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REVIEW

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NEDD4 family E3 ligases in osteoporosis: mechanisms and emerging potential therapeutic targets

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Abstract

Osteoporosis is a systemic skeletal disorder characterized by reduced bone density and an increased risk of fractures, particularly prevalent in the aging population. Osteoporotic complications, including vertebral compression fractures, hip fractures, and distal forearm fractures, affect over 8.9 million individuals globally, placing a significant economic strain on healthcare systems. Recent advances have expanded our understanding of the mechanisms underlying osteoporosis, particularly the intricate regulatory networks involved in bone metabolism. A central player in these processes is ubiquitin-mediated proteasomal degradation, a crucial post-translational modification system that involves ubiquitin, the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin ligase (E3), deubiquitinating enzymes, and the proteasome. Among the various E3 ligases, the NEDD4 family has emerged as a key regulator of both bone development and osteoporotic pathology. This review delineates the role of NEDD4 family in osteoporosis and identifies potential drug targets within these pathways, offering insights into novel therapeutic approaches for osteoporosis through targeted intervention.

Keywords Osteoporosis, Ubiquitination, E3 ubiquitin ligase, NEDD4 ubiquitin ligase family, Therapeutics

Introduction

Osteoporosis (OP), the most prevalent systemic skeletal disorder, is marked by significant loss of bone mass and deterioration of bone microarchitecture, leading to heightened risk of fragility fractures [1–3]. As the population ages, the global prevalence of OP has risen to 18.3%, posing a significant public health challenge [4, 5]. Osteoporosis vertebral compression fracture (OVCF), hip fracture and distal forearm fracture are most common osteoporotic fractures, which are recorded more than 8.9 billion worldwide, exerting substantial economic burdens both directly and indirectly [6–8]. Therefore, understanding OP pathogenesis is crucial for its diagnosis and treatment. The skeletal system is continually renewing, with a complete turnover approximately every 10 years [9]. Under physiological conditions, bone formation and resorption are in dynamic equilibrium, known as bone homeostasis. OP results from disruptions in this balance, critical to maintaining bone integrity. Bone turnover imbalance in OP can be assessed using bone turnover markers (BTMs). Markers of bone formation, such as alkaline phosphatase (bALP) and procollagen type I N-propeptide (PINP), reflect ossification, while bone resorption is indicated by serum cross-linked

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C-telopeptides of type I collagen (bCTx) and urinary cross-linked N-telopeptides (NTx). These markers are useful for both detecting bone turnover abnormalities and monitoring treatment response in OP [10–12].

Ubiquitin (Ub) is an evolutionarily conserved protein essential for protein degradation signaling, functioning through a cascade involving ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) [13, 14]. Ubiquitination, a post-translational modification, plays a crucial role in virtually all cellular processes and is vital for maintaining cellular homeostasis [15]. This process is catalyzed by a series of enzyme-catalyzed reactions. Ub is initially activated in an ATP-dependent manner by E1, transferred to E2's catalytic cysteine, and finally conjugated to target proteins' lysine residues via E3 ligases (Fig. 1). The E3 ligases, numbering between 600 and 1000 in humans, are pivotal for mediating substrate specificity and are implicated in various biological processes, including those critical for bone homeostasis [16–19]. Based on their structural domains and mechanisms, E3 ligases are classified into three main types [20]: HECT (Homology to E6AP C-Terminus) family, RING (Really Interesting New Gene) family, RBR (RING-between-RING) family [21]. Among them, the HECT domain E3 ligases are implicated in diverse pathologies, including bone disorders [22]. The conserved HECT domain is located at the C-terminal with a cysteine site within 350 amino acids. The N-terminal region interacts with E2 ligases, while the C-terminal HECT domain is involved in catalysis [23, 24]. The N-terminal part influences substrate specificity, leading to the classification of the HECT family into three subfamilies: the NEDD4 subfamily containing WW domains, the HECT and RCC1-like domain (HERC) subfamily with one or more RCC1-like domains, and various other HECT-containing subfamilies [25]. Among them, NEDD4 is the largest subfamily, including nine members: Smurf1 (Smad-specific E3 ubiquitin protein ligase 1), Smurf2 (Smad-specific E3 ubiquitin protein ligase 2), WWP1 (WW domain-containing E3 ubiquitin protein ligase 1), WWP2 (WW domain-containing E3 ubiquitin protein ligase 2), NEDD4, NEDD4L, NEDL1, NEDL2, and ITCH [26, 27]. These enzymes are widely expressed across various human organs and tissues, including those integral to the skeletal system, and play a critical role in modulating key signaling pathways that regulate cellular growth, proliferation, and differentiation, which are pivotal for bone health and homeostasis [28].

