Semaphorin 3A
A new player in bone remodeling

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Semaphorin 3A (Sema3A) is a protein identified originally as a diffusible axonal chemorepellent. Sema3A has multifunctional roles in embryonic development, immune regulation, vascularization, and oncogenesis. Bone remodeling consists of two phases: the removal of mineralized bone by osteoclasts and the formation of new bone by osteoblasts, and plays an essential role in skeletal diseases such as osteoporosis. Recent studies have shown that Sema3A is implicated in the regulation of osteoblastogenesis and osteoclastogenesis. Moreover, low bone mass in mice with specific knockout of Sema3A in the neurons indicates that Sema3A regulates bone remodeling indirectly. This review highlights recent advances on our understanding of the role of sema3A as a new player in the regulation of bone remodeling and proposes the potential of sema3A in the diagnosis and therapy of bone diseases.

Introduction

With the accelerated rate of population aging around the world, bone diseases, especially osteoporosis, have been a serious concern and cause an increase in the number of elderly people with sport disability and a reduction in the working population. In healthy adults, the continuous bone remodeling at a steady-state is mediated by osteoblastogenesis and osteoclastogenesis. An imbalance of bone remodeling can cause skeletal disorders such as osteoporosis. Therefore, it is important to elucidate molecular mechanisms of bone remodeling to develop better approaches for the prevention and treatment of skeletal diseases

Notably, increasing evidence suggests that bone remodeling is regulated by a variety of factors. Sema3A is a prototype of axonal guidance molecule in semaphorin family, and recent studies have demonstrated the implication of Sema3A in skeletal system. Furthermore, Sema3A-deficient mice have abnormal structures of bones and cartilaginous. In this review, we summarize recent advance on the investigation of the role of Sema3A in the modulation of bone remodeling.

Semaphorin signaling
Semaphorin family and the receptors

Semaphorin family proteins were originally identified in grasshopper as secreted or membrane-bound glycoproteins characterized by an N-terminal Sema domain followed by a short plexin–semaphorin–integrin (PSI) domain. Based on their C-terminal structure and similarity of amino acid, semaphorin family members are divided into eight subclasses (Fig. 1A). Classes 3–7 are present in vertebrates, whereas classes 1–2 are only found in the invertebrate so far. Classes V are encoded by viruses. In addition, different semaphorins contain different domains, such as an immunoglobulin-like domain (Ig domain) and a thrombospondin-like domain in all classes 5 and glycosylphosphatidylinositol (GPI) linkage in class 7.

The two major receptors of semaphorins are plexins family and neuropilins (Nrp1 and Nrp2). Nine plexins have been identified in the vertebrate so far and grouped into four subfamilies (A, B, C, and D), which show high structural homology with semaphorins. Moreover, the cytoplasmic domain of plexins contains two GTPase-activating protein (GAP) domains. Neuropilins contain two complement binding (CUB) domains, two coagulation factor domains (FV/VIII), and a Meprin, A5, Mu (MAM) domain. Most membrane-bound semaphorins can bind plexins directly, whereas some secreted Sema class 3 group such as Sema3A requires neuropilins to obligate as co-receptors, which function as the ligand-binding partner in complexes (Fig. 1B). In addition, several other molecules such as intergrins have been shown to participate in the formation of the receptor complex.

It is known that semaphorins play an important role as axonal guidance proteins in nervous system development; however, accumulated evidence has indicated that semaphorins have functions in a variety of tissues. Especially, several semaphorin family members are implicated in the regulation of key physiological processes, such as bone formation. For instance, Sema4D is secreted by osteoclasts and mediates communication between osteoclasts and osteoblasts. Sema7A regulates bone homeostasis. Interestingly, our recent study showed that Sema3A could regulate bone remodeling indirectly by modulating sensory nerve development. Therefore, in this review, we will focus on Sema3A as a example to highlight the key role of semaphorins in the regulation of bone remodeling.

Sema3A

Sema3A is the first semaphorin identified in the vertebrate and acts to induce the retraction and collapse of the structure on
Sema3A participates in the development of major structure of nervous system such as the brain, spinal cord, and peripheral nerves, and the regulation of the function of adult nervous system such as axon regeneration and neural repair. Sema3A has been implicated in additional physiological function such as immune responses, organogenesis, and oncogenesis. For example, Sema3A regulates the timing of tooth innervations and dental axon navigation and patterning. Since all three bones cell lineages (osteoclast, osteoblast, and osteocyte) express Sema3A and its receptors, Sema3A seems to be involved in bone development and bone homeostasis. Indeed, Behar et al. reported that sema3A-deficient mice displayed fusion of cervical bones, partial duplication of ribs, and poor alignment of the rib-sternum junctions.

Direct regulation of bone remodeling by Sema3A
Bone remodeling is a dynamic and continuous process in which osteoblast and osteoclast reshape and replace bone during growth and after injury such as a fracture. In addition to the interaction with humoral factors, osteoclasts break down calcified bone and the osteoblasts lay down new matrix.

