Review

EXPLORING YAP1-TEAD INTERACTION IN MULTI-PATHWAY REGULATION: POTENTIAL REPAIRING EFFICACY FOR BONE DEFECT

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Abstract

Bone defects present significant challenges, requiring timely restoration and regeneration in clinical practice. The restoration area of bone defects undergoes continuous bone formation and remodeling, regulated by a balance between bone generation and resorption. These processes of bone homeostasis are influenced by multi-pathway regulation. Yes-associated protein 1 (YAP1) and transcriptional enhanced associated domains (TEADs) are key components of the Hippo pathway. They serve as central regulators within these multi-pathway regulation networks, mediating both osteogenesis and osteoclastogenesis. YAP1 can be influenced by upstream Hippo pathway kinase modules, mechanical signals, and chemical cues, which regulate its interaction with TEAD through signaling pathways and post-translational modifications (PTMs), thereby modulating target protein transcription and various biological processes in bone homeostasis. In this review, we provide a current overview on YAP1-TEAD interactions as a crucial link in bone homeostasis, focusing on the upstream regulations, PTMs, and downstream target. Our aim is to elucidate the complex regulatory mechanisms of YAP1-TEAD and explore potential therapeutic strategies for bone defects.

Keywords: Bone defects, bone homeostasis, YAP1-TEAD interaction, Hippo pathway.

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Introduction

Managing bone defects is a considerable challenge in clinical practice. Such defects may arise from various causes, including resection of primary bone tumours, removal of invasive cancer margins from soft tissues, congenital malformations, trauma, and osteomyelitis, etc. [1, 2]. Effective management of bone defects is essential not only for anatomical reconstruction but also for functional restoration. For instance, reconstruction of bone defects in the oral and maxillofacial regions can rehabilitate mastication and preserve the patient's appearance [3]. These factors are crucial for a patient's psychological and social well-being. Therefore, timely intervention is of utmost importance.

Surgical treatment remains the most common approach for addressing bone defects, with methods such as autologous bone grafts, allograft bone grafts, and distraction osteogenesis frequently used in clinical practice [4]. From a biological perspective, these treatments primarily target bone regeneration by utilising osteoblasts, osteocytes, and chondrocytes, along with intracellular and extracellular molecular signalling pathways and biomechanical stimuli, to optimise skeletal repair. The bone defect area undergoes constant bone formation and remodeling, processes that are finely regulated by a balance between osteoblasts, which are responsible for bone formation, and osteoclasts for resorption [5]. This balance is crucial for maintaining bone homeostasis, ensuring that skeletal integrity is preserved and that damaged areas are repaired as needed.

The mechanisms underlying bone repair in defect areas involve complex interactions between cells and the extracellular matrix (ECM), influenced by factors such as mechanical stress, signaling pathways, and metabolic processes. With advancing knowledge in bone regeneration and drug discovery, key signalling pathways have been identified as essential for osteoblast differentiation and bone repair. For example, the Hedgehog (HH) pathway regulates osteoblast differentiation, a process linked to activation of the Wnt signalling pathway [6,7]. Conversely, activation of the Notch signaling pathway inhibits the Wnt pathway, thereby suppressing osteoblast differentiation [8,9]. Moreover, deficiency in bone morphogenetic protein receptor type 1A (BMPR1A) within the bone morphogenetic protein (BMP) signalling pathway in osteoblasts has been shown to impact bone material quality in both femoral cortical and trabecular compartments [10]. The activation and inhibition of these signalling pathways often involve complex, coordinated interactions to ensure proper bone development and repair. The Hippo pathway, along with its key components yes-associated protein 1 (YAP1) and transcriptional enhanced associated domains (TEADs), plays a crucial role in coordinating bone development and repair. Though often considered a "supporting actor" in osteogenesis, the Hippo pathway's influence is significant, requiring further investigation to fully understand its impact on bone regeneration.

The Hippo pathway is a highly conserved signaling cascade first discovered in *Drosophila melanogaster*, and plays a critical role in the regulation of cell proliferation, differentiation, and survival in mammals. Key components of the Hippo pathway include mammalian STE20-like kinase 1/2 (MST1/2), Salvador homologue 1 (SAV1), Mps one binder 1A and B (MOB1A/B), large tumour suppressor kinase 1/2 (LATS1/2), YAP1, transcriptional co-activator with PDZ-binding motif (TAZ), and TEAD [11]. Activation of the pathway involves phosphorylation of LATS1/2 and its scaffold MOB1A/B by mitogen-activated protein kinase kinase kinase kinases (MAP4Ks), MST1/2, and their scaffold protein SAV1. Phosphorylated LATS1/2 subsequently inhibits YAP1 by preventing its translocation into the nucleus, where it would otherwise interact with TEADs to activate downstream gene expression [12]. Therefore, YAP1 and TEADs are pivotal biomarkers within the Hippo pathway, essential for its regulatory functions. It has been reported that the mechanoactivation of YAP1/TAZ is vital for the matrix stiffness-dependent switch between adipogenic and osteogenic differentiation of bone marrow stromal cells [13]. However, the roles of YAP1 and TEADs in osteogenic differentiation remain ambiguous. Their interaction seems to promote both osteogenic differentiation and osteoclast formation *in vitro*, depending on the specific downstream signaling mechanisms involved.

Post-translational modifications (PTMs) of YAP1 and TEAD can regulate the functions of YAP1 and TEAD proteins, exerting varied effects. For YAP1, phosphorylation is the most classic modification method; for example, phosphorylation at Ser127 mediates the binding of YAP1 with 14-3-3, retaining YAP1 in the cytoplasm and preventing its nuclear entry. Various upstream kinases, including LATS1/2, c-Jun N-terminal kinase (JNK1/2), and cyclin-dependent kinases 1 (CDK1), can phosphorylate multiple serine, threonine, and tyrosine residues, leading to the same outcome. These sites include Thr119, Ser138, Thr154, Ser317, Thr362, Ser61, Ser109, Ser127, Ser164, Ser397, Thr119, Ser289, and Ser367 [14–17]. The retained YAP1 is then targeted for ubiquitin-proteasome system activation through ubiquitination at lysine residues, ultimately leading to degradation. For instance, phosphorylation at Ser381 recruits the Skp1-Cullin-F-box$^{\text{beta-transducin repeats-containing proteins}}$ ($SCF^{\beta-TRCP}$) E3 ubiquitin ligase, which mediates YAP1 ubiquitination and degradation. In addition, SUMOylation of Lys97 and Lys242 can stabilize YAP1, preventing its ubiquitination [18,19]. Methylation modifications at Lys342 and Lys494 inhibit YAP1 transcriptional activity, while acetylation at Lys494 and Lys497 reduces phosphorylation at Ser127, thus affecting YAP1's nuclear localization [20]. Furthermore, O-GlcNAcylation at Ser109 and Thr241 promotes YAP1's transcriptional activity. Regarding TEAD, its PTMs differ in type and function from those of YAP1. Autopalmitoylation at Cys53, Cys327, and Cys359 enhances TEAD's stability, facilitating its binding with YAP1 [21]. Ubiquitination at the Lys27 site of TEAD effectively promotes YAP1 binding [22]. Besides, lactylation at Lys108 enhances the transcriptional activity of the YAP1-TEAD complex [23].

Therefore, further basic research is required to elucidate the mechanisms of YAP1 and TEADs in bone repair. A deeper understanding of these mechanisms is crucial for developing future therapeutic strategies and drugs to treat bone defects. This review provides an overview of the YAP1-TEAD interaction, its upstream and downstream regulatory mechanisms, and their roles in bone development. Additionally, potential therapeutic targets, disruptors, and

