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Review

NOVEL POST-TRANSLATIONAL MODIFICATIONS IN BONE REMODELING: POTENTIAL THERAPEUTIC STRATEGIES FOR OSTEOPOROSIS

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Abstract

Osteoblast-induced bone formation and osteoclast-mediated bone resorption coordinate the balance of bone remodeling. However, the exact mechanisms underlying bone remodeling remain unknown. Several post-translational modifications (PTMs), including glycosylation, ubiquitination, sumoylation, lactylation, and palmitoylation, have been linked to bone remodeling. The core molecules involved in bone remodeling are widely modified by PTMs. An imbalance between bone resorption and formation is a prerequisite for osteoporosis. Therefore, targeting bone remodeling by modulating PTMs is a promising strategy for osteoporosis therapy. In this review we consider the roles of novel PTMs in bone remodeling, which may deepen our understanding of the mechanisms underlying bone remodeling and may also provide novel treatment targets for osteoporosis.

Keywords: Bone remodeling, osteoporosis, post-translational modifications, glycosylation, ubiquitination, sumoylation, lactylation, palmitoylation.

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Introduction

Bone is a highly dynamic and complex organ that undergoes continuous remodeling throughout the lifespan [1]. Bone remodeling consists of two basic processes, including the osteoblast-mediated bone formation and osteoclast-mediated bone resorption [2]. Osteoblasts produce osteocytes and extracellular matrix, which is further deposited and mineralized [3]. In contrast, to maintain homeostasis, osteoclasts dissolve the bone matrix and minerals via secreted acids and enzymes [4]. The equilibrium between these two processes is essential for several physiological functions, such as maintenance of bone renewal, repair of bone damage, adaption to various mechanical stresses, and systemic mineral homeostasis [5].

Dysregulation of bone remodeling is invloved in various bone-related diseases, such as arthritis, bone metastasis, osteopetrosis, and most importantly, osteoporosis [6]. Osteoporosis is one of the most common metabolic bone diseases worldwide and is characterized by reduced bone mineral density and an increased risk of pathological fractures [7]. An imbalance between bone resorption and formation is a prerequisite for osteoporosis [8]. Nevertheless, the exact molecular mechanisms underlying osteoporosis need to be further explored. Antiresorptive therapies such as bisphosphonates and denosumab, and osteoanabolic therapies such as teriparatide are the mainstream pharmacological treatment options for osteoporosis [9]. However, these pharmacological interventions may also lead to serious adverse reactions such as osteonecrosis of the jaw and electrolyte imbalance [9]. Therefore, exploring novel mechanisms of bone remodeling and developing new targets for osteoporosis with fewer adverse reactions are necessary.

Recent evidence suggests that post-translational modifications (PTMs) are closely involved in bone remodeling [10]. PTMs are the chemical modifications of specific amino acid side residues following protein biosynthesis and

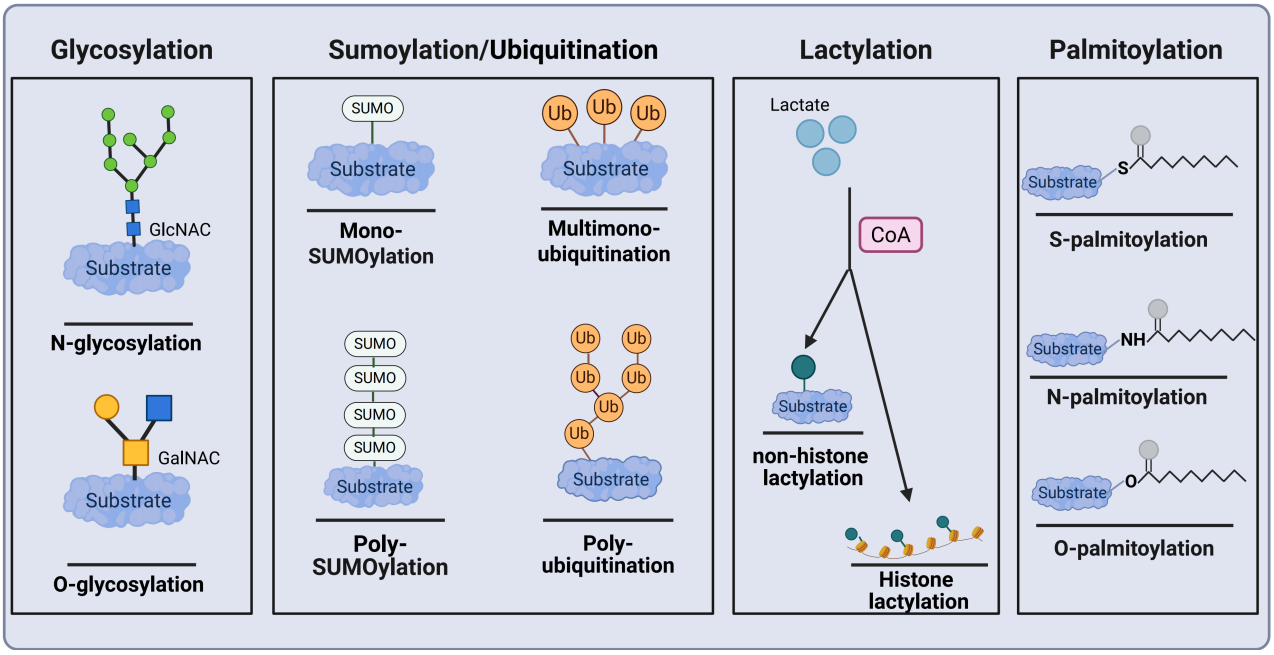

Fig. 1. Brief introduction of the post-translational modifications summarized in this review. GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; SUMO, small ubiquitin-related modifier; CoA, coenzyme A. Created in BioRender. Wang, Q. (2025) https://BioRender.com/jeg4msr.

play a predominant role in modulating protein conformation, activity, stability, localization, and interactions [11]. The chemical compositions of amino acids can be expanded by modifying or adding functional groups. PTMs are reversible processes in which the gain or loss of a protein modification site is mainly regulated by enzymes known as writers, readers, and erasers [12]. It was reported that there are more than 400 types of PTMs, including phosphorylation, methylation, glycosylation, acetylation, ubiquitination, and sumoylation [13]. Owing to the rapid development of PTM site prediction tools, a range of studies have focused on the relationship between PTMs and human diseases. PTMs have been strongly linked to numerous diseases such as cancer, cardiovascular diseases, and bone diseases [14].

There is a huge potential for drug development targeting PTM-related enzymes. Therefore, it is worthwhile to investigate whether PTMs are involved in the pathogenesis of osteoporosis. Therefore, this review aimed to summarize the functions of distinct PTM types in osteoporosis. As phosphorylation and methylation have been extensively studied in previous studies and reviews concerning osteoporosis, this article focuses on other PTMs, including glycosylation, ubiquitination, sumoylation, lactylation, and palmitoylation (Fig. 1).

In this review, we identified original studies published over the past 20 years in the PubMed database. The keywords ("Post-translational Modifications" OR "glycosylation" OR "ubiquitination" OR "sumoylation" OR "lactylation" OR "palmitoylation") AND ("bone remodeling" OR "osteoblast" OR "osteoclast" OR "osteoporosis") were used for search strategy, and articles relative to our topic are included in this review.

Glycosylation in Bone Remodeling

Protein glycosylation involves the covalent attachment of single sugars or glycans to target protein residues. In some cases, glycosylation is performed via direct addition of a single nucleotide-activated sugar to the target residue. This ligation is followed by the sequential attachment of additional nucleotide-activated sugars, eventually forming glycans in the presence or absence of a lipid carrier. N-glycosylation and O-glycosylation are the most frequently documented reactions. N-glycosylation is the connection of a glycan to select asparagine (N) or arginine (R) residues. O-glycosylation involves the attachment of a glycan to serine (S), tyrosine (Y), threonine (T), or other hydroxyl-containing residues. Other less common glycosylation types contain C-glycosylation, S-glycosylation, and P-glycosylation [15]. The relevant studies are summarized below.

