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The Multifaceted Effects of Osteocytic TGFβ Signaling on the Skeletal and Extraskeletal Functions of Bone

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Abstract

Purpose of Review To summarize the fundamental role of transforming growth factor beta (TGFβ) signaling in osteocytes and highlight the physiological and pathophysiological conditions stemming from the deregulation of this pathway in osteocytes.

Recent Findings Osteocytes perform a myriad of skeletal and extraskeletal functions, including mechanosensing, coordinating bone remodeling, local bone matrix turnover, and maintaining systemic mineral homeostasis and global energy balance. Transforming growth factor-beta (TGFβ) signaling, which is crucial for embryonic and postnatal bone development and maintenance, has been found to be essential for several osteocyte functions. There is some evidence that TGFβ might be accomplishing these functions through crosstalk with the Wnt, PTH, and YAP/TAZ pathways in osteocytes, and a better understanding of this complex molecular network can help identify the pivotal convergence points responsible for distinct osteocyte functions.

Summary This review provides recent updates on the interwoven signaling cascades coordinated by TGFβ signaling within osteocytes to support their skeletal and extraskeletal functions and highlights physiological and pathophysiological conditions implicating the role of TGFβ signaling in osteocytes.

Keywords Osteocytes · TGFβ signaling · Crosstalk · Mechanosensitivity · Endocrine metabolism

Introduction

The mammalian skeleton is one of the largest organs in the human body, accounting for 15% of body weight [1]. Bones serve as the building blocks of the skeleton and provide a structural framework for the body. The roles of bone are diverse; these include skeletal functions, like providing mechanical support, movement, blood cell production, and mineral storage, as well as extraskeletal functions, like endocrine regulation of mineral metabolism and whole-body energy metabolism, that are integral to a better quality of life [2–4]. Bone mechanical integrity is determined by the bone mass and quality. Bone mass is dynamically regulated by three primary cell types: osteoclasts, which dissolve and degrade the mineralized bone matrix; osteoblasts, which synthesize and secrete proteins forming the bone matrix; and osteocytes, which act as mechanosensory cells orchestrating activities of osteoblasts and osteoclasts [5]. Bone quality is dynamically regulated by osteocytes. The term bone quality encompasses all the parameters other than bone mass that influence bone's resistance to fractures, including trabecular microarchitecture, bone matrix composition and material properties, collagen quality, and mineralization [6].

Osteocytes, which represent the majority of adult skeletal cells, have the longest lifespan of any bone cells and are profoundly ingrained in bone [7]. Although derived from osteoblasts, osteocytes have a distinct stellate-shaped morphology similar to the neurons in the brain [8]. The osteocyte cell body resides within tiny cavities called lacunae in the calcified bone matrix, and their emanating dendritic projections extend through narrow tunnels called canaliculi [9]. Within the confines of the lacunocanalicular network (LCN), osteocytes occupy more than 215 m^2 of the total bone surface area [10]. The extensive LCN enables an osteocyte to connect with neighboring osteocytes as well as the cells lining

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the surfaces of bone (i.e., osteoblasts and osteoclasts), the vasculature, the adjacent marrow space, and articular cartilage. The intricate LCN also gives osteocytes the ability to sense the changes in the mechanical environment that stimulate fluid flow through the network [11]. Apart from the LCN, osteocytes are also endowed with cytoskeletal components, focal adhesions, gap junctions, the primary cilium, ion channels, and the glycocalyx [12] that collectively provide osteocytes enhanced capabilities of perceiving physical cues and initiating biochemical signals to modulate osteoblast and osteoclast behavior. Apart from mechanosensation, the extensive osteocyte LCN surface area also serves as a site of calcium release from bone [13]. Although the essential role of transforming growth factor-beta (TGFβ) in the skeleton has been studied for decades [14–20], this review focuses on the more recently discovered actions of TGFβ in the skeletal and extraskeletal functions of osteocytes.

