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REVIEW

Regulation of postnatal bone homeostasis by TGFβ

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Perhaps more so than any other tissue, bone has pivotal mechanical and biological functions. Underlying the ability of bone to execute these functions, whether providing structural support or preserving mineral homeostasis, is the dynamic remodeling of bone matrix. Cells within bone integrate multiple stimuli to balance the deposition and resorption of bone matrix. Transforming growth factor-β (TGFβ) uniquely coordinates bone cell activity to maintain bone homeostasis. TGFβ regulates the differentiation and function of both osteoblasts and osteoclasts, from lineage recruitment to terminal differentiation, to balance bone formation and resorption. TGFβ calibrates the synthesis and material quality of bone matrix and bone's responsiveness to applied mechanical loads. Therefore, by coupling the activity of bone forming and resorbing cells, and by sensing, responding to and defining physical cues, TGFβ integrates physical and biochemical stimuli to maintain bone homeostasis. Disruption of TGFβ signaling has significant consequences on bone mass and quality. Alternatively, TGFβ is a powerful lever that has the potential to yield therapeutic benefit in cases where bone homeostasis needs to be recalibrated.

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Postnatal Bone Homeostasis

Bone in the adult skeleton must continuously adapt to changing physical, endocrine and metabolic demands. The remarkable ability of the skeleton to support mechanical loads and resist fracture, while executing its role in mineral, nutritional, and endocrine homeostasis, is a balancing act that requires exceptional coordination. Transforming growth factor-β (TGFβ), a well-known regulator of skeletal development and maintenance, continues to emerge as a factor that facilitates the cellular integration of physical and biochemical inputs to maintain postnatal bone homeostasis. In bone and in many other tissues, TGFβ regulates cellular migration, proliferation, differentiation, matrix synthesis and apoptosis.[1,2] The activity of the TGFβ signaling pathway is influenced by crosstalk with many other pathways at the level of TGFβ ligands, receptors, agonists and antagonists.[3] The multiscale nature of this regulation allows the integration of diverse stimuli—including physical and biochemical—allowing bone to adapt to its dynamic environment.

Bone Remodeling and TGFβ

Bone homeostasis is critically dependent on the interactions between osteoblasts, the mesenchymally derived bone-forming cells, and osteoclasts, the hematopoietically derived bone resorbing cells, in a coupled process known as bone remodeling. Briefly, bone remodeling involves the sequence of osteoclast recruitment and differentiation, bone matrix resorption and a reversal phase in which osteoblasts are recruited to the resorption site where they deposit new bone matrix. Osteoblasts ultimately become embedded in the bone matrix as mature osteocytes. TGFβ is intimately involved in each stage of this process. TGFβ regulates the recruitment, differentiation and function of both osteoblasts (**Figure 1**) and osteoclasts (**Figure 2**), as well as the crosstalk mediating bone remodeling and the quality of bone matrix.

Regulation of Osteoblasts by TGFβ

At the cellular level, TGFβ acts on the osteoblast lineage to expand the pool of bone matrix-secreting cells. TGFβ has long been known to act as a chemoattractant for osteoprogenitor cells.[4] TGFβ recruits osteoprogenitors to the site of new bone formation or remodeling.[5] In bone fractures, osteoprogenitors migrate toward the site of fracture repair, following a gradient of TGFβ released from platelets and injured bone.[6–8] TGFβ also increases the number of osteogenic cells by stimulating osteoprogenitor proliferation, in part by promoting the degradation of the cell cycle inhibitor $p57_{Kip2}$.[9] As it does throughout the body, TGFβ regulates the synthesis of extracellular matrix proteins and proteases in bone, including