Direct glycosylation modification of bone marrow mesenchymal stem cells (BMSCs) or glycosylation modification of osteogenic-related factors has been confirmed to play fundamental roles in regulating bone remodeling. An intriguing study performed direct surface glycosylation of BMSCs [16]. Induced by the forced expression of fucosyltransferase VII, glycosylation of BMSCs increased their homing ability to bone defect sites, providing a novel biomedical engineering design for bone defect repair [16].

Other published data have focused on the glycosylation modification of osteogenic-related factors, such as sclerostin (SOST), bone morphogenetic protein-2 (BMP-2), dentin matrix protein 1 (DMP1), osteopontin (OPN), and osteocalcin (OCN). SOST is a glycoprotein secreted mainly by osteocytes and inhibits bone formation by inhibiting Wnt signaling [17]. In addition, SOST exerts protective effects on the cardiovascular system [18]. Although SOST inhibition is regarded as a promising strategy for osteoporosis intervention, its potential to increase the risk of cardiovascular disease may limit its clinical application [18]. The β4-N-acetylgalactosaminyltransferase 3 (β4GalNAcT3, coded by *B4GALNT3*) transfer N-acetylgalactosamine (GalNAc) to non-reducing terminal N-acetylglucosamine (GlcNAc) to form LacdiNAc (LDN) group on N- and O-glycans [19]. It has been shown that β4GalNAcT3 induces the LDN-glycosylation of SOST in osteocytes, while decreases circulating SOST levels, and thus exerts therapeutic effects on osteoporosis [20]. One possible explanation for this mechanism is that the LDN-glycosylated form of SOST may increase protein degradation or elimination, but this requires further study. Interestingly, the expression of *B4GALNT3* is seldom detected in the aorta, and interference with β4GalNAcT3 has limited effects on SOST levels in the aorta [20]. These results suggest that the activation or overexpression of β4GalNAcT3 are novel strategies for improving bone quality that may avoid the potential cardiovascular risks compared with SOST global inhibition. Secretion of BMP-2, an osteogenic promoter, is strictly regulated by N-glycosylation [21]. N-glycosylation at the N135 site helps BMP-2 fold as functional secreted protein to exert osteoblast differentiation functions [21]. Mutation of the N135 site leads to the retention of BMP-2 in endoplasmic reticulum (ER), which is consistent with elevated ER stress [21]. Glycosylation of DMP1 at S89 is also essential for the regulation of bone remodeling and mineralization and regulates bone repair dependent on BMP-Smad signaling [22,23]. N-glycosylation at the OPN N79 site bidirectionally regulates osteoblasts and osteoclasts [24]. N-glycosylation at N79 site in OPN promotes osteoclast proliferation and osteoclastogenesis but inhibits osteogenesis [24]. Further analysis showed that these effects were mediated through promoting nuclear factor-κB (NF-κB) signaling pathway [24]. O-glycosylation of OCN at S8 site prevents plasmin-mediated endoproteolysis, thus promoting its stability in mice [25]. Although the results demonstrated that the O-glycosylated form of OCN promoted insulin expression, its role in bone remodeling remains unknown [25]. Collectively, these studies reveal the regulatory roles of glycosylation in the functions of osteogenesis-related factors. However, the upstream events and the exact regulatory mechanisms of glycosylation during these processes remain unknown.

O-GlcNAcylation is a recently discovered post-translational modification. This refers to the formation of an O-glycosidic bond between a single GlcNAc and the hydroxyl group of serine/threonine in the protein [26]. This process is catalyzed by O-GlcNAc transferase (OGT), and the reverse reaction is mediated by O-GlcNAcase (OGA) [27]. Through the hexosamine biosynthetic pathway, glucose is converted into uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which serves as a substrate for O-GlcNAcylation modification [28]. Owing to its dependence on metabolic pathways, O-GlcNAcylation is regarded as a crucial nutritional sensor [29].

The global protein O-GlcNAcylation levels are gradually increased during osteogenic differentiation [30,31]. Moreover, inhibition of protein O-GlcNAcylation through either genetic or pharmacological suppression of OGT leads to impaired osteoblast activity and osteogenic differentiation [31,32]. As expected, OGA inhibitors had the opposite effects [30,32]. More specifically, the osteogenic factor runt-related transcription factor 2 (RUNX2) is modified by O-GlcNAcylation at S32, S33, and S371, which is essential for its transcriptional activity [30,31,33]. In addition to RUNX2, other proteins such as microtubule associated serine/threonine kinase family member 4 (MAST4), RNA binding protein ubiquitin-associated protein 2-like (UBAP2l), CREB-binding protein (CBP), and TGF-β-activated kinase 1/MAP3K7-binding protein 1 and 2 (TAB1 and TAB2) are also O-GlcNAcylated during osteoblast differentiation, but their causal effects on bone remodeling are still unknown [34]. Although most studies have reported that protein O-GlcNAcylation promotes osteogenesis, one study revealed the opposite effect during the osteogenic differentiation of periodontal ligament stem cells (PDLCs) [35]. At high glucose levels, OGT induced the Toll-like receptor 4 (TLR4) O-GlcNAcylation, which inhibited the osteogenic differentiation of PDLCs [35]. Another study found that BMP-2 induced elevated O-GlcNAcylation levels and osteogenic differentiation in the myoblast cell line C2C12 [36]. However, additional supplementation with a high concentration of glucose further increased O-GlcNAcylation levels, but inhibited the osteogenic capability of C2C12 cells, and these effects could be reversed by an OGT inhibitor [36]. Therefore, the elevated O-GlcNAcylation levels induced by pathological stimuli may inhibit osteoblast differentiation by modulating other effectors, suggesting complex regulatory roles for O-GlcNAcylation modification. Moreover, these results are also in accordance with the O-GlcNAcylation optimal zone hypothesis, in which the optimal protein O-GlcNAcylation levels as well as the coordination between OGT and OGA activity are essential in normal cellular functions [26].

The role of O-GlcNAcylation in osteoclastogenesis is complex. During macrophage colony-stimulating factor (M-CSF)- and receptor activator of nuclear factor-kappa B ligand (RANKL)-induced osteoclast differentiation, global protein O-GlcNAcylation gradually increases until 48 h and then steadily decreases over time, suggesting a tem-

Table 1. Roles of protein glycosylation in bone remodeling.

Cell line	Enzyme	Target	Effect	Mechanism	Reference
Saos-2	*β4GalNAcT3*	SOST	Enhances bone mass	*β4GalNAcT3* increases LDN-glycosylated SOST levels	[20]
MC3T3-E1	-	BMP-2	Promotes osteoblast differentiation	BMP-2 N-glycosylation at N135 site is essential in protein folding and function	[21]
Primary osteoblast	-	DMP1	Promotes osteoblast differentiation	DMP1 glycosylation of at S89 is essential in protein function	[22]
MC3T3-E1, RAW264.7	-	OPN	Inhibits osteoblast differentiation, promotes osteoclast differentiation	OPN glycosylation of at N79 promotes NF-κB nuclear translocation	[24]
Primary osteoblast	-	OCN	Promotes osteoblast differentiation	OCN glycosylation of at S8 increases its stability	[25]
MC3T3-E1	OGT	-	Promotes osteoblast differentiation	-	[32]
MC3T3-E1	OGA		Inhibits osteoblast differentiation	-	[32]
Primary BMSCs	-	RUNX2	Promotes osteoblast differentiation	RUNX2 glycosylation of at S32, S33, and S371 is essential for its transcriptional activity	[31]
Primary PDLCs	OGT	TLR4	Inhibits osteoblast differentiation	-	[35]
Primary BMMs	-	p65, NFATc1	Promotes osteoclast differentiation	RANKL promotes p65 and NFATc1 O-GlcNAcylation and nuclear translocation	[37]
Primary BMMs	-	NUP153	Promotes osteoclast differentiation	NUP153 O-GlcNAcylation promotes *MYC* nuclear translocation and transcriptional activity	[38]

BMSCs, bone marrow mesenchymal stem cells; PDLCs, periodontal ligament stem cells; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; SOST, sclerostin; BMP-2, bone morphogenetic protein-2; DMP1, dentin matrix protein 1; OPN, osteopontin; OCN, osteocalcin; TLR4, Toll-like receptor 4; NFATc1, nuclear factor of activated T cells 1; NUP153, nuclear pore protein nucleoporin 153; LDN, LacdiNAc; RANKL, receptor activator of nuclear factor-kappa B ligand; NF-κB, nuclear factor-κB; RUNX2, runt-related transcription factor 2; BMMs, bone marrow-derived macrophages.

