Bone Cell Communication Factors Provide a New Therapeutic Strategy for Osteoporosis

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Bone homeostasis is strictly regulated by the balance between bone resorption by osteoclasts and bone formation by osteoblasts. Many studies have shown that osteoclasts affect osteoblasts, and vice versa, through diffusible paracrine factors, cell-cell contact, and cell-bone matrix interactions to achieve the correct balance between osteoclastic and osteoblastic activities in the basic multicellular unit (BMU). The strict regulation that occurs during bone remodeling hinders the long-term use of the currently available antiresorptive agents and anabolic agents for the treatment of osteoporosis. To overcome these limitations, it is necessary to develop novel agents that simultaneously inhibit bone resorption, promote bone formation, and decouple resorption from formation. Therefore, a more detailed understanding of the mechanisms involved in osteoclast-osteoblast communication during bone remodeling is necessary.

Key Words: Osteoclasts; Osteoblasts; Cell Communication; Osteoporosis; Paracrine Communication

INTRODUCTION

The bone is a dynamic organ that undergoes continuous renewal through bone remodeling processes to maintain its mechanical characteristics and calcium homeostasis. Bone remodeling is a complex and sophisticated series of sequential events, which occur within a temporary anatomical structure called the basic multicellular unit (BMU), involving various cell types including osteoclasts, osteoblasts, osteocytes, T-cells, macrophages, pericytes, vascular endothelial cells, canopy bone lining cells, and precursor populations of osteoblasts and osteoclasts.\(^1,2\) In particular, osteoclasts and osteoblasts are the two major cells regulating bone remodeling processes. Osteoclasts and osteoblasts are responsible for old bone resorption and new bone formation, respectively. Bone remodeling in each BMU proceeds in cycles consisting of distinct phases: the recruitment of osteoclasts and bone resorption by osteoclasts; the coupling of resorption to formation or reversal from catabolism to anabolism; the recruitment of osteoblasts and new bone formation by osteoblasts; and the termination of these processes.\(^4,7\) As an imbalance between bone formation and bone resorption results in multiple metabolic bone diseases like osteoporosis and osteopetrosis, each phase of the bone remodeling process must be strictly regulated by various local or systemic factors and intracellular signals to maintain bone homeostasis.\(^8-11\)

Osteoporosis is the most common metabolic bone disease caused by excessive bone resorption relative to formation. It is characterized by low bone mass, the deterioration of bone tissue, and an increased risk of bone fracture. Osteoporosis-related fractures most commonly occur in the hip, wrist, spine, or shoulder, particularly in post-menopausal women.\(^8,11\)

Several drugs are currently available for osteoporosis treatment. These drugs target either the inhibition of bone resorption or the promotion of bone formation. However, certain limitations of antiresorptive agents and bone-forming drugs have been revealed. Antiresorptive drugs, such as alendronate, zoledronic acid, risedronate, and ibandronate, effectively block the formation and function of osteoclasts, but simultaneously reduce bone formation. In contrast, anabolic drugs, such as parathyroid hormones, teriparatide, and recombinant human parathyroid hormone, increase bone formation markers, but also increase bone resorption markers. These long-term adverse events...
induced by antiresorptives and bone-forming drugs suggest that the coupling process between bone resorption and formation plays a crucial role in the complete restoration of the bone removed during remodeling cycles. Therefore, an understanding of the signaling pathway involved in the coupling process will help develop novel drugs that simultaneously block bone resorption and promote bone formation without certain adverse events. Here, we have reviewed the coupling factors that may be an ideal target for the management of osteoporosis.

**RANKL/RANK SIGNALING**

The receptor activator of the nuclear factor kappa B ligand (RANKL) is an essential factor for osteoclast differentiation and function. It is secreted by osteoblasts and osteocytes, and binds to receptor activator of nuclear factor kappa B (RANK) on the surface of osteoclast precursors. In addition, the physiological roles of the RANKL in osteoblasts have recently been elucidated. The vesicular RANK, secreted from maturing osteoclasts, binds to the osteoblastic RANKL to promote bone formation by osteoblasts. The osteoblastic RANKL regulates bone formation through the activation of PI3K-Akt mTOR to induce the expression of runt-related transcription factor 2 (Runx2). Therefore, the RANKL-RANK system could regulate both bone resorption and bone formation by using RANKL forward signaling and RANKL reverse signaling, respectively.