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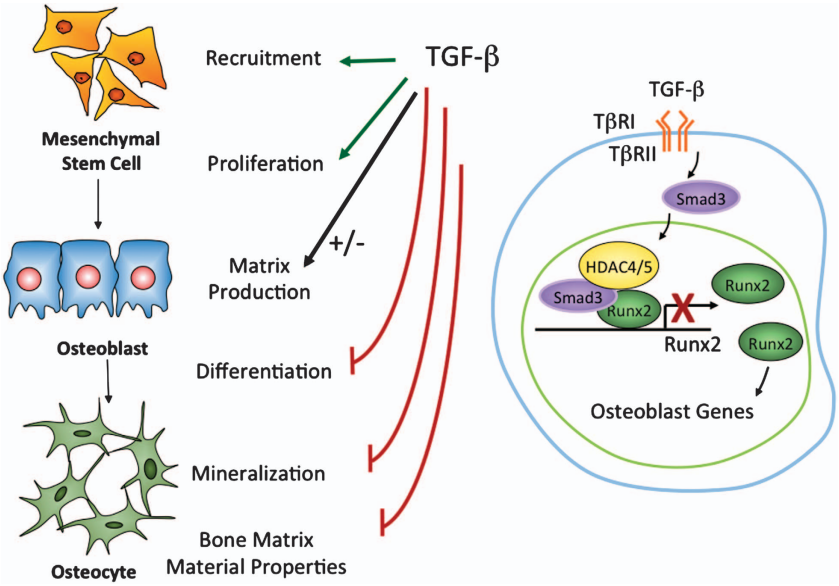

Figure 1 Transforming growth factor-β (TGFβ) regulates the recruitment, differentiation, and function of osteoblasts. The TGFβ-activated Smad3 represses Runx2 function to inhibit osteoblast differentiation. This osteoblast-dependent pathway is also responsible for the ability of TGFβ to regulate bone matrix elastic modulus, or stiffness, and it relies upon the action of Runx2.

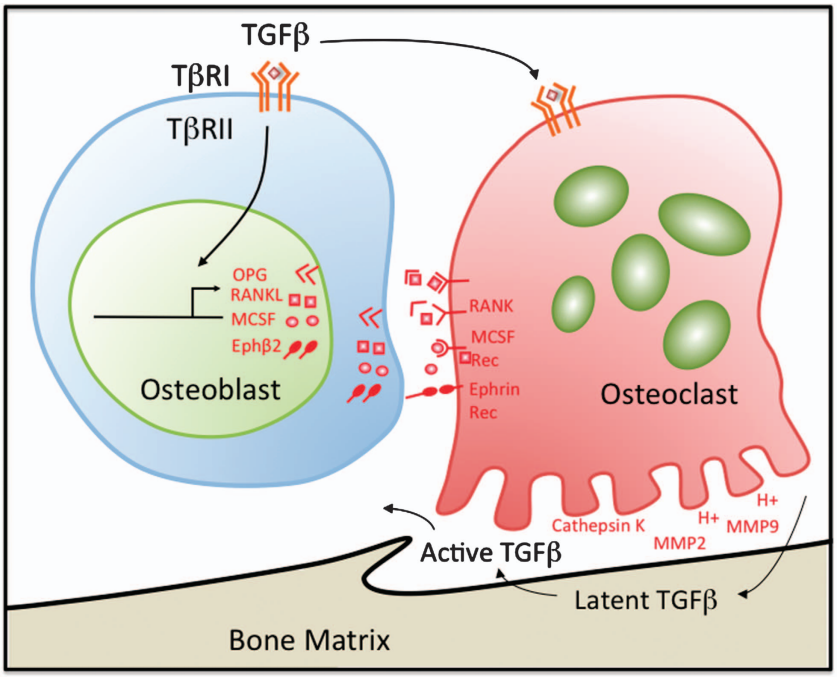

Figure 2 Transforming growth factor-β (TGFβ) couples bone formation with resorption through the regulation of osteoblast-derived osteoclast regulatory factors. Osteoclasts also release and activate TGFβ stored in latent form in the bone matrix during resorption through creating an acidic microenvironment as well as through the secretion of matrix metalloproteinases MMP-2 and MMP-9, which proteolytically activate TGFβ. The active TGFβ released during osteoclast-mediated bone resorption feeds back on osteoblasts. TGFβ 1-directed migration of bone-derived mesenchymal progenitors to resorptive sites is an essential step in the coupling process. By inducing osteoprogenitor recruitment and proliferation, TGFβ balances matrix resorption with new bone deposition.