porally dynamic change in O-GlcNAcylation during osteoclastogenesis [37,38]. Indeed, protein O-GlcNAcylation promotes immature osteoclast differentiation at the early stages of osteoclastogenesis [38]. Conditional knockout of OGT in osteoclast precursors significantly improves bone loss in inflammatory and non-inflammatory bone loss [38,39]. Mechanistically, RANKL induces NF-κB p65 and nuclear factor of activated T cells 1 (NFATc1) O-GlcNAcylation and nuclear translocation [37,39]. In addition, the nuclear pore protein nucleoporin 153 (NUP153) is O-GlcNAcylated during the early stage of osteoclastogenesis, which promotes the nuclear translocation and transcriptional activity of *MYC* [38]. However, a decrease in O-GlcNAcylation levels is essential for late-stage osteoclastogenesis. The OGA inhibitor thiamet-G impairs the number of multinucleated mature osteoclasts without affecting immature osteoclast precursors at the lates stage of osteoclastogenesis [38]. However, the exact mechanism by which decreased O-GlcNAcylation regulates osteoclast maturation remains largely unknown. Owing to the complicated roles of O-GlcNAcylation in bone remodeling, simply interfering with the overall O-GlcNAcylation levels may lead to unexpected confounding effects. It has been speculated that the modulation of O-GlcNAcylation levels in certain cell subtypes or even in cells at certain developmental stages may achieve ideal therapeutic effects. In addition, energy metabolism plays a central role in regulating bone remodeling, and it is unclear whether O-GlcNAcylation modification, as a nutritional sensor, is involved in this process [40]. Further studies are required to answer these questions. The roles of protein glycosylation in bone remodeling are listed in Table 1 (Ref. [20–22,24,25,31,32,35,37,38]).

Ubiquitination in Bone Remodeling

Ubiquitin is a highly conserved protein comprising 76 amino acids [41]. Protein ubiquitination is a dynamic PTM in which ubiquitin is added to a substrate protein [42]. Briefly, ubiquitination is catalyzed by several enzymes, including E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin ligases [43]. Under the action of these cascade reactions, a bond is formed between the COOH-terminal glycine of ubiquitin and the lysine (K) residue of the substrate protein [44]. Moreover, ubiquitin can be conjugated with other ubiquitin molecules through their lysine residues (K6, K11, K27, K29, K33, K48, and K63) or the N-terminal methionine residue (M1), forming highly dynamic ubiquitin chains, also known as ubiquitin codes [42]. According to the linking pattern of ubiquitin, ubiquitination can be classified as monoubiquitylation, multi-monoubiquitylation, homotypic polyubiqui-

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Table 2. Role of protein ubiquitination in bone homeostasis and osteoporosis.

Cell line	Enzyme	Target	Effect	Mechanism	Reference
C2C12, 2T3	Smurf1	RUNX2	Inhibits osteoblast differentiation	Smurf1 mediates RUNX2 ubiquitination and degradation	[50]
C3H10T1/2, primary osteoblast	RNF138	RUNX2	Inhibits osteoblast differentiation	RNF138 mediates RUNX2 ubiquitination and degradation	[51]
hPDLSCs	TRIM16	CHIP, RUNX2	Promotes osteoblast differentiation	TRIM16 inhibits CHIP-induced RUNX2 ubiquitination and degradation	[52]
C3H10T1/2	WWP2	RUNX2	Promotes osteoblast differentiation	WWP2 promotes RUNX2 mono-ubiquitination and transactivation	[56]
C3H10T1/2, primary osteoblast	HAUSP	RUNX2	Promotes osteoblast differentiation	HAUSP prevents RUNX2 from ubiquitination and degradation	[57]
Primary BMSCs	USP26	β-catenin	Promotes osteoblast differentiation	USP26 interacts and stabilizes β-catenin	[58]
Primary osteoblast	USP8	Fzd5, Wnt/β-catenin	Promotes osteoblast differentiation	USP8 activates Wnt/β-catenin signaling through preventing Fzd5 degradation	[59]
Primary osteoblast	USP15	β-catenin	Promotes osteoblast differentiation	β-catenin phosphorylation by MEKK2 at S675 recruits USP15 to stabilize β-catenin	[62]
MC3T3	USP7	YAP1, Wnt/β-catenin	Promotes osteoblast differentiation	USP7 deubiquitinates and stabilizes YAP1 and further promotes Wnt/β-catenin signaling	[63]
C3H10T1/2, ST2	USP7	Axin, Wnt/β-catenin	Inhibits osteoblast differentiation	USP7 stabilizes Axin and promotes β-catenin degradation	[64]
C2C12	USP4	Dvl, Wnt/β-catenin	Inhibits osteoblast differentiation	USP4 inhibits Wnt/β-catenin signaling through deubiquitinating Dvl	[61]
C3H10T1/2, BMSCs	β-TrCP	Wnt/β-catenin	Inhibits osteoblast differentiation	β-TrCP mediated ubiquitination and degradation of β-catenin	[65]
MSCs	Smurf1, Smurf2	Wnt/β-catenin	Inhibits osteoblast differentiation	Smurf1 and Smurf2 mediates RUNX2 ubiquitination and degradation	[66]
RAW264.7, BMMs	RNF146	Axin, Wnt/β-catenin	Promotes osteoclast differentiation	RNF146 promotes the ubiquitination and degradation of Axin, thus stabilize β-catenin	[67]
MLO-Y4, MC3T3-E1	USP10	p53	Promotes senescence of osteocytes and osteoblasts	USP10 deubiquitinates and stabilizes p53	[80]
MC3T3-E1	*MDM2*	p53	Inhibits osteoblast senescence	*MDM2* induces p53 ubiquitination	[83]
hFOB1.19, primary osteoblast	DCAF1	Nrf2	Dampens the anti-oxidative stress ability of osteoblasts	DCAF1 promotes Nrf2 degradation	[90]
C2C12	Smurf1	Smad1/5	Inhibits osteoblast differentiation	Smurf1 mediates Smad1/5 degradation	[92]
Primary osteoblast and BMMs	Smurf2	Smad3	Inhibits osteoclast differentiation	Smurf2 induces monoubiquitination of Smad3, interfering with Smad3-VDR interaction and RANKL expression	[96]
Primary BMMs	c-Cbl	TRAF6	Inhibits osteoclast differentiation	c-Cbl promotes TRAF6 degradation, thus suppressing NF-κB signaling	[71]
Primary BMMs	USP7	TRAF6	Inhibits osteoclast differentiation	USP7 impairs the K63-linked polyubiquitination of TRAF6	[74]
Primary BMMs	TANK	TRAF6	Inhibits osteoclast differentiation	TANK inhibits TRAF6 ubiquitination and NF-κB signaling	[75]
RAW264.7, primary BMMs	USP15	IκBα	Inhibits osteoclast differentiation	USP15 deubiquitinates and stabilizes IκBα	[76]
Primary BMMs	USP26	IκBα	Inhibits osteoclast differentiation	USP26 deubiquitinates and stabilizes IκBα	[58]
RAW264.7, primary BMMs	USP34	IκBα	Inhibits osteoclast differentiation	USP34 deubiquitinates and stabilizes IκBα	[77]
Primary BMSCs	CDC20	p65	Promotes osteoblast differentiation	CDC20 promotes the ubiquitination and degradation of p65	[78]
Primary BMSCs	Smurf1	JunB	Inhibits osteoblast differentiation	Smurf1 induces the ubiquitination and degradation of JunB	[98]
Primary BMSCs	WWP1	JunB	Inhibits osteoblast differentiation	WWP1 induces the ubiquitination and degradation of JunB	[99]
Primary BMSCs	Itch	JunB	Inhibits osteoblast differentiation	Itch induces the ubiquitination and degradation of JunB	[100]
RAW264.7	VHL	NFATc1	Inhibits osteoclast differentiation	VHL promotes the ubiquitination and degradation of NFATc1	[101]
RAW-D	Cullin3	NFATc1	Inhibits osteoclast differentiation	Cullin3 promotes the ubiquitination and degradation of NFATc1	[102]
Primary BMMs	Cbl-b	NFATc1	Inhibits olast differentiasteoction	Cbl-b promotes the ubiquitination and degradation of NFATc1	[103]

