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COMMENTARY

A functional role of sensory nerves in the control of bone remodeling

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Commentary on: T Fukuda, S Takeda, R Xu, H Ochi, S Sunamura, T Sato, S Shibata, Y Yoshida, Z Gu, A Kimura, C Ma, C Xu, W Bando, K Fujita, K Shinomiya, T Hirai, Y Asou, M Enomoto, H Okano, A Okawa, H Itoh. Sema3A regulates bone-mass accrual through sensory innervations. *Nature* 2013;**497**:490–493.

There are many nerves within the skeleton, and they come in many different types. Their presence in the bone microenvironment and the expression of a number of neuropeptides in bone were reported decades ago, and it is in fact through the detection of these neuropeptides that bone innervation has been mostly characterized (see Elefteriou[1] for review). Sympathetic and parasympathetic nerves innervate cancellous and cortical bones, and a growing number of studies over the last 10 years support the notion that these nerves contribute to the control of bone remodeling, downstream of hypothalamic and brainstem centers (see Elefteriou *et al.*[2] for review). In contrast, the density of sensory nerves is also very high in bone, and their functionality to inform higher brain centers regarding the existence of trauma, induced by fracture or lesions secondary to the growth of cancerous cells within bone, is evident. The paper by Drs Fukuda, Takeda, Xu and collaborators,[3] recently published in *Nature*, goes far beyond this evidence to show that the lack of sensory nerves within the bone microenvironment during development, due to Semaphorin 3A deficiency, has considerable repercussions on bone-mass accrual in mice and provides compelling genetic evidence to include sensory nerves as additional significant factors in the field of neuroskeletal biology.

Semaphorin 3A (Sema3A) belongs to one of the largest families of phylogenetically conserved guidance cues, initially characterized for their importance in the development of the nervous system and in axonal guidance, but since then shown to be expressed and required both inside and outside the nervous system.[4,5] Semaphorins are secreted, transmembrane and glycosylphosphatidylinositol (GPI)-linked proteins, signaling through the neuropilin and plexin families of receptors. Through analysis of mice globally deficient for Sema3A, Fukuda and collaborators[3] first confirmed that this molecule, mostly known for its axon-guidance properties, regulates bone homeostasis by a direct and autocrine action in osteoblasts. Differentiation (not proliferation) is indeed shown in this study to be impaired in osteoblast precursors prepared from mice globally deficient for Sema3A, and the activation of Rac1 is shown to be part of the mechanism, in agreement with an earlier study by Hayashi *et al.*[6] The authors also present evidence that Sema3A inhibits osteoclast progenitor differentiation *in vitro*, although no change in histologic indices of bone resorption was detected in Sema3A-deficient mice. This was also observed previously in the study by Hayashi *et al.*,[6] although the authors of this latter work did detect significant changes in osteoclast numbers in Sema3A-deficient mice. This study provided compelling evidence for an inhibitory effect of Sema3A on osteoclastogenesis and bone adipogenesis, and a stimulatory effect on osteoblast differentiation, based on the analysis of mice globally deficient for Sema3A and mice expressing a mutant *Nrp1* gene lacking the Sema-binding site (*Nrp1*$^{Sema-}$ mice).[6] One of the most notable contributions of the Fukuda study, and distinction from the Hayashi study, is the analysis of conditional mutant mice deficient for Sema3A. In this study, the lack of Sema3A in the osteoblast lineage surprisingly had no repercussion on bone mass, despite decreases in Sema3A expression in bone and the fact that osteoblasts extracted from Sema3A-deficient mice showed reduced differentiation when grown *in vitro*. These data, obtained from the analysis of two complementary animal models lacking Sema3A in osteoblasts or osteoprogenitor cells, thus suggested that the mechanism whereby Sema3A controls bone remodeling was more complex, and that other cell types expressing Sema3A might be involved. Of note is that these results did not exclude a possible role of Sema3A in osteoprogenitor cells, before their commitment to the osteoblast lineage and expression of Osterix, as the cre lines used target only committed osteoprogenitors and mature osteoblasts.[7] This putative role of Sema3A in early mesenchymal osteoprogenitors is supported by the impaired *in vitro* differentiation of nestin-cre-derived Sema3A-deficient osteoprogenitors shown by Fukuda *et al.*[3] and by the adipocyte phenotype reported in global Sema3A-deficient mice by Hayashi *et al.*,[6] both of which suggest the existence of an early mesenchymal commitment phenotype in absence of Sema3A.