Pharmacological treatments for osteoporosis include both anti-resorptive and anabolic medications. Anti-resorptive drugs, such as bisphosphonates (e.g. zoledronic acid) and RANK ligand inhibitors (e.g. denosumab), reduce bone resorption. The primary anabolic treatment is teriparatide, which promotes bone formation [29–32]. Despite significant advancements, concerns over side effects of anti-resorptive drugs, especially bisphosphonates, and lack of clear evidence for long-term efficacy have led many patients to avoid these treatments. Therefore, there is a critical need to improve patient adherence and acceptance of these effective therapies, while also developing new drugs that minimize side effects and offer sustained anabolic effects on bone [33]. Recent researches have increasingly connected bone remodeling with ubiquitination processes, underscoring the E3 ubiquitin ligase NEDD4 family's role in osteoblast function. The NEDD4 family plays a crucial role in maintaining the normal physiological functions of osteoblasts, and the inhibition of NEDD4 family members, such as Smurf1, leads to enhanced osteoblast differentiation, bone formation, and bone mass [34]. The E3 ubiquitin ligase NEDD4 family has emerged as a key focus in the study of osteoporotic metabolic mechanisms and drug targeting. Therefore, this review focuses on the NEDD4 family, summarizing how its members influence OP and discussing potential therapeutic targets. This provides a foundational understanding and novel therapeutic avenues for managing osteoporosis.

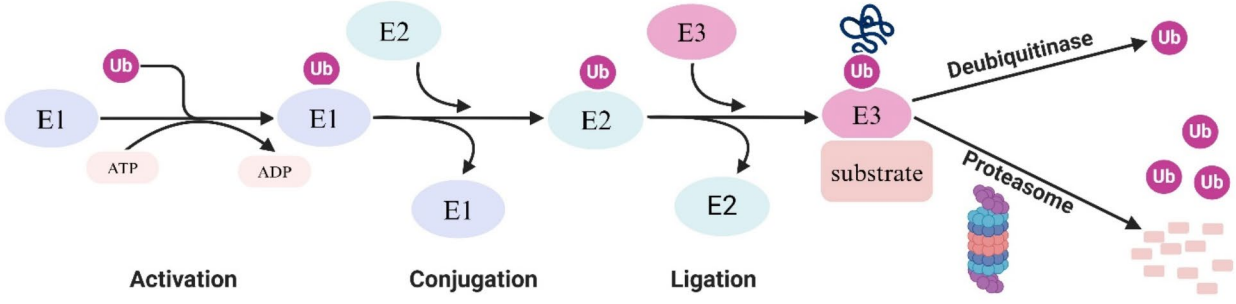

Fig. 1 The ubiquitination procedure. The ubiquitin process is mediated by E1 ubiquitin-activating enzyme, E2 ubiquitin conjugating enzyme, and E3 ubiquitin ligase. First, in the presence of ATP, E1 activates Ub and transfers it to E2. E2 then transfers the Ub to E3, which catalyzes the attachment of Ub to the target protein. The ubiquitinated proteins are subsequently recognized and degraded by the proteasome. The process can be reversed through deubiquitination by specific enzymes

Bone remodeling and its cellular regulation

Bone matrix is primarily composed of an organic matrix and mineral components, with minimal water content. The organic portion predominantly consists of collagen fibers and a modest amount of ground substance, which includes cell adhesion proteins, alkaline phosphatase, bone growth regulators, and various macromolecules [35, 36]. The ground substance features gel-like glycosaminoglycans, slightly alkaline or neutral, adhering to collagen fibrils. These non-collagenous proteins play crucial roles in collagen's synthesis, secretion, degradation, and the mineralization of the bone matrix. The inorganic component, mainly consisting of hydroxyapatite crystals (Ca10(PO4)6(OH)2), carbonates, calcium, magnesium, and citrates, integrates closely with the organic matrix. The tight integration of organic and inorganic components imparts rigidity to bone. Bone mineralization refers to the deposition of hydroxyapatite in the extracellular matrix of the tissue [37], which can be classified into physiological and pathological mineralization. Bone mineralization regulates mineral nucleation and constructs complex skeletal structures, being a cell-regulated process. Various substances regulate local changes at different stages of mineralization [38].

The skeletal system is dynamic, with bone formation and resorption regulated by various proteins and signaling molecules [39]. Three principal cell types are involved: osteoblasts, osteoclasts, and osteocytes. Osteoblasts mediate bone formation, while osteoclasts are responsible for bone resorption. Osteoblasts originate from undifferentiated mesenchymal stem cells (MSCs), which differentiate into osteoprogenitor cells, then into preosteoblasts, and finally into mature osteoblasts [40]. Bone marrow MSCs exhibit multilineage differentiation potential, forming not only osteoblasts but also chondrocytes and adipocytes, thereby promoting bone formation [41, 42]. Osteoblasts are primarily located on the bone surface, performing essential functions by secreting collagen and bone matrix proteins. Osteoclasts, multinucleated giant cells derived from the monocyte/macrophage lineage under the stimulation of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL), are the only cells responsible for bone resorption [43]. They contribute to bone remodeling by resorbing bone matrix and minerals, secreting various organic acids and proteolytic enzymes, thus maintaining healthy bone [44]. Osteocytes, mature osteoblasts, produce various signaling molecules and proteins to maintain bone metabolic balance [45].Bone homeostasis is fundamental to overall health, with osteoclasts and osteoblasts playing crucial roles in maintaining the dynamic balance between bone resorption and formation. From birth through to old age, the skeleton continually undergoes a remodeling process. Bone remodeling is a physiological process wherein osteoclasts first remove old or damaged bone, which is subsequently replaced by new bone formed by osteoblasts [46]. Bone remodeling can be divided into seven stages: resting phase, activation phase, resorption phase, reversal phase, formation phase, mineralization phase, and termination phase [47]. Various cell types involved in bone tissue participate in bone remodeling through complex interactions. Osteoblasts and osteoclasts maintain a dynamic equilibrium that is critical for the bone matrix microenvironment. Disruption of bone homeostasis can lead to bone-related diseases such as OP.