Basics of the Transforming Growth Factor Beta (TGFβ) Pathway

The TGFβ superfamily consists of an evolutionarily conserved family of proteins, including TGFβs, activins, bone morphogenetic proteins (BMPs), and other related proteins [18]. The members of the TGFβ family of proteins include three highly homologous isoforms—TGFβ1, β2, and β3 that demonstrate distinct spatial and temporal expression and exert mostly non-redundant functions. Together, the TGFβ family regulates a wide variety of biological processes, such as cellular migration, proliferation, commitment, and differentiation [21]. TGFβ ligand isoforms are secreted as polypeptide dimers formed from the tethering of conserved cysteine residues of the monomers into a single inter-chain disulfide bond [22]. The dimeric TGFβ ligand then associates with the pro-region-derived latency-associated protein (LAP), which in turn binds to latent TGFβ binding protein (LTBP) or glycoprotein-A repetitions predominant (GARP) proteins to form latent complexes [23, 24]. These latent complexes shield the active TGFβ and prevent it from binding to receptors. In bone, TGFβ1 is the most abundant isoform, and, once synthesized, it is bound to latent complexes and stored in the bone extracellular matrix (ECM) [25]. TGFβ is activated mechanically upon integrin binding to LAP [24] or by exposure to an acidic microenvironment, such as that created by osteoclasts during bone resorption [26]. Several other mechanisms of TGFβ activation exist in various tissues, and these distinct mechanisms add to the complexity and precision of regulating TGFβ signaling.

Once activated, the TGFβ ligands promote the assembly and activation of the heterotetrameric TGFβ receptor complexes consisting of two subunits of type I (TβRI or ALKs) and type II transmembrane receptors (TβRII), both of which possess serine/threonine kinase activity [14–20]. Activated TβRII-TβRI effectively phosphorylate Smad or non-Smad effectors to modulate target gene transcription [27]. The Smad-dependent canonical pathway of TGFβ involves the activation of three subgroups of Smad proteins by the TGFβ ligand-receptor complex: the receptor-activated Smads (R-Smads, Smad2/3 for TGFβ/activin receptors, and Smad1/5/8 for BMP receptors), the common mediator Smad (Co-Smad, Smad4) that translocates R-Smads into nuclei to control gene transcription, and the inhibitory Smads (I-Smads, Smad6, and Smad7) that dampen signal transduction by rerouting the R-Smad/Co-Smad trimeric complex towards proteasomal degradation via ubiquitin ligases such as Smurf2 [28, 29]. In the non-canonical, Smad-independent TGFβ signaling pathway, TβRI or TβRII phosphorylate non-Smad proteins, such as TGFβ activation kinase 1 (TAK1) and its binding protein (TAB1), protein kinase C (PKC), protein phosphatases 2 (PP2A), and phosphoinositide 3-kinase (PI3K) complexes that can, in turn, activate various signaling pathways to regulate cellular processes like migration, proliferation, differentiation, and apoptosis [13, 30]. Overall, the TGFβ pathway is precisely regulated at multiple levels, including ligand bioavailability and activation, permutation of receptor assembly, internalization, stabilization, selection of canonical vs. non-canonical effectors, and recruitment of I-Smads. Together, these mechanisms enable the TGFβ pathway to invariantly turn on and off gene expression programs in a cell-type specific and context-dependent manner to support diverse biological processes. Abnormalities in the regulation of the TGFβ pathways disrupt normal physiology and lead to the development of pathological diseases.

The Biological Role of the TGFβ Pathway in Bone

A large body of work has examined the role of members of the TGFβ family in skeletal development and maintenance [14–20, 30–33]. Briefly, TGFβ controls bone development through endochondral and intramembranous ossification [30]. TGFβ powerfully induces chondrogenic differentiation by stimulating the recruitment, proliferation, and condensation of mesenchymal cells, as well as the formation of a cartilage template, all of which are essential for the formation of mineralized bone by endochondral ossification. Accordingly, ablation in TGFβ signaling in early development leads to severe abnormalities in the axial and appendicular skeleton. Mice deficient in TGFβ2 and TGFβ3 have short ribs and craniofacial defects [34–36]. Similarly, TβRII deletion inhibited the proliferation and differentiation of osteo-chondrogenic progenitors leading to defects in the development of joints and long bone [37]. TGFβ signaling is equally important for the formation and maintenance of intramembranous bones of the craniofacial skeleton and tooth

eruption. Deletion of TβRII in Gli1 + osteogenic progenitors and Col1 + (3.2 kb) early osteoblasts leads to reduced alveolar bone development [38]. Inactivation of TβRII also impacts tooth development by targeting dental mesenchymal proliferation and odontoblast maturation [39].

