenesis [54–57]. In animal model studies, it has been demonstrated that upregulation of PPARγ results in high BMAT and low bone mass, while downregulation of PPARγ leads to a low BMAT and high bone mass phenotype [45, 58–61].

The differentiation of BMSCs to either adipocytes or osteoblasts is a two-step process, including lineage commitment and maturation. The lineage commitment of BMSCs is fine-tuned by the action of various extracellular matrix components, growth factors, cytokines, and chemokines, which in turn activate a cascade of signaling events regulating the key transcription factors such as PPARγ and C/EBPs or Runx2 and Osterix for adipogenesis or osteogenesis, respectively. The lineage commitment of BMSCs towards adipocytes or osteoblasts is regulated by a complex network of signaling pathways including transforming growth factor-beta (TGFβ)/bone morphogenic protein (BMP) signaling, wingless-type MMTV integration site family (Wnt) signaling, Hedgehogs (Hh), Notch, and fibroblast growth factors (FGFs) [62–70]. In general, the activation of Wnt signaling and Hedgehogs induces osteogenic differentiation, while activation of TGFβ/BMP, Notch, and FGFs signaling may exert a dual effect either favoring osteogenesis or adipogenesis depending on the ligand. These observations clearly show that all signaling pathways do not take place individually but rather act synergistically to promote BMSC's shift depending on the stimuli. Estrogen deficiency, increased glucocorticoid levels, oxidative stress, and immobilization favor adipogenesis over osteogenesis. Thus, treatment with estrogen, or intermittent PTH results in increased bone mass and reduced BMAT [29, 32••, 40]. Similarly, sclerostin, an inhibitor of bone formation expressed by osteocytes, stimulates adipogenesis [39], while OPG, an inhibitor of RANKL and bone resorption, inhibits adipocyte differentiation in vitro [71]. Considering that OPG functions as a blockage of RANKL activity, it is possible that RANKL regulates BMAT formation either directly or indirectly.

BMAT can interact with its microenvironment through the secretion of numerous factors, while the secretion profile of BMAT and its functional endocrine and paracrine implications

remain largely unexplored. So far, it has been shown that BMAT secretes endocrine factors designated as adipokines such as adiponectin, as well as other pro-inflammatory molecules, such as tumor necrosis factor (TNF) and interleukin-6, which affect bone homeostasis through the regulation of osteoblast and osteoclast functions. In vitro studies report that human adipocytes derived from BMSCs secrete cytokines, including macrophage inflammatory protein (MIP-1), granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor (GM-CSF) [72], whereas adipocytes from mouse BMSCs may also secrete chemokines such as chemokine (C-X-C motif) ligand 1 (CXCL1) and chemokine (C-X-C motif) ligand 2 (CXCL2) [73]. Adipose tissue secretes also a series of cytokines, which are termed adipokines including leptin, adiponectin, chemerin, omentin, and resistin. These have profound effects on surrounding and/or remote cell types [72, 74–79]. In the BM of osteoporotic postmenopausal women, the levels of leptin and adiponectin were significantly lower, whereas the effects of leptin on bone are not conclusive [74, 80–83].

The coexistence of increased BMAT and bone destruction with aging and osteoporosis, also suggests a mechanistic link between adipogenesis and osteoclastogenesis. Indeed, BM adipocytes produce RANKL, and thus can promote osteoclastogenesis [84–86]. A subpopulation of Pref-1^{+} pre-adipocytes was recently identified in BM that notably expresses RANKL and increased numbers of these cells coincide with aging [87]. A working hypothesis could be that RANKL[+]/Pref-1^{+} pre-adipocytes may contribute to bone loss through stimulation of osteoclastogenesis. In a recent study, mice lacking PTH receptors in mesenchymal stem cells develop high BMAT and reduced bone mass. In this model, BMAT was shown to produce high levels of RANKL, and there was also abundance of the RANKL[+]/Pref-1^{+} pre-adipocytes, suggesting that pre-adipocytes may contribute to bone loss [32••]. Therefore, BMAT could regulate osteoclast formation either directly through RANKL production or indirectly through adiponectin secretion, which stimulates osteoblasts to produce RANKL [77, 88–90]. Paradoxically, the impact of BMAT on bone resorption in vivo remains unclear and further studies are needed to take this further. In addition, the positive effect of numerous anti-resorptive therapies in BMAT attenuation suggests that osteoclasts are associated with BMAT formation. However, the underlying molecular mechanisms that connect osteoclasts with BMAT progression remain unknown.

Conclusion

A "neglected" feature of osteoporosis progression is BMAT development. BMAT is a "new tissue" with unique properties, which remains insufficiently understood. Animal and clinical studies have revealed that BMAT increases during aging and

is further enhanced in osteoporosis, emphasizing its potential impact in bone remodeling. The detrimental role of BMAT has been highlighted through the identification of secreted endocrine and/or paracrine factors (RANKL, pro-inflammatory cytokines and adipokines) that regulate bone metabolism. However, interventions targeting BMAT are limited and as a result the impact of BMAT on bone remodeling is far from conclusive. On the other hand, a positive effect of anti-resorptive therapies on BMAT progression has been established, while the underlying mechanisms have not been defined yet. Therefore, further studies are needed in order to shed light on the mechanistic basis of BMAT formation during osteoporosis and its pathophysiological role in bone remodeling.

Compliance with Ethical Standards

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