alkaline phosphatase, collagen I, osteocalcin, osteopontin, and matrix metalloproteinase-13 (MMP-13).[10] Initially, TGFβ stimulates bone matrix synthesis, perhaps as a function of its expansion of the osteoprogenitor population. However, TGFβ later inhibits terminal osteoblast differentiation and bone matrix synthesis by Smad3-dependent repression of Runx2 expression and function (**Figure 1**).[11] TGFβ also acts on terminally differentiated osteocytes by inhibiting osteocyte apoptosis, in part through a Smad3 and vitamin D receptor-dependent mechanism.[12] Therefore, TGFβ plays a distinct role at each stage of the osteoblast life cycle.

Regulation of Osteoclasts by TGFβ

The effect of TGFβ on osteoclasts also depends upon the stage of osteoclast differentiation.[13] TGFβ promotes the chemotaxis of isolated osteoclast precursors into bone,[14] and it later stimulates osteoclast precursor proliferation and differentiation.[15–18] TGFβ can act directly on osteoclasts and their precursors through its type I and type II TGFβ receptors. However, many of the effects of TGFβ on osteoclasts are indirect. For example, TGFβ acts on osteoblasts to regulate the expression of osteoclast regulatory proteins including M-CSF, RANKL, OPG, ephrin B2 and EphB4 (**Figure 2**).[19,20] At low doses, TGFβ treatment enhances osteoclastogenesis by increasing M-CSF expression and prostaglandin production, as well as the RANKL to OPG ratio.[21] In contrast, high TGFβ levels repress M-CSF and RANKL expression while increasing OPG expression.[22–24] Because high levels of TGFβ do not inhibit osteoclastogenesis in pure osteoclast cultures, the inhibitory effects of TGFβ at high doses are mediated by osteoblasts and thus may serve as a negative feedback loop to limit bone resorption.[13]

Coupling of Bone Resorption and Bone Formation by TGFβ

The ability of TGFβ to regulate osteoblast-derived osteoclast regulatory factors is one of the mechanisms by which TGFβ couples bone formation with resorption.[2,13] Moreover, during bone resorption, osteoclasts release and activate TGFβ stored in latent form in the bone matrix. The acidic microenvironment created by osteoclasts directly activates the TGFβ ligand from the latent complex. In addition to secreting TGFβ, osteoclasts also secrete matrix metalloproteinases MMP-2 and MMP-9 that, along with cathepsin K, can proteolytically activate TGFβ.[25–27] The active TGFβ released during osteoclast-mediated bone resorption feeds back on osteoblasts. The TGFβ 1-directed migration of bone-derived mesenchymal progenitors to resorptive sites is an essential step in the coupling process.[5] By inducing osteoprogenitor recruitment and proliferation, TGFβ balances matrix resorption with new bone deposition. Although many questions remain about the bone remodeling compartment canopy,[28,29] such a structure may create a microenvironment that limits the diffusion of TGFβ and other growth factors released from the bone matrix by osteoclasts while facilitating the local recruitment of progenitors.

Osteocyte-Mediated Perilacunar Remodeling and TGFβ

Osteocytes, the most abundant bone cells embedded in lacunae throughout the bone matrix, have recently been shown to modify their perilacunar matrix. Under physiological stress of lactation, cortical bone osteocytes express several proteins, including proteases, that allow these cells to resorb their local bone matrix to release mineral into the circulation for milk production. The lacunae around these TRAP-positive osteocytes enlarge with lactation and return to normal following weaning as the local bone matrix is replaced.[30] This lactation-mediated perilacunar remodeling requires MMP-13.[30,31] Even in normal conditions, perilacunar remodeling is required to maintain the collagen and mineral organization of cortical bone matrix and the ability of bone to resist fracture. The expression of MMP-13 is tightly regulated by TGFβ, parathyroid hormone, glucocorticoids and other factors.[32–35] TGFβ has also been shown to be important for the stability of osteocytes,[36] and it may be possible that osteocytes may exert their actions on the matrix through the TGFβ-mediated regulation of proteases such as MMP-13.[33,34] Taken together, maintaining the stability of osteocytes and the osteocytic-mediated dynamic remodeling of perilacunar bone matrix may be yet another way in which TGFβ regulates bone homeostasis.