Smurf1, Smad ubiquitylation regulatory factor 1; RNF138, RING finger protein 138; TRIM16, tripartite motif 16; WWP2, WW domain-containing E3 ubiquitin protein ligase 2; USPs, ubiquitin-specific proteases; β-TrCP, β-transducin repeat-containing protein; c-Cbl, casitas B-lineage lymphoma; TANK, TRAF family member-associated NF-κB activator; CDC20, coactivator cell division cycle 20; Itch, itchy E3 ubiquitin protein ligase; VHL, Von Hippel-Lindau tumor suppressor; CHIP, carboxy-terminus of Hsc70 interacting protein; Fzd5, frizzled 5; Dvl, Dishevelled; Nrf2, nuclear factor erythroid 2-related factor 2; TRAF6, TNF receptor-associated factor 6; MEKK2, mitogen-activated protein kinase kinase kinase 2; VDR, vitamin D receptor; IκB, inhibitor of κB; Cbl-b, Casitas B lymphoma-b; HAUSP, herpesvirus-associated ubiquitin-specific protease; YAP1, Yes1 associated transcriptional regulator; DCAF1, DDB1 and CUL4 associated factor 1; WWP1, WW domain containing E3 ubiquitin protein ligase 1; hPDLSC, human periodontal ligament stem cell.

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tylation, heterotypic polyubiquitylation, and atypical ubiquitylation [45]. K48 homotypic polyubiquitylation is the most abundant of the canonical ubiquitin chain, and enables protein to be degraded by the 26S proteasome, thus forming the well-known ubiquitin proteasome system (UPS) [46]. Other ubiquitination patterns are involved in several complex cellular functions such as DNA damage response, protein trafficking, and autophagy [45]. Deubiquitinating enzymes detach substrate proteins from ubiquitin [47]. Ubiquitin-specific proteases (USPs) are the largest and most well-studied family among the deubiquitinating enzymes [48].

Ubiquitination is an important area of research in the field of bone remodeling. The overall effects of the UPS on bone metabolism were revealed using the UPS inhibitor bortezomib. Bortezomib administration significantly promotes the bone formation in mice with ovariectomy induced osteoporosis [49]. Further studies have revealed that the ubiquitination affects bone remodeling through modulating several key signaling molecules during osteogenesis and osteoclastogenesis, such as RUNX2, Wnt/β-catenin, and NF-κB.

The ubiquitination of RUNX2 is regulated by several E3 ubiquitin ligases, such as Smad ubiquitylation regulatory factor 1 (Smurf1) [50], RING finger protein 138 (RNF138) [51], and carboxy-terminus of Hsc70 interacting protein (CHIP) [52]. The overall effects of these E3 ubiquitin ligases on RUNX2 induce protein ubiquitination and degradation through the UPS, thereby inhibiting osteogenic differentiation. Several recent studies have revealed specific mechanisms that regulate these processes. For example, Smurf1 can be modulated by both post-translational and transcriptional mechanisms. The phosphorylation of Smurf1 at S148, induced by the energy sensor AMP-activated protein kinase (AMPK), is essential for the ubiquitination of RUNX2 [53]. Glucose uptake inhibits AMPK activity, thereby inhibiting Smurf1-mediated RUNX2 degradation, revealing a complex connection between osteoblast differentiation and glucose metabolism [54]. Another study revealed that mechanical loading induces nuclear retention of Smurf1 messenger RNA (mRNA), which impairs Smurf1-mediated RUNX2 degradation and promotes bone formation [55]. This effect is mediated by the unique paraspeckle structure formed by the long non-coding RNA (lncRNA) nuclear enriched abundant transcript 1 (NEAT1) [55]. CHIP promotes RUNX2 K48 poly-ubiquitination and degradation [52]. However, another E3 ubiquitin ligase, tripartite motif 16 (TRIM16) competes with CHIP to interact with RUNX2, inducing RUNX2 K63 poly-ubiquitination and protecting RUNX2 from UPS-mediated protein degradation [52]. E3 ubiquitin ligases such as the WW domain-containing E3 ubiquitin protein ligase 2 (WWP2) also induce RUNX2 monoubiquitination, which increases RUNX2 transactivation instead of degradation [56]. These examples show that different ubiquitin codes exert diverse effects on substrate proteins. The ubiquitination and stability of RUNX2 are also regulated by deubiquitinating enzymes, such as USP7 [57]. Phosphorylation of RUNX2 induced by casein kinase 2 (CK2) recruits USP7, which inhibits the ubiquitination and stability of RUNX2, thus favoring osteogenic differentiation [57].

The ubiquitination modification of Wnt/β-catenin signaling is also important in bone remodeling. USPs regulate osteogenic differentiation by directly modulating the Wnt/β-catenin signaling pathway. For instance, USP26 can directly interact and stabilize β-catenin, which promotes osteoblast differentiation and bone formation [58]. USP8 controls the Wnt/β-catenin pathway in osteoblasts through stabilizing frizzled 5 (Fzd5), a Wnt pathway receptor [59]. Dishevelled (Dvl) is a core regulator of the Wnt/β-catenin signaling, and the K63-polyubiquitination of Dvl inactivates the destruction complex, leading to β-catenin stabilization and nuclear translocation [60]. USP4 deubiquitinates Dvl, thus inhibiting Wnt/β-catenin pathway during osteogenesis [61]. Moreover, phosphorylation of β-catenin at S675 induced by mitogen-activated protein kinase kinase kinase 2 (MEKK2) recruits USP15, which inhibits UPS-mediated β-catenin degradation and promotes bone formation [62]. USPs also affect Wnt/β-catenin signaling through indirect mechanisms. Wang *et al.* [63] found that USP7 promotes bone formation by stabilizing Yes1 associated transcriptional regulator (YAP1) and further inducing β-catenin nuclear translocation. However, a reverse effect of USP7 on Wnt/β-catenin pathway was reported by Ji *et al.* [64]. In this study, USP7 also inhibited Wnt/β-catenin pathway during osteogenesis through promoting the stabilization of Axin1, the scaffolding protein of the β-catenin degradation complex [64]. It has been speculated that other mediators determine the substrate specificity of USP7 in different settings, although no study has been conducted to test this hypothesis. Moreover, the E3 ubiquitin ligases such as β-transducin repeat-containing protein (β-TrCP), Smurf1, and Smurf2 mediate inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ)-NF-κB-induced β-catenin suppression, leading to impaired osteogenic differentiation [65,66]. In osteoclasts, RANKL-NF-κB suppresses the expression of the E3 ubiquitin ligase RNF146, leading to increased Axin1 stability and impaired Wnt/β-catenin signaling [67]. These results provide novel insights into the mechanisms and potential treatment strategies for inflammation-induced bone loss.

Under inflammatory conditions, canonical activation of NF-κB signaling is regulated by the E3 ubiquitin ligase tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) [68]. More specifically, TRAF6 cooperates with the ubiquitin-conjugating enzyme 13 (Ubc13)/ubiquitin-conjugating enzyme variant 1A (Uev1A) E2 complex to induce K63-polyubiquitin chain synthesis and TRAF6 auto-ubiquitination [69]. The unique K63-polyubiquitin chains help the activation of TGF-β-kinase 1 (TAK1) complex

