Bone marrow mesenchymal stem cells and TGF-β signaling in bone remodeling

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During bone resorption, abundant factors previously buried in the bone matrix are released into the bone marrow microenvironment, which results in recruitment and differentiation of bone marrow mesenchymal stem cells (MSCs) for subsequent bone formation, temporally and spatially coupling bone remodeling. Parathyroid hormone (PTH) orchestrates the signaling of many pathways that direct MSC fate. The spatiotemporal release and activation of matrix TGF-β during osteoclast bone resorption recruits MSCs to bone-resorptive sites. Dysregulation of TGF-β alters MSC fate, uncoupling bone remodeling and causing skeletal disorders. Modulation of TGF-β or PTH signaling may reestablish coupled bone remodeling and be a potential therapy.

Bone remodeling dynamically changes the bone marrow microenvironment

The fate of MSCs, including self-renewal, transient amplification, or differentiation, is regulated by the bone marrow microenvironment and systemic factors (20–22). In the reversal phase of bone remodeling, the bone-resorptive microenvironment provides signals that aid in the cessation of bone resorption and the promotion of bone formation by recruitment and differentiation of MSCs. Multiple cytokines, growth factors, and minerals are released from the bone matrix or secreted by local cells. For example, IGF-1 released from the bone matrix or secreted locally during bone remodeling stimulates osteoblast differentiation of MSCs by activation of mammalian target of rapamycin (mTOR) through the PI3K-Akt pathway (3). Semaphorin 4D (SEMA4D), expressed on the cell surface of osteoclasts, binds to its receptor, plexin B1, on osteoblasts to inhibit the RhoA/Rho-associated protein kinase (ROCK) pathway (23). The ROCK pathway normally phosphorylates IRS-1, a key factor in the PI3K-Akt-mTOR pathway (23); therefore, osteoclast expression of SEMA4D can inhibit MSC differentiation by cell-to-cell contact, creating a boundary between bone resorption and formation. Thus, the dynamic changes in the bone marrow microenvironment result in the coordination of the reversal phase during coupled bone remodeling.

Additional properties of the microenvironment also play a role in MSC fate. For example, at fresh resorptive sites, the bone mineral matrix is exposed and lacks a covering of lining cells, providing a stiff elastic microenvironment. The stiff matrix at remodeling sites directly promotes differentiation of MSCs into osteoblasts (24), which may promote the formation of lamellar bone rather than woven bone.

Blood vessels are in close proximity to bone remodeling, and a complex relationship exists between angiogenesis and osteogenesis (25). Factors from the vasculature appear to influence the bone marrow microenvironment. FGF2 is a potent mitogenic factor for several different cell types, including endothelial cells, and can induce angiogenesis by stimulating endothelial cell proliferation, migration, and expression of proteases, growth factors, and integrins involved in angiogenesis (26–29). TGF-β has been shown to enhance production of FGF2 in osteoblasts and positively modulate osteoblast differentiation and bone formation (30–32). In aortic endothelial cells, TGF-β stimulation of a complex con-
taining activin-like kinase receptor 5 (ALK5), TGF-β receptor II (TβRII), and SMAD2 enhances VEGF expression (33), but it is unclear whether there is similar activity at sites of bone remodeling. Hematopoietic stem and progenitor cells (HSPCs) are present in the same niche as MSCs (34). While MSCs and osteoblast precursors can influence the fate of HSPCs (35–37), it is unclear whether HSPCs reciprocally influence MSCs.

**PTH modulates the bone marrow microenvironment**

The parathyroid gland, the main production site of the calcium homeostasis regulator PTH, evolved in amphibians (38) and represents the transition of aquatic to terrestrial life. Permanent detection of osteoclasts and bone resorption also emerged as vertebrates transitioned to land (39–42), promoting survival by development of lighter cylindrical bones to aid in mobilization and release of calcium from the skeletal matrix. During PTH-mediated osteoclastic bone resorption, growth factors and cytokines are also released from the bone matrix. To protect the integrity of the skeleton in adapting to terrestrial life, PTH regulates bone remodeling by orchestrating signaling of local factors, including TGF-β, Wnts, bone morphogenetic protein (BMP), and IGF-1. Thus, the fate of MSCs and other cells in the microenvironment are indirectly regulated by PTH to integrate systemic control of bone remodeling (Figure 1 and refs. 7–15).

