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REVIEW

Bone marrow adiposity during pathologic bone loss: molecular mechanisms underlying the cellular events

Jiao Li[1] · Lingyun Lu[1,2] · Yi Liu[3] · Xijie Yu[1]

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Abstract

Bone marrow (BM) is a heterogeneous niche where bone marrow stromal cells (BMSCs), osteoblasts, osteoclasts, adipocytes, hematopoietic cells, and immune cells coexist. The cellular composition of BM changes with various pathophysiological states. A reduction in osteoblast number and a concomitant increase in adipocyte number in aging and pathological conditions put bone marrow adipose tissue (BMAT) into spotlight. Accumulating evidence strongly supports that an overwhelming production of BMAT is a major contributor to bone loss disorders. Therefore, BMAT-targeted therapy can be an efficient and feasible intervention for osteoporosis. However, compared to blocking bone-destroying molecules produced by BMAT, suppressing BMAT formation is theoretically a more effective and fundamental approach in treating osteoporotic bone diseases. Thus, a deep insight into the molecular basis underlying increased BM adiposity during pathologic bone loss is critical to formulate strategies for therapeutically manipulating BMAT. In this review, we comprehensively summarize the molecular mechanisms involved in adipocyte differentiation of BMSCs as well as the interaction between bone marrow adipocytes and osteoclasts. More importantly, we further discuss the potential clinical implications of therapeutically targeting the upstream of BMAT formation in bone loss diseases.

Keywords Bone marrow stromal cells · Bone marrow adipose tissue · Bone marrow adiposity · Bone loss · Molecular mechanism

Introduction

The development of osteoporosis involves two imbalances: one is imbalanced bone remodeling between bone formation by osteoblasts and bone resorption by osteoclasts, and the other is dysregulated equilibrium between adipogenesis and osteogenesis of bone marrow stromal cells (BMSCs) [1]. Generally, disruption of bone remodeling is the essential predisposing cause in osteoporotic diseases. For this reason, anti-resorptive drugs including bisphosphonates and denosumab and bone anabolic drugs including teriparatide and abaloparatide have been widely used for the treatment of osteoporosis [2, 3]. Nevertheless, currently available anti-osteoporotic agents are still limited by their side effects. In order to identify effective therapeutic modalities, the implications of osteo-adipogenesis imbalance in osteoporosis have attracted increasing attention.

Many pathological bone loss conditions, including aging, estrogen deficiency, obesity, and diabetes, are characterized by compromised bone formation and increased bone marrow adipose tissue (BMAT) accumulation [4–7]. This could be largely attributed to the shift in BMSC differentiation from osteogenesis to adipogenesis under pathological settings. Previous studies indicate that BMSCs isolated from aged or postmenopausal osteoporotic subjects have increased capacity to differentiate into adipocytes and reduced capacity to differentiate into osteoblasts [8–10]. However, the molecular mechanisms behind this reversed cellular event are not fully understood.

✉ Xijie Yu

[1] Department of Endocrinology and Metabolism, Laboratory of Endocrinology and Metabolism, Rare Disease Center, West China Hospital, Sichuan University, No.37 Guoxue Alley, Wuhou District, Chengdu 610041, Sichuan Province, China

[2] Department of Integrated Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu 610041, China

[3] Department of Rheumatology and Immunology, Rare Disease Center, West China Hospital, Sichuan University, Chengdu 610041, China

For the above-mentioned reasons, impeding BMSC differentiation into adipocytes may be a target for intervention with the aim of enhancing bone formation in bone loss disorders. Therefore, understanding the molecular mechanisms of adipocyte differentiation from BMSCs is quite conducive to the development of new-targeted biomarkers and drugs for bone-related disease such as osteoporosis. This review will summarize the current knowledge of upstream regulatory networks involved in bone marrow (BM) adipogenesis, including transcription factors, epigenetic regulators, noncoding RNAs (ncRNAs), and immunomodulatory properties inherent in BMSCs. Furthermore, as two major contributors of bone loss under pathological conditions, bone marrow adipocyte-osteoclast crosstalk is also reviewed. Finally, we will discuss strategies targeting BMAT formation to promote bone regeneration.

Transcriptional and epigenetic regulation of BM adipogenesis

BM adipogenesis, a process of lineage commitment and differentiation of BMSCs towards adipocytes, relies on the coordinated actions of a cascade of lineage-determining transcription factors and epigenetic regulators [11, 12]. First, upon adipogenic stimuli, transcription factors bind to the genome and activate the transcription of adipocyte-specific genes. Second, this genetic event can cause or respond to the epigenetic modifications that drive BMSC cell fate determination [13].

Transcriptional regulation

BMSC fate determination is controlled by multifarious transcription factors and signaling pathways. It is well established that peroxisome proliferator-activator receptor gamma (PPARγ) and the CAAT enhancer binding proteins (C/EBP) family serve as the master transcription factors responsible for the lineage commitment of BMSCs towards adipocytes [14–16], while runt-related transcript factor 2 (RUNX2) and osterix mainly govern BMSCs to differentiate down the osteoblastic lineage [17, 18]. Yet, these two distinct types of transcription factors suppress each other's action, as exemplified by the findings that inhibition and activation of PPARγ potentially facilitate and impede RUNX2-induced osteogenesis, respectively, and vice versa [19, 20]. Besides, using machine learning algorithms, Rauch et al. identified a transcriptional network of 202 transcription factors with osteogenesis-stimulated and adipogenesis-repressed activity, which are also defined as osteogenic stem cell factors [21]. Among them, 12 transcription factors including ELK4, SNAI2, MEF2A, NKX3-1, TEAD1, TEAD4, SMAD3, JUNB, PITX1, FLI1, HIF1A, and ARNT were selected for further investigation through siRNA-mediated knockdown assays. In line with the predicted anti-adipogenic activity, the results indicate that inhibition of these factors potentially blocks osteogenesis while accelerating adipogenesis. Furthermore, they demonstrated that osteoblast differentiation involves activation of enhancers preestablished in BMSCs, whereas adipocyte differentiation involves chromatin remodeling and enhancer de novo establishment and activation. Intriguingly, the different activation modes of enhancers between osteogenesis and adipogenesis can be ascribed to the control of osteogenic stem cell factors that are already active in undifferentiated BMSCs and dramatically declined during adipogenesis [22]. With advancements in this field, increasing numbers of new transcription factors determining BMSC lineage fate and the underlying mechanisms have been identified. For example, Yu et al. demonstrated that loss of PGC-1α in mice with bone loss induced by aging as well as ovariectomy (OVX) strongly primes BMSC to differentiate into adipocytes as opposed to osteoblasts via the repression of TAZ, a transcriptional coactivator of RUNX2 [23]. Moreover, Li et al. revealed that forkhead box P1 (FOXP1) orchestrates the fate decisions of BMSCs by targeting CEBPβ/δ and RBPjκ, key regulators of adipogenesis and osteogenesis, respectively. Accordingly, declined FOXP1 in BMSCs from aged mice results in enlarged bone marrow adiposity and reduced bone mass [24]. Additionally, by generating the adipocyte-specific signal transducer and activator of transcription factor 5 (STAT5) conditional knockout mice, Seong et al. demonstrated that STAT5 potentially inhibits BMSC adipogenesis through activating ATF3, a negative regulator of adipocyte differentiation [25]. In terms of adipogenesis, a subset of novel transcription factors, such as NLX1-2, EBF families, COUP-II, Twist-1, Dermo-1, Sox2, Oct4, and Zfp423, have been reported to exhibit pro-adipogenic capacity [26–28] (Fig. 1).