The development and progression of OP are regulated by various factors, including bone morphogenetic proteins (BMP), Transforming Growth Factor-β (TGF-β), Parathyroid Hormone (PTH), and Fibroblast Growth Factor (FGF) [48, 49]. Key signaling pathways involved in bone growth and development include the BMP/Smad, TGF-β/Smad, and Wnt/β-catenin pathways. Macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL) are two critical cytokines that stimulate the differentiation of osteoclasts from their precursors. The RANKL/RANK/MAPK and NF-κB pathways are the primary signaling routes regulating osteoclast formation and function. RANKL, a key receptor activator of NF-κB ligand, is essential for the survival, proliferation, and in vitro differentiation of osteoclast precursors. Upon binding of RANKL to RANK, tumor necrosis factor receptor-associated factor 6 (TRAF6) is recruited, leading to activation of downstream signaling pathways that stimulate osteoclast differentiation. These pathways include AKT-Gsk3β, NF-κB, and MAPKs (ERK, p38) [50, 51]. Research has shown that GPR125 is highly expressed in osteoclasts and acts as a positive regulator of osteoclastogenesis. GPR125 responds to RANKL stimulation by upregulating MAPKs and AKT-NF-κB (p-IKBα and p-P65) signaling pathways, thereby increasing the expression of osteoclast genes and promoting osteoclastogenesis [52]. Consequently, targeting GPR125 could represent a novel approach for the treatment of OP.

Mechanisms of bone remodeling linked to E3 ubiquitin ligase NEDD4 family and its potential targeting drugs

Smurf1 acts as an inhibitor of osteoblast differentiation and mineralization

Smurf1 is characterized by a catalytic HECT domain at its C-terminal, two WW domains (WW1 and WW2), and a phospholipid-binding C2 domain at the N-terminal [53]. It is widely expressed bone and cartilage, playing a crucial role in osteoblast differentiation and bone mass accumulation [54, 55]. Genetic variants in genes encoding Smurf1-related proteins have been implicated in the

risk of OP, as identified through hypothesis-free genome-wide association studies [56]. Mouse models have demonstrated that Smurf1 gene expression inversely affects osteoblast function and responsiveness to BMPs in a dose-dependent manner [57]. Notably, AI-Rawi et al. [58] identified OP in a child linked to a microduplication of the Smurf1 gene, highlighting its clinical significance. In experimental therapies, Rodríguez-Évora et al. [59] utilized poly(lactic-co-glycolic-acid) (PLGA) integrated with human recombinant BMP-2 enriched microspheres and MSCs with knocked-down Smurf1 expression to successfully repair rat calvarial defects. Furthermore, studies by Yamashita et al. [60] reported that Smurf1-deficient mice exhibited enhanced sensitivity to BMPs and an age-related increase in bone mass. The BMP/Smad signaling pathway operates through the signaling mothers against decapentaplegic (Smad) cascade, initiated when BMPs bind to their receptors, leading to the phosphorylation of Smads1, Smads5, and Smads8 (R-Smads). These phosphorylated Smads then form a transcriptional complex with Smad4 (Co-Smad), which translocates to the nucleus to bind BMP-targeted regulatory regions, activating key osteoblastogenic transcription factors such as Runx2 and Osterix [61, 62] (Fig. 2). This process facilitates osteoblast differentiation and maturation. Concurrently, BMP binding induces phosphorylation of mitogen-activated protein kinase kinase kinase 2 (MEKK2), activating the c-Jun N-terminal kinase (JNK) pathway, which enhances osteoblast responsiveness and activity toward BMPs [63]. Both BMPs and the JunB proto-oncogene (JunB) are vital factors that regulate the differentiation and function of MSCs as well as osteoblasts.

Smurf1 acts as an inhibitor of osteoblast differentiation and mineralization through various mechanisms. It promotes the ubiquitin-mediated degradation of Runx2 and interferes with the nuclear translocation of the phosphorylated R-Smad/Co-Smad complex. Additionally, Smurf1 ubiquitinates and regulates MEKK2, attenuating JNK activation and thereby suppressing osteoblast activity [62]. Furthermore, Smurf1 enhances the degradation of the JunB, inhibiting MSC proliferation and their differentiation into osteoblasts [64].