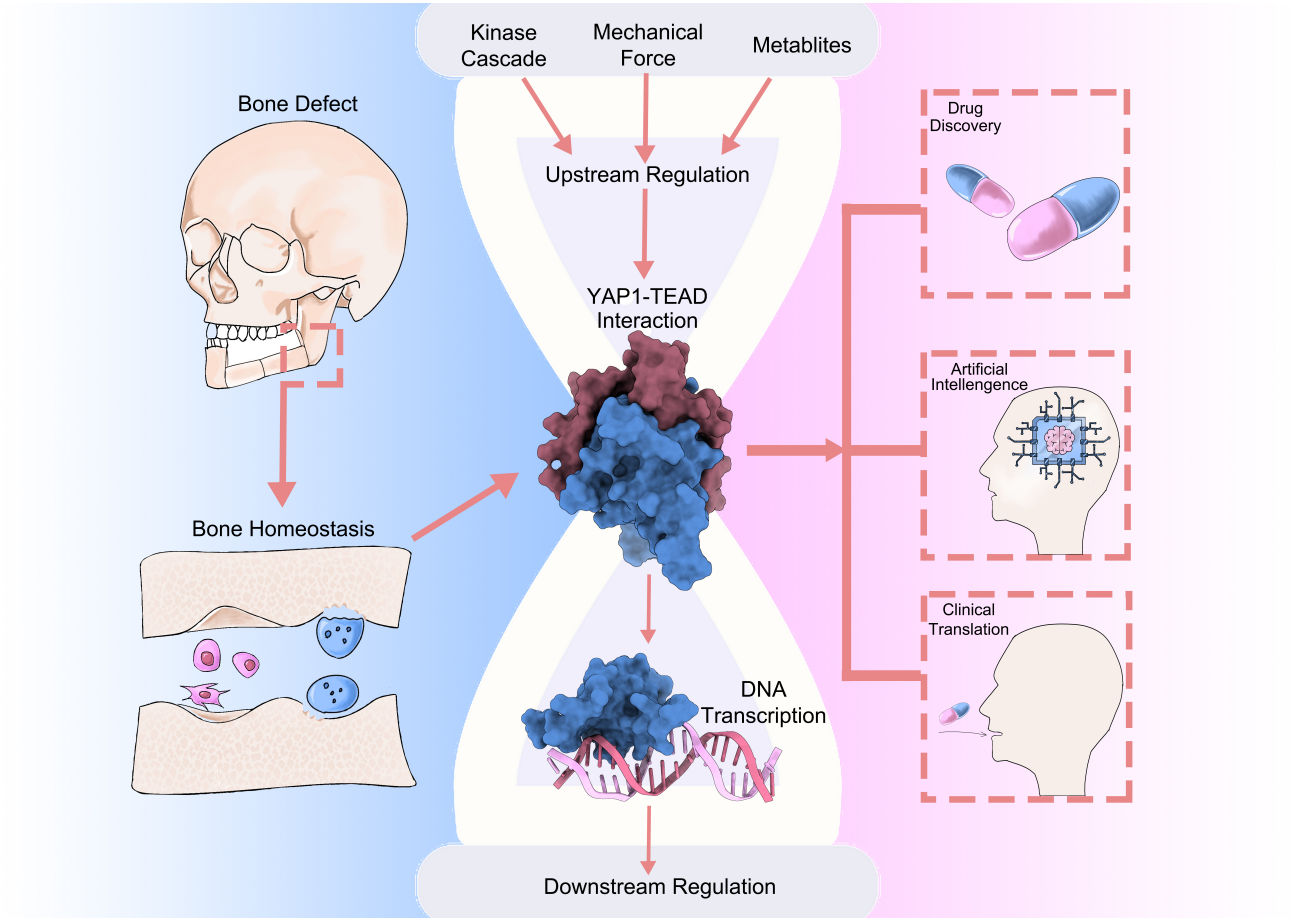

Fig. 1. A comphrehensive overview of this mini-review. This review aims to demonstrate YAP1-TEAD interaction and its upstream and downstream regulatory mechanisms in bone generation and resorption. Molecular mechanisms of YAP1-TEAD interaction in bone homeostasis and correlated research interests are also discussed on the purpose of promoting the current understanding and potential translation in the clinical treatment of bone defect. YAP1, yes-associated protein 1; TEAD, transcriptional enhanced associated domain. Figures were partially designed with Chimerax.

activators targeting the YAP1-TEAD interaction are summarized. By summarizing the molecular mechanisms of YAP1-TEAD in bone homeostasis, along with related research in drug discovery and artificial intelligence (AI), this review aims to enhance understanding and support potential clinical applications in treating bone defects (Fig. 1).

YAP1-TEAD Interactions in the Hippo Pathway

YAP1-TEAD interaction is generally considered a vital member of the Hippo pathway. The Hippo pathway typically consists of two core modules: the upstream kinase module and the downstream transcription module. In the upstream module, LATS1/2 can receive various extracellular stimuli, including mechanical signals related to osteogenic differentiation, cellular stress, and extracellular vesicles. Upon receiving extracellular signals, upstream effectors such as MST1/2, MAP4K, thousand-and-one amino acid kinase (TAOK), and others mediate the phosphorylation of LATS1/2, triggering the phosphorylation of conserved amino acid residues (e.g., Ser127) in YAP1 and TAZ in the cytoplasm [24]. Phosphorylated YAP1/TAZ then bind to the cytoplasmic protein 14-3-3, causing retention of YAP1 in the cytoplasm and preventing the nuclear entry process [25]. Eventually, YAP1 in the cytoplasm is degraded by the proteasome. Thus, the goal of the upstream kinase module of the Hippo pathway is to prevent YAP1 from entering the nucleus, thereby inhibiting the function of the downstream transcription module.

When the upstream kinase module is inhibited, unphosphorylated YAP1 enters the nucleus, binds to TEAD, and activates the downstream transcription module. TEAD itself has minimal transcriptional activity, and YAP1 lacks a DNA-binding domain, requiring YAP1 as a transcriptional co-activator to bind to TEAD and induce targeted gene expression [26]. Nuclear YAP1 interacts with TEAD's C-terminal transactivation domain, forming the YAP1-TEAD complex, which further mediates the transcription of downstream effectors, including the transcriptions of cysteine-rich angiogenic inducer 61 (CYR61, a member of integrin

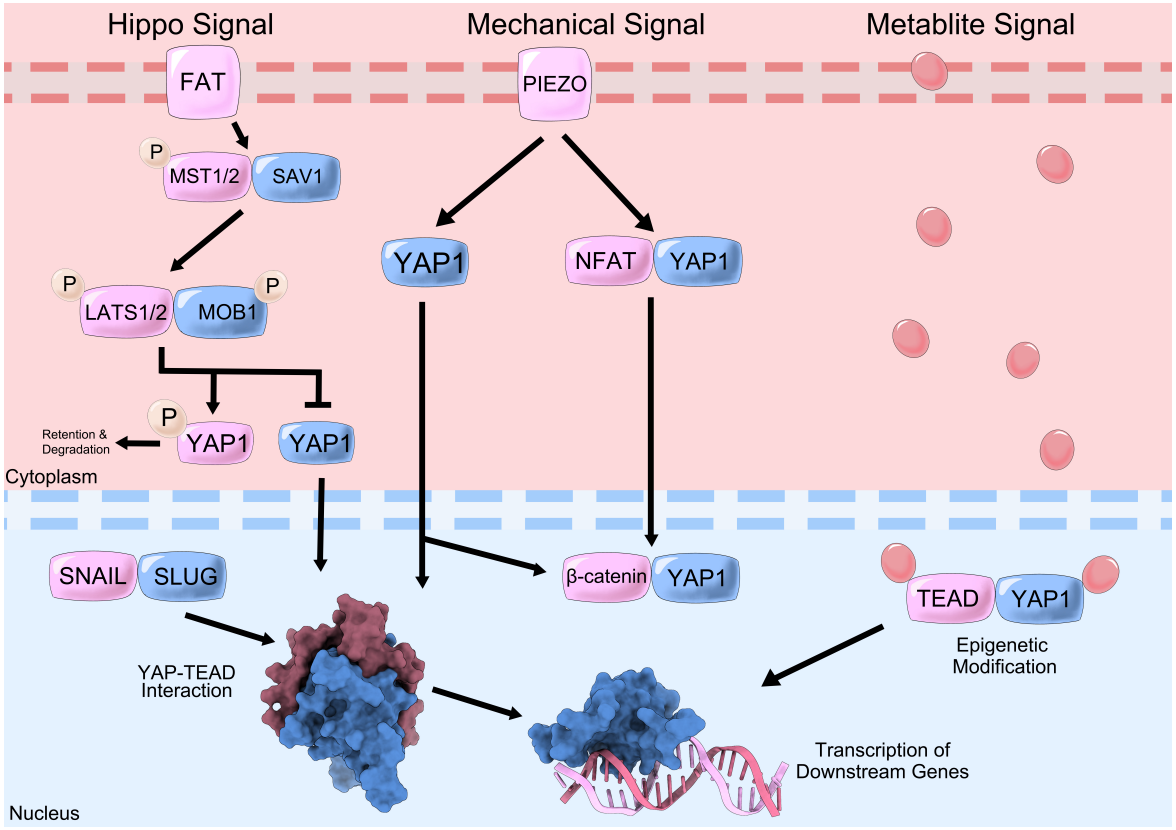

Fig. 2. The main upstreams of YAP1-TEAD interaction in bone formation. The Hippo pathway is involved inosteogenesis by promoting YAP1-TEAD interaction through the regulation of upstream kinases. Mechanical signals are clarified to promote YAP1-TEAD interaction effectively via a Hippo-independent axis. Additionally, metabolites can influence YAP1-TEAD transcription through both signaling regulation and epigenetic modifications. FAT, FAT atypical cadherin; MST1/2, mammalian STE20-like kinase 1/2; SAV1, Salvador homologue 1; LATS1/2, large tumour suppressor kinase 1/2; MOB1, Mps one binder kinase activator adaptor proteins; YAP1, yes-associated protein 1; TEAD, transcriptional enhanced associated domain; PIEZO, Piezo-type mchanosensitive ion channel component; NFAT, nuclear factor of activated T cells. Figures were partially designed with Chimerax.

ligand), connective tissue growth factor (CTGF, a factor of cellular communication), Myc (a transcriptional regulator), amphiregulin (AREG, a member of epidermal growth factor family), Vimentin (a cell adhesion-related protein), etc. [27–30]. These effectors are closely related to cell proliferation, differentiation, apoptosis, migration, and other functions. Consequently, the downstream transcription module of the Hippo pathway regulates various life processes, contributing to the diversity and complexity of the YAP1-TEAD roles in biological functions.

In fact, YAP1 is not limited to the downstream of the Hippo pathway. It can also be translocated to the nucleus through multiple mechanisms. Consequently, the physiological and pathological functions of YAP1 are highly complex and extend beyond the Hippo pathway. Some studies have shown that growth factors regulate YAP1's nuclear translocation. For instance, a study of Park *et al.* in 2015 [31] found that frizzled (FZD) receptors promote YAP1 activation and TEAD-mediated transcription, thereby promoting osteogenic differentiation and bone formation through a Hippo-independent mechanism. YAP1 is intricately linked to various metabolic pathways. For instance, glucose availability can sustain YAP/TAZ activity through the hexosamine biosynthesis pathway (HBP) [32]. Phosphofructokinase 1 (PFK1), which is a critical rate-limiting enzyme in the glycolytic cascade, can stabilise the YAP1-TEAD protein interaction [33]. A study has confirmed that glucose can promote YAP1's nuclear translocation in tumour models [34]. Glucose deprivation can induce cellular energy stress, leading to YAP1 phosphorylation and inhibiting nuclear translocation via the AMP-activated protein kinase (AMPK) signalling pathways [35]. Conversely, under high glucose conditions, YAP1 undergoes O-GlcNAcylation at Thr241, reducing its phosphorylation and enhancing nuclear translocation [36].

Additionally, the mevalonate/cholesterol biosynthetic pathway within lipid metabolism also influences YAP1 activity; through the production of geranylgeranylpyrophosphate, mevalonic acid can stabilise YAP1 activity [37], and YAP1 itself can impact lipid accumulation [38,39]. In amino acid metabolism, YAP1 primarily affects glutamine metabolism, controlling the transcription of

Table 1. Post-translational modifications of YAP1-TEAD.

