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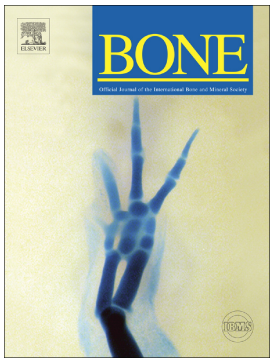

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Aging and lineage allocation changes of bone marrow skeletal (stromal) stem cells

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ABSTRACT

Aging is associated with decreased bone mass and accumulation of bone marrow adipocytes. Both bone forming osteoblastic cells and bone marrow adipocytes are derived from a stem cell population within the bone marrow stroma called bone marrow stromal (skeletal or mesenchymal) stem cells (BMSC). In the present review, we provide an overview, based on the current literature, regarding the physiological aging processes that cause changes in BMSC lineage allocation, enhancement of adipocyte and defective osteoblast differentiation, leading to gradual exhaustion of stem cell regenerative potential and defects in bone tissue homeostasis and metabolism. We discuss strategies to preserve the "youthful" state of BMSC, to reduce bone marrow age-associated adiposity, and to counteract the overall negative effects of aging on bone tissues with the aim of decreasing bone fragility and risk of fractures.

Keywords: bone marrow stromal stem cells; senescence; aging; adiposity; signaling pathways

Abbreviations: Alkaline phosphatase (ALP), Brown adipose tissue (BAT), Bone marrow (BM), Bone marrow stromal stem cells (BMSC), Bone marrow adipocyte (BMA), bone mineral density (BMD), CCAAT enhancer binding proteins (C/EBP), central nervous system (CNS), collagen type I (Col1), colony forming unit-fibroblast (CFU-F), Delta-like 1 (Dlk1), Dipeptidyl-peptidase-4 (DPP4), Distal-less homeobox 5 gene (Dlx5), Estrogen (E), Fibroblast growth factor 21 (FGF21), Forkhead box P1 (FOXP1), Glucagon-like-peptide-1 (GLP-1), Glucocorticoids (GC), Growth differentiation factors (GDF), kilodaltons (kDa), Human bone marrow stromal stem cells (hBMSC), Insulin-like growth factor 1 (IGF1), Insulin-like growth factor-binding protein (IGFBP1), Legumain (Lgmn), Magnetic resonance imaging (MRI), marrow adipose tissue (MAT), osteocalcin (OC), Ovariectomized (OVX), Peroxisome proliferator-activated receptor gamma (PPARγ), Parathyroid hormone (PTH), Receptor activator of nuclear factor kB ligand (RANKL), Reactive oxygen species (ROS), Runt-related transcription factor 2 (Runx2), Sclerostin (SOST), Strontium Ranelate (SrRN), Secreted frizzled-related protein 1 (sFRP), Thyroid hormone (TH), Tumor Necrosis Factor-alpha (TNFα), Transforming growth factor-beta (TGFβ), white adipose tissue (WAT).

1. Introduction

Bone is a dynamic tissue that is constantly remodeled and regenerated by the balancing act of osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Balance between bone formation and resorption maintains the skeletal integrity until pathological processes, commonly age-associated, disrupt this balance leading to bone fragility and increased risk for fracture manifesting as the osteoporotic syndrome (1). Histological studies of bone biopsies of aged and osteoporotic patients revealed an inverse relationship between decreased bone mass and increased marrow adipose tissue (MAT) formation suggesting a possible causal patho-physiological mechanism (2, 3).

Bone marrow stromal stem cells (BMSC) (also known as skeletal or mesenchymal stem cells) constitute a specific adult stem cell population within the bone marrow (BM) compartment that is required for bone tissue remodeling and regeneration. BMSC differentiate into mainly the mesoderm-type cells: osteoblasts and adipocytes (4). One hypothesis that has been examined in bone biology during the recent years, to explain the presence of an inverse relationship between age-related decreased in bone mass and increase MAT, is the increased commitment of BMSC to bone marrow adipocytes (BMA) and impaired osteoblast differentiation (5, 6).

The aim of this review is to present an analysis of the phenotypic and molecular characteristics of the age-related changes in BMSC and possible molecular causes mediated by age-related changes in local and systemic factors known to regulated BMSC biological functions. We will also discuss possible interventions to prevent these deleterious effects on BMSC biology, bone formation and bone fragility. We base our discussion on the concept that physiological aging leads to senescence-associated changes in BMSC as the general underlying mechanism for changes in lineage allocation and differentiation. In addition, we present concepts related to cellular senescence as examined in the biogerontology literature and that have previously been employed to discuss mechanisms of osteoblastic cell senescence (5, 6).