The importance of intact TGFβ signaling for postnatal growth of the appendicular skeleton is illustrated by studies reporting low trabecular and cortical bone mass in mice exhibiting TβRII deletion in Osx + mesenchymal stem cells [38]. Despite the marked increase in CAR + osteoprogenitors, the reduced number of osteoblasts accounts for the low bone mass phenotype in these mice [38]. A drastically opposite high bone mass phenotype is observed upon expression of a dominant negative TβRII or deletion of TβRII in Ocn + mature osteoblasts [15, 40•]. Interestingly, blockade of TGFβ signaling using a pharmacologic inhibitor of TβRI-kinase or antibodies against TGFβ ligand also leads to increased bone mass [41]. Although there is a consensus regarding TGFβ's importance in postnatal skeletal development and maintenance, the dramatic differences in bone phenotypes in different mouse models suggest that the role of TGFβ signaling in bone is highly cell type-specific and context-dependent. This has also been observed from a pathological perspective, particularly conditions of osteogenesis imperfecta and Camurati–Engelmann disease, where increased TGFβ ligand bioavailability has severe skeletal consequences, in part due to its effects on bone remodeling [42].

TGFβ signaling integrates the activity of multiple cell types involved in bone remodeling to maintain bone mass. TGFβ regulates RANKL-induced osteoclast formation and bone-resorbing activity [43]. Both TGFβ1 and TGFβ2 ligands control osteoclasts in a dose-dependent manner. Low concentrations of TGFβ ligands stimulated osteoclast development, whereas high levels of TGFβ attenuated osteoclastogenesis [44]. Time-dependent regulation of osteoclastogenesis by TGFβ signaling has been recently reported; TGFβ signaling promoted RANKL-mediated osteoclastogenesis in the early stages of differentiation and inhibited osteoclasts in the late stages [45]. Remarkably, TGFβ induces osteoclast apoptosis at later stages by upregulating Bim [46]. To maintain the close coordination between bone resorption and production, TGFβ signaling in osteoclasts couples their actions with those of osteoblasts in the bone [26].

The Function of TGFβ Signaling in Osteocytes

Over the last 5 years, new data has emerged that identifies the TGFβ pathway as a critical regulator of osteocyte functions [47•, 48•, 49, 50•, 51, 52•, 53]. Several breakthroughs have been made that greatly improved our knowledge of the mechanisms that upregulate or downregulate osteocytic TGFβ signaling through differential signaling pathways, as well as the pathophysiological response to multiple stimuli. These studies reinforce the notion of balanced TGFβ signaling and the context-dependent impact of TGFβ signaling in osteocytes. In this review, we summarize the several molecular mechanisms that crosstalk with TGFβ signaling in osteocytes and highlight the different skeletal and extraskeletal functions impaired in response to the ablation of TGFβ signaling within osteocytes (Table 1). In the following subsections, we review four main functions of osteocytes that TGFβ signaling contributes to, including- regulation of bone quality, mechanical loading, mineral metabolism, and energy metabolism as shown in Fig. 1.

Regulation of Bone Quality

Bone material properties, one of several bone quality parameters, are regulated by osteocytes, at least in part through the process of perilacunar/canalicular remodeling (PLR) [47•, 48•, 54, 55]. PLR involves resorption of the mineral and proteolysis of the organic matrix lining the lacunae and canaliculi, mediated by vacuolar H + ATPases (Atp6V1G1, Atp6V0D2, and Atp6V0B), carbonic anhydrases (CA1 and CA2), tartrate-resistant acid phosphatase (TRAP), cathepsin K (CTSK), and matrix metalloproteinases (MMPs) and replenishment of the resorbed matrix by secretion of collagen I, dentin matrix protein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), and a phosphate-regulating gene with homologies to endopeptidases on the X chromosome essential for phosphate metabolism (PHEX) [13, 56–60]. Phenotypic characterization of PLR can be conducted through visualization of the enlargement of lacunar volume and canalicular diameters of osteocytes or by monitoring PLR enzyme levels, among other strategies [13, 19, 54, 61].