Protein	PTM type	PTM site	Modifying enzymes	Demodifying enzymes	Function
YAP1	Acetylation	Lys494 Lys497	CBP p300	SIRT1 SIRT2	Reduce phosphorylation in Ser127, and the subsequent nuclear translocation of YAP1.
YAP1	Lactylation	Lys90	AARS1	SIRT1	Enhanced YAP1-TEAD transcriptional activity.
YAP1	Methylation	Lys342 Lys494	SETD7 SET1A	LSD1	Reduce YAP1 transcriptional activity (SETD7). Enhance YAP1 transcriptional activity (SET1A).
YAP1	O-GlcNAcylation	Ser109 Thr241 Thr381	OGT	-	Enhance YAP1 transcriptional activity.
YAP1	Phosphorylation	Ser61 Ser109 Thr119 Ser127 Ser138 Thr154 Ser164 Ser289 Ser317 Thr362 Ser367 Ser397	LATS1/2 JNK1/2 CDK1	S1P LPA SPC PP2A	Enhance binding of 14-3-3 proteins with YAP1, and the subsequent sequestration of YAP1 in the cytoplasm.
YAP1	SUMOylation	Lys830	SENPs	SENP3	Induce YAP nuclear localization.
YAP1	Ubiquitination	Lys63	$SCF^{\beta-TRCP}$ E3 ligase	OTUD1	Most ubiquitination leads to YAP1 degradation. Enhance YAP1 transcriptional activity (Lys63).
TEADs	Lactylation	Lys108	AARS1	SIRT1	Enhance YAP1-TEAD transcriptional activity.
TEADs	Palmitoylation	Cys53 Cys327 Cys359	PATs	APT2 ABHD17A	Improve the stability of TEADs.
TEADs	Ubiquitination	TEAD Lys27	RNF214	-	Promote YAP1-TEAD interaction and augment transcription.

YAP1, yes-associated protein 1; TEAD, transcriptional enhanced associated domain; CBP, CREB-binding protein; AARS1, alanyl-tRNA synthetase 1; SETD7, SET Domain Containing 7; SET1A, SET domain containing 1A; OGT, O-GlcNAc transferase; LATS1/2, large tumour suppressor kinase 1/2; JNK1/2, c-Jun N-terminal kinase; CDK1, cyclin-dependent kinases 1; SENP, SUMO specific peptidase; $SCF^{\beta-TRCP}$, Skp1-Cullin-F-box$^{\text{beta-transducin repeats-containing proteins}}$; PATs, palmitoyl acyltransferases; RNF214, ring finger protein 214; SIRT1, sirtuin 1; SIRT2, sirtuin 2; LSD1, lysine-specific demethylase 1; S1P, sphingosine-1-phosphate; LPA, lysophosphatidic acid; SPC, sphingosylphosphorylcholine; PP2A, protein phosphatase 2A; SENP3, SUMO specific peptidase 3; OTUD1, OTU deubiquitinase 1; APT2, acyl-protein thioesterase 2; ABHD17A, abhydrolase domain containing 17A; PTMs, post-translational modifications.

enzymes involved in this pathway, including glutaminase 1 (GLS1), glutamic-oxaloacetic transaminase 1 (GOT1), and phosphoserine aminotransferase 1 (PSAT1), which ultimately influence adenosine triphosphate (ATP), nucleotide, and amino acid synthesis [40,41]. Studies have also demonstrated a connection between YAP1 and the mTOR signalling pathway, which is activated by amino acids [42], suggesting that the interplay between YAP1 and mTOR is a crucial factor in cellular metabolism [43,44]. These findings indicate that YAP1 can be modulated by

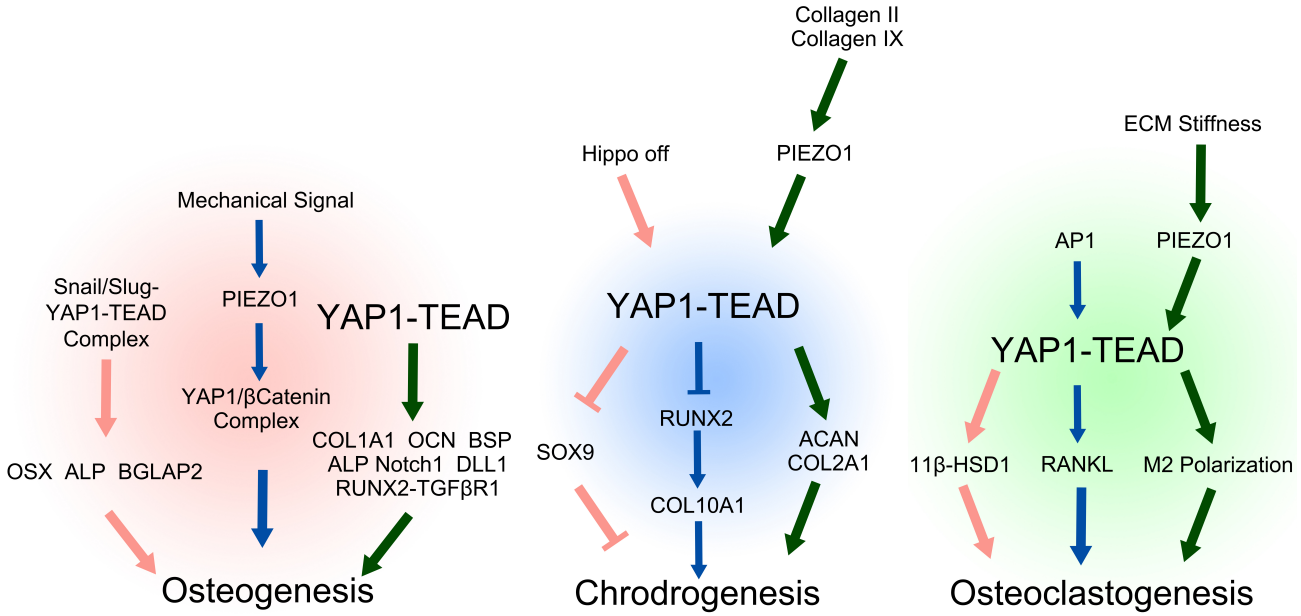

Fig. 3. Downstream regulation networks of YAP1-TEAD in bone homeostasis. YAP1 can be influenced by different upstream signals in different cell types. After interaction with TEAD through multiple signaling pathways, the YAP1-TEAD complex can affect target protein transcription and modulating osteogenesis, chrodrogenesis and osteoclastogenesis ultimately. YAP1, yes-associated protein 1; TEAD, transcriptional enhanced associated domain; PIEZO1, Piezo-type mchanosensitive ion channel component 1; OSX, Osterix; ALP, alkaline phosphatase; BGLAP2, bone gamma-carboxyglutamate proteins 2; COL1A1, collagen type I alpha 1; OCN, osteocalcin; BSP, blepharospasm; DLL1, delta-like ligand 1; RUNX2, runt-related transcription factor 2; TGFβR1, transforming growth factor beta receptor 1; SOX9, sex determining region Y-box 9; COL10A1, collagen type X alpha 1; ACAN, aggrecan; COL2A1, collagen type II alpha 1; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; AP1, activator protein-1; RANKL, receptor activator of nuclear factor-kappa B ligand; ECM, extracellular matrix.

various metabolic signals, serving as a key link between transcriptional and metabolic programmes.

Another important regulatory mechanism is mechanical force, which is considered independent of the Hippo signaling pathway. For example, Aragona *et al.* [45] discovered that mechanical stress acts as an overarching regulator of YAP1 in an epithelial monolayer, which is independent of the Hippo pathway. When cells perceive mechanical stress from the extracellular environment, F-actin cytoskeleton interacts with mechanosensory proteins like integrins, adherens junctions, and focal adhesion kinase (FAK), which regulate YAP1 in turn. Mesenchymal stem cells (MSCs) differentiation is influenced by this regulation [46]. Under low mechanical stress, MSCs differentiate into adipocytes by modulating endogenous RhoA (a member of RhoA/ROCK pathway) activity. When under high mechanical tension, YAP1 translocates to the nucleus, activating MSC differentiation into osteoblasts [47]. In this physiological process, YAP1 acts as a mechanosensor, which can capture information from the physical environment and respond to tensional forces in the skeletal system. This transduction regulates gene expression at the molecular level, bridging the ECM and intracellular gene transcription, and intervening in the cellular fate of cells involved in osteogenesis.

The TEADs family (TEAD1-TEAD4) are highly conserved and play a crucial role in this process. Briefly speaking, TEADs have a DNA-binding domain at the N-terminus for binding DNA fragments and initiating downstream gene transcription, while the C-terminus contains a transactivation domain for binding YAP1 [48]. It is generally believed that this domain contains three potential binding interfaces, defined as Interface 1, Interface 2, and Interface 3, which can serve as druggable sites for developing disruptors of the YAP1-TEAD interaction [49]. It has also been reported that there is a hydrophobic pocket in a deep site of TEADs for regulation of YAP1-TEAD interaction, which is originally demonstrated as a palmitate-binding pocket [21,50]. Activation of YAP1-TEAD interaction leads to increased downstream gene transcription and impacts bone formation

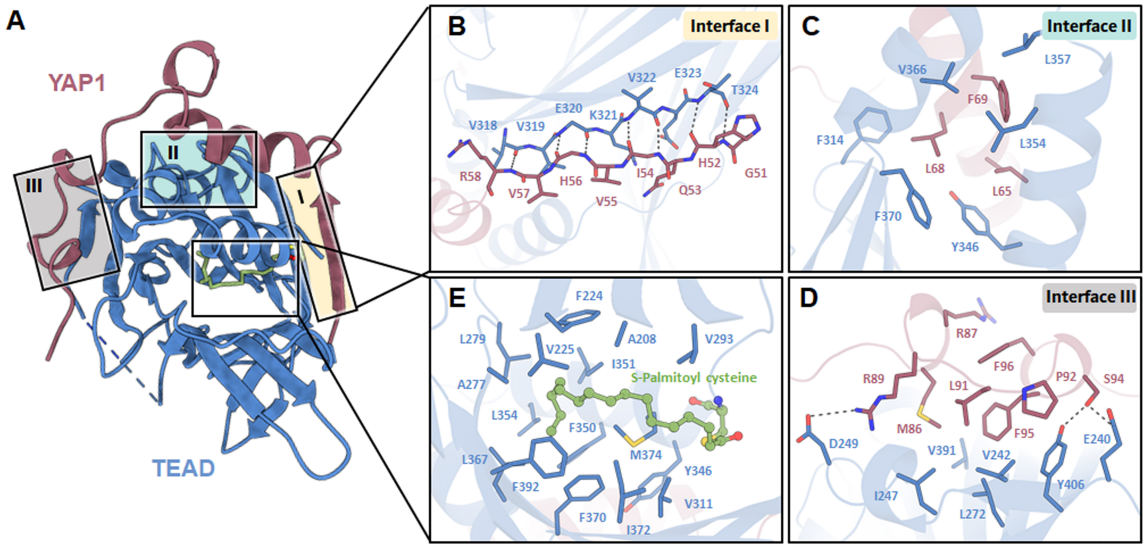

Fig. 4. A summary of protein-protein interaction and potential drugable sites in YAP-TEAD interactions. (**A**) YAP1-TEAD interaction: TEAD contains three potential binding surfaces with YAP1 and a palmitate-binding pocket inside it. (**B**) Interface 1: An anti-parallel β-sheet formed by YAP1 β1 (residues 52–58) and TEAD β7 (residues 318–324). (**C**) Interface 2: A binding groove formed by the YAP1 α1 helix (residues 61–73) and TEAD α3–α4 (residues 345–369). (**D**) Interface 3: an omega-shaped twisted-coil region. (**E**) Palmitate-binding pocket: a hydrophobic pocket in a deep site of TEAD, regulating YAP1-TEAD interaction allosterically. Figures were partially designed with Chimerax.

