Editorial review: *Journal of Bone and Mineral Research "CRMP 4 Inhibits Bone Formation by Negatively Regulating BMP and RhoA Signaling"*

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Collapsin response-mediated protein 4 (CRMP4) regulates the differentiation of mouse bone marrow skeletal stem cells (mBMSCs) into osteoblastic cells. CRMP4 was shown to control the early commitment of murine bone marrow-derived stromal stem cells into the osteogenic lineage. The inhibitory effect of CRMP4 on bone mass revealed its direct involvement in mediating BMP2-induced osteogenesis signaling and a role in osteoblast adhesion and proliferation via regulation of cytoskeletal dynamics in a RhoA-focal adhesion kinase (FAK)-dependent mechanism (1). In this editorial, we discuss the details of Rho and its downstream targets, especially Rho-kinase and CRMP families.

RhoA and its downstream targets

RhoA is the best characterized protein that circulates between inactive GDP-bound forms and active GTP-bound conformations, and functions as a molecular switch that interacts with downstream targets and triggers a variety of cellular responses. The activity of RhoA is controlled by a guanine nucleotide exchange factor that catalyzes the GDP exchange of GTP. In contrast, activation of GTPase protein stimulates endogenous activity of GTPase and inactivates RhoA. Guanine nucleotide dissociation inhibitors have been demonstrated to block spontaneous RhoA activation.

RhoA is a GTPase involved in signal transduction. Activation of Rho involves the regulation of actin filaments in the cell, showing the organization of stress fibers and focal adhesions (2,3). Contraction of actomyosin can be regulated by Rho-kinase in two ways. The first involves phosphorylation of the myosin-binding subunit (MBS) of myosin phosphatase, followed by phosphorylation of the myosin light chain (MLC), resulting in contraction of stress fibers in smooth muscle cells (4,5) and fibroblasts (5,6).

Non-muscle cell contractions including smooth muscle cells and fibroblastic cells are mediated in a calcium-dependent manner by phosphorylation of MLC by the calmodulin/myosin light chain kinase (MLCK) system. On the other hand, the Rho protein involved in intracellular signaling, Rho-related kinase, is known to be an effector of Rho small GTPase (7-10). Activation of Rho-kinase downstream of Rho regulates the formation of stress fibers and focal adhesions (2,6,10-12). In addition, Rho kinase was reported to directly phosphorylate MLC in a Ca^{2+}-independent manner *in vitro* (11,12). It is also known that Rho kinase promotes phosphorylation of MLC indirectly by lowering the activity of myosin phosphatase. Our recent experiments showed that the actin-myosin contractile system in the cell is regulated by at least two independent systems; the Ca^{2+}-dependent calmodulin/MLCK system and the Ca^{2+}-independent Rho kinase system. These two independent regulatory systems for myosin protein were confirmed to be present in the same cells. They are balanced with each other, and are involved in maintaining the morphology of the cell. Cytoplasmic tension in cells organized by stress fibers is essential for many cellular behaviors, such as cell movement, cell fixation in the extracellular matrix, embryonic development,

mechanosignaling to cells, etc. Rho and its related Rho-kinases appear to play important roles in the maintenance of normal cells *in vitro* and *in situ*.

The cell-substrate interface, which is called a focal adhesion or adhesion plaque, plays an essential role in many biological behaviors, such as cell migration, wound healing, and angiogenesis. These areas are composed of typical focal adhesion constituent proteins, such as vinculin, paxillin, talin, alpha-actinin, integrin, and FAK (13,14). Some signal transduction proteins, such as FAK, c-Src, Rho A, and integrin, are also localized along with these constituent proteins in close association with focal adhesions. These observations suggest that the focal adhesions play roles not only in connection between the plasma membrane of the cell and substrate, but also in signal transduction from outside to the inside of the cell. Interestingly, specific regulation and activation of FAK are thought to be important for focal adhesion formation and the associated stress fiber organization. Activation of Rho and Rho-kinase has been suggested to play a role in determining the effects of FAK on the formation of stress fibers and focal adhesions.

CRMPs as a therapeutic target for neuroregeneration

CRMP4 was initially identified as an isomorphic molecule involved in intracellular signaling derived from semaphorin 3A (Sema3A), which is a restitution factor of axons (15). CRMPs are cytoplasmic proteins, and five subtypes (CRMP1 to 5) have recently been identified (16). CRPMs could be detected in the developing nervous system, and they show a specific distribution within the cell. CRPMs are identical to *Caenorhabditis elegans* Unc-33, and mutation of Unc-33 was shown to cause axon outgrowth and abnormal guidance in nervous system development in nematodes (17). CRMPs are phosphorylated proteins, and regulation of phosphorylation plays an important role in nervous system development and maturation (18).

Studies using primary cultured neurons and knockout mice indicated that CRMPs are involved in axon formation and nerve cell migration, synaptogenesis, and synaptic plasticity. It was reported that the dendritic length and the number of branches were decreased in the hippocampus under CRMP4-deficient conditions. With deficiency or knockdown of CRMP4, the number of branch points of dendrites increases and dendrite elongation is promoted, while dendrite elongation is suppressed in nerve cells overexpressing CRMP4. It has been suggested that CRMP4 suppresses the branching and elongation of dendrites (19,20). CRMP4-deficient cells were also reported to show inhibition of axonal outgrowth and growth cone formation. It was suggested that CRMP4 regulates growth cone formation by promoting microtubule polymerization and bundling of actin filaments (21).

Abdallah and colleagues reported that p27 and cyclinD1 are two RhoA effectors that mediate the regulatory function of CRMP4 in osteoblast proliferation (1). They also reported direct evidence that CRMP4 regulates osteoblast proliferation via a RhoA-dependent mechanism. They also showed that the Rho-kinase inhibitor, Y-27632, significantly decreased the observed increase in cell proliferation rate, indicating the role of CRMP4 in regulating osteoblast proliferation by a RhoA-dependent mechanism via modulation of P27/cyclin D1 expression. CRMP4 deletion promotes osteoblast proliferation, and this effect is mediated by RhoA activation.

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Footnote

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