2. Phenotypic age-related changes in bone marrow tissue

The site of generation of BMSC is the bone marrow located between bone trabeculae of cancellous (spongy) bone of either axial or peripheral skeleton and can be phenotypically

classified as either red marrow, mainly filled with hematopoietic cells, and yellow marrow, characterized by increased formation of adipocytes (reviewed in (7)). Changes in marrow composition and proportion of MAT vary with gender, age, location within the bone, lifestyle, exposure to environmental factors (3, 8).

Studies of the qualitative changes in the marrow in humans have been reported since 1882, when Ernst Neumann recognized that with age, there is a trabecular bone atrophy and that most of the bone marrow in the axial skeleton consists of adipose tissue that does not take part in hematopoiesis. These findings were corroborated by many investigators (2, 9-11) .

More recent studies using alternative methodologies confirmed these initial histomorphometric findings in aged and osteoporotic patients (12, 13). Along gender lines, changes in marrow including a sharp increase in MAT content is observed in female subjects between 55 and 65 years of age while in male subjects the increase is much more gradual but steady, resulting in higher marrow fat content in females older than 60 years compared to males (13).

3. Mechanisms of age-associated changes in BMSC lineage allocation and differentiation

The underlying cellular and molecular mechanisms of age-related changes in bone and MAT are not fully characterized. However, these mechanisms can be classified as intrinsic BMSC age-associated changes (A) or age-related changes in bone microenvironment (B).

(A) Age-related intrinsic changes in BMSC

BMSC are identified by their clonogenic growth capacity, known as colony forming unit-fibroblast (CFU-F) and their ability to differentiate when cultured *in vitro*. The CFU-F assay has been employed as a surrogate marker of the number of BMSC *in vivo*. The number of CFU-F decreases with age in mice and rats (22, 23) but human studies revealed lack of an age-related effect when donor age is between 20-80 (reviewed in (5, 24)). More recent studies employed surface markers to define BMSC and to enumerate them. Murine BMSC defined by $CD45^-$ $CD31^-$ $Sca1^+$ $CD24^+$ exhibited a reduction in number with age, when comparing 2 months- vs. 15 months-old mice (25). Likewise, a decline in bone mass with age in 20-24 months old mice was reported to be the result of intrinsic defects in $Osx1^+$ osteoprogenitor cells, leading to decreased osteoblast numbers and increased support of osteoclast formation (21). In a human study, a certain decrease with age in the number of $CD271^+SSEA\text{-}4^+$ BMSC was reported, but bone marrow samples were not representative of

healthy aging as they were collected from femur heads of patients suffering femoral osteonecrosis or osteoarthritis (26). Thus, differences in bone marrow preparation methods, source of the bone marrow utilized, or definition of CFU-F or BMSC population can explain the reported discrepant results (reviewed in (5, 24)).

To determine the existence of intrinsic age-related changes in BMSC that lead to changes in BMSC lineage allocation, several models have been employed. Studies in senescent accelerated mouse model (SAMP-6) (27) and in normal aged mice (28), showed increased adipocyte differentiation (increased expression of adipocyte markers PPAR-γ2 and fatty acid binding protein aP2) along with a reduction in osteoblastogenesis (decreased expression of osteoblastic markers Runt-related transcription factor 2 (Runx2), Distal-less homeobox 5 gene (Dlx5), collagen and osteocalcin) in BMSC cultures (28). On the other hand, differentiation capacity of murine $CD45^-$ $CD31^-$ $Sca1^+$ BMSC was not altered by age (25) nor did plastic-adherent human bone marrow-derived stromal stem cells (hBMSC) (16, 29). Human $CD271^+SSEA\text{-}4^+$ BMSC obtained from elderly patients showed evidence of cellular senescence, as indicated by their low growth potential, high senescence-associated beta-galactosidase activity, and elevated p16 and p21 CDK inhibitor levels as well as weak osteogenic differentiation but higher adipogenic differentiation capacity (26). Interestingly, in some studies, cultured hBMSC and adipose tissue-derived MSC have been reported to be refractory to differentiation when they become senescent (30, 31). One of the consistent findings regarding intrinsic defects of hBMSC, is the presence of reduced proliferative potential of hBMSC in long term cultures (32).

A hallmark of aging-associated pathologies is the presence of senescent cells in tissues (14). In the bone microenvironment, subset of cells of various lineages become senescent in chronologically aged subjects, especially myeloid cells and osteocytes, but also a Lin^-/Leptin $receptor^+$ population that contains most bone- marrow-derived osteoblast progenitors. All senescent cell subsets develop a senescence-associated secretory phenotype (SASP) that is higher with aging in bone biopsies that may mediate age-related bone loss in mice and humans (15).