significantly from both osteogenesis and osteoclastogenesis. We briefly summarized the upstream of YAP1-TEAD in Hippo pathway and other regulatory mechanisms that may affect bone homeostasis (Fig. 2), and will introduce it in more detail in subsequent chapters.

Moreover, YAP1-TEAD also undergoes PTMs, which exhibit various effects. Concerning YAP1, a comprehensive summary has been provided previously [51]. The most classic modification is phosphorylation. In the Hippo pathway, the phosphorylation of LATS1/2 promotes the phosphorylation of Ser residues in YAP1, where phosphorylation at Ser127 mediates the binding of 14-3-3 to YAP1, retaining it in the cytoplasm. Phosphorylation at Ser61, Ser109, Ser127, Ser164, and Ser397 has also been confirmed to be mediated by LATS1/2. Notably, phosphorylation at Ser397 is associated with CK1δ/ε-mediated ubiquitination and subsequent degradation [14]. JNK1/2 can also catalyse phosphorylation at several sites in YAP1, including Thr119, Ser138, Thr154, Ser317, and Thr362 [16]. CDK1 can phosphorylate Thr119, Ser289, and Ser367 [17]. Sphingosine-1-phosphate (S1P), lysophosphatidic acid (LPA), sphingosylphosphorylcholine (SPC), and protein phosphatase 2A (PP2A) are considered to induce dephosphorylation of YAP1, promoting the upregulation of its target genes [52–54]. Overall, phosphorylation effectively activates serine or threonine residues in YAP1, preventing its nuclear entry. Retained YAP1 undergoes ubiquitination at lysine residues, triggering the activation of the ubiquitin-proteasome system and eventually degradation, with SCF$^{\beta-TRCP}$ mediating YAP1 degradation following phosphorylation at Ser381 [14]. Some non-proteolytic forms of ubiquitination, such as the ubiquitination of Lys63 by S-phase kinase associated protein 2 (SKP2), can promote YAP1 nuclear translocation and can be reversed by OTU deubiquitinase 1 (OTUD1) [55]. A similar function occurs with sumoylation, where SUMO-specific proteases (SENPs) modify Lys830, and SUMO specific peptidase 3 (SENP3) mediates the corresponding de-SUMOylation process [56,57]. Methylation is another modification affecting Lys residues. The main lysine methyltransferases for YAP1 are SET Domain Containing 7 (SETD7), which methylates Lys494, and SET domain containing 1A (SET1A), which methylates Lys342. The former promotes YAP1 retention in the cytoplasm [19], while the latter enhances YAP1-mediated transcriptional activity. Methylation at Lys342 can be reversed by lysine-specific demethylase 1 (LSD1) [18]. Acetylation of YAP1 occurs at Lys494 and Lys497 by CREB-binding protein (CBP) and p300, enhancing YAP1's nuclear localisation and transcriptional activity, with deacetylation mediated by sirtuin

Table 2. Regulation axis of YAP1-TEAD in osteogenesis and osteroclastogenesis.

Regulatory axis	Targeted cells	Effect	Mode of action
YAP-TEAD-AP1	Osteoclasts	YAP1-TEADs can interact with AP1 and result in regulating osteoclastogenesis.	YAP1 and YAP-TEAD interaction is essential for RANKL -induced osteoclast differentiation and bone resorption activity [93].
YAP1-Wnt/βcatenin	Osteoblasts	Promote progenitor cell proliferation and differentiation. Suppress MSC's adipogenic potential, and thus maintain trabecular bone mass.	YAP is required to maintain cytoplasmic and nuclear pools of β-catenin for maintenance of nuclear β-catenin level and Wnt/β-catenin signaling [79].
YAP1-TEAD-SOX6	Chondrocytes	Promote chondrocyte proliferation	YAP1 requires TEADs binding for direct regulation of SOX6 expression to promote chondrocyte proliferation [82].
YAP1-RUNX2-COL10A1	Chondrocytes	Inhibit chondrocyte maturation	YAP1 inhibits chondrocyte maturation by suppression of COL10A1 expression through interaction with RUNX2 [82].
YAP1/TAZ-TEAD	Mouse MSCs, Osteoblasts	Regulate osteoblast activity, matrix quality, and osteoclastic remodeling.	YAP/TAZ-TEAD inhibition results in reduced expressions of COL1A1 and COL1A2 mRNA, reduced OCN promoter activity, concomitant with reduced expression of BSP and ALP mRNA [75].
VGLL4-TEAD -RUNX2	MSCs	Inhibition of impaired bone ossification.	TEADs bind to RUNX2, inhibiting the transcriptional activity of RUNX2 in an independent way of YAP-TEAD interaction. VGLL4 relieve the inhibitory function of TEAD on RUNX2 [76].
Snail/Slug-YAP/ TAZ-TEAD	SSCs	Promote SSC homeostasis and osteogenesis.	Snail/Slug forms binary complexes with YAP/TAZ, activating YAP/TAZ/TEAD and RUNX2 downstream targets [77].
Snail/Slug-YAP/ TAZ-TEAD	MSCs	Maintain self-renewal properties as well as undergo osteogenesis.	Snail/Slug forms binary complexes with YAP/TAZ for maintenance of self-renewal properties in MSCs [78].
Rb1-YAP-TEAD -GLUT1	MSCs	Promote bone formation and remodeling.	Retinoblastoma 1 enhanced glucose uptake and lactate and ATP production in MSCs, enhancing the expression of GLUT1 and resulting in increased bone formation and remodeling [90].
MOB1-YAP-TEAD -SOX9	Chondrocytes	Impair chondrocyte proliferation and differentiation/maturation, leading to chondrodysplasia.	The hyperactive of YAP1/TAZ-TEAD complex function as a transcriptional repressor of SOX9 and thereby negatively regulates chondrogenesis [66].
Mechanical signal-YAP1/TAZ-TEAD-Notch1/ Delta like ligand 4	BMSCs	Promote segmental bone defect healing	Tensile stress activates of YAP/TAZ transcriptionally upregulates the expression of Notch1 and delta-like ligand 4 [81].
Mechanical signal -Rho GTPase-YAP -TEAD	MSCs	Promote osteoblast differentiation.	Mechanical signal exerted by ECM rigidity and cell shape induces nuclear relays of YAP/TAZ, thus promotes osteoblast differentiation [70].
Mechanical signal-PIEZO1-YAP-β-catenin	Periosteal stem cells	Upregulate osteogenic, chondrogenic and angiogenic factors.	PIEZO1 promotes expression and nuclear localization of YAP1. YAP1 combines with β-catenin and forms transcriptional YAP1/β-catenin complex [72].

Table 2. Continued.

Regulatory axis	Targeted cells	Effect	Mode of action
Mechanical signal -PIEZO1-YAP-TEAD -ACAN-COL2A1	Chondrocytes	Delay the degeneration of endplate cartilage.	YAP1 and TEAD1 overexpression increased the activity of the ACAN or COL2A1 promoter to enhance the transcriptional activity of human chondrocyte collagen [74].
Mechanical signal -PIEZO1-YAP-TEAD -Collagen	Osteoblastic cells	Regulate osteoclast differentiation.	PIEZO1 in osteoblastic cells controls the YAP-dependent expression of type II and IX collagens. In turn, these collagen isoforms regulate osteoclast differentiation [73].
Mechanical signal -PIEZO1-YAP	BMSCs	Inhibit osseointegration.	Matrix stiffening increases PIEZO1 expression, as well as leads to subsequent activation of the mechanotransduction signaling effector YAP1, thus favoring M1 polarization whilst suppressing M2 polarization [94].
Mechanical signal-PIEZO 1/2-NFAT-YAP-βcatenin	BMSCs	Maintain osteoblast differentiation and decrease bone resorption.	PIEZO1/2 promotes NFAT/YAP1/β-catenin complex formation and induces their dephosphorylation [71].
FZD9-Factin-YAP1	Osteoblasts	Rescue osteoblast dysfunction.	FZD9 overexpression regulated AKT and ERK phosphorylation and induced F-actin polymerization to form the actin cap, press the nuclei, and increase nuclear pore size, thereby promoting the nuclear translocation of YAP1 [80].
DCHS1/FAT4-Yap-TEAD-RUNX2	Osteoblasts	Regulate bone development within osteoblast progenitors via regulation of cell proliferation, survival and RUNX2 activity.	In the absence of DCHS1/ FAT4, YAP1 activity is increased, which regulates RUNX2-TGFβR1 [67].
β-HSD1-YAP	Osteoclasts	Promote osteoclastogenesis.	High expression of β-HSD1 enhances the expression of YAP and promotes osteoclastogenesis [92].