Denosumab, a monoclonal antibody against RANKL, is available for the management of osteoporosis and skeletal problems caused by the spread of cancers to bone. Denosumab binds to RANKL, thereby inhibiting osteoclast forward signaling. Despite its efficacy in the inhibition of bone resorption, adverse effects, such as low bone formation, may impede long-term use. Interestingly, Ikebuchi and colleagues developed an anti-RANKL antibody that reduces osteoclast formation and function by binding and inactivating multiple RANKL monomers, and stimulated osteoblast differentiation by binding to the cell-surface of the RANKL. Therefore, RANKL-RANK forward or reverse signaling offers a new strategy for the management of osteoporosis, which is able to trigger bone formation while inhibiting bone resorption.

**SCLEROSTIN**

Sclerostin is encoded by the SOST gene in humans. After discovering that the lack of SOST expression was the cause of the high bone mass in human Van Buchem disease and sclerosteosis, considerable evidence from in vitro, animal, and human studies has demonstrated that sclerostin plays an important role in bone homeostasis. Sclerostin is secreted primarily from osteocytes, but not osteoblasts. It has been identified as binding to LRPS/6 receptors and antagonizing the canonical Wnt pathway. The inhibition of the Wnt pathway by sclerostin leads to the inhibition of bone formation by osteoblasts. In addition, sclerostin stimulates bone resorption through its inhibitory action on the canonical Wnt pathway, because activation of the canonical Wnt pathway in osteoblasts increases the expression of osteoprotegerin (OPG), a decoy receptor for RANKL, and reduces bone resorption. Sclerostin expression is also detected in osteoclast precursors and its expression is decreased when osteoclasts are formed in vitro. Thus, Tnfsf11b(Opg)-/- and Tnfsf11 (Rankl)- transgenic mice with a high-bone turnover exhibited a low level of sclerostin, suggesting that the suppression of sclerostin was associated with bone resorption. Therefore, the dual roles of SLIT3 in both osteoblasts and osteoclasts result in osteoporotic bone phenotypes that involve a decrease in bone formation and an increase of bone resorption in mice lacking Slit3 or its receptor Robo1. Importantly, the injection of a truncated SLIT3 containing the ROBO-binding LRR2 domain into ovariectomized mice reversed ovariectomy-induced bone loss by simultaneously enhancing bone formation and reducing bone resorption.

**SEMAPHORINS**

Although semaphorins (SEMs) were first identified as axon guidance cues, they have been shown to play important roles in angiogenesis, tissue development, and the immune response. Of the eight classes of semaphorin family proteins, several studies have suggested important roles of SEMA4D and SEMA3A in bone metabolism. SEMA4D is a transmembrane semaphorin highly expressed in osteoclasts, but not in osteoblasts. FC-SEMA4D, a soluble FC receptor SEMA4D fusion protein, inhibits osteoblast differentiation and function without altering proliferation. The binding of SEMA4D to its receptor complex, consisting of ErbB2 and Plexin-B1, leads to activation of the small GTPase RhoA. Genetically altered mice with Semad and Plexb1 deletion, as well as mice expressing an
osteoblast-targeted dominant-negative RhoA, exhibited a high bone mass due to enhanced osteoblastic bone formation.\cite{45,46} However, the regulation of bone mass by SEMA4D may be more complicated. Dacquin et al.\cite{44} reported that the increased bone mass phenotype in \textit{Semadd}-deficient mice was primarily due to a functional defect in osteoclasts. The authors showed that \textit{Semadd}-deficient primary osteoclasts led to delayed osteoclast differentiation and reduced osteoclast resorption activity that was in part due to the unbalanced regulation of β3 integrin subunit signaling.\cite{44} Although the precise mechanisms through which SEMA4D contributes to bone homeostasis have not been elucidated, the injection of \textit{Sema4d} siRNA or SEMA4D-specific antibody into an ovariectomy-induced animal model of osteoporosis reversed bone mass, suggesting that SEMA4D was a beneficial target for osteoporosis treatment.\cite{45,47}

