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ABSTRACT

Bone metabolism is strictly regulated by osteoblasts and osteoclasts, responsible for bone formation and bone resorption, respectively. Nowadays, it is generally recognized that osteoblasts play a crucial role also in the regulation of bone resorption through receptor activator of nuclear factor κB ligand (RANKL) expression in response to a variety of bone resorptive stimuli. We previously reported that bone morphogenetic protein-4 (BMP-4) stimulates the synthesis of osteocalcin via p38 mitogen-activated protein (MAP) kinase in osteoblast-like MC3T3-E1 cells, whereas p44/p42 MAP kinase plays as a negative regulator in the synthesis. In the present study, we investigated whether Rho-kinase is involved in BMP-4-stimulated osteocalcin synthesis in MC3T3-E1 cells. The levels of osteocalcin were measured by ELISA. The phospholylation of each protein kinases was analyzed by Western blotting. The mRNA levels of osteocalcin were determined by real-time RT-PCR. BMP-4 induced the phosphorylation of myosin phosphatase targeting subunit-1 (MYPT-1), a substrate of Rho-kinase. Y27632 or fasudil, specific inhibitors of Rho-kinase, which attenuated the MYPT-1 phosphorylation, significantly amplified the BMP-4-stimulated osteocalcin synthesis in a dose-dependent manner. The osteocalcin mRNA expression levels induced by BMP-4 were enhanced by Y27632 or fasudil. BMP-4-stimulated osteocalcin release was significantly up-regulated in Rho-knocked down cells with Rho A-siRNA. Y27632 or fasudil failed to affect the BMP-4-induced phosphorylation of SMAD1 or p44/p42 MAP kinase. On the other hand, Y27632 or fasudil markedly strengthened

the phosphorylation levels of p38 MAP kinase induced by BMP-4. These results strongly suggest that Rho-kinase negatively regulates BMP-4-stimulated osteocalcin synthesis via the p38 MAP kinase pathway in osteoblasts.

Key words: Rho kinase, BMP-4, osteocalcin, MAP kinase, osteoblast.