BMSCs, bone marrow-derived macrophages; MSCs, mesenchymal stem cells; SSCs, skeletal stem cells; YAP1, yes-associated protein 1; TEAD, transcriptional enhanced associated domain; TAZ, transcriptional co-activator with PDZ-binding motif; PIEZO1, Piezo-type mchanosensitive ion channel component 1; OSX, Osterix; ALP, alkaline phosphatase; BGLAP2, bone gamma-carboxyglutamate proteins 2; COL1A1, collagen type I alpha 1; OCN, osteocalcin; BSP, blepharospasm; DLL1, delta-like ligand 1; RUNX2, runt-related transcription factor 2; TGFβR1, transforming growth factor beta receptor 1; SOX6, sex determining region Y-box 6; SOX9, sex determining region Y-box 9; COL10A1, collagen type X alpha 1; ACAN, aggrecan; COL2A1, collagen type II alpha 1; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; AP1, activator protein-1; RANKL, receptor activator of nuclear factor-kappa B ligand; VGLL4, vestigial-like family member 4; Rb1, retinoblastoma; NFAT, nuclear factor of activated T cells; FZD9, frizzled 9; DCHS1, dachsous cadherin related 1; FAT4, FAT atypical cadherin 4; MOB1, Mps one binder kinase activator adaptor proteins; GLUT1, glucose transporter type 1; COL1A2, collagen type I alpha 2; ECM, extracellular matrix; mRNA, messenger ribonucleic acid; AKT, protein kinase B; ERK, extracellular-signal-regulated kinases.

1 (SIRT1) and sirtuin 2 (SIRT2) [58,59]. Two metabolism-related PTMs also play crucial roles in regulating YAP1. O-GlcNAc transferase (OGT) can modify Ser109 and Thr241 under high glucose conditions, promoting YAP1's transcriptional activity, while GlcNAcylation at Thr381 can inhibit phosphorylation at Ser397, thereby enhancing transcriptional activity [60,61]. It has also shown that lactate can mediate lactylation of both YAP1 and TEAD, and the modification site of YAP1 is Lys90 [23].

Compared to YAP1, reports on PTMs in TEAD are less common and differ in mechanisms, mainly involving palmitoylation, ubiquitination, and lactylation. Palmitoylation in TEAD is primarily mediated by palmitoyl acyltransferases (PATs). Cys53, Cys327, and Cys359 have been identified as key modification sites, although it can also occur without PAT catalysis [21]. The main role of palmitoylation is to maintain TEAD protein stability, potentially coexisting with the protein throughout its lifecycle until degradation [62]. Depalmitoylases such as acyl-protein

thioesterase 2 (APT2) and abhydrolase domain containing 17A (ABHD17A), may regulate the dynamic control of TEAD palmitoylation [63]. The ubiquitin ligase ring finger protein 214 (RNF214) mediates non-proteolytic ubiquitylation at Lys27, promoting the interaction between YAP1 and TEAD, further enhancing the transcription of downstream targets such as angiomotin like 2 (AMOTL2), CTGF, and CYR61 [22]. TEAD's lactylation modification occurs at Lys108, with alanyl-tRNA synthetase 1 (AARS1) sensing and modifying lactate, promoting the transcriptional function of the YAP1-TEAD complex. The downstream genes include *AARS1* itself, forming a positive feedback loop [23]. We have summarised the relevant information on YAP1-TEAD modifications in the table below (Table 1).

YAP1-TEAD Interactions in Bone Homeostasis

Hippo Pathway-YAP1-TEAD Axis in Osteogenesis

In the classical Hippo pathway, the upstream of YAP1-TEAD is a kinase cascade pathway. The regulation of upstream genes or proteins alters the phosphorylation level of YAP1 through this kinase module, thereby determining whether YAP1 translocates into the nucleus to promote TEAD transcription or remains in the cytoplasm for degradation. Vanyai *et al*. [64] found that the deletion of Lats1/2, leads to catastrophic malformations resembling chondrodysplasia or achondrogenesis. MST2, another core component of the Hippo pathway, has been shown to cause attenuated osteoblast differentiation and function when knocked-out [65]. Similarly, Goto *et al*. [66] discovered that the knockout of another key Hippo component, Mps one binder kinase activator adaptor proteins (MOB1), induces hyperactivation of YAP1 and the YAP1-TEAD complex. It represses the expression of sex determining region Y-box 9 (SOX9), and thus negatively regulating chondrogenesis [66]. Additionally, the transcriptional activity of YAP1-TEAD is found to be enhanced in the absence of both FAT atypical cadherin 4 (FAT4, another regulator of the upstream Hippo signaling pathway) and dachsous cadherin related 1 (DCHS1). Bone development is then facilitated by the regulation of runt-related transcription factor 2-transforming growth factor beta receptor 1 (RUNX2-TGFβR1) axis [67].

It is evident that research on the upstream elements of the Hippo pathway primarily focuses on the development of bone and cartilage, which involves crosstalks with other signaling pathways. Given that YAP1-TEAD transcription can also be activated through Hippo-independent mechanisms, studies on the upstream regulators of YAP1-TEAD are no longer confined to the classical kinase members, but have expanded to include a broader range of components.

Mechanical Signaling-YAP1-TEAD Axis in Osteogenesis

Mechanoregulation from the extracellular matrix also influences the nuclear translocation of YAP1. Mechanical signals, such as matrix stiffness, stretch, and fluid shear stress, exist between cells and the ECM as well as between different cell types [68]. These signals are sensed by the cell membrane and transduced intracellularly through both physical and biochemical means. Although it is suggested that mechanoregulation may regulate YAP1 activity via the classical Hippo pathway [69], Dupont *et al*. [70] have identified that ECM rigidity and cell shape can lead to nuclear translocation of YAP1/TAZ in MSCs independently of the Hippo/LATS cascade.

Among the mechanically activated proteins regulating YAP1, Piezo-type mchanosensitive ion channel component 1 (PIEZO1) has garnered significant attention in recent research. Upon receiving mechanical signals from the extracellular environment, PIEZO1 promotes the formation of the NFAT/YAP1/β-catenin (nuclear factor of activated T cells, NFAT) complex and induces dephosphorylation [71]. This process maintains osteoblast differentiation and reduces bone resorption as a result. Similarly, it has been reported that in periosteal stem cells (PSCs), PIEZO1 promotes the formation of the YAP1/β-catenin complex, which upregulates osteogenic, chondrogenic, and angiogenic factors [72]. It is also found that type II and IX collagens can promote osteoclast differentiation through the PIEZO1-YAP1 axis [73]. In cartilage formation, mechanical stimulation has been shown to mediate hyperactivation of YAP1-TEAD via PIEZO1, increasing the transcriptional activity of aggrecan (ACAN) and collagen type II alpha 1 (COL2A1), ultimately enhancing the transcriptional activity of human chondrocyte collagen [74].

It is foreseeable that an increasing number of studies on osteogenesis will focus on the activation of the YAP1-TEAD complex by mechanoregulation and the corresponding subsequent transcription of downstream targets. These downstream targets differ from those associated with the Hippo pathway typically, making the YAP1-TEAD complex a central focus in the study of osteogenesis.

YAP1-TEAD Transcriptional Targets in Osteogenesis

The formation of the YAP1-TEAD complex is not only the endpoint of upstream kinase signals or mechanical signals but also the starting point for the transcription of a series of downstream targets. In MSCs, directly inhibiting the interaction between YAP1 and TEAD proteins reduces the expression of collagen-related messenger ribonucleic acids (mRNAs) such as collagen type I alpha 1 (COL1A1) and collagen type I alpha 2 (COL1A2), as well as osteogenic markers like osteocalcin (OCN), blepharospasm (BSP), and alkaline phosphatase (ALP) [75]. Another study using vestigial-like family member 4 (VGLL4) demonstrates that it inhibits the binding of TEAD to RUNX2, preventing impaired bone ossification [76]. Additionally, research has shown that Snail/Slug forms binary complexes with YAP1-TEAD, activating osteogenic markers including Osterix (OSX), ALP, and bone gamma-carboxyglutamate

Table 3. Activators and disruptors targeting YAP1-TEAD interaction.

Name	Targeting site	Stage	Biomodels	Categories	References
Verteporfin	Inhibites YAP1-TEAD formation	FDA approved	Hepatic cell carcinoma	Disruptor	Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP
Compound 6	Interface 2	Preclinical experiments	Breast cancer	Disruptor	Discovery of a cryptic site at the interface 2 of TEAD—Towards a new family of YAP/TAZ-TEAD inhibitors
Cyclic Peptide	Interface 3	Molecular biology experiment	Protein-protein Interaction	Disruptor	Structure-based design and synthesis of potent cyclic peptides inhibiting the YAP-TEAD protein–protein interaction
IAG933	Interface 3	Clinical trail (phase 1)	Malignant mesothelioma	Disruptor	NCT04857372, direct and selective pharmacological disruption of the YAP-TEAD interface by IAG933 inhibits Hippo-dependent and RAS-MAPK-altered cancers
YAP-TEAD-IN-2	Interface 3	Preclinical experiments	Malignant mesothelioma	Disruptor	The first class of small molecules potently disrupting the YAP -TEAD interaction by direct competition
GNE7883	Palmitate site	Preclinical experiments	Bronchioalveolar carcinoma, lung adenocarcinoma	Disruptor	An allosteric pan-TEAD inhibitor blocks oncogenic YAP/TAZ signaling and overcomes KRAS G12C inhibitor resistance
IK-930	Palmitate site	Clinical trail (phase 1)	Malignant mesothelioma, epithelioid hemangioendothelioma	Disruptor	NCT05228015
K-975	Palmitate site	Preclinical experiments	Malignant mesothelioma	Disruptor	The novel potent TEAD inhibitor, K-975, inhibits YAP1/TAZ-TEAD protein-protein interactions and exerts an anti-tumour effect on malignant pleural mesothelioma
Kojic acid analogue 19	Palmitate site	Preclinical experiments	Colon cancer	Disruptor	Discovery of covalent inhibitors targeting the transcriptional enhanced associate domain central pocket
LM-41	Palmitate site	Preclinical experiments	Breast cancer	Disruptor	Development of LM-41 and AF-2112, two flufenamic acid-derived TEAD inhibitors obtained through the replacement of the trifluoromethyl group by aryl rings
MSC-4106	Palmitate site	Preclinical experiments	Lung squamous cell carcinoma	Disruptor	Optimization of TEAD P-site binding fragment hit into *in vivo* active lead MSC-4106
MYF-01–37	Palmitate site	Preclinical experiments	Lung adenocarcinoma	Disruptor	Treatment-induced tumour dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway
MYF-03-69	Palmitate site	Preclinical experiments	Malignant mesothelioma	Disruptor	Covalent disruptor of YAP-TEAD association suppresses defective Hippo signaling
SW-682	Palmitate site	Clinical trail (phase 1)	Malignant mesothelioma	Disruptor	NCT06251310
TEAD-IN-9	Palmitate site	Preclinical experiments	Intestinal epithelial cells	Disruptor	WO2024061366A1
TED-347	Palmitate site	Preclinical experiments	Glioblastoma	Disruptor	Small-molecule covalent modification of conserved cysteine leads to allosteric inhibition of the TEAD-Yap protein-protein interaction
VT103, VT104, VT107	Palmitate site	Preclinical experiments	NF2-deficient Mesothelioma	Disruptor	Small molecule inhibitors of TEAD auto-palmitoylation selectively inhibit proliferation and tumour growth of NF2-deficient Mesothelioma

Table 3. Continued.