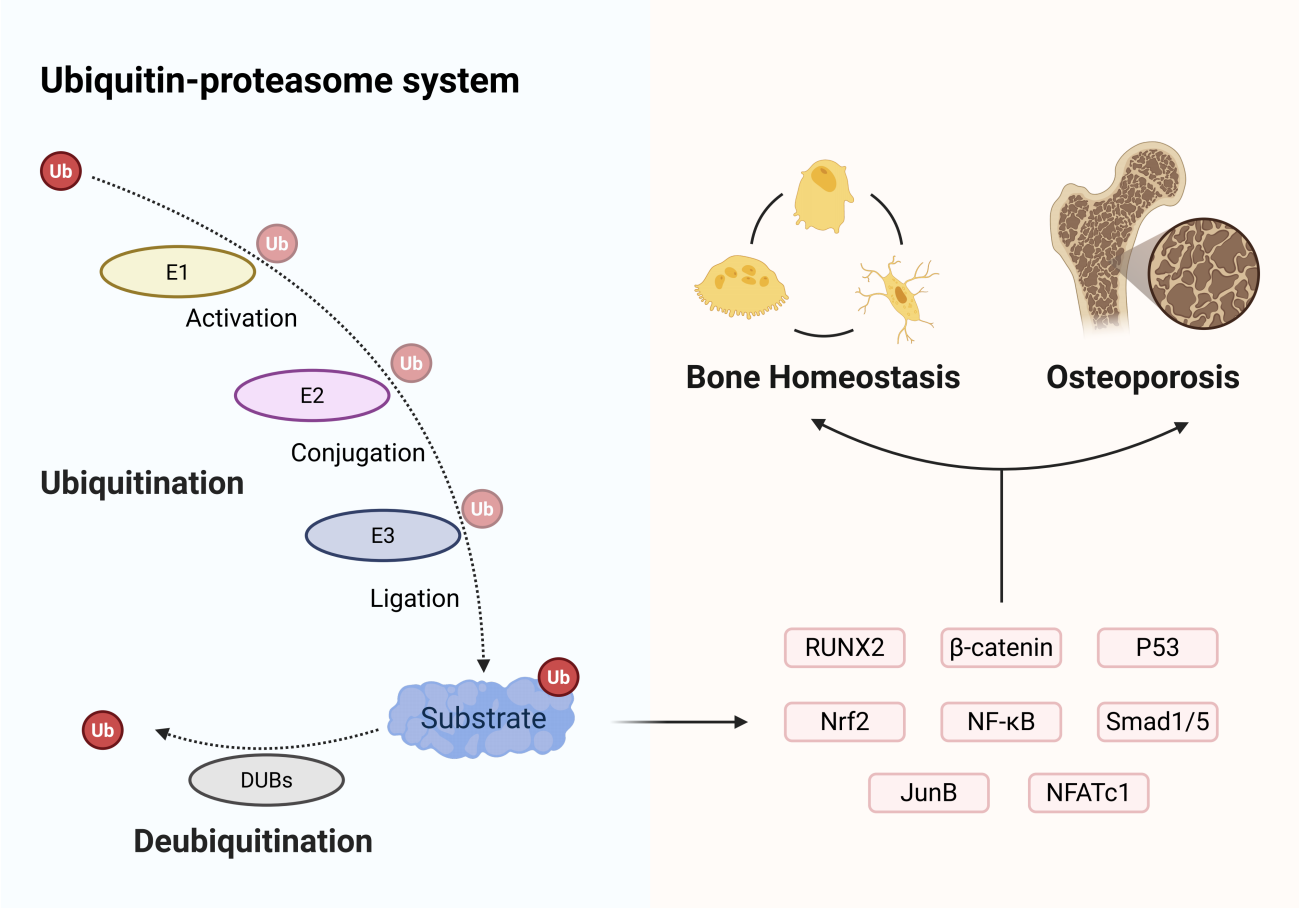

Fig. 2. Functions of ubiquitin-proteasome system in the regulation of bone homeostasis and osteoporosis. Nrf2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor-κB; NFATc1, nuclear factor of activated T cells 1; RUNX2, runt-related transcription factor 2; DUBs, deubiquitinating enzymes. Created in BioRender. Wang, Q. (2025) https://BioRender.com/s4hpstp.

independent of proteasome-mediated protein degradation, which further phosphorylates IKK and activates NF-κB [70]. Given the essential role of NF-κB signaling in osteoclastogenesis, manipulation of its upstream molecule TRAF6 may provide novel insights into the treatment of bone-related diseases. Recent studies have found that TRAF6 is ubiquitinated and destabilized by several other E3 ubiquitin ligase, such as casitas B-lineage lymphoma (c-Cbl) [71], and 14-3-3ζ [72], thereby inhibiting NF-κB signaling in osteoclasts. Mechanistically, c-Cbl induced K48-linked polyubiquitination of TRAF6 [71]. However, the specific ubiquitination pattern that determines the role of 14-3-3ζ on TRAF6 is still not known. In addition, deconjugating the K63-linked polyubiquitin chains from TRAF6 by deubiquitination enzymes, such as cylindromatosis (CYLD) [73] and USP7 [74], also inhibits downstream NF-κB signaling and osteoclastogenesis. Interestingly, the NF-κB downstream protein itchy E3 ubiquitin protein ligase (Itch) promotes the interaction between CYLD and TRAF6, which inhibits NF-κB in turn, suggesting a negative feedback loop of Itch-NF-κB signaling in osteoclasts [73]. Similar to Itch, TRAF family member-associated NF-κB activator (TANK) also promotes TRAF6 deubiquitination and inhibits NF-κB signaling [75]. However, it remains unclear whether these effects depend on other K63-linked specific deubiquitination enzymes. The NF-κB inhibitor, IκBα is regulated by several USPs, such as USP15 [76], USP26 [58], and USP34 [77]. These USPs deubiquitinate and stabilize IκBα, thereby suppressing NF-κB signaling and osteoclast-mediated bone loss. The ubiquitination of NF-κB signaling is also involved in the osteogenic differentiation of BMSCs, although only a few studies have focused on this field. Study of Du *et al.* [78] showed that the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) and its coactivator cell division cycle 20 (CDC20) facilitate the ubiquitination and degradation of the NF-κB component p65, promoting osteogenic differentiation of BMSCs. Overall, these studies emphasize the finely-tuned regulation of NF-κB by ubiquitination in bone remodeling, providing a promising therapeutic approach for inflammation-related bone loss.

The p53 protein is a transcription factor implicated in cellular responses to stress and is linked to cellular senescence and apoptosis [79]. It also regulates senescence and differentiation of osteoblasts and is modulated by protein ubiquitination. USP10 deubiquitinates and stabilizes p53,

leading to p53-mediated osteoblast senescence [80]. Moreover, the expression of USP10 is inhibited by estrogen signaling, revealing a potential mechanism of estrogen deficiency in osteoporosis [80]. Moreover, miR-203-3p/PDZ-linked kinase (PBK) is implicated in senescence of BMSCs by inhibiting p53 ubiquitination and degradation [81]. However, the exact mechanism underlying this process remains unclear. Moreover, p53 ubiquitination and degradation are regulated by the oncoprotein *MDM2* [82]. The anti-apoptotic protein BRE promotes osteoblast differentiation through *MDM2*-induced p53 ubiquitination and degradation [83].

Nuclear factor erythroid 2-related factor 2 (Nrf2) modulates transcriptional activation of antioxidant response genes in response to oxidative stress [84]. However, Nrf2 interacts with Kelch-like ECH-associated protein 1 (Keap1), which serves as a substrate adaptor for the cullin 3 (CUL3)-RING-box protein 1 (RBX1) E3 ubiquitin ligase complex, mediating the ubiquitination and degradation of Nrf2 [85]. The Keap1-Nrf2 system is implicated in bone remodeling and is fine-tuned by the UPS [86]. Several pharmacological interventions, such as bitopertin [87], carnitine [88], and *Dendrobium officinale* polysaccharides (DOP) [89] can disrupt the Keap1-Nrf2 interaction, leading to suppressed Nrf2 ubiquitination and improved bone quality in osteoporosis. In addition, miR-3175 inhibits Nrf2 ubiquitination and degradation, thus preventing osteoblasts from dexamethasone-induced oxidative stress and cellular injury [90].

The polyubiquitination and subsequent degradation of Smad1/5 are regulated by Smurf1 [91]. In support of this result, Cao *et al.* [92] screened and identified the small molecule compounds A01 and A17 that inhibited Smurf1-induced Smad1/5 degradation and promoted osteogenic differentiation. In addition, TNF-α facilitates Smurf1-mediated Smad1 ubiquitination and degradation, resulting in inflammatory bone loss [93]. These effects can be reversed by melatonin, implying its strong potential in the treatment of osteoporosis [93]. Moreover, transmembrane anterior posterior transformation 1 (TAPT1) [94] and LIM and cysteine-rich domains 1 (LMCD1) [95] have been identified as novel upstream modulators that promote and inhibit Sumrf1-induced Smad1/5 ubiquitination, respectively. Collectively, these results suggest that Smurf1 acts as a potential suppressor of osteogenic differentiation by modulating Smad1/5. As a close homologue of Smurf1, Smurf2 has also been identified as an osteogenic differentiation suppressor [96]. However, Smurf2 also exerts non-redundant effects by inhibiting osteoblast RANKL production and osteoblast-induced osteoclastogenesis [96]. Mechanistically, Smurf2 mediates Smad3 monoubiquitination in osteoblasts, which disrupts the Smad3-vitamin D receptor (VDR) interaction, leading to decreased RANKL expression and impaired osteoblast-osteoclast coupling [96]. As a result, Smurf2 knockout mice displayed the opposite bone phenotype compared to Smurf1 knockout mice, i.e., the former exhibited osteopenia, while the latter showed increased bone mass [96,97].