TGFβ is a key regulator of PLR [47•, 48•, 49]. In vitro, TGFβ promotes osteocytic expression of resorptive genes, and its blockade leads to reduced osteocyte-mediated acidification. In vivo, inhibition of TGFβ signaling using a pharmacologic inhibitor (TβRI) or in a mouse model with osteocyte-intrinsic ablation of TβRII (TβRII$^{ocy-/-}$) led to suppressed PLR. Although PLR suppression did not impact bone mass, it led to a marked reduction in bone material properties and increased fragility of cortical bone of TβRII$^{ocy-/-}$ mice. Besides regulating PLR gene expression, TGFβ signaling also impacts the LCN. Whether through systemic pharmacologic inhibition of TGFβ signaling or in TβRII$^{ocy-/-}$ mice, osteocyte dendricity and canalicular length were reduced [47•, 49]. The underlying mechanism behind the disruption of osteocytic dendrites with ablation of the TGFβ pathway is unclear. Expression of at least two

genes essential for dendrite formation in early osteocytes, such as podoplanin (E11/gp38) and MT1-MMP (MMP14), are controlled by TGFβ signaling and could likely be responsible for altered LCN. Whether the regulation of E11/gp38 and MMP14 by TGFβ signaling is direct requires further investigation. Several proteins that are transcriptionally regulated by TGFβ signaling, including sclerostin (SOST), parathyroid hormone receptor (PTH1R), yes-associated protein (YAP), and transcriptional coactivator with PDZ-binding motif (TAZ), have been identified as mediators of PLR and bone quality [13, 56, 62]. Additional research will be needed to determine if these factors act in an epistatic fashion to control bone quality.

Regulation of Osteocyte Mechanosensitivity

TGFβ signaling, which is integral for mechanical load-induced bone anabolism, can be suppressed in bone via hindlimb loading [63•]. In fact, loss of sensitivity to TGFβ signaling blunts the anabolic effects of mechanical loading on bone, as seen in mice expressing the dominant negative version of TβRII under the control of an osteocalcin promoter. Unlike loading, the role of TGFβ signaling in unloading models has been quite controversial and contradictory. Bone unloading, characterized by disuse-associated bone loss, has been studied using spinal cord injury or denervation models in rodents. In the murine spinal cord injury model, inhibition of TGFβ mitigated the disuse-associated bone loss [64]. While in the murine denervation model, exogenous administration of TGFβ partially relieved denervation-induced bone loss by supporting osteoblastic differentiation and activity and mitigating the effects of glucocorticoids on the bone [65, 66]. Which of these effects result from mechanoregulation of TGFβ, relative to innervation or other factors, remains unknown.

At the cellular level, TGFβ's role in osteocyte mechanobiology has been attributed to several molecular cascades. Several studies show a close link between the regulation of TGFβ signaling and the expression of sclerostin in osteoblasts and osteocytes [50•, 63•, 67•]. TGFβ signaling transcriptionally regulates sclerostin expression in osteocytes and loss of sensitivity to TGFβ impairs load-induced suppression of sclerostin, thereby causing loss of bone anabolism. The deregulated sclerostin expression has also been detected in subchondral bone osteocytes of mice expressing ablated TGFβ receptor under the control of the Dmp1-Cre promoter [50•]. Apart from sclerostin, TGFβ can indirectly promote Wnt signaling in osteocytes through the suppression of microRNA-100, a negative regulator of the Wnt/βcatenin pathway [51]. Other than Wnt, TGFβ also induces expression of gap junction protein, connexin 43 (Cx43), and channel protein pannexin (Panx1) through non-canonical activation of mitogen-activated protein kinase (ERK1/2) [52•]. Both Cx43 and Panx1 are large-pore channel proteins located on the dendritic processes of osteocytes and mediate permeation of ions (i.e., Ca2 +) and key metabolites (ATP and prostaglandins), which are crucial for mechanotransduction in bone [68, 69]. Similarly, YAP and TAZ serve as key mechanosensors in various cell types. In bone, YAP/TAZ was recently found to support osteocyte dendricity; TGFβ signaling acts upstream of YAP/TAZ to modulate osteocyte behavior [62]. While TGFβ-YAP/TAZ dictates PLR, what remains unclear is how this molecular mechanism coordinates the mechanobiology of osteocytes. Studies using finite element modeling have postulated that load will induce changes in perilacunar and canalicular volume within osteocytes and that lacunar and canalicular structures will experience different strain distributions [49, 70]. Furthermore, changes in canalicular density are also predicted to alter the corresponding shear stress experienced by osteocytes [49]. Based on the predictions from the computational model, the mechanosensitivity of the osteocytes will be negatively affected as the LCN and canalicular density are known to be reduced in mice with osteocytic TGFβ-signaling ablation. Experimental studies in the TβRII$^{ocy-/-}$ mice will be useful in understanding the relevance of LCN in mediating cellular mechanosensitivity and load-induced bone anabolism.