Regarding signaling pathways involved in BM adipogenesis, as the leading regulator of adipocyte formation, PPARγ acts as the key target of almost all pro-adipogenic signaling pathways [29]. Over the past decades, huge bodies of evidence have shown that several key signaling pathways including Wnt signaling, TGF-β/BMPs signaling, Notch signaling, and Hedgehogs signaling participate in the regulation of differentiation of BMSCs [30]. In particular, the canonical Wnt/βcatenin signaling pathway has been regarded as the most important pathway governing the osteogenesis of BMSCs [31]. Parallel to its pro-osteogenic action, activation of canonical Wnt signaling contributes to the suppression of BM adipogenesis [32, 33]. Thus, expanded bone marrow adiposity is inevitably involved in the repression of canonical Wnt signaling [34]. In addition, other signaling pathways present complicated bi-directional regulatory roles in osteoadipogenic differentiation, which require further studies for elucidation [19].

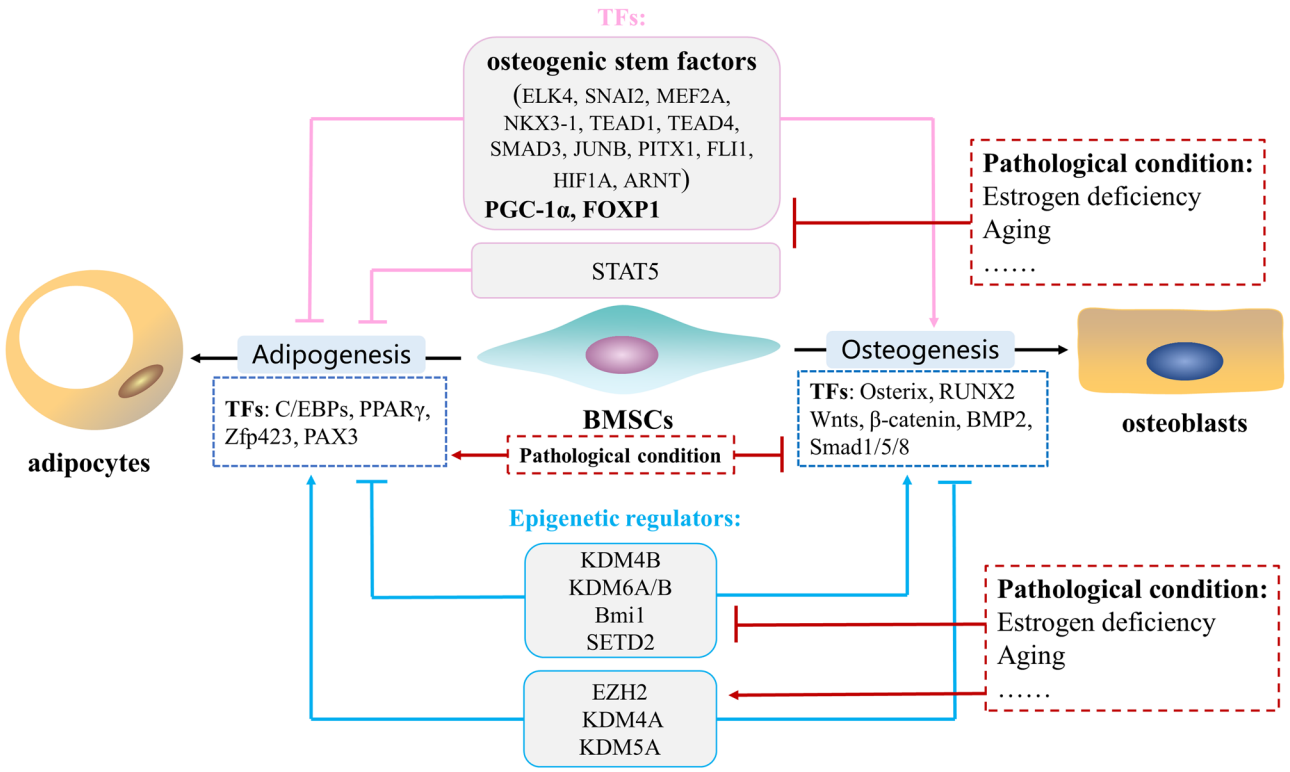

Fig. 1 Transcriptional and epigenetic regulation of bone marrow adipogenesis. Under pathological conditions such as estrogen withdrawal and aging, bone marrow stromal cells (BMSCs) exhibit an increased propensity toward adipocyte differentiation accompanied by a reduction in osteogenic commitment. The molecular mechanisms behind this cellular event involve activation of adipogenic transcription factors (TFs) as well as repression of osteogenic TFs. Importantly, numerous epigenetic regulators also play crucial roles. BMSCs, bone marrow mesenchymal stem cells; TFs, transcription factors

Epigenetic regulation

Emerging evidence has revealed that epigenetic modification of gene transcription is an indispensable component in the lineage commitment and differentiation of BMSCs. Notably, the event of transcription factors binding to genomes can be preceded by or bring about epigenetic modifications, as evidenced by the fact that activation of adipogenic transcription factors is preceded by chromatin remodeling during adipocyte differentiation [13, 21]. Epigenetic regulation mainly involves DNA methylation; histone modifications including methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation; and chromatin remodeling, all of which are modulated by specific enzymes referred to as epigenetic regulators [35, 36]. Generally speaking, DNA methylation usually causes gene silencing, whereas histone modifications are related to either transcription activation or repression [28].