SEMA3A was first identified in the involvement of patterned neuronal connections and is now recognized as a mediator linking osteoclasts and osteoblasts.\cite{48} SEMA3A is mainly expressed by osteoblasts and its receptor, Nrp1, is expressed by osteoclast precursors.\cite{48,50} \textit{Sema3a}-deficient osteoclasts showed a defect in osteoclast differentiation owing to the inhibition of β-catenin activation, whereas SEMA3A treatment caused a decrease in the differentiation of osteoclast precursors through the inhibition of RhoA activation.\cite{51} Hayashi et al.\cite{51} reported that a global \textit{Sema3a} deletion in mice caused a severe osteopenic phenotype that was associated with a decrease in osteoblastic bone formation and an increase in osteoclastic bone resorption. Interestingly, mice with osteoblast-specific deletion of \textit{Sema3a} did not undergo any change in bone parameters, whereas mice with neuron-specific deletion of \textit{Sema3a} exhibited a markedly low bone mass, similar to mice with global deletion of \textit{Sema3a}.\cite{52} These results were indicative of the indirect effects of SEMA3A on bone metabolism through the nervous system. Furthermore, the injection of SEMA3A into ovariecotized mice prevented ovariectomy-induced bone loss, both through the promotion of bone formation and the suppression of bone resorption.\cite{51}

**CONCLUSIONS**

Generally, coupling factors are the molecules that are involved in the stimulation of osteoblastic bone formation in response to osteoclastic bone resorption to preserve normal bone mass.