Aging is associated with many cellular and molecular changes leading to cellular senescence that contribute to the aging phenotype. General mechanisms underlying the bone aging phenotype have been shown including dysfunctional telomeres, age-related increased in DNA damage and impaired DNA repair mechanisms (15-21).

Age-related changes in intracellular signaling pathways and transcriptional factors

Differentiation of BMSC into osteoblastic or adipocytic cells is regulated by number of genetic signaling pathways that converge on lineage-specific transcriptional factors and that exhibit age-related changes (33)(Fig. 1 and Table 1).

Wnt signaling

Two independent Wnt signaling pathways—the canonical Wnt/β-catenin pathway and the non-canonical Wnt pathway—induce osteoblastogenesis and suppress adipogenesis (34). On the other hand, excessive activation of the Wnt/β-catenin signaling pathway, resulted in activation of the DNA damage response and the p53/p21 pathway inducing the aging of BMSC (35).

Peroxisome proliferated-activated receptor gamma (PPARγ)

The most characterized transcription factor and a key regulator of adipogenesis is PPARγ, that belongs to a nuclear receptor superfamily activated by lipophilic ligands. The activation of PPARγ is necessary and sufficient for adipocyte differentiation and, also, required for maintenance of a differentiated state in BMSC (36, 37). Inhibition of PPARγ *in vitro* impairs adipogenesis, while enhancing osteoblast differentiation in BMSC. In mice, PPARγ deficiency leads to impaired development of adipose tissue and enhanced bone mineral density (BMD) (36). Also, recent analysis of a PPARγS112 mutation in mice, that prevented MAPK phosphorylation and inhibition of PPARγ transcriptional activity, was associated with reduced trabecular bone, reduced bone formation, increased MAT and BMSC preferentially differentiated to adipocytes. Moreover, protein levels of total and phosphorylated RUNX2 declined (38).

The role of PPARγ activation in age-related increase of MAT and decreased osteoblastogenesis has been shown in several studies. PPARγ and CCAAT/enhancer-binding protein alpha (C/EBPα) were found to be increased in the BM of aged mice (39). An increase in age-associated production of endogenous ligands of PPAR-γ2 could exert the observed phenotype that enhances bone marrow adipogenesis at the expense of osteoblastogenesis

e.g. dietary lipids, dyslipidemia, hyperlipidemia, use of medications like lipid lowering agents, glucocorticoids and thiazolinediones (40).

CCAAT-enhancer binding proteins (C/EBP) family

Additional transcription factors involved in BMSC function and adipocyte differentiation are members of the C/EBP family: C/EBPα, C/EBPβ, C/EBPγ and C/EBPδ. In BMSC, the function and activation of individual transcription factors exhibited a different pattern (41). Moreover, it has been shown that an isoform of C/EBPβ, liver-enriched inhibitory protein (LIP), which lacks transcriptional binding domain, induces activation of Runx2 and promotes osteoblast differentiation of BMSC (42). C/EBPs crosstalk with PPARγ and regulate each other via a feedback loop (43). C/EBPβ-deficient mice displayed reduced bone mineral density with decreased trabecular number (44, 45). These findings corroborate an important role of C/EBPs in the early stage of MSC differentiation and their commitment (46).

Forkhead box P1 (FOXP1)

In BMSC, FOXP1 expression levels decreased with age in an inverse manner with those of the senescence marker *p16INK4A*. Conditional depletion of *Foxp1* in BMSC leads to premature aging phenotype including increased bone marrow adiposity, decreased bone mass, and impaired BMSC self-renewal capacity in mice (47). On the contrary, overexpression of FOXP1 augmented the replication capacity of BMSC irrespective of their age. FOXP1 influenced adipose cell-fate through interactions with the C/EBPβ/δ key modulators of adipogenesis and directly repressed transcription of *p16INK4A (47)* (47).

(B) Age-related changes in the extrinsic factors in the BMSC micro-environment

The role of age-related changes of bone micro-environment in influencing BMSC biology has been demonstrated in transplantation experiments where BMSC of young donors favored adipogenesis and not osteoblastogenesis when transplanted *in vivo* in old mice (48). Several factors present within the bone microenvironment are subject to age-associated changes and thus may affect BMSC differentiation capacity (49). Examples of these factors are discussed here and summarized in Table 1.

Receptor activator of nuclear factor kappa-B ligand (RANKL)

RANKL present within the bone microenvironment has recently been demonstrated to be secreted by MAT (39, 50). Age-related changes in marrow adiposity are associated with increased RANKL expression. Takeshita et al (2014) reported increased expression of RANKL by lineage committed BMSC in bone marrow of aged mice, leading to enhanced osteoclastogenesis (39).