In recent years, various epigenetic factors and their regulatory mechanisms have been illustrated. As an example, EZH2 is a histone 3 lysine 27 (H3K27) methyltransferase that acts to suppress BMSC osteogenesis whilst promoting adipogenesis through trimethylation of H3K27 (H3K27me3) of Wnt and RUNX2 [37, 38]. In addition, the upregulation of KDM5A is confirmed in osteoporotic BMSCs. KDM5A, a H3K4me3 demethylase, impedes osteogenic differentiation of BMSCs, thus enhancing adipogenic differentiation by demethylating H3K4me3 on the RUNX2 promoter region [39]. Similarly, the H3K9me3 demethylase KDM4A functions to facilitate adipocyte formation by removing repressive H3K9me3 on the promoters of C/EBPα and SFRP4, two adipogenic transcription factors [40]. To the contrary, another H3K9me3 demethylase, KDM4B, is shown to participate in PTH-mediated bone anabolism and inhibit marrow adiposity through decreasing H3K9me3 levels in the promoters of β-catenin and Smad1, eventually inducing osteogenic gene transcription. Consistently, conditional deletion of KDM4B in murine BMSCs exacerbates bone loss and BMAT accumulation during aging, OVX-mimicked postmenopausal osteoporosis, and a high fat diet [41]. Moreover, the H3K27me3 demethylase property of KDM6A/B makes them potential repressors of adipocyte differentiation owing to downregulation of repressive mark H3K27me3 on RUNX2 [37, 42]. Furthermore, Hu et al. uncovered that Bmi1 in BMSCs shows a dramatic decline with differentiation stimuli for adipocytes and during aging and that conditionally deleting Bmi1 in

BMSCs significantly accelerates marrow fat accumulation [43]. Mechanistically, Bmi1, a component of the polycomb repressive complex 1, functions to inhibit multiple developmental programs of BMSCs by maintaining repressive histone H2A ubiquitylation (H2Aubi) and H3K27me3 on the promoter of PAX3, a transcription factor mediating adipogenic differentiation. Correspondingly, derepression of H2Aubi and H3K27me3 on PAX3 in the absence of Bmi1 contributes to increased BM adipogenesis. Moreover, an in vivo study suggests that the histone methyltransferase SETD2 trimethylates H3K36 on the lipopolysaccharide-binding protein (LBP) gene, a novel BMSC fate regulator with anti-adipogenic activity. In turn, this modification leads to a decrease in differentiation of BMSCs towards adipocytes. Accordingly, ablation of SETD2 leads to markedly reduced bone mass and excessive marrow fat accumulation [44]. Furthermore, post-transcriptional processes mediated by ncRNAs also take part in epigenetic regulation of BMSCs, and long non-coding RNA (lncRNA) acts as an epigenetic regulator [45–47], which will be reviewed later (Fig. 1).

Involvement of ncRNAs in BM adipogenesis

It is known that about 80% of the human genome is transcribed into RNA, whereas only 2% of the genome is translated into proteins, suggesting that ncRNA constitutes the majority of the transcriptome [48]. Indeed, the crucial roles of ncRNAs in BMSC lineage allocation and occurrence and the development of osteoporotic diseases have been well investigated. Here we will focus on the regulation of BM adipogenesis by microRNA and lncRNA.

MicroRNA

MicroRNAs are a class of endogenous small single-stranded ncRNAs with 19–25 nucleotides in length, which regulate the expression of their target genes at the post-transcriptional level [49, 50]. They function as negative regulators of gene expression through binding to the 3′-untranslated regions (3′ UTR) of the target genes and thus leading to mRNA degradation and/or translational repression [51, 52]. Many studies suggest that miRNAs are involved in the regulation of lineage commitment and differentiation of BMSCs [53, 54]. A variety of miRNAs have been shown to be implicated in the balance between adipogenic and osteogenic differentiation of BMSCs, and the alterations of these miRNAs are closely related to the increase in bone marrow adiposity during pathological bone loss such as aging, estrogen deficiency, obesity, and glucocorticoid administration. Some of the miRNAs with pro-adipogenic or anti-adipogenic effects are listed as below.

Pro-adipogenic miRNAs

By sorting Sca-1$^+$ CD29$^+$ CD45$^-$ CD11b$^-$ BMSCs of young and aged mice and humans using FACS for miRNA microarray analysis, Li et al. found that miR-188 is highly expressed in BMSCs with aging, implying the involvement of miR-188 in the lineage commitment shift of BMSCs from osteoblasts to adipocytes [55]. As predicted, miR-188 knockout in mice counteracts age-associated bone marrow fat accumulation and bone loss; meanwhile transgenic mice overexpressing miR-188 in osterix$^+$ osteoprogenitors and mice with BMSC-specific overexpression of miR-188 exhibit significantly increased bone marrow fat and reduced bone formation. Furthermore, they demonstrated that miR-188 promotes adipogenic differentiation of BMSCs through directly targeting HDAC9 and RICTOR, both of which are known as repressors of PPARγ activity [55].

MiRNA expression profiling and bioinformatic analysis of human BMSCs during adipocytic differentiation indicate that miR-320 functions as a crucial regulator promoting lineage commitment of BMSCs into adipocytes through suppressing the expression of RUNX2 [56]. The overexpression of miR-214 has been shown to effectively facilitate the adipocytic differentiation of BMSCs and attenuate osteoblastic differentiation by inhibiting the JNK and p38 pathways [57]. In addition, Xi et al. reported that miR-214-3p enhances preadipocyte proliferation and differentiation through targeting the 3′ UTR of Ctnnb1, a key transcriptional regulatory factor of the Wnt/β-Catenin pathway [58]. Yet, another study showed that miR-214-5p can improve adipogenic differentiation of BMSCs involving the TGF-β/Smad2/COL4A1 signaling pathway [59]. The pro-adipogenic and anti-osteogenic effects of miR-204 in BMSCs are owing to its negative regulation of RUNX2 expression [60]. Moreover, miR-204-5p has been shown to positively regulate adipogenesis of human adipose-derived mesenchymal stem cells by modulating disheveled segment polarity protein 3 (DVL3) expression and subsequently suppressing the activation of the Wnt/β-catenin signaling [61]. MiR-199a-3p has been shown to be upregulated during adipocytic differentiation of BMSCs. Mechanistically, miR-199a-3p inhibits the activity of KDM6A that epigenetically modulates H3K27 as a type of histone demethylases and subsequently induces the inactivation of Wnt signaling, ultimately leading to enhanced adipogenesis of BMSCs [62]. Wang et al. revealed that miR-363-3p promotes adipocyte commitment of rat BMSCs by downregulating tumor necrosis factor receptor-associated factor 3 (TRAF3) expression [63] (Table 1).

Anti-adipogenic miRNAs

The level of miR-130a in BMSCs significantly declines in aged mice and humans compared with young subjects. Lin