Regulation of Mineral Metabolism

Osteocyte-mediated regulation of mineral metabolism is attributed to PLR [57], osteoclast governing functions of osteocytes [57, 71], and to the osteocytic secretion of FGF23, a phosphaturic hormone targeting endocrine regulation of phosphate metabolism [72]. In physiological conditions of mineral stress, such as lactation, where bouts of calcium are needed to support milk production, osteocytes rapidly resorb and release calcium and phosphate from the bone matrix lining the lacunae and canaliculi, leading to increased lacunar volume and canalicular diameter. With the cessation of mineral demand, the geometry of the osteocyte lacunar and canalicular structures fully recovers, along with an increase in bone mass [73]. While osteocytes support maternal calcium needs, deregulated PLR fails to detectably impact milk production. Maternal calcium demands are met through other compensatory mechanisms, such as increased intestinal calcium absorption [73, 74].

TGFβ signaling is intimately linked to the activation of osteocytic PLR during lactation [48•]. Under basal conditions, female TβRII$^{ocy-/-}$ mice do not exhibit any phenotypic differences relative to controls. However, with lactation, the typical trabecular and cortical bone loss phenotype observed in control mice is mitigated in the TβRII$^{ocy-/-}$bones. The impaired lactation-induced bone loss in TβRII$^{ocy-/-}$ mice stems from reduced PLR and reduced type I receptor (PTH1R) expression in osteocytes. PTH1R, the common