Secreted frizzled-related protein 1 (sFRP)

We have previously identified sFRP1 as a secreted protein from adipocyte committed BMSC (51). sFRP inhibits osteoblastogenesis and promotes adipogenesis of BMSC by blocking the Wnt signaling (52). Similar effects of sFRP1 have been reported in preadipocytes and primary adipose tissue-derived cells (53, 54). The expression of sFRP1 mRNA levels was significantly increased in the biopsies from old as compared to young women (55). With age, the increase in intrinsic stiffness of human trabecular meshwork cells is associated with increased sFRP1 expression (56).

Fibroblast growth factor 21 (FGF21)

FGF21 has been identified as a circulating hepatokine with effects on glucose and lipid metabolism and is secreted from adipose tissue and skeletal muscle (57). FGF21 exerts a positive effect on adipogenesis via activation of PPARγ in BMSC and adipose tissue progenitors. FGF21 deficient mice exhibit high bone mass and decreased fat formation. Reciprocally, mice overexpressing FGF21 exhibit reduced bone mass (58, 59). In older men, high serum levels of FGF21 are associated with low bone mass (60) . FGF21 can induce Insulin-like growth factor-binding protein 1 (IGFBP1), to activate Integrin beta 1 receptor and to stimulate osteoclast differentiation and bone resorption. IGFBP1 blockade is a potential strategy to prevent FGF21-induced bone loss (61).

Sclerostin (SOST)

SOST, a secreted protein of osteocytes and Wnt signaling inhibitor. It has been reported that serum levels of SOST increases in elderly people and is positively associated with increased MAT (62). It is plausible that SOST inhibits bone formation and increases MAT through regulating differentiation of BMSC via inhibiting Wnt signaling (63, 64).

Dipeptidyl-peptidase-4 (DPP4)

DPP4 is an exopeptidase shed from the plasma membrane, and its membrane-bound form, known as CD26. It is a ubiquitously expressed glycoprotein of 110 kDa on the surface of a variety of cells (65). DPP4 selectively cleaves the N-terminal dipeptides from a variety of substrates e.g. incretin hormones that stimulate a decrease in blood glucose levels. Soluble DPP4 was found to originate primarily from the bone marrow (66). Recently, Ambrosi et al. showed that MAT produced DPP4, inhibiting bone fracture healing, suggesting a potential mechanism for the impairment of BMSC differentiation and bone regeneration (25). DPP4 has been found to be selectively expressed on the surface of senescent but not proliferating human fibroblasts, its expression is increased in peripheral blood cells from elderly individuals and it can promote the senescence program (67).

Delta-like 1 (Dlk1)

Dlk1 (also known as fetal antigen 1 (FA1), or pre-adipocyte factor 1 (Pref-1) is a factor secreted by committed BMSC progenitor cells in the bone microenvironment (68). It is a member of the epidermal growth factor (EGF)-like protein family that includes proteins characterized by the presence of EGF-like motifs (69). These proteins are involved in cell fate decision and cell differentiation processes in a variety of tissues.

Our group intensively examined the role of Dlk1/FA1 in lineage commitment and differentiation of hBMSC. Overexpressing Dlk1/FA1 in hBMSC resulted in inhibition of differentiation into mature osteoblasts and adipocytes and thus maintenance of the undifferentiated hBMSC population (68). The effect of Dlk1/FA1 on osteoblastic differentiation is mediated through a pathway downstream of Cbfa1/Runx2 since expression of Cbfa1/Runx2 was not affected and mature osteoblastic markers (alkaline phosphatase (ALP), collagen type I (Col1) and osteocalcin (OC) were inhibited by over-expression of Dlk1/FA1. Similarly, Dlk1/FA1 overexpression in hBMSC, inhibited adipocyte differentiation by acting downstream on the early adipogenic commitment marker C/EBPβ. Osteoblast-specific-Dlk1 over-expressing mice (Col1-Dlk1) (70) exhibited reduced total fat mass and bone mass, reduced bone formation rate, osteoblast surfaces and increased osteoclast surfaces. Interestingly, estrogen (E) deficiency which is the principal factor mediating post-menopausal bone loss, is associated with increased serum levels of Dlk1. Dlk1-deficient mice

were significantly protected from OVX-induced bone loss. (71). Thus, our data suggest that Dlk1/FA1 may mediate some of the E-deficiency-related bone loss.