Name	Targeting site	Stage	Biomodels	Categories	References
PY-60	Upstream kinase (ANXA2)	Preclinical experiments	Epidermal keratinocyte	Activator	YAP-dependent proliferation by a small molecule targeting annexin A2
GA-017	Upstream kinase (LATS)	Preclinical experiments	Intestinal organoid	Activator	Small molecule LATS kinase inhibitors block the Hippo signaling pathway and promote cell growth under 3D culture conditions
NIBR-LTSi	Upstream kinase (LATS)	Preclinical experiments	Liver regeneration	Activator	NIBR-LTSi is a selective LATS kinase inhibitor activating YAP signaling and expanding tissue stem cells *in vitro* and *in vivo*
YTT-10	Not clarified	Preclinical experiments	Cardiomyocytes proliferation	Activator	Characterization of a small molecule that promotes cell cycle activation of human induced pluripotent stem cell-derived cardiomyocytes

ANXA2, Annexin A2; LATS, large tumour suppressor kinase; FDA, food and drug administration; 3D, three-dimensional; RAS, rat sarcoma; MAPK, mitogen-activated protein kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog.

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proteins 2 (BGLAP2), thereby promoting osteogenesis [77, 78].

Moreover, the transcriptional activity of the YAP1-TEAD complex can interact with other signaling pathways. For instance, YAP1 can interact with β-catenin, which is necessary for maintaining nuclear β-catenin levels and Wnt/β-catenin signaling, thus promoting the proliferation and differentiation of osteoblast progenitor cells [79]. Another Wnt receptor FZD9 can induce F-actin polymerization, resulting in the nuclear translocation of YAP1 and the formation of the YAP1-TEAD complex [80]. The Notch pathway can also be activated by YAP1-TEAD, upregulating the expression of Notch1 and delta-like ligand 4 (DLL4), which promotes the healing of segmental bone defects [81].

In chondrocytes, the interaction between YAP1 and TEAD proteins regulates sex determining region Y-box 6 (SOX6), promoting chondrocyte proliferation [82]. The interaction with RUNX2 regulates collagen type X alpha 1 (COL10A1) and influences chondrocyte maturation [82]. Knockout of YAP1/TAZ also impairs normal cartilage morphogenesis [64].

In summary, the role of the YAP1-TEAD complex as a starting point for downstream regulation is of particular interest. The complexity of its downstream regulatory network warrants the design of AI models combined with multi-omics data for more efficient and cost-effective predictions. This approach may help determine the role of YAP1-TEAD in bone formation comprehensively.

Chemical Cues-YAP1-TEAD Axis in Osteogenesis

As previously discussed, YAP1 can sense various cellular environmental stimuli, including nutrients and metabolic products as a transcriptional co-activator, thereby reprogramming cellular metabolic pathways. It serves as a crucial link between transcriptional and metabolic programmes. A detailed description of the relationship has been reported between YAP1 and metabolic pathways [44]. It is also shown that glucose sustains YAP1 activity through the hexosamine biosynthesis pathway (HBP) [32]. However, when glucose levels in the cellular microenvironment decrease, or when glycolysis is inhibited using 2-deoxyglucose, YAP1 phosphorylation is induced, which reduces its transcriptional activity [33,83]. The potential mechanism underlying this may involve the promotion of YAP1-TEAD interaction through PFK1, which mediates transcription. Several glucose-sensitive enzymes also impact YAP1. For example, AMPK can directly phosphorylate YAP1, inhibiting YAP1-TEAD protein interaction, or indirectly activate the Hippo upstream pathway by phosphorylating AMOTL1, leading to LATS activation and reduced YAP1 activity ultimately [35,84]. In high-glucose environments, YAP1 may also undergo O-GlcNAcylation or experience reduced phosphorylation, enhancing its translocation into the nucleus [34–36]. Certain fatty acids may also induce YAP1 activity. Yuan *et al.* [85] discovered that palmitic acid, a saturated fatty acid, can mediate YAP1 phosphorylation via MST1, suggesting that the Hippo upstream kinase pathway can sense changes in palmitic acid levels. Interestingly, TEAD has a deep pocket that can be palmitoylated, and palmitoylation stabilises TEAD, promoting YAP1-TEAD interaction and transcriptional activity [62]. In addition, it is also shown that serum-derived phospholipids, such as LPA and S1P, can inhibit LATS1/2 via G protein-coupled receptors (GPCRs), leading to YAP1 activation [86]. In the mevalonate/cholesterol biosynthetic pathway, researchers have found that mevalonic acid can stabilise YAP1 activity through the production of geranylgeranyl-pyrophosphate [37].

In addition to sensing metabolic cues in the cellular environment, the YAP1-TEAD complex can actively remodel intracellular metabolic programmes through transcriptional regulation. In glucose metabolism, high YAP1 activity alters cellular metabolism by enhancing glucose uptake and utilisation. For example, it promotes the transcription of glucose transporter 3 (GLUT3) to accelerate glucose uptake [87], or indirectly enhances the expression of BCAR4, which activates key glycolytic enzymes such as hexokinase 2 (HK2) and phosphofructokinase B3 (PFKB3) [88]. In fatty acid metabolism, YAP1's role is more complex. Another study suggests that YAP1 accelerates fatty acid accumulation in the liver [39]. Another research indicates that it promotes fatty acid consumption via uncoupling protein 1 (UCP1), reducing lipid deposition [89]. Regarding amino acid metabolism, glutamine, a fuel for the tricarboxylic acid (TCA) cycle, is closely related to YAP1 transcriptional regulation. For example, YAP1-TEAD can transcribe glutaminase 1 (GLS1), promoting the conversion of glutamine to glutamate [41]. YAP1 also enhances the transcription of glutamic-oxaloacetic transaminase 1 (GOT1), phosphoserine aminotransferase 1 (PSAT1), and other enzymes involved in the breakdown of glutamine into other products [40]. Additionally, high-affinity amino acid transporters are also regulated by YAP1 transcription. In summary, YAP1-TEAD is influenced by various metabolic signals and, in turn, modulates cellular metabolic programmes, playing diverse regulatory roles under different physiological and pathological conditions.

When referring to the field of osteogenesis, some studies have started to explore the regulatory relationship between metabolic products or metabolites-related proteins and YAP1-TEAD interaction. For example, research conducted by Li *et al.* [90] revealed that the deficiency of retinoblastoma (Rb1) enhances glucose uptake and increases the production of lactate and adenosine triphosphate (ATP) in MSCs. This metabolic shift promotes the formation of the YAP1-TEAD complex, which in turn mediates the expression of glucose transporter type 1 (GLUT1, a glucose transporter). This phenomenon results in increased bone formation and remodeling. This finding highlights

the significant impact that metabolites such as glucose may have on bone repair processes. Similarly, it is also discovered that lactate, another metabolic byproduct, inhibits the nuclear translocation of YAP1 in macrophages. This inhibition ultimately suppresses the biological functions of macrophages [91].

Despite the emerging evidence of the interplay between metabolism and YAP1-TEAD signaling in bone repair, research in this area remains limited. Further investigation into the relationship between metabolism-related small molecules and the YAP1-TEAD complex might uncover new therapeutic targets for enhancing bone repair. In recent years, there has been growing interest in the role of PTMs in regulating protein function. Some PTMs, including glycosylation, acetylation, and lactylation, are driven by metabolites. These modifications can have significant effects on protein networks, influencing from protein stability to interaction with other cellular components. A recent study has reported that the YAP1-TEAD complex itself undergoes PTMs, which can alter its ability to regulate downstream gene expression [23]. This epigenetic regulation could have important implications for bone repair, as changes in YAP1-TEAD activity could lead to alterations in the expression of key genes involved in osteogenesis and osteoclastogenesis. Understanding how these metabolites-triggered transcription of YAP1-TEAD activity in the context of bone repair could provide new insights into the development of therapies, with the aim of enhancing bone regeneration.

YAP1-TEAD Interactions in Osteoclastogenesis

In addition to the crucial role in osteogenesis, the YAP1-TEAD complex also makes an impact on osteoclastogenesis. This is mainly due to the different downstream targets activated by the YAP1-TEAD complex in various cell lineages, even when triggered by the same intervention. For instance, Li *et al.* [92] found that YAP1 activation mediated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) leads to the formation and maturation of osteoclasts. Another study indicated that activator protein-1 (AP1) can interact with the YAP1-TEADs complex, promoting receptor activator of nuclear factor-kappa B ligand (RANKL)-induced osteoclast differentiation and bone resorption activity [93]. Consequently, using YAP1 inhibition reduces the YAP1/TEAD/AP-1 complex and nuclear factor-kappa B (NF-κB) signaling pathway, thereby decreasing osteoclast formation. It is worth noting that ECM stiffening activates PIEZO1-YAP1 can inhibit osseointegration by promoting M1 polarization while suppressing M2 polarization, which inhibits osseointegration [94]. Another study has shown that soft materials reduce the expression of inflammatory markers and YAP1, and the depletion of YAP1 inhibits macrophage inflammation [95].