PTH modifies many cells that are important in the bone marrow microenvironment. In a lineage tracing study, intermittent PTH treatment increased osteoblast number by converting lining cells to active osteoblasts (43). Lining cells are primarily defined by their location on the bone surface. Further characterization of the nature of lining cells will help to elucidate the relevant cellular mechanisms. PTH stimulates bone marrow CD8+ T cells to produce large amounts of Wnt10b, which activates Wnt signaling in MSCs and osteoblast precursors, thereby increasing osteoblast proliferation and differentiation (44). PTH has also been shown to alter the bone marrow microenvironment by spatially relocating small blood vessels closer to

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**Figure 1**

Modulation of the bone marrow microenvironment by PTH-stimulated bone remodeling. PTH enhances osteoclast bone resorption through direct activation of cells in the osteoblastic cell lineage. During bone remodeling, active TGF-β, IGF-1, and many other bone matrix factors are released to the marrow. PTH orchestrates signaling of local factors, including but not limited to TGF-β, Wnts, and BMP. Thus, PTH regulates cellular activities including those of MSCs, T cells, and other PTH-responsive cells in the bone marrow to integrate systemic control of bone remodeling. PTH-stimulated bone remodeling expands nestin+ MSC populations, spatially relocates blood vessels closer to sites of new bone formation, and orchestrates the osteogenic bone marrow microenvironment.
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Figure 2
Activation of TGF-β recruits MSCs during bone remodeling. TGF-β1 is released from the bone matrix and activated during osteoclast-mediated bone resorption, creating a gradient. TGF-β1 induces migration of MSCs to the bone remodeling sites to couple bone resorption and formation. The bone-resorptive microenvironment also provides signals that direct the cell lineage–specific differentiation of MSCs.

sites of new bone formation, likely secondary to PTH-mediated upregulation of VEGFA and neuropilin 1 and 2 (45). The closer proximity of blood vessels allows more efficient delivery of nutrients to support new bone formation.

The PTH1R is responsible for the spatial regulation of MSC recruitment, differentiation, and function is a part of the integration of the signaling networks of local factors for the spatiotemporal regulation of bone remodeling. PTH can expand nestin + MSC populations

1 induces migration, it does not induce osteoblast differentiation (68). The specific effects of TGF-β signaling are largely determined by the interaction of SMAD2/3 proteins with cell type–specific master transcription factors that specify and maintain specific effects (67). Whereas the master transcription factors in embryonic stem cells, myotubes, and pro-B cells have been determined (Oct4, MyoD1, and PU.1, respectively; ref. 67), MSC master transcription factors have not yet been identified.

Active TGF-β released into the microenvironment can exert specific effects, including proliferation, differentiation, migration, and apoptosis, depending on the cell type and duration of action (51). For example, although TGF-β signaling induces MSC migration, it does not induce osteoblast differentiation (68). The gradient of TGF-β created during osteoclast bone resorption can limit further osteoclast activity. In the short term, low concentrations of active TGF-β can induce macrophage migration via activation of RhoA; however, high concentrations or prolonged exposure of macrophages/monocytes to active TGF-β have been shown to inhibit migration of osteoclast precursors (69). Both high concentrations of and prolonged exposure to TGF-β activate SMAD3-SMAD4 complexes, which in turn activate PKA, resulting in phosphorylation and inactivation of RhoA (69, 70). Thus, the gradient of TGF-β generated at the resorption sites likely prohibits its further recruitment of osteoclast precursors, protecting it from further resorption during the reversal phase of bone remodeling.

Disorders associated with abnormalities in bone remodeling
High levels of active TGF-β in the bone marrow and abnormalities in bone remodeling are associated with multiple skeletal disorders. Genetic mutations in the TGF-β signaling pathway cause
premature activation of matrix latent TGF-β and may manifest with various skeletal defects. There are additional diseases that result in high levels of active TGF-β, which may contribute to the pathology. Here, we discuss how abnormal TGF-β signaling results in uncoupled bone remodeling, mainly by loss of site-directed recruitment of MSCs that causes aberrant bone formation. Direct or indirect inhibition of TGF-β signaling may provide potential therapeutic options for these disorders.