Legumain (Lgmn)

We have recently identified legumain (Lgmn) as a novel regulator of BMSC differentiation (72). Lgmn is a secreted cysteine protease involved in regulation of diverse physiological and pathological processes by remodeling tissue-specific targets (e.g. extracellular matrix (ECM) components, enzymes, receptors). We showed that Lgmn enhances adipocyte differentiation of hBMSC through degradation of the extracellular protein fibronectin which is an inhibitor of adipogenesis (73-75). In addition, levels of Lgmn in hBMSC cultured from osteoporotic patients, are increased compared to young donors, and in bone biopsies high levels of Lgmn were associated with increased MAT and decreased trabecular bone mass (72).

(C) Age-related changes in systemic hormones

Aging is associated with significant changes in the endocrine system that exerts significant changes on BMSC biology. The role of some of the individual hormones will be presented here.

Glucocorticoids (GC)

GC are known to inhibit bone formation and high levels of GC in human (Cushing syndrome) are associated with increased risk of fractures and increased MAT formation. GC stimulates the synthesis of collagenase III, which is an enzyme involved in bone matrix degradation, inhibits the anabolic effect of IGF hormones and induces significant changes in neuropeptide Y that may contribute to osteoporosis and increased MAT phenotype (76). Life-course time-point analysis has shown that circulating GC follow an age-related trajectory, which can be affected by both the external environment and internal physiological events, which varies between gender and individuals (77).

Growth hormone (GH) and insulin-like growth factors

GH is an important hormone regulating bone growth and energy metabolism (78). GH levels decrease with age and are associated with bone loss. It has been shown that GH is a negative

regulator of bone adiposity. GH increases osteoblast differentiation while suppressing bone marrow lipid accumulation in GH deficient animal model (79).

Insulin-like growth factor 1 (IGF1) is considered one of the most important mediators of bone growth. Systemic IGF1 is synthesized primarily in the liver, where its synthesis is GH dependent. IGF1 plasma half-life and the interaction of IGF1 with its receptor (IGF1R) is regulated either positively or negatively by a family of six high-affinity IGF binding proteins (IGFBPs; IGFBP1 to IGFBP6). Tissue responsiveness to IGF1 is altered with aging in many tissues (80). In bone, IGF1 responsiveness decreased with aging (81, 82). The mitogenic response of osteoblasts derived from patients of different ages require higher concentration of IGF1 with increasing age (82). Increased serum IGF1 levels protect the musculoskeletal system but are associated with elevated oxidative stress markers and increased mortality independent of tissue *IGF1* gene expression (83). These studies suggest that IGF1 may play a protective role in the young skeleton and its age-related decline leads to bone fragility and an increased fracture risk.

Thyroid hormones (TH)

TH exert a negative effect on MAT. Mice deficient in TH exhibit growth retardation, lower BMD and increased bone adiposity (84, 85). Circulating free TH levels usually decrease with aging, leading to hypothyroidism which is among the most frequent chronic diseases in the elderly (86). However, the incidence of mild hyperthyroidism also increases with increasing age, especially in >70 year old populations with historical or current iodine deficiency (87). Hyperthyroidism was shown to have a negative effect on bone metabolism via accelerating bone turnover and decrease of BMD through increased osteoclast activation and dysregulation of calcium metabolism (88, 89). However, it is not known whether higher TH levels causes increase MAT formation.

(D) Age-related changes in metabolism and metabolic stimuli

Obesity increases with aging and is associated with low grade inflammation, lipotoxicity and impaired glucose metabolism. We have recently demonstrated a negative impact of obesity-induced diet on BMSC with increased MAT, decreased bone formation, skeletal stem cell exhaustion (90). Another animal study reported impairment of mitochondrial function and apoptosis of BMSC in obese mice (91). Patients with type 1 and type 2 diabetes exhibit

increased MAT formation and a risk for bone fractures (92). Thus, defective systemic metabolism affects differentiation capacity and function of BMSC. Several factors have been studied in relation to BMSC biology and bone fragility. Some of them are discussed here below and depicted in Fig. 1 and Table 1.

Fatty acids (FA)

An *in vitro* study showed that BMSC incubated with sera obtained from overweight persons enhances adipocyte differentiation and diminishes osteoblast differentiation (93), suggesting that secreted factors/nutrients present in circulation can affect the differentiation of BMSC. Saturated FA affect cell survival and proliferation of hBMSC (94, 95). Also, saturated FA peroxidation produce reactive oxygen species (ROS) and activate signaling pathways leading to cellular senescence and apoptosis (95-97). Additional suggestive evidence for the role of FA metabolism in regulating BMSC functions is the presence of lower FA saturation index as determined by high resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy, in the MAT of subjects with lower BMD (98).