Thus, targeting Smurf1 for inhibition could potentially prevent OP. Various factors, including non-coding RNAs, deoxyribonucleotide, specific compounds, inflammatory mediators, and others, have been shown to influence Smurf1 expression, positioning them as potential therapeutic targets. For instance, Ye et al. [65] demonstrated that overexpression of miR-195-5p activates the BMP-2/SMAD/Akt/Runx2 signaling pathway by suppressing Smurf1, thereby alleviating the progression of OP. Similarly, high levels of miR-25 in exosomes from bone MSCs promote osteogenic differentiation, proliferation, and migration of osteoblasts by inhibiting Smurf1-mediated ubiquitination and degradation of Runx2 [66]. Other miRNAs, such as miR-503 [67], miR-19b-3p [68], miR-672-5p [69] and miR-15b [70] have also demonstrated potential in suppressing Smurf1 expression, underscoring their therapeutic potential for OP. GapmeRs is known as a particular type of antisense oligonucleotides, typically 14–20 bp long, which can selectively bind to its target mRNA and promote its catalytic degradation via the action of RNase H, an endonuclease that specifically recognizes DNA/RNA heteroduplexes and cleaves the RNA strand. In animal models, GapmeRs have been shown to enhance osteogenic differentiation by silencing Smurf1 expression [71]. Targeted compounds like B06 and B75, which interact with the ubiquitin-binding site within Smurf1's HECT domain [72], as well as chalcone derivatives such as 2-(4-cinnamoylphenoxy) acetic acid [73], have been found to inhibit Smurf1 activity and enhance osteoblast differentiation by activating BMP signaling. Additionally, LIM and cysteine-rich domains-1 (LMCD1), a member of the LIM protein family, could protect Runx2 and Smad1 proteins from Smurf1-induced ubiquitination and degradation, thereby facilitating the osteogenic differentiation of BMSCs [74]. Furthermore, melatonin treatment has been shown to downregulate TNFα-induced Smurf1 expression, restoring TNFα-impaired osteogenesis by maintaining BMP-Smad1 signaling activity [75]. Collectively, these findings highlight the multifaceted role of Smurf1 in bone metabolism and suggest that targeting its activity could offer a novel therapeutic approach for osteoporosis.

Smurf2 inhibits osteoblast differentiation and promotes bone resorption

Smurf2, which shares over 70% sequence identity with Smurf1 and possesses an additional WW domain, exhibits significant structural similarity to Smurf1 [76]. However, Smurf2 functions oppositely to Smurf1 in several key aspects. Xu et al. [77]. demonstrated that Smurf2 can ubiquitinate Smad3, thereby disrupting its interaction with the vitamin D receptor. This disruption enhances osteoclast activity through increased expression of RANKL, a crucial factor in osteoclast differentiation and activation. In contrast to the increased bone mass phenotype observed in Smurf1$^{-/-}$ mice, Smurf2$^{-/-}$ mice display reduced bone mass and elevated bone resorption [78]. TGF-β plays a pivotal role in maintaining bone homeostasis by promoting osteoblast differentiation and proliferation while simultaneously inhibiting osteoclast formation [79]. Smurf2 primarily acts as a negative regulator of the TGF-β/Smad and BMP/Smad signaling pathways [80]. Upon TGF-β binding to its receptor, TGF-β receptor II phosphorylates TGF-β receptor I, initiating a downstream intracellular signaling cascade. This cascade commences with the phosphorylation of Smad2 and