In healthy adults, bone formation and bone resorption are maintained at a steady-state under mechanical and homeostatic regulation. As an imbalance of bone remodeling results in skeletal disorders such as osteoporosis in women after menopause, it is important to address the precise molecular mechanisms that regulate the balance of bone remodeling from a therapeutic view.

So far, most drugs target osteoclastogenesis by reducing the rate of bone resorption such as RANKL inhibitor. However, these drugs often cause the reduction in new bone formation because of the consequent disruption of the linkage between osteoclast and osteoblast activity. Recently, Hayashi et al. found that Sema3A plays a dual role in osteoclasts and osteoblasts and is a potential therapeutic agent in bone diseases.

Sema3A signaling in osteoclastogenesis
Osteoclasts, originated from the monocyte/macrophage lineage of hematopoietic stem cells, are large and multinucleated cells, which destroy the bone extracellular matrix. The discovery of receptor activator of nuclear factor κ-B ligand (RANKL)/receptor activator of nuclear factor κ-B (RANK)/osteoprotegerin (OPG) system led to major advances in our understanding the molecular mechanism of bone resorption. OPG is an important osteoclastogenesis inhibitor secreted from osteoblasts. Sema3A was contained in conditioned medium from OPG-deficient mouse calvarial cells and could inhibit osteoclast formation. Interestingly, Sema3A-deficient mice have a severe osteopenic phenotype. The discovery of receptor activator of nuclear factor κ-B ligand (RANKL)/receptor activator of nuclear factor κ-B (RANK)/osteoprotegerin (OPG) system led to major advances in our understanding the molecular mechanism of bone resorption. OPG is an important osteoclastogenesis inhibitor secreted from osteoblasts. Sema3A was contained in conditioned medium from OPG-deficient mouse calvarial cells and could inhibit osteoclast formation. Interestingly, Sema3A-deficient mice have a severe osteoporosis phenotype. In addition, the mutant Nrp1 knockin mice (Nrp1<sup>sem</sup> mice), which lacked the Sema-binding ability, exhibited a similar phenotype to Sema3A-deficient mice. These in vivo data suggest that Nrp1 is the functional receptor of Sema3A in bone cells.

PlexinA family is the primary receptor of Sema3A and they form receptor complex with Nrp1. Takegahara et al. found that in response to Sema6D, plexinA1 associated with the triggering receptor expressed on myeloid cells-2 (TREM2) and DNAX-activating protein of 12 kDa (DAP12) to enhance osteoclastogenesis by activating the immunoreceptor tyrosine-based activation motif (ITAM) signaling.
signaling is known to regulate cytoskeletal rearrangement and the Rho family of small GTPases. Interestingly, Sema3A-Nrp1 axis repelled osteoclast precursor cells through affecting small GTPase RhoA activation but not Rac activation. Our preliminary experiments confirmed that Rac activation was not involved in Sema3A-dependent inhibition of osteoclast differentiation by using dominant-negative Rac1 constructs (unpublished data).

**Sema3A signaling in osteoblastogenesis**

Osteoblasts originated from mesenchymal stem cells, they can differentiate into chondrocytes, adipocytes, and myoblasts, and are responsible for synthesis of bone matrix. In addition to the severe osteoclastic phenotype, both Sema3A-deficient and Nrp1 mice had decreased amounts of the factors of bone formation based on histomorphometric analyses. In accord with the osteoblastic phenotype in vivo, primary osteoblasts from Sema-deficient mice had a defect in differentiation with decreased expression of osteoblast markers, such as Runx2 and Bglap. We found that Sema3A accelerated osteoblastic differentiation. In contrast, the knockdown of Sema3A in osteoblasts hampered the differentiation, and treatment with Sema3A restored the defective differentiation in Sema3A-knockdown osteoblasts. In addition, Sema3A expression is very high in osteoblasts (unpublished data). Taken together, these findings suggest that Sema3A is a positive regulator in osteoblastogenesis in an autocrine manner.

Intriguingly, using primary osteoblasts from sema-deficient mice, it was shown that Wnt signaling is involved in the molecular pathway by which Sema3A regulates osteoblastogenesis. Canonical Wnt signaling is well-known to regulate the differentiation of osteobalsts and adipocytes. Rac1, which promotes β-catenin localization in the nucleus in response to Wnt ligands, is required for the action of Sema3A on the collapse of the growth cone. Indeed, treatment of osteoblasts with recombinant Sema3A activated Rac1 via FERM, RhoGEF, and pleckstrin domain-containing protein 2 (FARP2), leading to nuclear accumulation of β-catenin induced by Wnt3a (Fig. 2B). These results are consistent with our recent report that ectopic expression of a dominant-negative form of Rac1 inhibited Sema3A-dependent osteoblast differentiation.