On the basis of these results and because Sema3A was first identified as a neuronal molecule, Fukuda and collaborators

then ablated *Sema3A* in nestin- and synapsin I-positive neurons using the cre/lox system again. This genetic alteration led to a reduction in *Sema3A* expression in bone and a low-bone mass phenotype caused by reduced osteoblast activity. Importantly, all of these mutant mice lacking Sema3A in osteoblasts or neurons showed a similar reduction in bone Sema3A levels, but only the neuron-deficient mice had a low bone mass, indicating that Sema3A level in bone is not the causal determinant of this bone phenotype. Rather, the authors show that bone innervation, and specifically sensory nerve innervation, was significantly reduced in mice lacking Sema3A in neurons, in line with the chemorepellent and axon-guidance function of this molecule. This reduction in bone sensory innervation was shown by no less than three independent methods, including the analysis of sensory nerve markers and reporters, retrograde tracing and functional pain assays, firmly demonstrating the role of Sema3A in the process of bone innervation by sensory nerves. Not surprisingly, this defect of bone sensory innervation was observed early during the development and in a concomitant manner with their low bone mass, supporting the notion that bone mass accrual during development requires sensory bone innervation. In addition, mice deficient for Sema3A in osteoblasts did not show a reduction in bone sensory innervation and had a normal bone mass, suggesting that it is the reduced projections of sensory nerve fibers within the bone environment, not Sema3A levels in bone, that are causing the low bone mass of neuron-specific and global Sema3A-deficient mice. Bone sympathetic innervation did not seem to be overtly affected in neuron-specific Sema3A-deficient mice, as shown by the normal density of *Dbh*-positive nerves in their bones. Deleting Sema3A in neurons or bone selectively after birth did not affect bone mass (assuming gene recombination was efficient), further supporting the idea that embryonic sensory bone innervation is important for the acquisition of a normal bone mass.

This study is the first to provide genetic evidence for a role of sensory nerves, not only in bone pain perception but also in bone mass accrual. A big question left unanswered, however, is how sensory nerves promote osteoblast differentiation and bone formation. Although osteoblast-specific Sema3A-deficient mice did not show a low-bone mass phenotype and neuron-specific mutants mice did, osteoblasts from both models clearly expressed Sema3D. There is thus another mediator causing the low bone mass of the neuron-specific SemaA-deficient mice.

Questions pertaining to the most relevant cellular sources of Sema3A for bone sensory innervation and bone accrual may also be tightly linked to the mechanism of action of this molecule on nerves and bones. Uncertain is how neurons distinguish between osteoblast- and neuron-derived Sema3A to grow within bone during development. One can speculate that proteolytic processing, time/site of secretion and/or receptor expression patterns during development are differentially regulated in bone cells and neurons, all of which remain to be investigated. Nevertheless, a clear bone anabolic response has been measured following injection of recombinant Sema3A in adult mice in the study by Hayashi *et al.*,[6] that showed that Sema3A stimulates the canonical Wnt/β-catenin signaling pathway in osteoblasts, at least in part, through FARP2-mediated activation of Rac1 during osteoblast differentiation. Hence, collectively, these two studies suggest that, during development, Sema3A in sensory neurons is necessary for bone sensory innervation and bone mass accrual, whereas, in adults with normal sensory innervation, Sema3A in Osx-negative mesenchymal progenitor cells is required for differentiation to the osteoblast lineage and normal bone remodeling.

Finally, it is also interesting to note that the repulsive properties of Sema3A for nerve cone elongation and guidance are critical to the development of the central nervous system (CNS). It is thus possible that neuronal projections and connectivity could be altered in the CNS of the neuron-specific mutant mice described in this study, and consequently the production of neurohormonal and neuronal cues that could have repercussions on bone remodeling. This is supported by the absence of a bone phenotype in mice in which Sema3A is ablated postnatally, after formation of the CNS.

Thus, there are now data from genetic and pharmacological studies to attribute a functional role of sensory, sympathetic and parasympathetic nerves in the control of bone accrual and remodeling, in addition to pituitary neurohormones like follicle-stimulating hormone.[8,9] The interactions between the CNS and the skeleton are thus multidirectional and are of various natures. How these systems interact under physiologic and diseased conditions, and how they apply to human biology, remains unknown. Nevertheless, this study by Fukuda and collaborators is a tour de force that further extends our understanding of the interactions between the neuronal and skeletal systems.

Conflict of Interest

The author declares no conflict of interest.

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