Glucose

There is an age-related increase in glucose levels that may have effects on BMSC biology. I*n vitro* studies have shown that high glucose levels induce increased adipogenesis and decreased proliferation capacity of BMSC in mice and human (99-101). One proposed mechanism is changes in the levels of transforming growth factor-β (TGF-β) signaling leading to changes of lineage allocation of BMSC from osteoblast to adipocyte differentiation in murine BMSC (102). High glucose levels induce cell senescence by activating autophagy in hBMSC (103). Chronic exposure to high levels of glucose and insulin stimulate BMSC adipogenic differentiation (104).

Glucagon-like-peptide-1 (GLP-1)

Activation of BMSC-expressing GLP-1 receptor by exendin-4, a GLP-1 agonist, promote the osteogenic differentiation and inhibited adipocytic differentiation through regulating PKA/β-catenin and PKA/PI3K/AKT/GSK3β signaling(105). Exendin-4 also improved BMSC proliferation and cell motility (106). Glucagon-like peptide-1 receptor agonists also known as

GLP-1 receptor agonists or incretin mimetics significantly increased trabecular bone mass, in OVX mice (107). The role of GLP1 in regulating BMSC biology remains to be determined.

4. Possible approaches for pharmacological/therapeutic targeting of the BMA

The clinical use of BMSC in clinical regenerative medicine protocols to treat bone fragility and impaired fracture healing in osteoporotic elderly patients, has been hampered by several challenges (108). Interventions to *in vivo* targeting of BMSC may prove beneficial to enhance bone formation and skeletal repair. Several possible interventions are described below

Estrogen (E)

Evidence that BMSC is targeted by E is derived from clinical studies showing that MAT increases during menopause and is inversely related to bone mass due to E deficiency (13). Also, MAT mass is greater in postmenopausal women compared to premenopausal women and men (109). E has been shown to affect adipocyte development and function at multiple levels (reviewed in (110)) and to directly inhibit adipocyte differentiation (111, 112). Also, E exerts an inhibitory effect on adipose tissue formation through regulation of energy expenditure and food consumption (113). One-year treatment of postmenopausal osteoporotic women with transdermal E increased bone mass and decreased the MAT mass and BMA number as compared to placebo-treated controls (114). Also, treatment of 6 postmenopausal women with oral 17β-estradiol (2 mg/day) resulted in a reduction of the vertebral MAT fraction (109).

Parathyroid hormone (PTH)

Several studies have demonstrated that PTH treatment targets BMSC lineage allocation and differentiation fate (50). Intermittent treatment with PTH has anabolic effect on bone formation, enhances Wnt signaling and increases osteoblast differentiation (115). Treatment of post-menopausal women, with fragility fractures and low BMD, with daily injections of PTH for 18 to 24 months, resulted in decreased MAT without change in adipocyte number, as assessed in bone biopsies (116). In another clinical study, PTH treatment decreased MAT in postmenopausal women following 12-month therapy, as judged by T1 weighted MRI scanning (117). A recent paper by Fan et al. reported that PTH inhibits expansion of pro-

adipogenic progenitors and that in PTHr1 KO mice there exists an impaired bone formation and enhanced bone adiposity (50).

Strontium Ranelate (SrRN)

Strontium Ranelate (SrRN) has been used for treatment of osteoporosis (118). SrRN increases bone formation and decreases bone resorption through multiple mechanisms, such as increasing osteoblast proliferation, differentiation, and survival, as well as decreasing osteoclast differentiation and function (reviewed in (118)). In addition, SrRN exerts a dose-dependent inhibitory effect on adipocyte differentiation of BMSC *in vitro* and *in vivo* (119-121), and prevents lipotoxicity within the bone marrow milieu (119). SrRN treatment increases bone mass and reduces MAT in SAMP6 accelerated senescent mice through stimulation of Wnt and NFATc/Maf pathways, and consequent inhibition of adipogenesis due to repressed PPARγ expression (121).

Rejuvenation factors

Several systemic factors with potential rejuvenating effects have been identified from studies of heterochronic parabiosis. Recent works on the subject, reviewed in (122), have shed a perspective on the intricacies pertaining these factors.

One of the growth differentiation factors (GDF) which is subfamily of proteins belonging to the TGFβ superfamily, GDF11 has been reported as a potent regulator of bone metabolism (123). It does so by inhibiting the activity of PPARγ, promoting osteoblastogenesis and inhibiting adipogenesis from BMSC. Serum levels of GDF11 are decreased in patients with osteoporosis compared to normal age-matched donors (123). Other studies reported contradictory effects as injection of GDF11 into mice inhibited bone formation and accelerated age-related bone loss (124). Thus, whether GDF11 can be employed to target lineage fate of BMSC awaits further studies.

Phytochemicals and natural compounds

Several bioactive molecules potentially inhibiting adipogenic differentiation and enhancing osteogenic differentiation have been identified in animal and cell culture models (125). For example, resveratrol, a phytoalexin known to be a caloric restriction mimetic, is long known to inhibit adipogenic differentiation and increase osteogenesis of BMSC (126, 127).