JunB has been identified as a novel regulator of osteoblasts, and its ubiquitination is regulated by several ubiquitin E3 ligases such as Smurf1 [98], WW domain containing E3 ubiquitin protein ligase 1 (WWP1) [99], and Itch [100]. These ubiquitin E3 ligases demonstrated similar effects on JunB, i.e., inducing the ubiquitination and degradation of JunB and impairing the osteogenic differentiation of BMSCs. NFATc1, an essential mediator of osteoclast differentiation, is also regulated by ubiquitination modification. Jumonji C domain-containing 5 (JMJD5) facilitates the interaction between NFATc1 and the Von Hippel-Lindau tumor suppressor (VHL) E3 ligase, thus promoting the ubiquitination and degradation of NFATc1 [101]. Kelch repeat and BTB domain-containing protein 11 (KBTBD11) interacts with CUL3 to promote NFATc1 ubiquitination [102]. Moreover, Casitas B lymphoma-b (Cbl-b) promoted NFATc1 ubiquitination and impaired osteoclastogenesis [103]. The functions of protein ubiquitination in bone remodeling are illustrated in Fig. 2 and Table 2 (Ref. [50–52,56–59,61–67,71,74–78,80,83,90,92,96,98–103]).

Sumoylation in Bone Remodeling

Sumoylation has been identified over the last two decades as a post-translational modification. Small ubiquitin-related modifier (SUMO), an analog of ubiquitin, is a tag protein approximately 100 amino acids long [104]. Five SUMO isoforms (i.e., SUMO1-5) have been identified in mammals [105]. Similar to protein ubiquitination, sumoylation requires a three-enzyme cascade, followed by ligation to the lysine residue of the substrate protein [106]. However, the precursor SUMO must first be converted into mature SUMO by SUMO-specific protease [107]. Briefly, sumoylation involves sequential processes, including SUMO maturation catalyzed by SUMO-specific proteases (SENPs); SUMO activation induced by the SUMO1 activating enzyme subunit 1 (SAE1)/SAE2 complex, i.e., the E1-activating enzyme, and SUMO conjugation by the E2-conjugation enzyme ubiquitin carrier protein 9 (Ubc9), E3 ligases facilitate the specificity and efficiency of sumoylation, and SENPs also mediate the deconjugation of SUMO from target proteins [106]. Sumoylation can change the fate of substrate proteins and broadly regulate biological and pathological processes such as DNA replication and repair, cell cycle progression, and cell metabolism [108]. Sumoylation has also been implicated in the regulation of bone remodeling.

Androgen and androgen receptor (AR) have been implicated in maintaining bone homeostasis in males [109]. The sumoylation of AR at residues K381 and K500 is essential for its protein activity, including the regulation of sperm maturation [110] and bone development [111]. Male mice with mutant AR sumoylation sites (i.e., K381 and K500)

display an osteoporotic phenotype [111]. A study showed that AR sumoylation affects its interaction with chromatin and target gene selection in prostate cancer cells [112]. However, whether these mechanisms apply to the cellular events during osteogenic differentiation remains unknown.

Studies have identified Smad4 as an important SUMO substrate in osteoblasts [113–115]. Smad4, as the vital mediator of transforming growth factor-β (TGF-β)/BMP signaling, participates in the formation of the Smad complex, and promotes the transcription of the osteoblast differentiation-related genes [116]. The E2-conjugation enzyme Ubc9 promotes Smad4 sumoylation, which increases its stability and nuclear translocation in osteoblasts [113]. Moreover, Smad4 sumoylation enhanced the resistance of osteoblasts to oxidative stress-induced cell injury [114]. These results are consistent with those of early studies in other settings, in which sumoylation modification showed positive regulatory roles in the protein functions of Smad4 [117]. However, another study revealed the opposite role of Ubc9-induced Smad4 sumoylation in osteoblasts [115]. Sumoylation of Smad4 at K158 inhibited its transcriptional activity without affecting protein stability or cellular localization [115]. One possible explanation for these distinct results is that the diverse Smad4 sumoylation modification sites may have different functions. The different osteoblast cell lines used in these studies may also account for these discrepancies. However, current evidence is not sufficient to confirm these views and needs to be further explored.

The sumoylation of E3 ligase protein inhibitor of activated STAT-xβ (PIASxβ) increased the transcriptional activity of osterix in osteoblasts [118]. However, owing to the lack of the sumoylation motif in osterix, the above-mentioned mechanism seems to be indirect. Another E3 ligase, PIAS3, increases Smurf1 sumoylation and activity, thereby impairing BMP-2-induced osteogenic differentiation [119]. This study provides an example of the interplay between ubiquitination and sumoylation in the regulation of bone remodeling. Peroxisome proliferator-activated receptor γ (PPARγ) is the nuclear receptor that controls the differentiation of BMSCs [120]. The activation of PPARγ promotes the differentiation to adipocytes, but inhibits osteogenic differentiation [121]. As early as 2004, Yamashita *et al.* [122] found that PPARγ2 can by modified by sumoylation at the K107 site, which impairs its transactivating function. However, the pathological roles of PPARγ sumoylation in bone remodeling have been only recently discovered [123,124]. Growth and differentiation factor 11 (GDF11) promotes PPARγ sumoylation and facilitates osteogenic differentiation [123]. However, GDF11 levels are regulated by ageing and rosiglitazone (the PPARγ agonist approved for diabetes treatment), suggesting a novel mechanism for senile osteoporosis and the rosiglitazone-related bone loss [123]. In addition, glucocorticoids increased the expression of SUMO-specific protease SENP3, which deSUMOylated PPARγ2 at K107, leading to impaired osteogenic differentiation and glucocorticoids-induced osteoporosis [124].

Another study found a distinctive role for SENP3 in promoting osteogenic differentiation of MSCs through the epigenetic regulation of distal-less homeobox 3 (DLX3), an essential transcription factor for osteogenic differentiation [125]. More specifically, SENP3 induces the desumoylation of RB-binding protein 5 (RbBP5), which promotes the activity of the MLL1/MLL2 complex as a methyltransferase at H3K4 and elevates the recruitment of active RNA polymerase II on DLX3 [125]. SENP3 also suppresses the differentiation of osteoclasts [126]. SENP3 deSUMOylates interferon regulatory factor 8 (IRF8) at the K310 site, which sustains the inhibitory roles of IRF8 on NFATc1 expression and osteoclastogenesis [126]. Another deSUMOylase, SENP6, inhibits senescence and apoptosis of osteochondroprogenitors (OCPs), thereby maintaining normal skeletal development [127]. SENP6 deSUMOylates and stabilizes SUMO ligase E3 tripartite motif-containing protein 28 (TRIM28), inhibiting p53-mediated OCPs ageing [127]. Overall, these studies revealed that sumoylation regulates bone remodeling through various mechanisms, such as modulation of protein stability, subcellular localization, transcription regulation, and enzyme activity. These results suggest a complex regulatory role of sumoylation in bone remodeling under both normal and pathological conditions.

Lactylation in Bone Remodeling

First reported in 2019, lactylation is a recently discovered protein modification related to cell metabolism [128]. Certain conditions, such as hypoxia and cellular glycolysis promote the formation of endogenous lactate [129]. Lactylation occurs when L- or D-lactyl is transferred to lysine residues of various proteins [130]. Lactylation modifications were initially discovered to act on histones and regulate gene transcription levels [128]. Dysregulated histone modifications impair gene transcription and play crucial roles in the onset and development of various diseases [131]. Recently, non-histone proteins involved in various biological processes, such as modulating protein activity and interactions, have also been found to be substrates of lactylation modifications [132]. Histone and non-histone protein lactylation further indicate intricate connections between energy metabolism and diseases.