Table 1 MicroRNA associated with adipogenic differentiation

MicroRNA	Functional role	Target mRNA	Reference
miR-188	Promotion	HDAC9, RICTOR (repressor of PPARγ)	[55]
miR-320	Promotion	RUNX2	[56]
miR-214	Promotion	JNK, p38	[57]
miR-214-3p	Promotion	Ctnnb1 (Wnt signaling)	[58]
miR-214-5p	Promotion	TGFβ/Smad2/COL4A1	[59]
miR-204	Promotion	RUNX2	[60]
miR-204-5p	Promotion	DVL3	[61]
miR-199a-3p	Promotion	KDM6A, Wnt signaling	[62]
miR-363-3p	Promotion	TRAF3	[63]
miR-130a	Inhibition	PPARγ, Smurf2	[64]
miR-149-3p	Inhibition	FTO	[65]
miR-194	Inhibition	COUP-TFII	[66]
miR-27	Inhibition	Mef2c	[67–69]
miR-203	Inhibition	DKK1	[71]

et al. suggested that miR-130a impedes adipogenic differentiation but stimulates osteogenic differentiation of BMSCs via downregulation of PPARγ and Smad ubiquitination regulatory factors 2 (Smurf2) [64]. It has been reported that miR-149-3p suppresses the adipocytic differentiation of BMSCs through binding to the 3′ UTR of fat mass and obesity-associated protein (FTO) and thus inhibiting FTO expression [65]. Jeong et al. found that miR-194 directly targeted chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) that enhances PPARγ expression and concomitantly represses RUNX2 activity, thus contributing to significantly decreased adipogenesis of BMSCs [66]. You et al. demonstrated that miR-27a is downregulated in BMSCs after adipogenic differentiation and upregulated after osteogenic differentiation. Furthermore, they identified myocyte enhancer factor 2c (Mef2c), an osteogenesis-associated transcription factor, as the target gene of miR-27a for the switch of BMSCs from osteoblastic commitment to adipocytic commitment in osteoporosis [67]. The negative effect of miR-27 during adipogenesis of BMSCs has also been verified by other studies [68–70]. The study by Qiao et al. showed that miR-203 is markedly lower in postmenopausal osteoporosis patients than that in healthy individuals and miR-203 abates adipocytic differentiation of BMSCs by downregulating Dickkopf1 (DKK1), an inhibitor of the classical Wnt signaling pathway [71] (Table 1).

LncRNA

LncRNAs are a type of non-protein coding RNA transcript of more than 200 nucleotides in length [72]. Being initially regarded as transcriptional trash with no biological function, lncRNAs are now known to participate in multiple biological processes and have thus been implicated in many diseases such as osteoporosis [73]. Notably, emerging evidence has established that lncRNAs are involved in the regulation of cell fate determination of BMSCs [30, 74]. Furthermore, lncRNAs function through a diverse range of mechanisms including acting as scaffolds, decoys, guides, and signals [75, 76].

The upregulated expression of lncRNA-ORLNC1 has been observed in osteoporotic humans and mice induced by OVX [77]. BMSCs with overexpression of lncRNA ORLNC1 present increased adipogenic capacity and decreased osteogenic capacity, as corroborated by the opposite observation in BMSCs treated with shRNA-ORLNC1. Further, the mechanism by which lncRNA-ORLNC1 controls adipo-osteogenic differentiation of BMSCs could be attributed to the inhibitory effect of ORLNC1 on miR-296 that was previously established to favor BMSC osteogenesis. Moreover, PTEN, an anti-osteogenic factor, was demonstrated to act as the target of miR-296 and mediate the role of lncRNA-ORLNC1-miR-296 axis in adipo-osteogenic commitment of BMSCs [77]. By conducting microarray analysis of BMSCs isolated from young and aged mice, Li et al. identified a novel lncRNA, Bmncr, which exhibits a significant reduction with aging, suggesting its essential role in the age-related lineage fate switch from osteogenesis to adipogenesis of BMSCs [78]. Further research showed that Bmncr overexpression ameliorates bone loss and bone marrow fat accumulation induced by aging. On the one hand, Bmncr positively maintains the osteogenic niche of BMSCs through fibromodulin (FMOD)-mediated BMSC adherence to the bone surface matrix as well as activation of the BMP2 signaling pathway. On the other hand, Bmncr might function as a scaffold to facilitate the interaction of TAZ and ABL, and the subsequent formation of TAZ-RUNX2 and TAZ-PPARγ transcription complexes, consequently stimulating bone formation and inhibiting bone marrow adipogenesis [78]. Shen et al. revealed that lncRNA-GAS5 acts as a competing endogenous RNA (ceRNA) to sponge miR-18a, which abrogates connective tissue growth factor (CTGF) expression, eventually contributing to impaired adipocyte differentiation [79]. Later, this research group congruously found that GAS5 interacts with UPF1 to induce degradation of SMAD7 mRNA, resulting in enhanced BMSC differentiation into osteoblasts [80]. Wang et al. reported that lncRNA-GAS5 promotes the differentiation of BMSCs into osteoblasts through functioning as a sponge for miR-135a-5p and upregulating FOXO1 expression [81], further supporting the reciprocal regulation of GAS5 in the balance between adipogenesis and osteogenesis of BMSCs. Similarly, Kalwa et al. elucidated that upregulation and downregulation of lncRNA-HOTAIR attenuates or favors adipogenic differentiation of bone marrow-derived stromal cells, respectively. The unfavorable impact of HOTAIR on

adipogenic differentiation of BMSCs is possibly mediated by the modification of RNA-DNA-DNA triple helix formation; furthermore, replicative senescent BMSCs exhibit hypermethylation of the HOTAIR binding sites in the genome which stimulates this triple helix formation [82]. Besides, lncRNA-H19 was shown to negatively affect BMSC differentiation into adipocytes and promote osteogenic differentiation in mice via sponging miR-188 to upregulate ligand-dependent corepressor (LCoR), a previously characterized transcriptional corepressor with anti-adipogenic activity [83]. This effect of H19 has also been demonstrated in other studies [84–86]. MEG3, another lncRNA involved in BMSC differentiation, may favor osteogenic differentiation of BMSCs through facilitating BMP4 transcription as well as targeting miR-140-5p, in parallel leading to compromised adipogenesis [87, 88]. In addition, some other lncRNAs, such as lncRNA-OG [89], lncRNA-KCNQ1OT1 [90], lncRNA-MIR22HG [91], lncRNA-DANCR [92], and lncRNA-linc-ROR [93], have been illustrated to potentiate the osteogenic commitment of BMSCs via diverse signaling pathways, yet whether they concurrently blunt adipogenesis of BMSCs remains to be clarified (Table 2).

Collectively, the aforementioned evidence highlights that BMSC lineage determination is a fine-tuned event. The balance between osteoblastogenesis and adipogenesis of BMSCs is maintained by orchestrated actions of a network of lineage-specific transcription factors, epigenetic regulators, and ncRNAs including miRNA and lncRNA. The dysregulation of these molecules under pathologic conditions may underline the skewed differentiation of BMSCs into adipocytes over osteoblasts, contributing to an increase in BM adiposity and a decrease in bone mass. Therefore, a clear understanding of upstream molecular regulatory mechanisms of BMSC fate decisions could significantly help clarify the pathogenesis of osteoporotic diseases and pave new avenues for the treatment of osteoporosis.