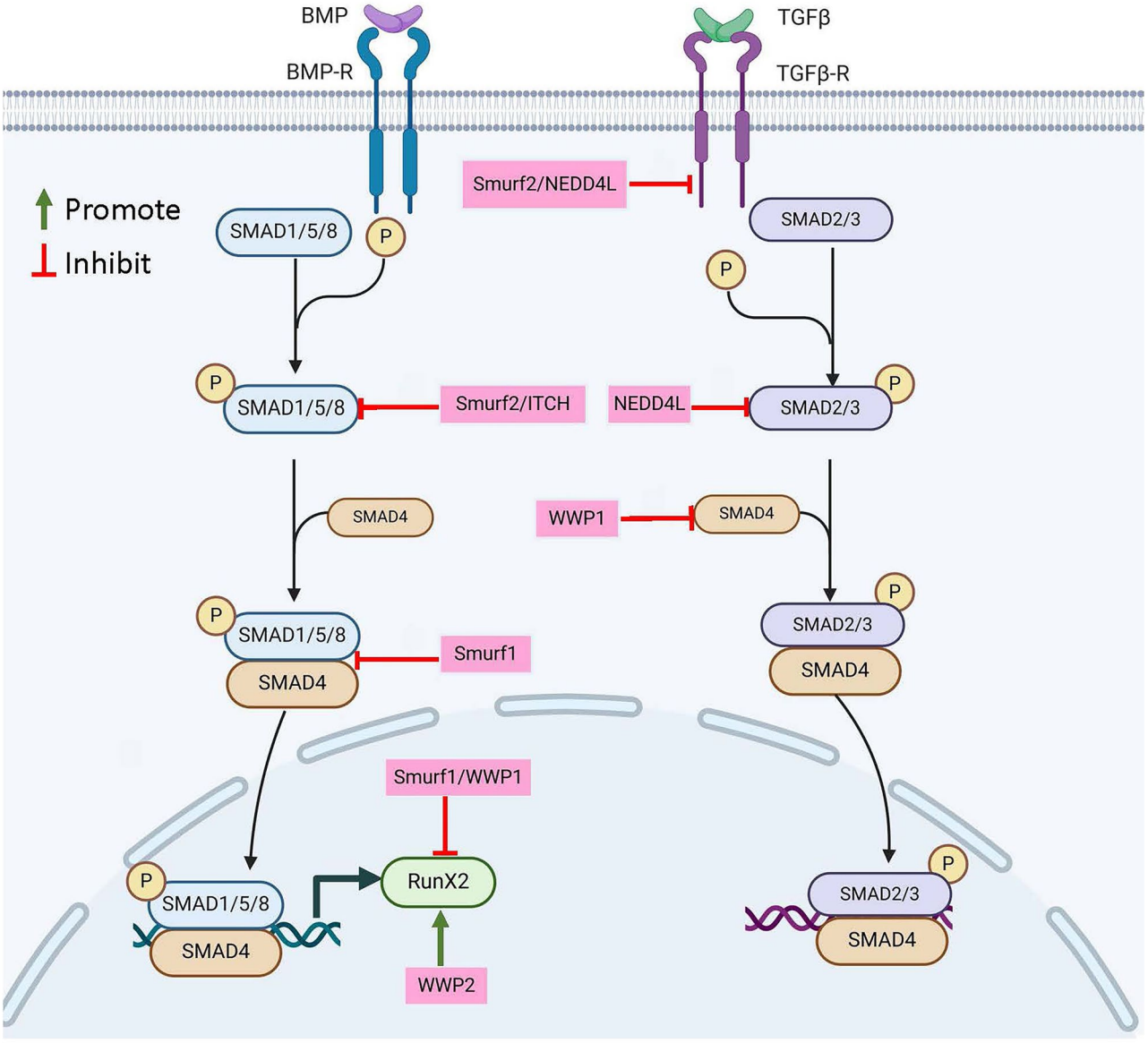

Fig. 2 NEDD4 family members in the BMP/Smad and TGF-β/Smad pathways. BMP/Smad pathway: BMP binding to its receptors activates the phosphorylation of Smads1, 5, and 8 (R-Smads), which can be inhibited by Smurf2 and ITCH. Phosphorylated R-Smads form a complex with Smad4 (Co-Smad), translocating to the nucleus to activate key osteoblastogenic transcription factors, such as Runx2 and Osterix. Smurf1 inhibits Co-Smad, while Runx2 is regulated by Smurf1(inhibition), WWP1 (inhibition), and WWP2 (activation). TGF-β/Smad pathway: TGF-β binding to its receptor phosphorylates Smad2 and Smad3 (R-Smads), which then associate with Smad4 (Co-Smad) and translocate to the nucleus, where they recruit co-factors to activate TGF-β target gene transcription. R-Smads are inhibited by NEDD4L, and Smad4 is inhibited by WWP1

Smad3 (R-Smads), which subsequently form a complex with Smad4 (Co-Smad). This complex is subsequently translocated to the nucleus, where it recruits additional transcriptional co-factors and activates the transcription of TGF-β target genes (Fig. 2). In addition, Smad6 and Smad7 (I-Smads) serve as inhibitory Smads that can suppress the Smad signaling pathways of TGF-β and BMP, thereby establishing a negative feedback loop. Smurf2 promotes the ubiquitination of TGF-β receptor, Smad1 and Smad5, leading to the inhibition of the TGF-β and BMP/Smad signaling pathways [78, 81].

Hence, factors that inhibit the activity of Smurf2 could act as potential therapeutic targets for OP. Upadhyay et al. [82]. demonstrated that Hakai, an E3 ubiquitin ligase, can protect Runx2 from Smurf2-mediated ubiquitin-proteasomal degradation, thereby enhancing its protein stability. Similarly, the serine/threonine protein kinase Akt has been shown to increase the transcriptional activity and protein stability of Runx2 by suppressing

Smurf2-mediated degradation [83]. Moreover, Smad7 is known to inhibit osteoblast differentiation through Smurf2-mediated degradation of Runx2. Vishal et al. [84]. found that miR-590-5p can upregulate Smad7 expression, indirectly stabilizing Runx2 and promoting osteoblast differentiation. Other studies have indicated that miR-19b [85], miR-130a [86] and lncRNA RAD51-AS1 [87] could promote the differentiation of human BMSCs into osteoblasts by downregulating expression of Smurf2. Additionally, TNF receptor-associated factor 4 (TRAF4) [88] positively regulates the osteogenic differentiation of MSCs by acting as an E3 ubiquitin ligase that targets Smurf2 for degradation. By modulating the pathways that regulate Smurf2, it may be possible to enhance osteoblast function and improve bone health, offering new hope for individuals affected by OP.

WWP1 negatively regulates osteoblast differentiation and bone formation

WWP1 is a multifunctional protein that features an N-terminal C2 domain, four tandem WW domains responsible for substrate binding, and a C-terminal HECT domain that facilitates ubiquitin transfer [89]. This protein has been implicated in negatively regulating bone homeostasis. The role of WWP1 in maintaining bone health was first proposed by Jones et al. [90]., who highlighted its significance in bone metabolism. Subsequent research by Shu et al. [91]. demonstrated that WWP1$^{-/-}$ mice exhibited a notable increase in bone mass as they aged. This increase was correlated with heightened rates of bone formation and normal parameters of bone resorption, suggesting that WWP1 plays a critical role in bone remodeling. Mechanistically, WWP1 negatively regulates osteoblastic bone formation through the TGF-β signaling pathway by inhibiting the phosphorylation of Smad2 [92]. Additionally, Morén et al. [93]. revealed that WWP1 employs Smad7 to facilitate the ubiquitination and degradation of Smad4, further elucidating its role in bone metabolism. Furthermore, WWP1 has been shown to negatively regulate Runx2 at the protein level, a key transcription factor in osteoblast differentiation [90]. JunB, another important regulator of osteoblast proliferation and function, is also subjected to ubiquitin-mediated degradation by WWP1 [89, 94].