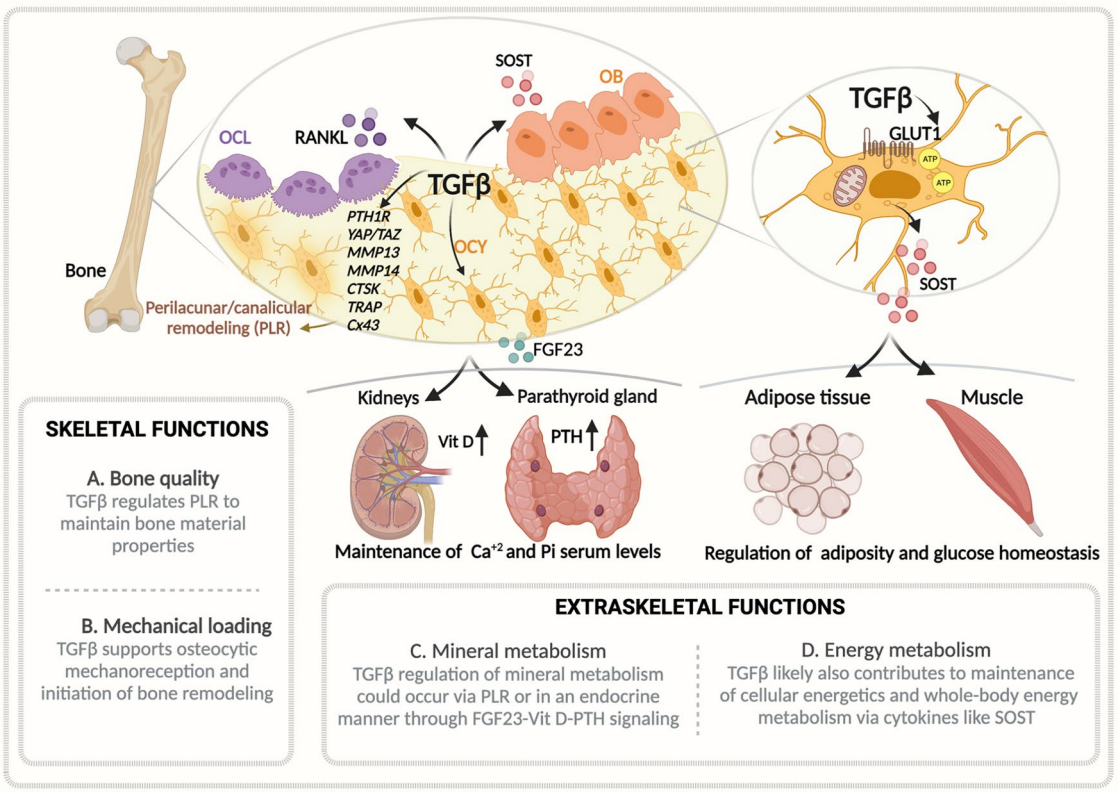

Fig. 1 The multifaceted role of osteocytic TGFβ signaling. Osteocytic TGFβ signaling regulates both the conventional skeletal and extraskeletal functions of bone. **A** In basal conditions, osteocytic TGFβ signaling supports the maintenance of bone quality; **B** in mechanical stress conditions, osteocytic TGFβ signaling integrates and converts mechanical cues into biological signals (such as RANKL and SOST) that modulate the number and activity of osteoclasts and osteoblasts; **C** in conditions of mineral metabolic stress, osteocytic TGFβ signaling controls calcium homeostasis by locally lysing and then remodeling perilacunar and canalicular bone matrix (e.g., during lactation); although untested, it is possible that osteocytic TGFβ signaling also functions in an endocrine manner by releasing factors like fibroblast growth factor 23 (FGF23), which will act on the kidney and parathyroid glands to modulate systemic calcium and phosphate homeostasis; **D** TGFβ signaling can also directly impact osteocyte energetics and affect the production of other cytokines like sclerostin to modulate whole-body energy metabolism through regulation of adipogenesis, muscle activity, and glucose metabolism. Figure created with BioRender.com

G-protein-coupled receptor for PTH and PTHrP ligand, is crucial in osteocytes for lactation-induced PLR, such that its ablation block PLR and induction of osteoclasts during lactation [13].

Although osteocyte-specific ablation of PTH1R in mice can lead to hypocalcemia [75], systemic calcium levels are maintained in mice with osteocytic TβRII deficiency [48•]. It is possible that with a deficiency in PLR, systemic mineral needs in the TβRII knockout mice are met in an endocrine manner via increased osteocytic secretion of FGF23 and vitamin D3 (1,25(OH)2D3).

Interestingly, in osteoblasts, TGFβ can stimulate FGF23 production and enhance cellular calcium levels [76]. Similarly, intense crosstalk between TGFβ and vitamin D3 signaling has been previously characterized [77], and newer studies indicate transcriptional induction of vitamin D receptors by TGFβ in non-bone cells [78, 79]. Although osteocytic TGFβ signaling is crucial for PLR-mediated regulation of systemic mineral metabolism, it is worthwhile to understand how osteocytic TGFβ signaling influences the endocrine arm of systemic mineral metabolism regulated by osteocytes when considering the crosstalk between TGFβ-FGF23-PTH-1,25(OH)2D3.

Pathological conditions associated with defective mineral metabolism, chronic kidney disease (CKD), and renal osteodystrophy have been widely studied recently. Elevated ligands, receptors, and downstream targets of TGFβ signaling have been observed in animal models of CKD and CKD patients [80]. Given the link of TGFβ with PTH and FGF23 signaling, both mechanisms could be implicated in disrupted mineral metabolism. In fact, the resistance of CKD bones to the calcemic action of PTH, reported in many studies [81, 82], could be attributed to the attenuation of PTH1R by TGFβ signaling [40•, 83]. Similarly, the increased serum FGF23 levels in CKD patients track with increased TGFβ ligands and further strengthen the notion

that TGFβ signaling is upstream of FGF23 signaling and could be targeted for restoration of impaired bone turnover and mineral metabolism in CKD. Deducing the role of osteocyte-intrinsic TGFβ in the regulation of FGF23 and PTH-mediated calcium and phosphate metabolism in CKD will provide a unique opportunity of targeting a new cell type that as per new evidence, is implicated in the development of CKD and vascular calcification [84–86].

Regulation of Energy Metabolism

The contribution of osteocytes in the regulation of whole-body metabolism has been well-recognized. Since the function of a cell is tightly linked to its metabolism, much interest has risen in understanding the metabolic pathways and substrates in osteocytes [87].

The first evidence that TGFβ regulates glucose metabolism in the skeleton came from a study in the articular chondrocytes [88]. TGFβ stimulates glucose consumption and lactate production in human articular chondrocytes. Increased glucose consumption was attributed to increased cellular glucose transport in articular chondrocytes due to TGFβ-mediated upregulation of glucose transporter type 1 (Glut1). Apart from Glut1, TGFβ1 also induced the expression of hexokinases I and II and upregulated glycolysis-mediated lactate production in articular chondrocytes. Similarly, a recently presented abstract provided at the ASBMR 2022 annual conference also implicated the positive role of TGFβ signaling in promoting glycolysis in chondrocytes through Glut1/3 upregulation during embryonic joint development [89]. Interestingly, TGFβ mediated upregulation in glucose uptake and induction of Glut 1 and hexokinases (HKI and HKII) has also been reported in fibroblasts [90–92]. In murine and human lung fibroblasts, TGFβ-stimulated glycolysis is crucial for profibrotic gene expression, cell migration, colony formation, and activation of the transcription factors YAP/TAZ [91].