Regulation of Bone Mass and Bone Quality by TGFβ

Several mouse models demonstrate the consequences of altering TGFβ signaling on bone mass. Given the multiplicity of TGFβ activities on osteoblasts, osteoclasts and osteocytes, it should not be surprising that these phenotypes fail to reveal a simple anabolic or catabolic role for TGFβ in bone. For example, a low bone mass phenotype results from mutations that increase TGFβ signaling (by overexpression of the activated ligand[37]), as well as from those that reduce it (by ablation of the key TGFβ effector Smad3).[12] Rather, these mouse models clearly illustrate the key role of TGFβ in the intricate coupling of osteoblast and osteoclast activity during bone remodeling, and the subsequent effects on bone quality and fracture resistance.[38] Many of the bone phenotypes become more severe with age, consistent with the importance of TGFβ in maintaining postnatal bone mass.[12,37,39]

In addition to bone mass, the fracture resistance of bone is determined by several other factors including the bone microarchitecture, geometry and extracellular matrix material properties.[40,41] Each of these factors is biologically defined by a number of key signaling molecules. Consequently, mice with mutations in the TGFβ pathway exhibit alterations in other aspects of bone quality as well. In particular, TGFβ regulates bone matrix material properties.[38] Unlike bone mass, this effect is dose dependent, such that the elastic modulus of bone matrix is reduced in genetically modified mice with elevated TGFβ-signaling; but increased in those that have lower TGFβ signaling activity.[38] Similarly, postnatal pharmacologic inhibition of TGFβ type I receptor function is sufficient to increase the elastic modulus of bone matrix, demonstrating that these properties are regulated postnatally and must be maintained.[20,38] TGFβ regulates bone matrix elastic modulus, or stiffness, through an osteoblast-dependent mechanism that relies upon the action of Runx2.[42] Just as TGFβ-activated Smad3 represses Runx2 function to inhibit osteoblast differentiation,[11,42] this pathway is also employed to control bone matrix stiffness (**Figure 1**). Although TGFβ also regulates the material quality of skin, tendon and dentin matrix,[43–45] the extent to which it also targets master transcriptional regulators to do so remains unclear.

TGFβ as an Integrator of Physical and Biochemical Stimuli in Bone

The ability of TGFβ to regulate bone matrix material properties may be important for the mechanical and biological function of bone. Bone matrix material properties are biologically regulated and anatomically distinct, suggesting that this regulation offers a selective advantage to the organism. The functional significance of this regulation for long bone fracture resistance has so far proven difficult to distinguish from the many other factors that affect bone quality. Nonetheless, analyses of a unique bone, the cochlea, revealed that each GPa decrement in

elastic modulus was responsible for nearly 2 dB of hearing loss, which may contribute to the hearing loss in patients with cleidocranial dysplasia.[42,46,47] Although the ability of osteocytes to regulate bone matrix stiffness in response to TGFβ is yet unknown, in light of recent studies, it is intriguing to consider the possibility that osteocytes have the capacity to quickly change the material quality of the perilacunar bone matrix. This could be particularly important as extracellular matrix stiffness itself is a potent regulator of osteoblast gene expression and cellular apoptosis.[12,48,49] Factors that change extracellular matrix stiffness may in turn alter the physical cues in the cellular microenvironment to direct bone cell behavior.