Given these insights, targeting factors that inhibit WWP1 activity presents a promising therapeutic strategy for OP. siRNA therapeutics have shown great potential in treating OP, however, their clinical application has been hindered by challenges such as susceptibility to degradation, low cellular uptake, and poor tissue-specific localization. To address these limitations, Wang et al. [95]. developed an innovative hybrid nanoparticle (NP)/hydrogel system designed to deliver siRNA/NP complexes directly to fracture calluses. This targeted approach effectively silences WWP1, thereby accelerating bone formation and enhancing biomechanical strength. In vivo experiments conducted by Guo et al. [96]., demonstrated that miR-19b-3p could be transmitted via extracellular vesicles to the nucleus, leading to the inhibition of WWP1 expression and promoting the osteogenic differentiation of BMSCs. This mechanism ultimately facilitated bone repair in rats with induced bone defects. In vitro studies further revealed that WWP1 was targeted by miR-142-5p, which enhanced osteoblast activity and matrix mineralization [97]. Similarly, the miR-19 derived from MSCs represses the expression of WWP1 through Wnt/β-catenin signaling pathway, thus facilitating fracture healing [85]. Nucleic acid aptamers offer a therapeutic advantage by providing specific and tailored inhibition of protein targets. Tucker et al. [98]. developed a particular DNA aptamer called C3A, which was proved to inhibit WWP1 ubiquitination of Runx2 and promote extracellular matrix deposition. This innovative approach highlights the potential of nucleic acid-based therapies in the treatment of bone-related disorders, paving the way for novel interventions aimed at enhancing bone health and preventing OP.

WWP2 enhances osteogenic differentiation and bone mass regulation

WWP2 plays a positive role in the regulation of osteogenesis. It features three distinct domains: a phospholipid-binding domain, four tandem WW domains that facilitate substrate recognition, and a C-terminal HECT domain responsible for ubiquitin ligation [99]. Initially, it was designated as atrophin-1 interacting protein 2 (AIP2) by Wood et al. [100]. through yeast two-hybrid screening and in vitro binding analysis. Unlike the phenotype observed in WWP1$^{-/-}$mice, WWP2-deficient mice exhibit notable craniofacial malformations [101]. Zhu et al. [102] further demonstrated that the knockdown of WWP2 in MSCs results in significant osteogenic deficiencies, evidenced by decreased mineral deposition and the down-regulation of osteogenic marker genes. Importantly, the mono-ubiquitination of WWP2 has been shown to enhance Runx2 transactivation, thereby promoting Runx2-responsive reporter activity. Moreover, the BMP signaling pathway has been established to enhance the interaction between WWP2 and Runx2, ultimately facilitating WWP2-dependent ubiquitination and transactivation of Runx2 [102]. WWP1 generally exists in an autoinhibited state, suggesting that factors capable of relieving this autoinhibition may serve as potential therapies for OP. Notably, it has been demonstrated that NDFIP1/2 (NEDD4 family-interacting proteins) [103] and Dishevelled protein 2 (Dvl2) [104] have been demonstrated to alleviate WWP2 from its autoinhibition, thereby activating its function.

Dual roles of ITCH in osteogenesis and bone mass homeostasis

ITCH exhibits dual roles in the regulation of osteogenesis. Zhang et al. [105] found that ITCH $^{-/-}$ mice at 1-month-old age display high bone mass but subsequently develop OP by 1 year of age. Liu et al. [106] also found that young ITCH knockout mice exhibit increased bone mass and enhanced osteoblast differentiation, correlating with elevated expression levels of Runx2 mRNA. Conversely, Zhong et al. [107] suggested that knockdown of Circ-ITCH, a well-known circular RNA, inhibits its ALP activity, mineralized nodule formation, and the expression levels of Runx2, osteopontin (OPN), and osteocalcin (OCN) during osteogenic induction. ITCH negatively regulates osteogenic differentiation through the modulation of the Wnt/β-catenin and BMP signaling pathways (Figs. 2 and 3). Wnt ligands interact with Wnt receptors and LRP5/6, activating CKI and recruiting Dishevelled (DVL) to the cell membrane for phosphorylation. This leads to the stabilization and accumulation of β-catenin. Subsequently, β-catenin enters the nucleus, where it binds to T-cell factor/Lymphoid Enhancer Factor (TCF/LEF) and initiates the transcription of Wnt pathway target genes. Specifically, it phosphorylates Dvl protein, a critical component in Wnt signaling transduction, thereby inhibiting this pathway [108]. Additionally, ITCH negatively regulates BMP signaling by promoting the degradation of Smad1, which is essential for osteogenic differentiation [109]. Furthermore, ITCH controls osteoblast differentiation from bone marrow mesenchymal precursor cells (BM-MPCs) through the proteasomal degradation of JunB, a positive regulator of osteoblast activity [105]. Conversely, ITCH may inhibit osteoclastogenesis by interacting with cylindromatosis and promoting the deubiquitination of TNF receptor-associated factor 6 (TRAF6) [106]. Notably, in vivo studies have shown that Circ-ITCH promotes osteogenic differentiation in OP by sponging miR-214 [107].