Although the mechanism underlying TGFβ-stimulated aerobic glycolysis is an active line of investigation, it will be interesting to if conservation in TGFβ's action on cellular metabolism persists across different skeletal tissues. It is remarkable to consider that both chondrocytes and mature osteocytes sustain in a hypoxic environment [93–95]. Remarkably, hypoxia is a known inducer of TGFβ signaling, and this may be integral for metabolic reprogramming in hypoxic environments. Further studies need to be conducted to understand the relevance of aerobic glycolysis in osteocyte survival and function.

Whether an osteocyte's metabolic program (normoxic vs. hypoxic environment) is affected by where they are located inside the cortical bone (closer to the bone surface vs. deeply embedded in the calcified bone matrix) needs to be investigated. Notably, elevated glucose levels stimulate TGFβ signaling in non-osteocyte cells [89]. A positive feedback loop may exist to support the upregulation of TGFβ signaling in conditions of high glucose to mediate glucose uptake and continue cell reliance on glycolysis. Whether such a feedback loop exists in osteocytes and the relevance of TGFβ mediated metabolic reprogramming for osteocyte function in physiological and pathological conditions requires further investigation. Unlike osteoblasts or osteoclasts, where the relationship of glucose utilization to cell differentiation and function is clear [96–99], the functional consequences of control of osteocytic glucose utilization remain unresolved. The sequestration of osteocytes within the mineralized bone matrix makes it challenging to characterize their metabolism in vivo. As a result, we rely primarily on ex vivo metabolic profiling techniques despite their inability to recapitulate the osteocytic environment. With these challenges, many gaps remain regarding the role of the osteocytic TGFβ pathway in regulating cellular fatty acids, glucose, and glutamine metabolism, and the impact of changes in the cellular metabolism of osteocytes on the regulation of whole-body energy metabolism.

The notion that bone is a driver of energy metabolism has been strengthened by the growing list of bone-derived factors coordinating systemic energy intake and expenditure. Recent reports in conference proceedings have opened the possibility that TGFβ signaling within bone cells could contribute to the maintenance of whole-body (organismal) energy metabolism [53, 100, 101]. Although this is still a subject of active investigation, it is not far-fetched to construe that the effects of bone intrinsic TGFβ signaling on energy metabolism could possibly be mediated through its molecular partners like sclerostin. Sclerostin, a direct target of the TGFβ pathway, has been shown to increase in the bones of mice that were fed a high-fat diet (a model of type 2 diabetes) [102]. In addition, sclerostin overexpression results in increased adiposity and impaired glucose homeostasis in mice [103, 104]. Moreover, both TGFβ and sclerostin are recognized as mediators of bone-muscle crosstalk [105–107]. In fact, the regulation of skeletal muscles by bone-derived TGFβ has been elegantly described in the context of cancer cachexia following tumor metastasis to bone. Cancer metastasis increases TGFβ release from the bone extracellular matrix and upregulates NADPH oxidase 4-mediated RyR1 oxidation in muscle cells. Oxidized RyR1 leads to calcium leak from the sarcoplasmic reticulum and impairs muscle contraction and muscle wasting. Inhibition of TGFβ signaling using TGFβ receptor I kinase inhibitor (SD-208) or TGFβ neutralizing antibody (1D11) improved muscle weight and function and increased body weight [105, 108]. Similarly, osteocytic Cx43, which is a target of TGFβ, has also been recognized as a regulator of bone-muscle crosstalk [109]. While these studies highlight the role of bone-derived TGFβ signaling in regulating muscle mass

and function, the key point to take away is that some of the effects of TGFβ signaling on whole-body energy metabolism could be mediated directly or indirectly through the regulation of skeletal muscle and adipose tissue functions. Apart from cancer, increased TGFβ signaling has been reported in the pathophysiology of obesity and type 2 diabetes. Bridging the gap between how TGFβ signaling within bone cells drives whole-body energy metabolism could contribute to improving declining metabolic health associated with obesity and T2D.