Resveratrol has many other beneficial anti-aging functions e.g. regulating lipolysis, mitochondrial biogenesis and thermogenesis. The effects are mediated in part by activation of SIRT1, a member of the sirtuin family of histone deacetylases (126). Preclinical studies in aged OVX female rats treated with vitamin D combined with genistein, quercetin, and resveratrol exhibited increased BMD, reduced body weight gain and a significant decrease in BMA (128). Puerarin, a phytoestrogen extracted from Pueraria lobata (Willd.) Ohwi, a plant used in Chinese traditional medicine, is known to have anti-osteoporotic effects. Puerarin and zinc promoted the serological level of osteocalcin, BMSC proliferation, and the expression of alkaline phosphatase, and decreased MAT mass (129).

Asiatic acid (AA), a triterpenoid found in *Centella asiatica*, inhibited the adipogenic induction of lipid accumulation, the activity of glycerol-3-phosphate dehydrogenase, and expression of of BMSC through inhibition of PPARγ through C/EBPβ-independent mechanisms (130). Isosporalen (ISO) is the main active ingredient extracted from the seeds of *Psoralea corylifolia*, a Chinese herb used in the treatment of fractures, and bone and joint diseases. ISO attenuated bone marrow adipogenesis, caused increased RUNX2 and decreased PPARγ levels, resulting in a BMSC shift in lineage differentiation from adipocytes to osteoblasts, involving the mTORC1 signaling pathway (131). Sodium butyrate, a known histone deacetylase (HDAC) inhibitor, decreases proliferation potential and multilineage differentiation capability of BMSC (132). Also, genistein, epigallocatechin and various oxysterols have been found to inhibit PPARγ expression and adipogenic differentiation of BMSC (125, 133).

Probiotics and the role of age-related changes in microbiota

Many reports have revealed the importance of the gut microbiota on healthy aging (134) and human longevity, as reviewed in (135). Although the core communities, belonging to the Ruminococcaceae, Lachnospiraceae and Bacteroidaceae families, decrease with age, a longevity adaptation in some individuals promote the enrichment of health-associated taxa, such as Akkermansia and Bifidobacterium, known to activate immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis, that might support extreme aging and longevity (136). A summary of gut microbiota composition and its role in bone loss during aging and osteoporosis has been published (137).

The gut microbiota is known to induce IGF1 which promotes bone formation and skeletal growth (138). In another study, the microbiota contributed to increase adipogenesis, inhibit osteogenesis and enhance the immunomodulatory capacity of BMSC. Probiotic and prebiotic treatments were able to increase bone mass and BMD, indicating that intestinal microbiota may impact on BMSC biological functions and differentiation (139).

5. Conclusions and future perspectives

The mechanisms of age-related changes in BMSC are currently an important topic of bone biology studies as they may uncover novel approaches for treating age-related bone fragility and osteoporosis. While the *in vitro* mechanistic studies have identified several principal age-related pathways, their targeting *in vivo* to determine relevance and efficacy need to be performed. The development of non-invasive imaging technologies that can monitor MAT formation and bone mass changes in humans will allow translation of results obtained in pre-clinical animal models to the clinic. Targeted elimination of bone-specific senescent cell subsets is a promising therapeutic approach to prevent or delay osteoporosis. We expect that in the coming decade, we will witness the development of several novel approaches to target age-related changes in BMSC to treat age-related bone fragility and risk.

Disclosures

The authors do not have any conflicts of interests to disclose.

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Figure legend

Fig. 1. Scheme illustrating the intrinsic and extrinsic factors influencing BMA and the possible functional roles of BMA during aging

Table 1: List of selected regulatory factors promoting adipocyte differentiation in the bone marrow

Name	Symbol	Category	References
Peroxisome proliferated-activated receptor γ	PPARγ	Transcription factor	(36, 37, 43)
CCAAT enhancer binding proteins	C/EBPα/β/γ/δ	Transcription factors	(41)
Secreted frizzled related protein 1	sFRP1	Secreted factor	(52)
Fibroblast growth factor 21	FGF21	Secreted factor	(58, 59)
Sclerostin	SOST	Secreted factor	(64, 140)
Dipeptidyl-peptidase-4 inhibitor	DPP4	Enzyme	(25)
Receptor activator of nuclear factor kappa-B ligand	RANKL	Secreted factor	(39, 50)
Glucocorticoids	GC	Hormone	(141)
Glucose	Glu	Metabolite	(99-101)
Saturated fatty acids	FA	Metabolite	(94, 95)
Reactive oxygen species	ROS	Metabolite	(142)