Herein, we provide a summary figure (Fig. 3) and a table to demonstrate regulation axis of YAP1-TEAD in bone homeostasis (Table 2, Ref. [66,67,70–82,90,92–94]). Accordingly, the activation of YAP1 by the same upstream signals (such as mechanical signals) can lead to completely opposite outcomes in different cell types. Therefore, even if the interaction between YAP1 and TEAD proteins is activated or inhibited at the cellular level, interventions *in vivo* may still result in unpredictable outcomes. Hence, the development of YAP1-TEAD interaction activators or disruptors must assess the potential for clinical translation in early research carefully.

Therapeutic Potential of Targeting YAP1-TEAD Interactions

As summarized earlier, targeting the YAP1-TEAD protein interaction is central to the study of both osteogenesis and osteoclastogenesis, which might also be one of the key targets in development bone defect therapies in the future. Therefore, the development of disruptors and activators targeting YAP1-TEAD interaction holds significant importance both in basic research and clinical translation.

The discovery of small-molecule disruptors targeting YAP1-TEAD has been a challenging. For now, Verteporfin is the only food and drug administration (FDA)-approved inhibitor of the YAP1-TEAD protein interaction. Originally used as a photosensitizer in photodynamic therapy, Verteporfin assists in treating superficial tumours such as choroidal melanoma [96]. In 2012, Liu-Chittenden *et al.* [97] first demonstrated that Verteporfin, along with another compound, Protoporphyrin (an intermediate in the heme biosynthetic pathway), could bind to YAP1 and inhibit the formation of the YAP1-TEAD complex without light activation. Wang *et al.* [98] also confirm that Verteporfin increases levels of 14-3-3σ, which keeps YAP1 sequestered in the cytoplasm. Unfortunately, due to its off-target effects, Verteporfin may not be the best tool for disrupting the YAP1-TEAD protein interaction [99].

The development of YAP1-TEAD protein interaction disruptors has been particularly challenging due to the lack of druggable pockets on the YAP1 surface. Efforts have mainly focused on the heterodimer formed by YAP1 and TEAD in the nucleus (Fig. 4). It is generally believed that TEAD's transactivation domain contains three potential binding interfaces, defined as Interface 1, Interface 2, and Interface 3. Interface 1 (Fig. 4**A**) is an anti-parallel β-sheet formed by YAP1 β1 (residues 52–58) and TEAD β7 (residues 318–324). Interface 2 (Fig. 4**B**) is a binding groove formed by the YAP1 α1 helix (residues 61–73) and TEAD α3–α4 (residues 345–369). According to Fig. 4**C**, Leu65, Leu68, and Leu69 in YAP1 form a hydrophobic patch (LXXLF motif) that binds to a hydrophobic groove in TEAD, formed by Phe314, Val366, Phe370, Tyr346, Leu354, and Leu357. Interface 3 is located in an omega-shaped twisted-coil region. Met86, Leu91, and Phe95 in YAP1 form hydrophobic side chains that interact with Ile247, Val242, Leu272, Val391, and Tyr406 in TEAD.

In Fig. 4**D**, Arg89 in YAP1 forms a hydrogen bond with Asp249 in TEAD, while Ser94 in YAP1 forms two hydrogen bonds with both Glu240 and Tyr406 in TEAD, strengthening the interaction of Interface 3. By designing mutations at different amino acid sites within these three interfaces, Li *et al.* [49] have identified that the most critical interaction between YAP1 and TEAD is located in the omega loop of Interface 3.

Another potential druggable site is the hydrophobic pocket within the TEAD protein (Fig. 4**E**). Chan *et al.* [21] reported that certain evolutionarily conserved cysteine residues in TEAD, such as Cys359 in TEAD1, can be specifically autopalmitoylated. Additionally, Cys348 and Cys380 are also believed to undergo palmitoylation. These residues are intended to bind with palmitate, stabilizing the conformations of TEAD. By targeting the pockets formed by these sites, small molecules can be screened that allosterically regulate the YAP1-TEAD interaction, thus influencing subsequent transcriptional regulation. Compared to the surface pockets mentioned earlier, the palmitate-binding pocket of TEAD is deep and hydrophobic, making it a mainstream target in the discovery of drugs aimed at regulating YAP1-TEAD transcriptional activity. Some of these drugs have even entered clinical trials.

TEAD also contains three potential drug-binding pockets within its DNA-binding domain. Although no inhibitors targeting these sites have been reported to date, Liberelle *et al.* [100] suggest that the pockets, composed of three α-helices, have significant potential for drug development. In addition to targeting surface sites on TEAD, YAP1's structure includes a conserved module known as the WW domain, which contains two conserved and consistently positioned tryptophan residues [101]. The WW domain facilitates YAP1's interaction with proteins that contain PPXY motifs effectively, such as LATS1/2. The only FDA-approved drug that can block the formation of the YAP1-TEAD complex, Verteporfin, is thought to bind to the WW domain [102]. However, this remains supported only by computational modelling. No biological experimental evidence is available so far. Another site located at the N-terminus of YAP1 contains HXRXXS motifs, which are primarily responsible for binding to TEAD. The serine residue at position 127 (Ser127) within the HXRXXS motif can be phosphorylated by LATS1/2 [103], which then mediates the binding of 14-3-3 to YAP1, leading to its sequestration in the cytoplasm and subsequent degradation. Therefore, targeting the HXRXXS motifs within YAP1, or the phosphorylation of Ser127, could serve as potential sites for blocking the YAP1-TEAD interaction [104,105]. Verteporfin has been reported to bind to YAP1, promoting the association of 14-3-3 with YAP1 and retaining it in the cytoplasm [98]. After Verteporfin treatment, a significant reduction in YAP1 levels within the nucleus is observed. Although some of these targets within YAP1 are distinct from those at the YAP1-TEAD binding interface, the downstream targets examined remain primarily consistent, such as CCN1 and CCN2, which are common downstream effectors of YAP1-TEAD complex [106].

On the other hand, activators of the YAP1-TEAD protein-protein interaction primarily focus on inhibiting the function of the Hippo kinase module. For example, NIBR-LTSi and GA-017 inhibit LATS kinase, preventing the phosphorylation of conserved amino acid residues in YAP1 and TAZ, thereby stopping YAP1 from being sequestered in the cytoplasm and thus activating YAP1 signaling [107,108]. Another activator, PY-60, can bind to annexin A2 and associate with YAP1 directly at the cell membrane [109]. Afterward, the transcriptionally active form of YAP1 enters the nucleus to bind with TEAD instead of being phosphorylated in the cytoplasm. Additionally, an activator named TT-10 has been reported to activate YAP1-TEAD complex-mediated transcription *in vivo*, improving cardiac function [110], although its regulatory mechanism remains unclear.

Here, we provide a summary table based on different druggable sites of the YAP1-TEAD complex, listing both activators and disruptors (Table 3). Given the controversies surrounding the role of YAP1-TEAD in bone formation and the possibility that it functions independently of the upstream kinase module, the transcription of downstream target genes regulated by YAP1-TEAD, as well as its crosstalk with other pathways, may become the focus of future research. Therefore, the development of precise disruptors and activators targeting protein-protein interaction of YAP1-TEAD holds great significance in exploring the mechanisms of bone formation and developing potential therapies for bone defects.

Discussions

Endpoints and Starting Points

YAP1-TEAD interaction serves as both the endpoint of upstream Hippo kinase modules and the starting point for the transcription of numerous target genes. Given that mechanical signals, metabolites and other factors independent of the Hippo kinase modules exist, and considering that YAP1 activation can lead to completely opposite outcomes in osteoblasts, osteoclasts, and immune-related cells (sometimes under the influence of the same upstream signals), the role of YAP1-TEAD protein interaction as a starting point in regulating subsequent targets may become a focal point of future research in bone repair. Drug development in the management of bone defect will also need to clarify the specific regulatory mechanisms that follow the promotion or inhibition of protein-protein interaction of YAP1-TEAD interactions to proceed more effectively. From this perspective, YAP1-TEAD represents a promising starting point for exploring potential bone defect therapies.

Disruptors and Activators

In clinical practice, there is still a lack of effective targeted drugs for bone defects. Unfortunately, despite the important and complex role of YAP1-TEAD in osteogenesis, inhibitors and activators of YAP1-TEAD have not yet entered the clinical translation phase for the treatment of bone defects. They are primarily used in the laboratory to study the mechanisms of osteogenesis and osteoclastogenesis. There are potentially two main reasons for this clinical translation bottleneck.

Firstly, the upstream signalling of YAP1-TEAD and its downstream transcriptional targets are relatively complex, and this ambiguity in the mechanisms presents a significant barrier to drug discovery and translation. YAP1 can be activated by various signals, not only from the Hippo pathway but also by mechanical and chemical cues from the surrounding cellular environment. Additionally, non-proteolytic ubiquitination, acetylation, glycosylation, and lactylation are among the various PTMs that occur, which mediate different downstream targets and effects. Therefore, precisely inhibiting or activating the YAP1-TEAD interaction, or even blocking specific PTMs, is crucial for studying the downstream targets and phenomena. This approach is vital for a deeper understanding of the role of YAP1-TEAD in bone homeostasis and for advancing therapeutic strategies.

Another reason is that there are few drugs targeting the YAP1-TEAD interaction in clinical practice currently. The discovery of activators and disruptors targeting YAP1-TEAD interactions is crucial for drug therapy for bone defects and for studying the mechanisms of osteogenesis and osteoclastogenesis. Regarding to disruptors, the currently FDA-approved Verteporfin does not adequately meet the requirements for mechanistic research or clinical translation. Although some disruptors or allosteric modulators targeting the YAP1-TEAD protein interface have been developed, they are primarily intended for anti-cancer therapy or drug applications. On the other hand, given that researches have demonstrated the effectiveness of activating YAP1-TEAD transcriptional activity in promoting osteogenesis, which has already summarized in the above content, the development of YAP1-TEAD activators may hold greater significance for bone formation. Unfortunately, reports on YAP1-TEAD activators are still limited, and target upstream kinases mostly. This indirect regulation often results in unpredictable downstream effects. In this context, molecular glue could represent a potential direction for future development of activators. Molecular glue aims to use monovalent small molecules to bridge two proteins by orchestrating or catalyzing protein-protein interactions [111]. Although molecular glue is mainly used to design protein degraders to hold a protein to an E3 ligase, and result in the degradation of targeted proteins, it is also believed to act as a chemical stabilizer, inducing interactions between two targeted proteins [112]. Mechanistically, the initiation of downstream transcriptional modules requires YAP1-TEAD protein interactions. Developing novel molecular glues to strengthen these protein interactions may represent one approach to precisely activating YAP1-TEAD downstream regulation.