**Indirect regulation of skeletal sensory nerves by Sema3A**

Historically, most bone biologists believe that bone remodeling is regulated in an endocrine manner. However, the discovery that leptin regulates bone mass through a hypothalamic relay shed new light on the mechanism underlying bone remodeling. The sympathetic nervous system (SNS) has been shown to mediate the regulation of bone remodeling by β-adrenergic receptor-2 (Adrb2). Thanks to the use of excited fluorescent nerve-specific markers and genetic mutant mice models, great advances have been achieved in the field of neuroskeletal biology.

**Skeleton innervations**

More than 50 years ago, bone innervations were shown to affect bone homeostasis in peroneal denervation experiments. Later it was found that sympathetic nerves exist in the bone marrow and they are positive for dopamine β-hydroxylase (DBH), tyrosine hydroxylase (TH), and neuropeptide Y (NPY) by immunofluorescence staining. On the other hand, sensory fibers are detected in vertebral bones, long bones, and bone marrow. In particular, an extremely dense distribution of calcitonin gene-related peptide (CGRP) and substance P (SP) is observed primarily in periosteal innervations. In addition, rapid neural growth of CGRP-positive nerves occurs during bone regeneration in the periosteum of adult rodents. However, so far, the molecular mechanism by which skeletal innervations regulate bone remodeling is elusive.

Recent evidence suggests that both central and peripheral nervous systems regulate bone remodeling. Sema3A is a well-known axon guidance molecule and functions as a chemorepellent in nervous systems. Indeed, Sema3A-deficient mice have multiple phenotypes due to abnormal neuronal innervations. Moreover, Sema3A exhibits temporal and spatial expression patterns in parallel with the establishment of innervations. Therefore, we speculate that Sema3A may modulate bone remodeling indirectly via regulating nervous systems.
The link between bone remodeling and sensory innervations by Sema3A

Sema3A is ubiquitously expressed in a variety of tissues, including the bone. To specifically dissect the role of Sema3A in nervous systems without the disruption from other organs, we generated neuro-specific Sema3a-deficient mice (Sema3A neuron−/− mice) based on synapsin I-cre mice or nestin-cre mice. These Sema3A neuron−/− mice exhibited similar phenotype to Sema3A−/− mice, such as low bone mass. In contrast, osteoblast-specific Sema3A-deficient mice (Sema3A osb−/− mice) did not develop any bone abnormalities. These results indicate that Sema3A in osteoblasts is not the sole cause of bone phenotype in Sema3A−/− mice in vivo.

Surprisingly, skeleton innervations are significantly decreased in both Sema3A−/− mice and Sema3A neuron−/− mice, but not in wild-type mice and Sema3A osb−/− mice. Furthermore, sensory-positive nerves makers such as CGRP were decreased in bones of Sema3A−/− mice and Sema3A neuron−/− mice. In contrast, DBH-positive sympathetic nerve fibers, which inhibit bone formation, were not affected significantly in Sema3A−/− mice and Sema3A neuron−/− mice. Therefore, low sensory innervations we observed are consistent with low bone mass in Sema3A neuron−/− mice in vivo.

Clinical potential of Sema3A in bone diseases

To investigate the therapeutic potential of Sema3A, Hayashi et al. performed three experiments. First, intravenous injection of recombinant Sema3A led to increased osteoblastic parameters and decreased osteoclastic parameters in wild-type mice. Second, Sema3A treatment enhanced bone regeneration in a mouse model of cortical bone defect. Third, Sema3A treatment rescued bone loss in ovariecctomized mice. Moreover, in Sema3A neuron−/− mice bone regeneration was reduced with defective nerve innervations after bone marrow ablation. These data suggest that sema3A has the potential to be used for the treatment of bone diseases.

Notably, Sema3A expression is proposed as a marker for systemic lupus erythematosus and rheumatoid arthritis. Similarly, Sema3A may be a marker for skeleton disorders such as osteoporosis. In fact, familial dysautonomia patients who have loss of sensory nerves suffer from osteoporosis.

In conclusion, Sema3A regulates bone remodeling directly by regulating the activities of osteoclasts and osteoblasts, and indirectly by engaging in sensory nerve innervations. These findings provide new insight into the role of sema3A in bone biology. Consequently, sema3A represents a novel target for the diagnosis and therapy of skeletal disorders.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
osteocatobic

Adrb2

Bone remodeling

osteocatobic

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Sympathetic nerve system

Interactions

Sensory nerve system

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Figure 4. The hypothetical balance between osteo-anabolic afferent sensory nerves and osteo-catabolic efferent sympathetic nerves. The sympathetic nervous system regulates osteo-catabolic (bone resorbing) response via β-adrenergic receptor-2 (Adrb2) in the bone cells, while sensory nerve system regulates osteo-anabolic (bone forming) response via unidentified receptors. The balance of these actions modulate bone remodeling.
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