Conclusion and Future Perspectives

All the preclinical studies highlighted in this review emphasize the multifaceted role played by osteocytic TGFβ signaling in bone, where its regulation is linked to the maintenance of bone mass and quality, bone anabolic response to loading, and facilitating systemic mineral metabolism (Fig. 1). We believe that the interactions of the TGFβ pathway with SOST, PTH1R, Cx43, and YAP/TAZ serve at the interface of many of these functions and epistatically fine-tune osteocyte responses. Our understanding regarding the choice or the sequence of the molecular partners of TGFβ signaling during a particular physiological or pathophysiological context (Table 1) is very limited.

The emerging line of research implicating TGFβ signaling in the coordination of cellular energy metabolism adds another level of complexity to the already confounding effects of TGFβ in bone. We believe that understanding how TGFβ signaling within osteocytes regulates metabolic flexibility will help us comprehend, to some extent, the context-dependent effects of the TGFβ pathway on bone during homeostasis vs. in a physically (exercise) or metabolically (lactation) challenging situation. It is worth noting that cellular energy metabolism also actively regulates TGFβ signaling. This has been evidently shown in the context of cancer, where intracellular metabolites and metabolic proteins affect the production or bioactivity of TGFβ ligands, influence the expression of TGFβ receptors, and regulate the activation and abundance of Smad proteins [110–114]. With the recent push towards mapping metabolomic signatures linked to poor skeletal health, delineating metabolites associated with deregulated osteocytic TGFβ signaling could offer newer options for improving skeletal health [115–117]. For example, in obesity where lipid metabolites are in abundance, understanding the impact of increased intracellular lipid metabolites on TGFβ signaling could help us target obesity-associated bone fragility.

Table 1 Molecular partners of osteocytic TGFβ signaling and its direct and indirect role in supporting skeletal and extraskeletal functions of bone

TGFβ molecular partners	Skeletal and extraskeletal functions of bone supported by TGFβ molecular partners	Physiological and Pathophysiological conditions implicating TGFβ molecular partners
Sclerostin (SOST) [50•, 63•, 67•]	Regulation of osteoblastogenesis [118] Osteocyte mechanosensitive response and load-induced bone formation [119]	Bone mechanosensitivity [63•, 67•] Age-induced bone loss and decline in bones mechanosensitivity [119] Osteoarthritis [50•]
	Regulation of adiposity and body composition [103, 104] Bone-muscle crosstalk [106, 120, 121]	Obesity and T2D risk [103, 104] Osteosarcopenia [122]
PTH type 1 receptor (PTH1R) [40•, 48•, 83]	Bone remodeling [123] Osteocyte dendricity [124] Perilacunar/ canalicular remodeling [13] Osteocyte mechanosensation [125]	Increased bone mass and high turnover [123, 126] Lactation-induced bone loss [13, 48•] Age-associated bone loss [127]
	Maintaining mineral homeostasis through FGF23 regulation [128]	Kidney-Bone mineral disorders [81, 82]
Connexin 43 (Cx43) [52•]	Osteocyte formation and viability [69] Regulation of cell–cell communication via gap junction formation and cellular mechanosensitivity [52•, 69]	Bone response to mechanical loading/unloading [52•]
	Bone-muscle crosstalk [109]	Age-associated decline in bone mechanosensitivity and muscle mass [109]
YAP/TAZ [62, 91]	Osteocyte dendricity and Perilacunar/canalicular remodeling [62] Mechanosensitive response to load-induced bone formation [129] Glucose uptake and cell-intrinsic energy metabolism [91]	Bone material properties and bone quality maintenance [62]
Glut1 and HKI/HKII [88, 91]	Metabolic reprogramming to support anaerobic glycolysis [88]	Osteoarthritis [88]

Lastly, since TGFβ signaling is a growth factor with non-linear effects, a thorough mechanistic analysis of the new anticipated extraskeletal functions of osteocytic TGFβ is necessary. TGFβ ligand neutralizing antibodies and TGFβ receptor antagonists have proved to be beneficial for the restoration of skeletal health in diseases like osteogenesis imperfecta. However, in light of the new findings indicating that the effects of TGFβ can extend beyond bone, it will be crucial to carefully weigh the benefits and drawbacks of modulating this pathway on the overall physiology of the organism prior to developing novel strategies for refining TGFβ signaling.

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Declarations

Conflict of Interest The authors have no conflicts of interest to disclose.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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