Considering the close relationship between energy metabolism and bone remodeling, protein lactylation is speculated to be involved in the regulation of bone homeostasis [133]. Lactate levels gradually increase during osteoblast differentiation [134]. Lactate dehydrogenase A (LDHA) promotes cellular lactate levels and the lactylation of histone 3 on lysine residue 18 (H3K18la) of the JunB promoter, thereby increasing JunB expression and osteogenic differentiation [134]. Another study conducted by Wu *et al.* [135] revealed a similar role for lactylation in bone. Lactate secreted by endothelial cells promotes H3K18la expression

in BMSCs, which in turn increases osteogenic differentiation [135]. These results suggest that histone lactation plays a positive role in bone formation and is a promising strategy for the treatment of osteoporosis.

Lactylation of non-histone proteins is also involved in bone remodeling. For instance, αB-crystallin (CRYAB) interacts with and stabilizes ferritin heavy chain 1 (FTH1) in a lactylation-dependent manner [136]. FTH1 further suppresses ferroptosis and promotes osteogenic differentiation of BMSCs [136]. However, the specific writer protein mediating FTH1 lactylation remains unknown. In addition, a recent study found that proanthocyanidins promote the lactylation of RUNX2 at K176, which prevented the ubiquitination and degradation of RUNX2, thereby promoting the osteogenic differentiation of stem cells [137]. In conclusion, protein lactylation is a novel PTM pattern discovered in recent years, and only a few studies focused on the roles of lactylation in the bone. However, based on the current studies, this field shows promising prospects that merit further exploration.

Palmitoylation in Bone Remodeling

Protein palmitoylation is a reversible lipid modification that has been extensively studied in hundreds of mammalian proteins [138]. According to different connection methods, protein palmitoylation can be classified as S-palmitoylation, N-palmitoylation, and O-palmitoylation [139]. S-palmitoylation, the most common modification, is achieved by generating a thioester bond between palmitic acid and a cysteine residue [140]. Protein S-palmitoylation is catalyzed by the zinc finger DHHC-type containing (ZDHHC) protein family, whereas de-palmitoylation is mediated by Acyl protein thioesterase [141]. Functionally, palmitoylation enhances the hydrophobicity of substrate proteins, thereby affecting protein interactions, membrane structures, and protein localization [142]. Palmitoylation also determines protein structure, assembly, maturation, and stability [138].

Several recent studies have indicated the potential role of protein palmitoylation in bone remodeling. The cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element binding protein (CREB) signaling pathway is involved in promoting osteogenic differentiation [143]. PKA-induced CREB phosphorylation mediates CREB nuclear translocation and downstream osteogenic gene expression [143]. However, ZDHHC16 promotes CREB palmitoylation while inhibiting its phosphorylation, thereby suppressing the osteogenic differentiation of stem cells [144,145]. However, another palmitoyl acyltransferase, ZDHHC13, is positively linked to bone development. A nonsense mutation in ZDHHC13 in mice results in an osteoporosis-like phenotype [146]. ZDHHC13 induces palmitoylation and nuclear translocation of membrane type 1 matrix metalloproteinase (MT1-MMP), which regulates endochondral bone formation [147]. The palmitoylation inhibitor 2-bromopalmitic acid (2-BP) inhibited both osteogenic and osteoclast differentiation [148,149]. Interestingly, it seems that the inhibitory effects of 2-BP on osteoclast differentiation overwhelmed its inhibitory role in osteogenic differentiation, therefore it exerted an overall effect to delay bone loss in ovariectomy-induced osteoporosis [149]. Owing to the non-selective and off-target effects of 2-BP, the extent to which its regulatory role in bone is achieved by inhibiting protein palmitoylation remains largely unknown.

Future Perspectives

A growing number of studies have focused on the role of PTMs in regulating bone remodeling, which deepens our understanding of bone biology. These results provide a foundation for future clinical applications. Osteoporosis is characterized by impaired osteogenic and excessive osteoclast differentiation. PTM modulation is also a promising target for the treatment of osteoporosis because of its essential regulatory role in bone remodeling. The potential efficacy of several specific PTM modulators for the treatment of osteoporosis has been evaluated in recent studies.

The UPS inhibitor bortezomib, as an example, has been approved for treating multiple myeloma and improving osteolytic bone lesions in patients, which inspires further research on the correlation between the UPS and bone remodeling and suggests a potential treatment effect on osteoporosis [150]. Indeed, bortezomib significantly improved bone loss induced by ovariectomy [49]. Other UPS inhibitors, such as MG132 and ixazomib also favor bone formation, but these effects have not been verified in *in vivo* studies [151,152].

Targeting O-GlcNAcylation is another potent strategy for treating osteoclast-mediated bone loss. However, considering the dynamic roles of O-GlcNAcylation at different stages of osteoclast differentiation, opposing interventions may be utilized at different stages of the disease [38]. Therefore, there are several questions regarding this issue: (i) is it possible to determine the optimal timing for different interventions during disease progression? (ii) Is it possible to identify feasible biomarkers that reflect dynamic roles of O-GlcNAcylation in osteoclast? And (iii) is it possible to determine the core mechanisms of the dynamic roles of O-GlcNAcylation in osteoclast? Further studies addressing these issues may help in the selection of an appropriate treatment method for osteoporosis.

Several multi-target drugs also improve bone mass and quality in osteoporosis by modulating PTMs. Beraprost is a prostaglandin I2 (PGI2) analogue used to treat peripheral arterial diseases [153]. A recent study found that beraprost improves osteoporosis though inhibiting the E3 ubiquitin ligase neural precursor cell expressed developmentally downregulated 4 (Nedd4)-mediated RUNX2 ubiquitination and degradation [154]. Lansoprazole, a proton pump inhibitor, upregulates the K63-linked polyubiqui-

tination of TRAF6, which promotes RUNX2-mediated osteoblast differentiation by activating non-canonical BMP-TAK1-p38 signaling [155]. An *in vivo* study further emphasized the promoting effect of lansoprazole on bone healing during bone fractures [155]. Natural compounds can also ameliorate bone loss during osteoporosis by modulating PTMs. DOPs are biologically active compounds that extracted from the traditional Chinese medicine *Dendrobium officinale* [156]. DOP can improve osteoporosis by suppressing Nrf2-Keap1 interaction and Nrf2 ubiquitination [89]. Higenamine is the active compound isolated from the traditional Chinese herbal medicine *Aconitum carmichaelii*, and has been used in cardiovascular diseases due to its β receptor agonist activity [157]. A recent study found that higenamine improves estrogen deficiency and ageing-related osteoporosis by inhibiting Smad4 ubiquitination and degradation [158]. Overall, these multitarget drugs and active compounds represent potentially innovative therapies for osteoporosis. However, future studies are also needed to address the following issues: (i) owing to the relatively low specificity and multiple targets of these drugs, their side effects should also be carefully explored; (ii) the efficacy and side effects of these drugs should be compared with the current osteoporosis treatments; (iii) chemical modification-based methods may help improve the bioavailability and therapeutic activity of natural compounds.

Proteins and stem cells modified by certain PTMs may exert stronger antiosteoporotic effects, offering promising prospects for bioengineering applications. Freire *et al.* [159] initially developed a strategy for immobilizing anti-BMP-2 antibodies on an absorbable collagen sponge to capture endogenous BMP-2 and exert osteogenic function, which was termed as antibody-mediated bone regeneration. Another study found that the sialylation of anti-BMP-2 IgG enhanced its pro-osteogenic capacity while avoiding the potential side effects such as pro-osteoclastogenic effects and inflammatory infiltration induced by the Fc-Fc fragment receptor interaction [160]. Moreover, surface glycosylation modification increased the homing ability of BMSCs towards bone defects, providing novel insights into stem cell-based therapy for bone diseases [16]. Hydrogel serving as drug-loading systems can encapsulate agents that modulate PTMs to exert anti-osteoporotic and bone-regeneration effects [161]. These agents comprise PTM inhibitors or agonists, lipid nanoparticles containing PTM enzyme mRNA, and stem cells modified by PTMs [58,162].