Genetic disorders. The critical role of TGF-β1 in the reversal phase of bone remodeling is demonstrated by the range of skeletal disorders resulting from mutations in genes involved in TGF-β1 signaling. Camurati-Engelmann disease (CED), characterized by a fusiform thickening of the diaphysis of the long bones and skull, is caused by mutations in TGFBR1 that result in premature activation of TGF-β1 (71–74). Approximately 11 different TGFBR1 mutations have been identified from families affected by CED (75, 76). All of the mutations are located in the region encoding LAP, either destabilizing LAP disulfide bridging or affecting secretion of the protein, both of which increase TGF-β1 signaling, as confirmed by in vitro cell cultures and mouse models. Bone histology sections from patients with CED show decreased trabecular connectivity despite normal bone histomorphometric parameters with respect to osteoblast and osteoclast numbers (76, 77), suggestive of uncoupled bone remodeling. In vitro, the ratio of active to total TGF-β1 in conditioned medium from cells expressing the CED mutant TGF-β1 is significantly higher and enhances MSC migration (18). Targeted recruitment of MSCs to the bone-remodeling site is likely disrupted, secondary to loss of a TGF-β1 gradient.

Elevations in TGF-β signaling have also been observed in many genetic connective tissue disorders with craniofacial, skeletal, skin, and cardiovascular manifestations, including Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and Shprintzen-Goldberg syndrome (SGS). MFS is caused by mutations in fibrillin and often results in aortic dilation, myopia, bone overgrowth, and joint laxity. Fibrillin is deposited in the ECM and normally binds TGF-β, rendering it inactive. In MFS, the decreased level of fibrillin enhances TGF-β activity (78). LDS is caused by inactivating mutations in genes encoding TBR1 and TBR11 (79). Physical manifestations include arterial aneurysms, hypertelorism, bifid uvula/cleft palate, and bone overgrowth resulting in arachnodactyly, joint laxity, and scoliosis. Pathologic analyses of affected tissue suggest chronically elevated TGF-β signaling, despite the inactivating mutation (79). The mechanism of enhanced TGF-β signaling remains under investigation. SGS is caused by mutations in the v-ski avian sarcoma viral oncogene homolog (SKI; refs. 80, 81) and causes physical features similar to those of MFS or mutations in genes involved in activation of TGF-β, such as small leucine-rich proteoglycans (87) and fibrillin (88), or mutations in genes involved in activation of TGF-β, such as in CED (76) and LDS (89), are associated with high osteoarthritis incidence. Osteoblast differentiation of MSCs in aberrant locations appears histologically as subchondral bone osteoid islets and alters the thickness of the subchondral plate and calcified cartilage zone, changes known to be associated with osteoarthritis (90, 91). A computer-simulated model found that a minor increase in the size of the subchondral bone (1%–2%) causes significant changes in the mechanical load properties on articular cartilage, which likely leads to degeneration (86). Importantly, inhibition of the TGF-β signaling pathway delayed the development of osteoarthritis in both mouse and rat models (86).

MSCs in bone loss. Aging leads to deterioration of tissue and organ function. Skeletal aging is especially dramatic: bone loss in both women and men begins as early as the third decade, immediately after peak bone mass. Aging bone loss occurs when bone formation does not adequately compensate for osteoclast bone resorption during remodeling. Age-associated osteoporosis was previously believed to be due to a decline in survival and function of osteoblasts and osteoprogenitors; however, recent work by Park and colleagues found that mature osteoblasts and osteoprogenitors are actually nonreplicative cells and require constant replenishment from bone marrow MSCs (92). When MSCs fail to migrate to bone-resorptive sites or are unable to commit and differentiate into osteoblasts, new bone formation is impaired. Therefore, insufficient recruitment of MSCs, or their differentiation to osteoblasts, at the bone remodeling surface may contribute to the decline in bone formation in the elderly.

There are multiple hypotheses regarding the decreased osteogenic potential of MSCs during aging. For example, during aging, the bone marrow environment has an increased concentration of ROS and lipid oxidation that may decrease osteoblast differentiation, yet increase osteoclast activity (93, 94). MSCs also undergo senescence, which decreases proliferative capacity and contributes to decreased bone formation (95, 96). Cellular senescence involves the secretion of a plethora of factors, including TGF-β, which induces expression of cyclin-dependent kinase inhibitors 2A and 2B (p16INK4a and p15INK4b, respectively; refs. 97).
Microgravity experienced by astronauts during spaceflight causes severe physiological alterations in the human body, including a 1%–2% loss of bone mass every month during spaceflight (98). Several studies have shown decreases in osteostatic markers of bone formation and increases in bone resorption (99–101). The underlying molecular mechanisms responsible for the apparent concurrent decrease in bone formation and increase in bone resorption remain under investigation. Work by the McDonald group suggests that bone remodeling may become uncoupled under zero-gravity conditions secondary to decreased RhoA activity and resultant changes in actin stress fiber formation (102).