AI Prediction and Clinical Translation

Clinicians often encounter bone defects that are more intricate than those studied in laboratory settings. These defects involve a complex interplay of osteoblasts, osteoclasts, macrophages, and other cellular components, which together maintain bone homeostasis in the human body [5]. Exogenous interventions can elicit unpredictable responses from these diverse cell types. Mechanistically, key regulatory factors such as YAP1-TEAD act as critical junctions within the complex networks of signaling pathways, influencing various biological outcomes across different cell lineages. Consequently, laboratory observations may offer only a limited view of the full clinical scenario. The integration of multi-omics data through deep learning presents a promising strategy to address this complexity [113].

Currently, multi-omics data are predominantly used in the discovery of anticancer drugs. Given the large patient population, multi-dimensional omics analysis frameworks can effectively identify new therapeutic targets and personalized treatment strategies for cancer, by recognizing changes in cancer genes, proteins, metabolites, and epigenetic markers. For instance, Migliozzi *et al.* [114] combined proteomics, phosphoproteomics, acetylomics, metabolomics, and lipidomics data to reconstruct four functional subtypes of glioblastoma (GBM), and developed a novel computational approach, Substrate Phosphosite-Based Inference for Network of Kinases (SPHINKS), which identified protein kinase C delta (PKCδ) and DNA-dependent protein kinase catalytic subunit (DNA-PKCS) as master kinases driving GBM subtypes. Additionally, AI models have been reported to predict molecular biological processes. Researchers have developed AI prediction tools to identify PTM substrates and sites across various PTMs in the proteome. For example, Chen *et al.* [115] used deep bidirectional long short-term memory recurrent neural networks (RNNs) to predict lysine modification sites and types based on both full-sequence and fragment models. Yan *et al.* [116] developed MIND-S, a graph neural network (GNN)-based tool to predict various PTMs on amino acids and study the impact of PTM-induced single nucleotide polymorphism mutations at the proteome level. Recently, Shrestha *et al.* [117] introduced PTMGPT2, an interpretable protein language model using generative pre-trained transformers (GPT) to generate models representing modified protein sequences.

Given that YAP1-TEAD interactions are influenced by cell lineage differences, signaling variations, metabolic cues, and PTMs, integrating data from spatial transcriptomics, metabolomics, and proteomics could provide a more comprehensive understanding. By harnessing arti-

ficial intelligence to analyze these complex datasets, researchers can uncover dynamic interactions and regulatory networks that govern bone formation. Indeed, similar applications have already been demonstrated. Recently, Zhou *et al.* [118] integrated publicly available RNA-seq data related to the tri-lineage differentiation (osteogenesis, chondrogenesis, and adipogenesis) of human MSCs and developed a model to predict the differentiation spectrum of human MSCs.

In addition to integrating multi-omics data for modeling and analysis, AI can be applied to drug development after constructing networks and identifying key genes, proteins, metabolites, and pathways in the biological networks. AI-based drug screening, drug design, efficacy prediction, and safety prediction are critical factors in accelerating clinical translation. In drug screening, AI models can predict the physical properties (such as melting point, distribution coefficient), biological activity, and toxicity of drugs based on molecular characterization. Toxicity optimization, in particular, is one of the most costly and time-consuming tasks in the preclinical phase of drug discovery. AI can reduce the experimental costs of new drugs in the real world by predicting toxicological profiles [119]. In drug design, accurate three dimensional (3D) structure predictions of target proteins using models like AlphaFold 3 are foundational [120]. On this basis, AI can further simulate the interactions between target proteins and drugs at the atomic level, predict their electrostatic properties, and assess small molecule potential [121]. AI can also be employed for retrosynthetic pathway prediction. For example, Segler *et al.* [122] combined Monte Carlo tree search and symbolic AI to discover retrosynthetic routes, aiming to identify simple, usable precursor molecules. In addition to drug synthesis routes, some AI models have been developed to predict organic reaction products and yields, using experimental results to effectively model and simulate expected drug synthesis yields in silico [123]. The development of these AI models undoubtedly reduces the cost and time of drug development cycles, and promises to make drug discovery faster, cheaper, and more efficient. AI serves as a powerful facilitator in translating laboratory insights into clinical practice, potentially bridging the gap between experimental findings and real-world clinical applications.

Conclusions

Despite the predominant reliance on surgical interventions for the treatment of bone defects, there is an increasing focus on elucidating the fundamental mechanisms of bone regeneration to enhance bone repair and skeletal function restoration. The YAP1-TEAD interaction, a central effector within the Hippo signaling pathway, exerts a critical yet intricate influence on both osteogenesis and osteoclastogenesis. Achieving a comprehensive understanding of the YAP1-TEAD binding mechanism is vital for advancing research in these areas and for the development of targeted therapies that can effectively address bone defects. The emergence of technologies such as artificial intelligence (AI) offers the potential to predict regulatory networks, thereby facilitating the clinical application of YAP1-TEAD-targeted therapies. In summary, the investigation of YAP1-TEAD interaction mechanisms in the context of bone defects and regeneration, in conjunction with advancements in drug discovery, computational modeling, and clinical application, remains a significant research priority.

List of Abbreviations

ABHD17A, abhydrolase domain containing 17A; ACAN, aggrecan; AI, artificial intelligence; ALP, alkaline phosphatase; AMOTL2, angiomotin like 2; AARS1, alanyl-tRNA synthetase 1; AMPK, AMP-activated protein kinase; AP1, activator protein-1; APT2, acyl-protein thioesterase 2; AREG, amphiregulin; ATP, adenosine triphosphate; BGLAP2, bone gamma-carboxyglutamate proteins 2; BMP, bone morphogenetic protein; BMPR1A, bone morphogenetic protein receptor type 1A; BSP, blepharospasm; CBP, CREB-binding protein; CDK1, cyclin-dependent kinases 1; COL10A1, collagen type X alpha 1; COL1A1, collagen type I alpha 1; COL1A2, collagen type I alpha 2; COL2A1, collagen type II alpha 1; CTGF, connective tissue growth factor; CYR61, cysteine-rich angiogenic inducer 61; DCHS1, dachsous cadherin related 1; DLL4, delta-like ligand 4; DNA-PKCS, DNA-dependent protein kinase catalytic subunit; ECM, extracellular matrix; FAK, focal adhesion kinase; FAT4, FAT atypical cadherin 4; FDA, food and drug administration; FZD, frizzled; GBM, glioblastoma; GLS1, glutaminase 1; GLUT1, glucose transporter type 1; GLUT3, glucose transporter 3; GNN, graph neural network; GOT1, glutamic-oxaloacetic transaminase 1; GPT, generative pretrained transformers; HK2, hexokinase 2; HH, Hedgehog; JNK1/2, c-Jun N-terminal kinase; LATS1/2, large tumour suppressor kinase 1/2; LPA, lysophosphatidic acid; LSD1, lysine-specific demethylase 1; MAP4Ks, mitogen-activated protein kinase kinase kinase kinases; MOB1, Mps one binder kinase activator adaptor proteins; MOB1A/B, Mps one binder 1A and B; MSC, mesenchymal stem cell; MST1/2, mammalian STE20-like kinase 1/2; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-kappa B; OCN, osteocalcin; OSX, Osterix; OGT, O-GlcNAc transferase; OTUD1, OTU deubiquitinase 1; PATs, palmitoyl acyltransferases; PIEZO1, Piezo-type mchanosensitive ion channel component 1; PP2A, protein phosphatase 2A; PSAT1, phosphoserine aminotransferase 1; PSC, periosteal stem cell; PKCδ, protein kinase C delta; PFKB3, phosphofructokinase B3; RANKL, receptor activator of nuclear factor-kappa B ligand; Rb1, retinoblastoma; RNF214, ring finger protein 214; RNN, recurrent neural network; RUNX2, runt-related transcription factor 2; S1P, sphingosine-1-phosphate; SAV1, Salvador homologue 1; $SCF^{\beta-TRCP}$, Skp1-Cullin-F-box$^{\text{beta-transducin repeats-containing proteins}}$; SETD7, SET Domain

Containing 7; SENP3, SUMO specific peptidase 3; SET1A, SET domain containing 1A; SIRT1, sirtuin 1; SIRT2, sirtuin 2; SPHINKS, Substrate Phosphosite-Based Inference for Network of Kinases; SKP2, S-phase kinase associated protein 2; SOX6, sex determining region Y-box 6; SOX9, sex determining region Y-box 9; SPC, sphingosylphosphorylcholine; TAOK, thousand-and-one amino acid kinase; TAZ, transcriptional co-activator with PDZ-binding motif; TCA, tricarboxylic acid; TEAD, transcriptional enhanced associated domain; TGFβR1, transforming growth factor beta receptor 1; UCP1, uncoupling protein 1; VGLL4, vestigial-like family member 4; YAP1, yes-associated protein 1; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; BMSCs, bone marrow-derived macrophages; SSCs, skeletal stem cells; PTMs, post-translational modifications; HBP, hexosamine biosynthesis pathway; PFK1, phosphofructokinase 1; mRNA, messenger ribonucleic acid; AKT, protein kinase B; ERK, extracellular-signal-regulated kinases; 3D, three-dimensional; RAS, rat sarcoma; MAPK, mitogen-activated protein kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; GPCRs, G protein-coupled receptors.

Declaration of AI and AI-Assisted Technologies in the Writing Process

In this manuscript, AI-assisted technology was used for writing assistance, including the grammar polish and expression in British English. We assert that our manuscript is free of plagiarism.

Availability of Data and Materials

The data of this study are available from the corresponding authors.

Author Contributions

CPZ, FB, XY and XLZ contributed to the design of this work and revised critically for important intellectual content. YW, HY, LL, CX, KLW, GYF and WJX contributed to the interpretation of data and analyzed the data. YW and HY 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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