Immunological properties of BMSCs and enhanced BM adiposity

The coexistence of BMSCs, hematopoietic stem cells (HSCs), T cells, and B cells in the bone marrow accounts for the interplay between BMSCs and immune cells, which reminds us of the intimate association between immunological characteristics and enhanced BM adiposity under osteoporotic conditions. The innate immunomodulatory properties of BMSCs have been studied [94]. BMSCs with low expression of immune co-stimulatory molecules and secretion of anti-inflammatory cytokines manifest as low immunogenicity, thus repressing the activation of immune cells such as T cells and B cells [95]. In addition, the ability of BMSCs to repress CD3 mAb-stimulated T-cell proliferation has been investigated in previous studies [96]. Moreover, Huang et al. reported that BMSCs from ovariectomized osteoporotic rats exhibit attenuated osteogenic and chondrogenic capacities but enhanced adipogenic capacity compared with sham-operated rats. Further, they revealed that the expression of CD40 and CD80, the second signaling molecules for T cell activation, is higher in BMSCs isolated from osteoporotic subjects than normal group. Conversely, PD-L1, a negative co-stimulator for T-cell activation, is lowly expressed in BMSCs under osteoporotic condition. Correspondingly, co-culture assay substantiates the suppressive role for non-osteoporotic BMSCs in T cell activation, but not for osteoporotic BMSCs. Overall, BMSCs under osteoporotic conditions can be endowed with increased immunogenicity, causing an inflammatory microenvironment that favors BMSCs to preferentially differentiate into adipocytes rather than osteoblasts [97]. In addition, a study by Li et al. suggested that OVX-induced estrogen deprivation leads to expansion of T cells mediated by the T-cell costimulatory molecule CD40L, which interacts with CD40 on BMSCs to produce more inflammatory

Table 2 LncRNA associated with adipogenic differentiation

LncRNA	Function	Molecular mechanism	Reference
ORLNC1	Promotion	miR-296-PTEN axis	[77]
Bmncr	Inhibition	1) FMOD-mediated BMSC adherence 2) BMP2 signaling pathway 3) Scaffold for TAZ-RUNX2/PPARγ transcriptional complex	[78]
GAS5	Inhibition	1) miR-18a/CTGF axis 2) Interacting with UPF1 to degrade SMAD7 3) miR-135a-5p/FOXO1 axis	[79–81]
HOTAIR	Inhibition	DNA methylation	[82]
H19	Inhibition	1) miR-188/LCoR axis 2) miR-149/SDF1 axis 3) miR-138/FAK axis 4) Epigenetic modulation of histone deacetylases (HDAC4-6)	[83–86]
MEG3	Inhibition	1) Upregulating BMP4 expression 2) Targeting miR-140-5p	[87, 88]

factors via activation of NF-κB signaling [98]. Therefore, the characterization of BMSCs by immunological markers is required in order to take full advantage of the immunomodulatory abilities of BMSCs. It is worth noting that the expression of surface markers CD29, CD73, CD105, CD90, and CD146, in the absence of CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-DR, is recommended for the immunological characterization of BMSCs [94, 99]. For example, a decline in the expression of CD90 by BMSCs was observed with aging, indicating the loss of CD90; this is therefore a potential immunological marker for BMSCs aging [100, 101]. Congruously, in vitro cultured BMSCs from CD90 knockout mice exhibit decreased osteogenic differentiation with a concomitant increase in adipogenic differentiation compared with wild-type mice. CD90 knockout mice show increased BMAT volume when compared to controls [102]. Similarly, loss of CD146 in BMSCs associated with advanced age has also been reported [100, 103] (Fig. 2).

Taken together, alterations in the intrinsic immunological characteristics of BMSCs under osteoporotic states may link to, or partially contribute to, their increased adipogenic propensity, despite the reported inconsistences on characterization, lineage markers, and immunological features of BMSCs. Further studies of the activity and function of BMSCs are required to test this hypothesis.

BMAT biology

BMSC-derived adipocytes resided within the bone marrow are collectively referred to as BMAT. While the well-established inverse relationship between BMAT and bone mineral density (BMD) has been interpreted to indicate that BMAT is a negative regulator of bone mass, caution should be taken in drawing the conclusion in that within BMAT there exists a significant degree of heterogeneity [104, 105]. In 1976, Tavassoli et al. firstly characterized two histochemically distinct populations of BMAT, which have since been termed regulated BMAT (rBMAT) and constitutive BMAT (cBMAT) [106]. The later-forming rBMAT adipocytes that exist at proximal tibia and femur are histologically defined as single cells interspersed within the red, hematopoietic bone marrow. By contrast, cBMAT adipocytes enriched in distal tibia and caudal vertebra develop early at or slightly before birth, are larger in size, and appear histologically as convergent groups of cells that are devoid of hematopoiesis [107]. Importantly, the two BMAT subtypes potentially differ in their lipid composition and response to external stimuli. Compared with cBMAT, rBMAT have lower degree of unsaturated fatty acid composition (unsaturation), which is associated with lower BMD and increased fracture risk [108]. Additionally, rBMAT is found to be more responsive to external stimuli than cBMAT. In this regard, both physiological challenges, excess of calories in obesity and

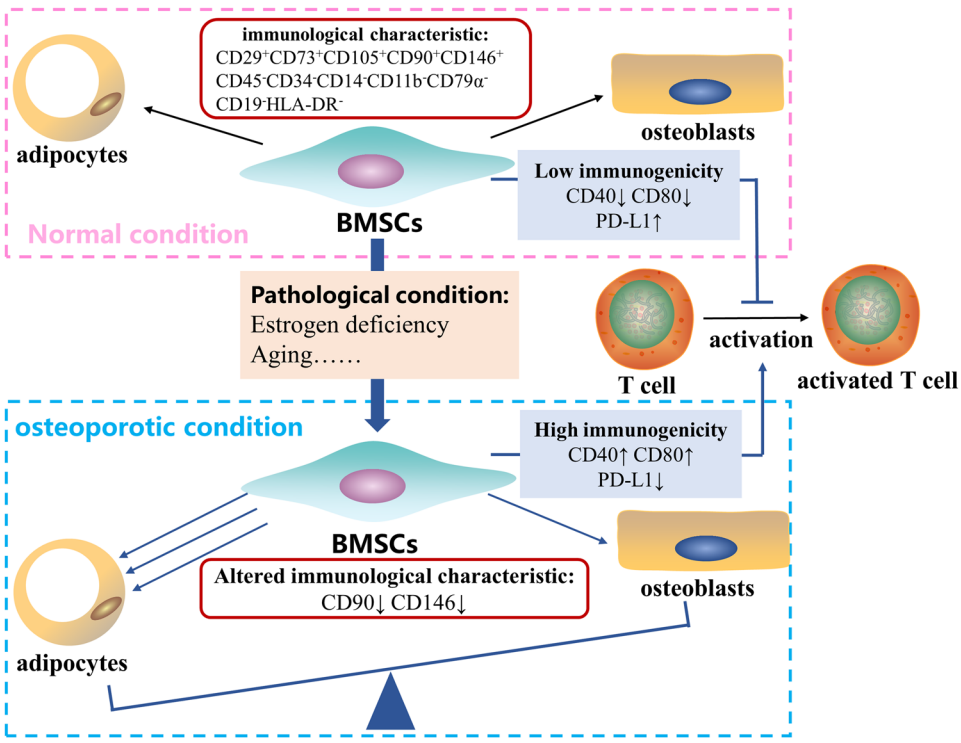

Fig. 2 Immunological properties of BMSCs and enhanced bone marrow adiposity BMSCs from osteoporotic subjects become inclined to commitment into adipocytes than osteoblasts. In addition, BMSCs under osteoporotic condition manifest high immunogenicity with enhanced expression of CD40 and CD80 whereas declined expression of PD-L1, which significantly promote T cell activation. Besides, the immunological characteristics are changed in osteoporotic BMSCs, including loss of surface markers CD90 and CD146