Just as TGFβ maintains bone mass and quality in response to changing biological conditions, it also participates in the anabolic response of bone to mechanical load. In bone and many other tissues, the expression of TGFβ ligands responds to physical stimuli including fluid flow and compressive loads.[50] Using an *in vivo* hind-limb loading model, we recently found that mechanical loading of bone represses TGFβ signaling through Smad2/3.[51] This mechanosensitive regulation of TGFβ is essential for the anabolic response of bone to mechanical loads. In this way, TGFβ is critical for the ability of bone to continuously adapt to changing stimuli—both biological and physical.

Human Pathologies

Given its critical roles in directing bone cell fate and the coupling of osteoblast/osteoclast interactions, it is perhaps not surprising that disruptions in TGFβ signaling have been implicated in deregulation of bone mass and quality in several human pathologies as it does in several mouse models.[12,20,37–39,52] These studies have shown that increased TGFβ signaling reduces bone mass and bone quality, producing a bone phenotype that is similar to osteoporosis. Other skeletal pathologies due to deregulation of TGFβ signaling include Camurati–Engelmann disease and osteopoikilosis. Camurati–Engelmann disease, also known as progressive diaphyseal dysplasia, is characterized by hyperostosis and sclerosis of the base of the skull and the long-bone diaphyses. Camurati–Engelmann disease is the result of *TGFB1* mutations in either a signal peptide or the latency-associated propeptide,[53–55] and these mutations result in increased levels of bioactive TGFβ1 and subsequently increased TGFβ signaling.[56] In this case, the deregulated activation of TGFβ1 results in hyperactive formation of fragile bone.[5,55,56] Osteopoikilosis is a skeletal dysplasia characterized by symmetric but unequal distribution of hyperostotic regions across the skeleton, with variants that also include sclerosis of the skin. Osteopoikilosis can be attributed to the loss of *LEMD3* (*LEM domain-containing 3*), which confers increased bone morphogenetic protein and/or TGFβ signaling.[57–59] These diseases illustrate the essential role of TGFβ in bone homeostasis, and the dramatic effects that result from the deregulation of TGFβ.

Therapeutic Potential

Many studies have investigated the therapeutic utility of manipulating the TGFβ pathway in the skeleton, through the direct administration of TGFβ or the regulation of TGFβ effectors. For example, the inhibition of TGFβ signaling, either by the pharmacologic inhibition of the TGFβ type I receptor[5,20] or by TGFβ blockade using antibodies,[28,29,60] exerts positive effects on both bone mass and bone matrix quality that culminate in improved fracture resistance. Antagonizing TGFβ also protects bone from breast cancer metastasis.[61] Importantly, the effect of TGFβ critically depends on the cell and tissue context.[3] In particular, TGFβ signaling has distinct effects during different stages of the bone regenerative response and has critical roles in the intramembranous ossification process during fracture healing. The exogenous delivery of TGFβ enhances bone regeneration around bone implants over a 4-week period with increased regenerated bone volume fraction, bone contact area and reduced implant-tissue gap,[62] likely because of the ability of TGFβ to recruit osteoprogenitors to the implant site. Numerous studies have explored mechanisms by which TGFβ enhances the bone regenerative response and bone union during fracture healing. These result in part from the ability of TGFβ to promote chondrogenesis. This complements the ability of TGFβ released from bone and platelets following trauma to mobilize mesenchymal stem cells and osteoprogenitors to the injury site.[63] In addition, TGFβ calibrates the timing of chondrocyte and osteoblast differentiation in order to ensure an adequate cell population for repair before ossification of the fracture site.[64]

Conclusion

As bone remodeling is critical for the metabolic and mechanical roles of the bone, TGFβ maintains bone homeostasis by coupling the osteoblast and osteoclast activity, mediating the biological responsiveness towards mechanical stimuli, mobilizing stem cells, and calibrating the differentiation of bone forming and resorbing cells. Disruption of TGFβ signaling has adverse consequences in bone mass, quality, and regeneration. Because of the potent effects of TGFβ, leveraging TGFβ signaling for the directed homeostatic and regenerative response of bone may provide valuable therapeutic benefits.

Conflict of Interest

The authors declare no conflict of interest.

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