Other NEDD4 family members

NEDD4, also known as RPF1, was first isolated from mouse neural precursor cells [110]. It promotes osteoblast proliferation and osteogenesis by degrading pSMAD1 activated by TGF-β1 [111]. Wiszniak et al. [112] demonstrated that NEDD4$^{-/-}$mice exhibit craniofacial defects, characterized by severe hypoplasia of neural crest-derived intramembranous bone. Additionally, lncRNA SNHG1 negatively regulates p38 MAPK signal pathway though ubiquitination through NEDD4-mediated ubiquitination, thereby inhibiting the osteogenic differentiation of MSCs [113].

NEDD4L, also known as RSP5, plays a dual role in bone metabolism. It positively regulates the osteogenic capacity of MSCs through inducing K63-linked polyubiquitination and activation of the Akt pathway [114]. Adversely, Conversely, NEDD4L overexpression enhances the degradation of Smad2/3 and TβR-I receptor in the TGF-β signaling pathway, inhibiting TGF-β and BMP-induced transcriptional activities [115, 116]. Furthermore, small nucleolar RNA host gene 14 (SNHG14), a type of lncRNA, promotes the osteogenic differentiation of BMSCs through HuR-mediated upregulation of NEDD4L [117]. Moreover, Morinda officinalis polysaccharides (MOP), a traditional Chinese medicine, effectively prevent postmenopausal OP via the miR-214-3p/NEDD4L pathway in mouse models [118].

Summary and perspectives

In recent years, the role of E3 ubiquitin ligase NEDD4 family, has garnered significant attention in the context of OP. Members of the NEDD4 family have been identified as critical regulators of key signaling pathways involved in bone metabolism, including the BMP/Smad, TGF-β/Smad, and Wnt/β-catenin pathways. These pathways are essential for maintaining bone homeostasis, and their dysregulation is implicated in the pathogenesis of OP.

The BMP/Smad signaling pathway is well-known for its role in promoting osteoblast differentiation and bone formation, with WWP2 exhibiting a positive regulatory effect on this pathway. In contrast, Smurf1, Smurf2, WWP1, and ITCH negatively regulate BMP signaling (Fig. 2). Similarly, the TGF-β/Smad pathway, which is crucial for bone remodeling, is negatively regulated by Smurf2, WWP1, and NEDD4L (Fig. 2). Furthermore, the Wnt/β-catenin pathway, a key regulator of osteoblast function, is also negatively influenced by ITCH (Fig. 3). JunB, a positive regulator of osteoblast activity, is subject to degradation by Smurf1, WWP1, and ITCH, thereby affecting osteogenic processes. Additionally, NEDD4L enhances the osteogenic capacity of MSCs by activating the Akt pathway, while NEDD4 promotes osteoblast proliferation and osteogenesis through the degradation of pSMAD1. Notably, ITCH inhibits osteoclastogenesis by facilitating the deubiquitination of TRAF6. Thus, pharmacological targeting of NEDD4 family members presents a novel therapeutic approach for the treatment of OP (Table 1).

Despite the advancements in our understanding of the functions of NEDD4 family members, significant knowledge gaps persist. Future research should aim to elucidate the precise molecular mechanisms by which these E3 ligases influence bone metabolism and their interactions with other signaling pathways. The potential for targeting NEDD4 family members in therapeutic interventions for OP is promising, as current studies indicate that modulation of these ligases could lead to innovative treatments aimed at enhancing bone formation and preventing bone loss. A comprehensive understanding of their roles in