restriction of calories in anorexia nervosa, induce BMAT expansion. The paradox that peripheral WAT is lost whereas BMAT increases in animals under caloric restriction (CR) and humans with anorexia nervosa leads to a line of studies of investigating the mechanisms. Notably, Scheller et al. demonstrated that CR-induced BMAT expansion predominantly occurs within the rBMAT-enriched sites, whereas cBMAT remains almost unchanged [109, 110]. Similarly, the divergent responses between these subtypes are replicated in states of estrogen deficiency, aging, high-fat diet, and cold exposure [111]. Furthermore, the group found that BMAT expansion contributes significantly to increased circulating adiponectin during CR, and possibly, cBMAT expresses higher level of adiponectin than rBMAT. The fact that adiponectin improves metabolic and cardiovascular health, to some extent, determines the pathogenicity of rBMAT [109]. In addition, they showed that rBMAT is more responsive to β-adrenergic stimulation while cBMAT relatively resists lipolysis, which maybe explain why rBMAT reduces whereas cBMAT is preserved upon cold exposure, as well as the seemingly contradictory studies demonstrating resistance to bone loss at sites of high BMAT [112, 113]. On the other hand, rBMAT and cBMAT may also respond differently to hematopoietic demands, as evidenced by Tavassoli's finding that rBMAT is depleted in response to phenylhydrazine-induced hemolysis while the cBMAT is preserved [106]. Moreover, a recent study by Zhou et al. revealed that BMAT adipocytes promote HSC maintenance and hematopoietic regeneration by secreting stem cell factor, but except for caudal vertebra, strongly supporting the presence of different types of BMAT [114]. In summary, perhaps it is not simply the presence of BMAT but rather a specific type of BMAT that mediates detrimental effect during pathological bone loss, with rBMAT more involved.

Bone marrow adipocyte-osteoclast crosstalk

In view of the competitive lineage allocation between adipogenesis and osteogenesis of BMSCs, increased BM adiposity inevitably results in impaired osteoblastic bone formation. Notably, a recent important finding to mention is that bone marrow adipocytes secrete the specific BMPR antagonists such as chordin-like1 (Chrdl1) and gremlin1 (Grem1), which significantly suppress bone formation [115, 116]. In addition, emerging evidence indicates that accumulated bone marrow fat also contributes to enhance osteoclastic bone resorption [117]. The pro-osteoclastogenic effects of marrow adipocytes are achieved through transcription factors that interweave the adipogenic and osteoclastogenic programs as well as the paracrine manner of bone marrow adipocytes.

On the one hand, several key transcription factors such as PPARγ, C/EBPα, and C/EBPβ that are required for the adipogenesis of BMSCs are expressed in HSCs and promote HSC osteoclastogenesis. Using PPARγ-tTA TRE-H2BGFP reporter mice, Wei et al. revealed that osteoclast progenitors reside within the PPARγ-expressing bone marrow subpopulation [118]. Furthermore, both Notch activation in PPARγ + cells and selective ablation of PPARγ + cells by diphtheria toxin lead to suppression of osteoclast differentiation and high bone mass. Mechanistically, PPARγ acts to bind to cis-regulatory elements within the promoter regions of osteoclast-specific transcription factors such as GATA2, c-fos, and NFATc1, activating the transcription of these factors and stimulating osteoclastogenesis [118]. Moreover, PGC1β, a transcriptional co-activator for ERR1α, is induced by suppression of Wnt/β-catenin signaling by PPARγ and functions to support osteoclast function through stimulating mitochondrial biogenesis [117, 119]. Similar to PPARγ, C/EBPα and C/EBPβ can also serve as osteoclastogenic transcription factors to induce osteoclast differentiation by activating the transcription of osteoclast-specific genes including NFATc1, c-fos, Ctsk, and Atp6i [117, 120]. Additionally, activation of C/EBPβ and C/EBPδ upon adipogenic stimuli contributes to the upregulated expression of receptor activator of nuclear factor-κB ligand (RANKL) through binding to the RANKL promoter [121]. In this fashion, the normal crosstalk between osteoblasts and osteoclasts is decoupled by hyperactivated pro-adipogenic transcription factors during pathological conditions, leading to expansion of both adipogenesis and osteoclastogenesis (Fig. 3).

On the other hand, BMAT-secreted cytokines play an essential role in promoting osteoclast differentiation. RANKL, a major pro-osteoclastogenic cytokine, is highly expressed in bone marrow adipocytes under a variety of pathological conditions such as aging [121], estrogen deficiency [122], glucocorticoid administration [123], and type 1 diabetes [124]. By performing FACS using Pref-1 as the preadipocyte marker, Takeshita et al. determined the potential of Pref-1-positive bone marrow preadipocytes to express RANKL and found that Pref-1 and RANKL double-positive cell population was increased with aging [121]. As expected, bone marrow macrophages are induced to differentiate into TRAP-positive osteoclasts upon coculturing with these Pref-1 + RANKL + cells in the absence of osteoclastogenic cytokines such as RANKL, indicating the induction of osteoclastogenesis by RANKL-expressing preadipocytes in bone marrow [121]. Moreover, Fan et al. demonstrated that conditional deletion of PTH1R signaling in BMSCs leads to markedly increased BMAT and low bone mass along with enhanced osteoclast formation and bone resorption. Importantly, RANKL is known to be largely secreted by bone marrow adipocytes to favor bone resorption [10, 125, 126]. Of note, Zhong et al. recently unveiled a new adipose lineage cell population named as marrow adipogenic lineage precursors (MALPs) using the technique of large-scale single-cell