In modeled microgravity, cultured human MSCs exhibit disruption of F-actin stress fibers within three hours of initiation of microgravity; the fibers are completely absent after seven days. RhoA activity is significantly reduced, and introduction of an adenoviral construct expressing constitutively active RhoA can reverse the elimination of stress fibers, significantly increasing markers of osteoblast differentiation (102). Under zero-gravity conditions, RhoA is unable to bind to its receptor, and a sufficient number of MSCs may not be able to migrate correctly to the bone-resorptive site for osteoblast differentiation, ultimately leading to bone loss with every cycle of remodeling.

Bone metastases are a frequent complication of cancer and often have both osteolytic and osteoblastic features, indicative of dysregulated bone remodeling. The importance of the bone marrow microenvironment contributing to the spread of cancer was first described in 1889 (103), postulating that tumor cells can grow only if they are in a conducive environment. Activation of matrix TGF-β during bone remodeling plays a central role in the initiation of bone metastases and tumor expansion by regulating osteolytic and pro-metastatic factors (reviewed in refs. 104–110). For example, TGF-β1 can induce osteoclastic bone destruction by upregulating tumor cell expression of PTHrP and IL-11. Additionally, upregulation of CXCRI4 by TGF-β mayhome cancer cells to bones.

**Potential treatment of uncoupled bone remodeling disorders**

Genetically modified mouse models have been used to demonstrate that increased TGF-β signaling leads to diminished mineral concentration and inferior mechanical properties, whereas decreased TGF-β signaling enhances these properties (111). Wild-type mice injected with a relatively low concentration of an antibody against TGF-β show increased bone mineral density, trabecular thickness, and bone volume as a result of elevated osteoblast numbers and decreased osteoclasts (112). In the ACLT rodent model of osteoarthritis, inhibition of TGF-β signaling attenuated articular cartilage damage, delaying osteoarthritis onset (86). Active TGF-β1 concentrations are also high in the subchondral bone in humans with osteoarthritis, suggestive of a similar pathogenesis and a potential role for TGF-β signaling inhibition as a disease-modifying therapy.

Several clinical trials have or are being conducted to evaluate the efficacy of inhibiting TGF-β signaling in metastatic cancers, focal segmental glomerulosclerosis, and idiopathic pulmonary fibrosis (112–115). Because abnormal bone remodeling also generates high levels of active TGF-β and leads to skeletal disorders, inhibition of TGF-β signaling could be a potential treatment.

The bone marrow microenvironment may change in disease states and affect TGF-β1-mediated coupled bone remodeling. Improving the osteogenic microenvironment may help restore coupling by enhancing the osteogenic potential of MSCs during the reversal phase of bone remodeling, and therefore may be another potential therapeutic target. PTH modifies the bone marrow microenvironment by orchestrating the signaling of local factors for bone remodeling, reducing ROS, and stimulating Wnt signaling in the bones of old mice (7–16). PTH is an FDA-approved anabolic therapy for osteoporosis, and daily injection of PTH increases bone formation with normal microarchitecture by coupling bone remodeling (7–15). PTH treatment has been shown to attenuate osteoarthritis progression in animal models (116, 117). Translational studies expanding the use of PTH for the treatment of skeletal remodeling disorders are in the beginning phases.

**Conclusion**

In order for bone homeostasis to be maintained through adulthood, bone remodeling must be spatially and temporally regulated. TGF-β1 is one of the key cytokines responsible for coupling bone resorption with formation, largely by recruitment of MSCs to bone-resorptive sites. Aberrant TGF-β signaling or increased TGF-β activation can result in uncoupled remodeling and cause skeletal diseases/disorders. Therapies that can attenuate TGF-β signaling, either directly by neutralizing TGF-β activity or indirectly by PTH-mediated modulation of the bone marrow microenvironment, may serve as potential therapies for these bone and joint disorders.

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