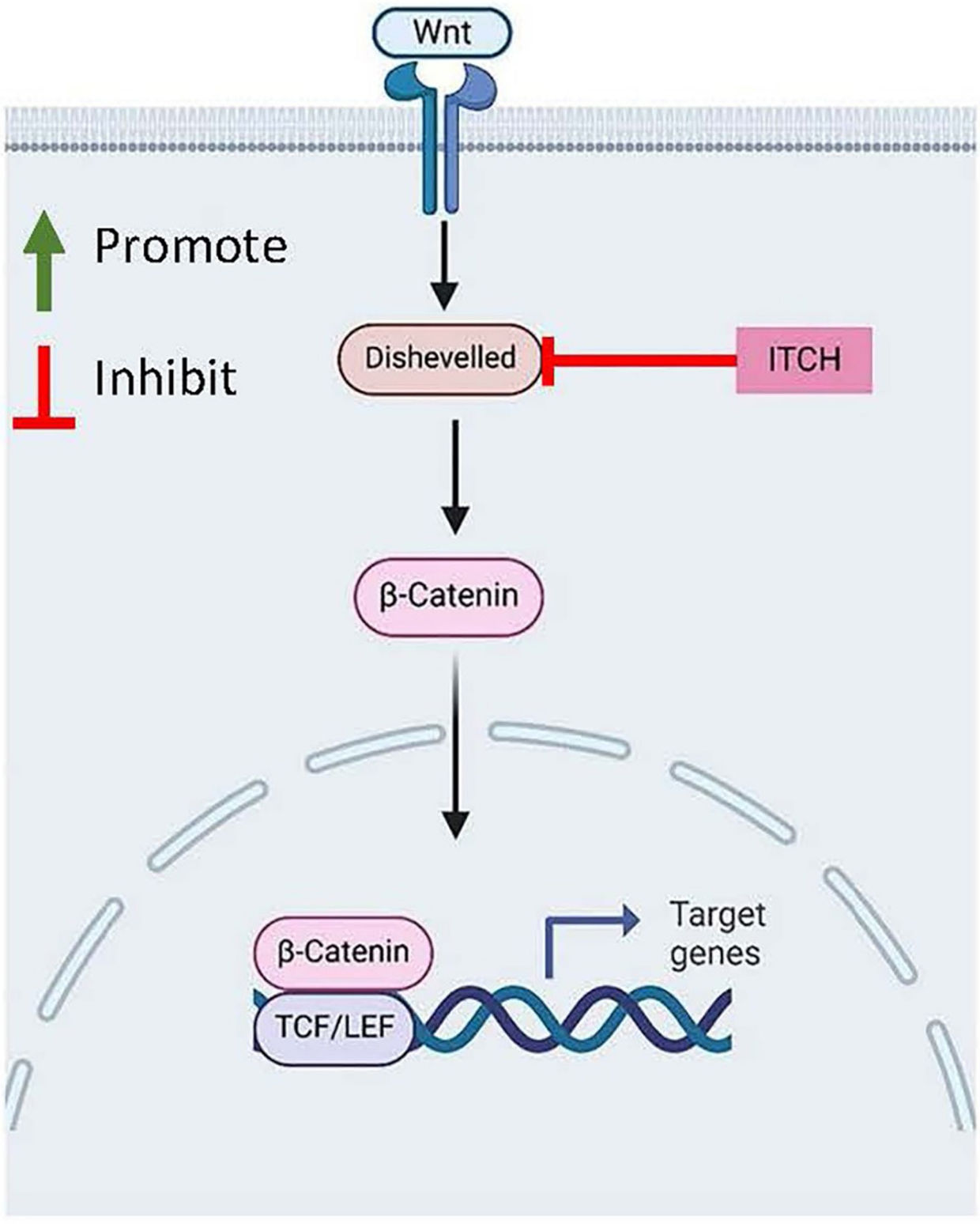

Fig. 3 NEDD4 family members in the Wnt/β-catenin pathways. Wnt ligands bind to Wnt receptors, recruiting Dishevelled (DVL) to the cell membrane, where it undergoes phosphorylation. This process stabilizes and accumulates β-catenin, which then translocates to the nucleus. There, β-catenin binds to T-cell factor/Lymphoid Enhancer Factor (TCF/LEF) and activates the transcription of Wnt target genes. DVL activity can be inhibited by ITCH

Table 1 The functions, mechanisms, and potential drug targets of NEDD4 family in OP

Name	Functions	Mechanisms	Potential drug targets	Refs
Smurf1	Inhibits osteoblast differentiation	Degrades BMP/Smad signaling pathway molecules	miR-195-5p, miR-25, miR-503, miR-19b-3p, miR-672-5p, miR-15b, GapmeRs, B06 and B75 compunds, chalcone derivatives, LMCD1, melatonin	[65–75]
		Ubiquitinates JunB		
		Attenuates JNK activation		
Smurf2	Inhibits osteoblast differentiation	Ubiquitinates receptor of BMP and TGF-β signaling pathway	Hakai, serine/threonine protein kinase Akt, miR-590-5p, miR-19b, miR-130a, lncRNA RAD51-AS1, TRAF4	[82–88]
WWP1	Negatively regulates bone homeostasis	Ubiquitinates molecules in BMP and TGF-β signaling pathway	siRNA/NP complexes, miR-19b-3p, miR-142-5p, C3A DNA aptamer	[95–98]
		Repressed by Wnt/β-catenin signaling pathway	miR-19b	[85]
		Ubiquitinates JunB		
WWP2	Positively regulates osteogenesis	Relieves autoinhibition	NDFIP1/2, Dvl2	[103, 104]
		Enhances Runx2 transactivation		
ITCH	Bidirectionally regulates osteogenesis	Phosphorylates Dvl in Wnt/β signaling pathway	Circ-ITCH	[107]
		Degrades Smad1 in BMP signaling pathway		
		Degrades JunB		
		Deubiquitinates TRAF6		
NEDD4	Positively regulates osteogenesis	Degrades pSMAD1	Unclear	Unclear
NEDD4L	Bidirectionally regulates osteogenesis	HuR-mediated upregulation of NEDD4L	lncRNA SNHG14	[117]
		miR-214-3p/NEDD4L pathway	MOP	[118]
		Degrades Smad2/3 and TβR-I receptor in BMP and TGF-β pathway		
		Activates Akt pathway		

bone metabolism not only enriches our knowledge of the underlying disease mechanisms but also opens new avenues for therapeutic intervention, ultimately contributing to improved management strategies for OP.

Author contributions

HW, JHZ, YD and SW conceived the study. HW, JHZ, YD drafted the manuscript. HW, YD and HRL performed the literature search and collected the data. SW helped with the final revision of this manuscript. All authors reviewed and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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