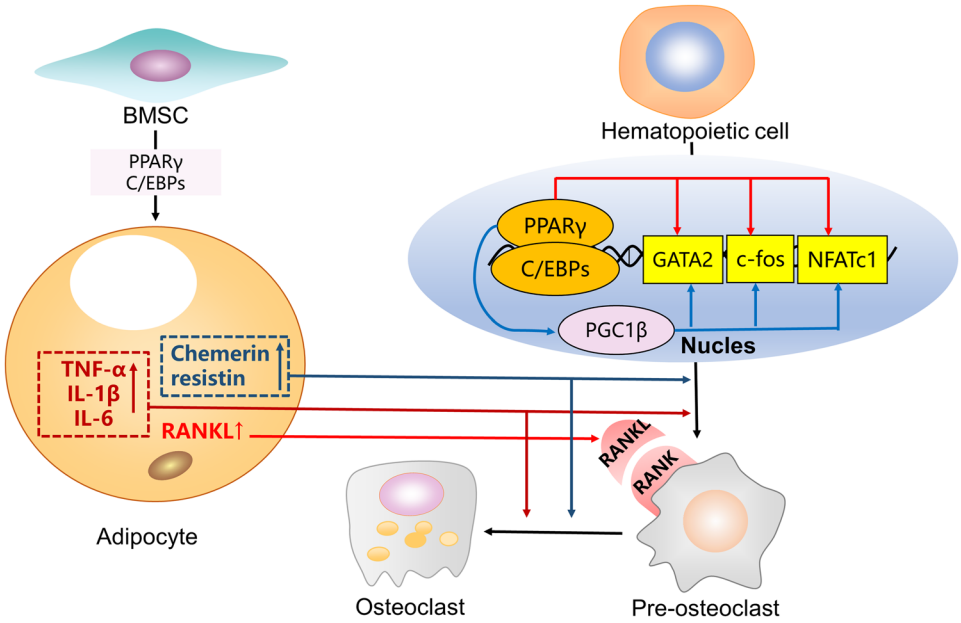

Fig. 3 Bone marrow adipocyte-osteoclast crosstalk Adipogenic transcription factors such as PPARγ and C/EBPs interweave adipocyte and osteoclast differentiation programs. During pathological conditions, hyperactivation of PPARγ and C/EBPs contributes to remarkably enhanced bone marrow adipocyte and osteoclast formation. In addition, accumulated MAT secretes increased RANKL, inflammatory cytokines such as TNF-α, IL-1β, IL-6, and adipokines such as chemerin and resistin, all of which significantly promote osteoclast differentiation and function

RNA sequencing [116]. MALPs are unique in that they are non-proliferative cells that express adipocyte markers but contain no lipid droplets. MALPs exist abundantly as stromal cells and pericytes, forming a ubiquitous 3D network within the bone marrow [116]. MALPs are identified as the major source of RANKL, as evidenced by the finding that conditional deletion of RANKL in MALPs labeled by adipocyte-specific adiponectin-Cre results in drastically increased trabecular bone mass accompanied by suppressed bone resorption. This is also true in pathological conditions. For instance, RANKL deficiency in MALPs can prevent osteolytic lesions induced by LPS treatment and partially attenuate bone resorption induced by OVX [127]. As such, MALPs, as a critical component of BMAT, play a pivotal role in bone remodeling under both normal and pathological conditions via the production of RANKL. In addition to RANKL, BMAT also has the capacity to produce inflammatory cytokines including IL-1β, IL-6, TNF-α, and MCP-1 [128–130]. Intriguingly, by performing microarray analysis, Liu et al. demonstrated that BMAT surpassed epididymal white adipose tissue as a source of inflammatory factors [131]. Under pathological conditions, excessive BMAT becomes one of the major sources of inflammatory factors, significantly contributing to a BM inflammatory microenvironment. Importantly, these inflammatory factors have long been recognized as pro-osteoclastogenic cytokines. For instance, TNF-α not only acts to induce RANKL production, but also promotes RANKL-induced osteoclastogenesis by activating the PI3K/Akt/NF-κB pathway [132, 133] (Fig. 3).

Moreover, BMAT-secreted adipokines, such as chemerin and resistin, are also known to promote osteoclast differentiation. For example, chemerin binds to the receptor CMKLR1 in HSCs to induce their differentiation into osteoclasts via activation of NFATc1 [134, 135]. Consistent with this, chemerin neutralization causes the blockade of HSC osteoclastogenesis, supporting a key role for chemerin in osteoclast formation [136]. Similarly, resistin has been shown to positively affect osteoclast differentiation through activating nuclear factor of kappa B (NF-κB) signaling [137]. As for other adipokines such as adiponectin, leptin, visfatin, and omentin, their dual and complex regulatory roles in osteoclast development remain to be elucidated (Fig. 3).

Taken together, expanded BM adipogenesis not only results in enervated osteoblast differentiation of BMSCs, but also immensely favors osteoclast differentiation and function, both of which synergistically accelerate bone loss. Based on the cellular triad of adipocyte, osteoblast and osteoclast interactions, targeting the reduction of BM adiposity may exert dual effects: one is to promote osteoblastic bone formation, and the other is to diminish osteoclastic bone resorption. As such, therapeutic strategies targeting bone marrow adipocyte differentiation, which combine the advantages of bone anabolic and anti-resorptive agents, appear to be highly effective and encouraging for the treatment of osteoporosis.

Targeting BMAT upstream to prevent bone loss

Considering the adverse effects of conventional anti-osteoporotic drugs and the roles of BMAT accumulation during pathological bone loss, suppressing BMSC adipogenic differentiation while promoting osteogenesis may be a potential

approach for combating osteoporosis. As mentioned above, the well-orchestrated molecular networks composed of transcription factors, epigenetic regulators, and ncRNAs including miRNA and lncRNA play a crucial role in the lineage fate decisions of BMSCs, which makes them promising therapeutic targets for osteoporosis.

Targeting adipo-osteogenic transcription factors

PPARγ serves as the master transcription factor for the adipogenic differentiation of BMSCs. Other transcription factors including the C/EBP family, Zfp423, Sox2, and Oct4 are also involved in the regulation of adipogenesis. Thereby, antagonists targeting these factors may be utilized to prevent BMAT accumulation and promote bone formation. Moreover, as previously mentioned, inhibition of PPARγ also contributes to reduced osteoclast differentiation, so much so that BADGE, a PPARγ antagonist, is known to significantly attenuate BM adiposity and induce bone formation in C57BL/6 mice, without affecting glucose metabolism [138]. The osteoanabolic effect exerted by pharmacological inhibition of PPARγ is corroborated by another in vitro study [139]. However, the major issue that hinders the use of PPARγ antagonists is the coupled regulation of bone homeostasis and energy metabolism by PPARγ. PPARγ agonists such as thiazolidinediones are routinely used for the treatment of diabetes mellitus owing to their ability to enhance insulin sensitivity. Thus, balancing the dual effects of PPARγ signaling in bone and glucose homeostasis is essential for PPARγ antagonists. On the other hand, RUNX2 and Wnt are the key transcription factors responsible for BMSC osteogenesis. Considering the mutually suppressive actions between PPARγ and Wnt, Wnt inhibitors such as sclerostin (SOST), Dkk1, and sRFP-1 that are expressed in the bone are amenable to targeting for their production of osteoanabolic effects [140, 141]. For instance, romosozumab, a SOST antibody, is approved for the treatment of postmenopausal osteoporosis with anti-adipogenic and pro-osteogenic effects [142]. Nevertheless, studies concerning agonists of RUNX2 and the Wnt signaling pathway are scarce. Although a growing body of adipo-osteogenic transcription factors can be ideal targets owing to their crucial roles in the regulation of osteo-adipogenesis, specifically targeting their activity in BMSCs without affecting other cellular processes is extremely challenging.