Although many experimental studies have explored the roles of PTMs in bone remodeling, only a few have focused on the potential utilization of protein PTMs as disease biomarkers for osteoporosis. For instance, Yang *et al.* [163] used mass-spectrometry-based proteomics to identify differentially ubiquitinated sites and proteins in the whole blood of patients with ostmenopausal osteoporosis. However, few studies have explored the correlation between PTM protein levels and severity, risk of complications, and prognosis of osteoporosis. The potential utilization of PTM protein levels as novel bone turnover markers needs to be studied further.

Another intriguing topic is the interactions among diverse PTMs during bone remodeling. Indeed, the crosstalk between multiple PTMs may result in synergistic or antagonistic effects on protein biological functions [164]. These interactions of PTMs form a complex regulatory network that affects bone homeostasis. Among these PTM interactions, the ubiquitination-phosphorylation interaction is the most widely studied and comprises several paradigms. (i) Protein phosphorylation antagonizes its ubiquitination. CK2 phosphorylates RUNX2 at T340, S354, and S387, which further inhibiting UPS-mediated protein degradation by recruiting USP7 [57]. (ii) Protein phosphorylation promotes ubiquitination. For example, IKKβ phosphorylates β-catenin at S33, S37, and S45, which favors β-TrCP-mediated β-catenin ubiquitination and degradation, leading to inhibited osteogenic differentiation [65]. These results indicate that the phosphorylation of target proteins may affect PTMs through modulating protein-protein interactions. (iii) Phosphorylation of E3 ubiquitin ligases and USPs may determine their enzymatic activities. For instance, Smurf1 phosphorylation at S148 by AMPK is essential for its ability to target RUNX2 for degradation [53]. In osteocytes, CK2 phosphorylates USP4, which further deubiquitinates and stabilizes sirtuin 1 to modulate SOST expression [165]. In addition to protein phosphorylation, citrullination can also antagonize UPS-mediated protein degradation. As seen in study of Kim *et al.* [166], citrullination of RUNX2 at arginine (R) 381 protects it from UPS-mediated degradation, although the specific mechanism remains unknown.

Conclusions

This review highlights the roles of several novel PTMs including glycosylation, ubiquitination, sumoylation, lactylation, and palmitoylation, in the regulation of bone remodeling. In particular, several core molecules of osteoblast differentiation signaling such as Wnt/β-catenin, BMP/Smad, RUNX2, SOST, osterix, and those of osteoclast differentiation signaling NF-κB and NFATc1 have been found to be widely modified by post-translational mechanisms. These PTMs exert their roles through modulating protein functions, such as stability, activity, subcellular localization, and interaction. These results deepen our understanding of the mechanisms underlying bone remodeling and may provide novel treatment targets for bone diseases, especially osteoporosis. However, it should be pointed out that current studies have largely explored the roles of PTMs on bone remodeling from a laboratory perspective, whereas clinical studies on this issue are still scarce. Future studies that link PTMs with clinical parameters of osteoporosis may favor the potential utilization of PTMs in clinical settings. In addition, post-translationally modified proteins can be delivered through bone tissue en-

gineering and targeted drug delivery strategies, thereby exerting functional effects and improving bone remodeling. Several natural medicines can improve bone loss during osteoporosis by modulating PTMs. However, owing to the low bioavailability and multiple targets of these natural products, further studies are required. Finally, it should be noted that this review, as a narrative review, is potentially subject to selection bias. This limitation may be addressed in future studies by conducting systematic reviews with strict inclusion and exclusion criteria and rigorous quality assessments.

List of Abbreviations

AMPK, AMP-activated protein kinase; APC/C, anaphase-promoting complex/cyclosome; AR, androgen receptor; β-TrCP, β-transducin repeat-containing protein; β4GalNAcT3, β4-N-acetylgalactosaminyltransferase 3; BMP-2, bone morphogenetic protein-2; BMSCs, bone marrow mesenchymal stem cells; 2-BP, 2-bromopalmitic acid; cAMP, cyclic adenosine monophosphate; c-Cbl, casitas B-lineage lymphoma; CBP, CREB-binding protein; CDC20, coactivator cell division cycle 20; CHIP, carboxy-terminus of Hsc70 interacting protein; CK2, casein kinase 2; CREB, cAMP response element binding protein; CRYAB, αB-crystallin; CYLD, cylindromatosis; DMP1, dentin matrix protein 1; DOP, *Dendrobium officinale* polysaccharides; DLX3, distal-less homeobox 3; Dvl, Dishevelled; FTH1, ferritin heavy chain 1; Fzd5, frizzled 5; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; IκB, inhibitor of κB; Itch, itchy E3 ubiquitin protein ligase; IRF8, interferon regulatory factor 8; JMJD5, Jumonji C domain-containing 5; KBTBD11, Kelch repeat and BTB domain-containing protein 11; Keap1, Kelch-like ECH-associated protein 1; LDHA, lactate dehydrogenase A; LMCD1, LIM and cysteine-rich domains 1; lncRNA, long non-coding RNA; MAST4, microtubule associated serine/threonine kinase family member 4; M-CSF, macrophage colony-stimulating factor; MEKK2, mitogen-activated protein kinase kinase kinase 2; MT1-MMP, membrane type 1 matrix metalloproteinase; NEAT1, nuclear enriched abundant transcript 1; Nrf2, nuclear factor erythroid 2-related factor 2; OCPs, osteochondroprogenitors; OCN, osteocalcin; OGT, O-GlcNAc transferase; OPN, osteopontin; PDLCs, periodontal ligament stem cells; PGI2, prostaglandin I2; PIASxβ, protein inhibitor of activated STAT-xβ; PKA, protein kinase A; PTM, post-translational modification; RANKL, receptor activator of nuclear factor-kappa B ligand; RbBP5, RB-binding protein 5; RBX1, RING-box protein 1; RNF138, RING finger protein 138; SAE1, SUMO1 activating enzyme subunit 1; SENPs, SUMO-specific proteases; Smurf1, Smad ubiquitylation regulatory factor 1; SOST, sclerostin; SUMO, small ubiquitin-related modifier; TAB1, TGF-β-activated kinase 1/MAP3K7-binding protein 1; TAK1, TGF-β-kinase 1; TANK, TRAF family member-associated NF-κB activator; TAPT1, transmembrane anterior posterior transformation 1; TGF-β, transforming growth factor-β; TLR4, Toll-like receptor 4; TRAF6, TNF receptor-associated factor 6; TRIM16, tripartite motif 16; UBAP2l, ubiquitin-associated protein 2-like; Ubc9, ubiquitin carrier protein 9; Ubc13, ubiquitin-conjugating enzyme 13; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; Uev1A, ubiquitin-conjugating enzyme variant 1A; UPS, ubiquitin proteasome system; VDR, vitamin D receptor; VHL, Von Hippel-Lindau tumor suppressor; WWP2, WW domain-containing E3 ubiquitin protein ligase 2; LDN, LacdiNAc; ER, endoplasmic reticulum; OGA, O-GlcNAcase; NFATc1, nuclear factor of activated T cells 1; NUP153, nuclear pore protein nucleoporin 153; USPs, ubiquitin-specific proteases; TNF, tumor necrosis factor; PBK, PDZ-linked kinase; CUL3, cullin 3; Cbl-b, Casitas B lymphoma-b; PPARγ, peroxisome proliferator-activated receptor γ; GDF11, growth and differentiation factor 11; H3K18la, histone 3 on lysine residue 18; NF-κB, nuclear factor-κB; ZDHHC, zinc finger DHHC-type containing; RUNX2, runt-related transcription factor 2; BMMs, bone marrow-derived macrophages; hPDLSC, human periodontal ligament stem cell; HAUSP, herpesvirus-associated ubiquitin-specific protease; YAP1, Yes1 associated transcriptional regulator; DCAF1, DDB1 and CUL4 associated factor 1; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; mRNA, messenger RNA; DUBs, deubiquitinating enzymes; WWP1, WW domain containing E3 ubiquitin protein ligase 1.

Availability of Data and Materials

Not applicable.

Author Contributions

QZW and JP contributed to the design of this work and revised critically for important intellectual content. ZJP and HHL contributed to the interpretation of data. ZJP, HHL and LYL analyzed the data and drafted the work. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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