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Highlights

- Aging is associated with decreased bone mass and accumulation of bone marrow adipocytes
- Impaired osteoblastogenesis and an increase in bone marrow adiposity is correlated with advancing age
- The mechanisms of age-associated changes in bone marrow stromal stem cells (BMSC) lineage allocation and differentiation are characterized by intrinsic changes, extrinsic changes in the bone microenvironment, systemic hormones and changes in metabolism and metabolic stimuli
- Possible approaches for pharmacological/therapeutic targeting of bone marrow adiposity are described. Interventions to *in vivo* targeting of BMSC may prove beneficial to enhance bone formation and skeletal repair

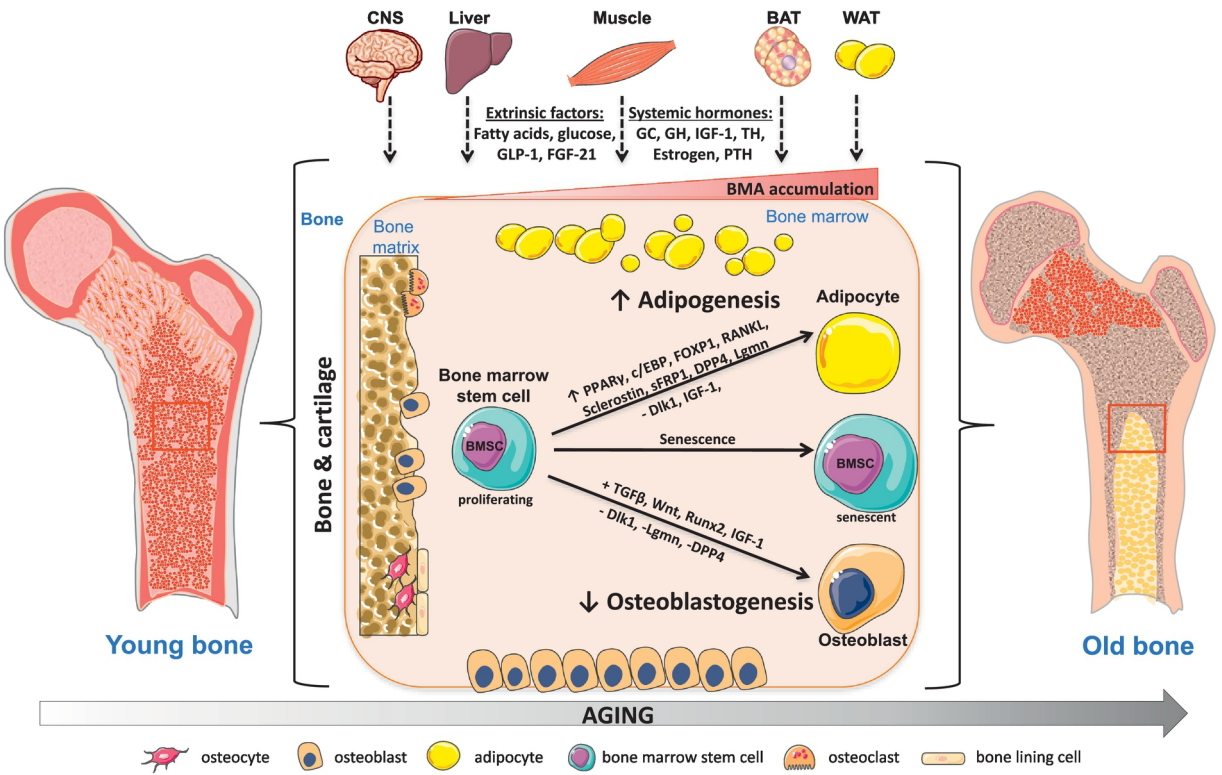
CNS
Liver
Muscle
BAT
WAT
Extrinsic factors:
Fatty acids, glucose,
GLP-1, FGF-21
Systemic hormones:
GC, GH, IGF-1, TH,
Estrogen, PTH
BMA accumulation
Bone
Bone matrix
Bone marrow
Bone & cartilage
↑ Adipogenesis
Adipocyte
↑ PPARγ, c/EBP, FOXP1, RANKL,
Sclerostin, sFRP1, DPP4, Lgmn
- Dlk1, IGF-1,
Bone marrow
stem cell
BMSC
proliferating
Senescence
BMSC
senescent
+ TGFβ, Wnt, Runx2, IGF-1
- Dlk1, -Lgmn, -DPP4
↓ Osteoblastogenesis
Osteoblast
Young bone
Old bone
AGING
osteocyte
osteoblast
adipocyte
bone marrow stem cell
osteoclast
bone lining cell

Figure 1