Targeting adipo-osteogenic epigenetic regulators

Despite still being at the preclinical stage, great efforts have been made in BMSC-dependent epigenetic therapeutics aimed at alleviating marrow adiposity and improving bone mass. GSKJ126, an EZH2 inhibitor, was found to enhance bone formation by reducing repressive H3K27me3 on Wnt and RUNX2 in MC3T3-E1 cells and mice [143]. With one accord, the use of the EZH2 chemical inhibitor DZnep was able to reverse the osteoporotic BMSCs lineage fate from adipogenesis to osteogenesis [144]. Moreover, KDM5A-mediated bone loss and marrow fat accumulation during osteoporosis were partially rescued following pretreatment with the KDM5A inhibitor JIB-04 [39]. Pargyline, an inhibitor of lysine-specific demethylase 1 (LSD1), could improve the osteogenic ability of BMSCs under osteoporosis conditions by decreasing H3K4 methylation levels on the promoters of osteogenesis-related genes [145]. Similarly, the HDAC inhibitors, trichostatin and vorinostat, function to enhance osteoblast differentiation of BMSCs by modulating histone acetylation on the promoter region of OCN and RUNX2 [146, 147].

Non-coding RNA-based therapeutic strategy

Recently, miRNA-based therapeutics have gained increasing attention and already entered Phase 2a clinical trials [148]. However, systemic administration of miRNA mimics or antagonists could exert adverse effects in non-skeletal tissues [149]. A variety of bone targeting delivery systems have emerged. By virtue of the long-lasting local effect and lower cost, delivery of therapeutic nucleic acids into BMSCs to improve their osteogenic capacity shows tremendous potential for bone regeneration [150]. Non-viral vectors, such as nanomaterials with high biocompatibility are increasingly utilized for the study of bone defect repair [151]. A typical example is exosomes, naturally derived nanocarriers, that show promising prospects for drug delivery with low biotoxicity and high barrier penetrating capacity [152]. In Hu's study, CXCR4-expressing exosomes from genetically engineered NIH-3T3 cells were constructed to achieve targeted accumulation in SDF1-rich bone marrow in chemotaxis behavior. Subsequently, the CXCR4-positive exosomes were fused with liposomes carrying antagomir-188 to obtain hybrid NPs, which specifically reduce bone marrow miRNA-188 levels and reverse age-related bone loss through suppressing adipogenic differentiation and enhancing osteogenic differentiation of BMSCs [153]. Moreover, a chitosan-based non-viral sustained delivery system was developed to entrap CTH nanoparticles loaded with antagomiR133a/b, which remarkably enhanced osteogenic differentiation of BMSCs and bone regeneration [154]. In addition, considering the risk of toxicity, tumorigenesis, and the adverse immune response of viral vectors, Bu et al. synthesized ascorbic acid-PEI carbon dots (CD) carrying osteoinductive miR-2861 using the microwave-assisted pyrolysis method. The CDs with high biocompatibility and no cytotoxicity could be efficiently internalized into BMSCs by the clathrin-mediated endocytosis pathway.

Moreover, it was revealed that CD alone had pro-osteogenic effects in vitro and CD loaded with miR-2861 presented a much stronger capacity for promoting osteogenic differentiation of BMSCs [155]. Furthermore, lipidoids, a general term of numerous cationic lipid-like materials, have emerged as a promising gene delivery platform with high efficiency and safety. In vivo delivery of miR335-5p by lipidoids has been shown to significantly induce bone regeneration [156].

In addition to miRNA, mounting evidence supports the importance of lncRNA in clinical application [30]. For example, Tao et al. demonstrated that extracellular vesicle-mimetic nanovesicles (EMNVs) serve as an effective nano-drug delivery system for lncRNA and EMNVs carrying LncRNA-H19 function to counteract hyperglycemia-induced impaired angiogenesis and remarkably accelerate diabetic wounds healing via the ceRNA effect [157]. Moreover, an EpDT3 aptamer-linked poly(amidoamine) (PAMAM) dendrimer confers the capacity of targeting EpCAM that is highly expressed on the surfaces of prostate cancer cells. Furthermore, PAMAM-PEG-EpDT3 serves as a novel carrier for the targeted delivery of plasmid-encoding lncRNA MEG3 (pMEG3) into castration-resistant prostate cancer (CRPC) cells, which demonstrates a significant anti-CRPC effect both in vivo and in vitro [158]. However, compared to the progress made with miRNA, there are few reports regarding the application of lncRNA in gene therapy, particularly for bone diseases.

Conflicting evidence

Despite immense supportive evidence that blocking BMAT formation contributes significantly to the prevention of bone loss, there are still some contradictory conclusions. For example, Botolin et al. demonstrated that BADGE, a PPARγ antagonist, dramatically attenuates type I diabetes-induced hyperlipidemia and bone marrow adiposity, with the inability to rescue bone loss [159]. Similar phenotype was observed in another bone loss model: BADGE treatment mitigates bone marrow adiposity both in sham and OVX rats and promotes bone formation in the sham group, but does not prevent bone loss induced by OVX [160]. In addition to pharmacological inhibition of PPARγ, Almeida et al. revealed that conditional deletion of PPARγ in BMSCs of the murine appendicular skeleton markedly abolishes BM adipogenesis, but fails to reverse rosiglitazone-induced trabecular or cortical bone loss in male mice, or age-dependent bone deterioration in female mice [161]. Furthermore, work by Keune et al. showed that instead of bone-protective effect, disuse-induced cancellous bone loss is exacerbated in genetic model of BMAT deficiency [162]. Thus, with regard to the feasibility and validity of blocking BM adipogenesis for bone loss prevention, further studies are required due to inconsistencies of available data so far.

Conclusions

Osteo-adipogenesis commitment of BMSCs is a fine-tuned process that depends on the coordinated action of transcription factors, epigenetic regulators, and ncRNAs; in addition, the immunological properties of BMSCs also play a role. Over the past few decades, a growing number of signaling molecules have been identified to be aberrantly expressed in bone-related diseases. Importantly, many of them have been shown to possess crucial biological functions and participate in the occurrence and development of osteoporosis. This review provides an overview of the different roles of multiple regulatory factors in BMSC fate decisions and osteoporosis, as well as revealing new prospects for their clinical application. Meanwhile, the investigation into crossroads of bone marrow adipocyte and osteoclast differentiation programs further replenishes the cellular and molecular basis of osteoporosis, which is of great significance for the precision treatment of bone loss diseases. However, there is still much that is unknown regarding the detailed molecular mechanisms accounting for enhanced BM adipogenesis during pathologic bone loss. Additionally, clinical translation of therapeutic targeting of the above molecules is still in its infancy, even though they hold great promise. Therefore, a comprehensive understanding of the molecular network regulating BMAT formation as well as broad exploration of biological therapeutic technologies are laying the foundation for the development of novel potent medications with no side effects for the treatment of osteoporosis.

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Declarations

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Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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