**Fig. 1.** The dual roles of bone cell communication factors during bone remodeling. The forward Receptor activator of nuclear factor kappa-B ligand (RANKL) signaling pathway originating from osteoblasts is known to induce osteoclast differentiation, and reverse RANKL signaling from osteoclasts also induces osteoblast formation. Several \textit{in vitro} and \textit{in vivo} studies have shown that some bone cell communication factors, such as semaphorin 3A (SEMA3A), slit guidance ligand 3 (SLIT3), and collagen triple-helix repeat-containing 1 (CTHRC1), stimulate bone formation while suppressing bone resorption, and other factors, such as semaphorin 4D (SEMA4D) and sclerostin, inhibit bone formation while increasing bone formation. The roles of these bone cell communication factors in both osteoclasts and osteoblasts offer a new strategy for the development of bone disease therapies.
bone mass. However, recent studies have shown that some molecules, such as sclerostin, SEMA4D, and SEMA3A, control bone remodeling through cell-cell communication between bone cells rather than a classical coupling process. Negishi-Koga et al. proposed that such factors should be called bone cell communication factors, as they participate in the bone remodeling process by regulating intercellular cross-talk among bone cells. Herein, we have discussed bone cell communication factors that are likely to be ideal therapeutic targets for osteoporosis (Fig. 1). As the orchestration of bone remodeling is strictly regulated by various known and as yet unknown bone communication factors, future investigations should be focused on the discovery of additional coupling signals and elucidate how these factors coordinate resorption and formation coupling in concert.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. Bonekey Rep 2014;3:481.
2. Sims NA, Walsh NC. Intercellular cross-talk among bone cells: new factors and pathways. Curr Osteoporos Rep 2012;10:109-17.
3. Kim BJ, Koh JM. Coupling factors involved in preserving bone balance. Cell Mol Life Sci 2019;76:1243-53.
4. Frost HM. The pathomechanics of osteoporoses. Clin Orthop Relat Res 1985;(200):198-225.
5. Matsuo K, Irie N. Osteoclast-osteoblast communication. Arch Biochem Biophys 2008;473:201-9.
6. Zaidi M. Skeletal remodeling in health and disease. Nat Med 2007;13:791-801.
7. Ikeda K, Takeshita S. Factors and mechanisms involved in the coupling from bone resorption to formation: how osteoclasts talk to osteoblasts. J Bone Metab 2014;21:163-7.
8. Chang B, Quan Q, Li Y, Qiu H, Peng J, Gu Y. Treatment of osteoporosis, with a focus on 2 monoclonal antibodies. Med Sci Monit 2018;24:855-66.
9. Siris ES, Miller PD, Barrett-Connor E, Faulkner KG, Wehren LE, Abbott TA, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. JAMA 2001;286:2815-22.
10. Kanis JA, Cooper C, Rizzoli R, Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 2019;30:3-44.
11. Forcea MA, McLean RM, Qaseem A. Treatment of low bone density or osteoporosis to prevent fractures in men and women. Ann Intern Med 2017;167:904.
12. Cotts KG, Cifu AS. Treatment of osteoporosis. JAMA 2018;319:1040-1.
13. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377:1276-87.
14. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician’s guide to prevention and treatment of osteoporosis. Osteoporos Int 2014;25:2359-81.
15. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999;397:315-23.
16. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O’Brien CA. Matrix-embedded cells control osteoclast formation. Nat Med 2011;17:1235-41.
17. Ikebuchi Y, Aoki S, Honma N, Hayashi M, Sugamori Y, Khan M, et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature 2018;561:190-1.
18. Zaidi M, Cardozo CP. Receptor becomes a ligand to control bone remodelling. Nature 2018;561:180-1.
19. McClung MR, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH, et al. Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 2006;354:821-31.
20. Leder BZ, Tsai JN, Uhlein AV, Wallace PM, Lee H, Neer RM, et al. Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised controlled trial. Lancet 2015;386:1147-55.
21. Bone HG, Wagan RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol 2017;5:513-23.
22. Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, et al. Increased bone density in sclerostosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 2001;10:537-43.
23. Chen X, Wang Z, Duan N, Zhu G, Schwarz EM, Xie C. Osteoblast-osteoclast interactions. Connect Tissue Res 2018;59:99-107.
24. Delgado-Calle J, Sato AY, Bellido T. Role and mechanism of action of sclerostin in bone. Bone 2017;96:29-37.
25. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 2018;397:315-23.
26. Leder BZ, Tsai JN, Uhlein AV, Wallace PM, Lee H, Neer RM, et al. Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised controlled trial. Lancet 2015;386:1147-55.
27. Bone HG, Wagan RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol 2017;5:513-23.
28. Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, et al. Increased bone density in sclerostosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 2001;10:537-43.
29. Chen X, Wang Z, Duan N, Zhu G, Schwarz EM, Xie C. Osteoblast-osteoclast interactions. Connect Tissue Res 2018;59:99-107.
30. Delgado-Calle J, Sato AY, Bellido T. Role and mechanism of action of sclerostin in bone. Bone 2017;96:29-37.
et al. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. Mol Cell Biol 2010;30:3071-85.
31. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U S A 2008;105:20764-9.
32. Koide M, Kobayashi Y, Yamashita T, Uehara S, Nakamura M, Hiraoka BY, et al. Bone formation is coupled to resorption via suppression of sclerostin expression by osteoclasts. J Bone Miner Res 2017;32:2074-86.
33. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 2014;370:412-20.
34. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Osteoclast-derived SLIT3 coordinates bone resorption and formation. J Clin Invest 2018;128:1429-41.
35. Koh JM. Osteoclast-derived SLIT3 is a coupling factor linking bone resorption to bone formation. BMB Rep 2018;51:263-4.
36. Iqbal J, Yuen T, Kim SM, Zaidi M. Opening windows for bone remodeling through a SLIT. J Clin Invest 2018;128:1255-7.
37. Hayashi M, Nakashima T, Taniguchi M, Kodama T, Kumanogoh A, Takayanagi H. Osteoprotection by semaphorin 3A. Nature 2012;485:69-74.
38. Fukuda T, Takeda S, Xu R, Ochi H, Sunamuro S, Sato T, et al. Sema3A regulates bone-mass accrual through sensory innervations. Nature 2013;497:490-3.
39. Takeshita S, Matsuoka K, Park KA, Aburatani H, Kato S, et al. Osteoclast-secreted CTHRC1 in the coupling of bone resorption to formation. J Clin Invest 2013;123:3914-24.
40. Jin YR, Stohn JP, Wang Q, Nagano K, Baron R, Bouxsein ML, et al. Inhibition of osteoclast differentiation and collagen antibody-induced arthritis by CTHRC1. Bone 2017;97:153-67.