学位論文の要旨

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学位論文名　Parathyroid Hormone Upregulates BMP-2 mRNA Expression Through Mevalonate Kinase and Rho Kinase Inhibition in Osteoblastic MC3T3-E1 Cells

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論文内容の要旨

INTRODUCTION

Parathyroid hormone (PTH) has clinically been introduced in many countries to treat osteoporosis. Although it is well-known that PTH possesses anabolic action on bone, the mechanisms have not fully been understood. The anabolic action has been reported to be mediated by PTH/PTH-related protein receptor (PTH1R) followed by stimulating differentiation and mineralization of osteoblasts, suppressing mature osteoblastic apoptosis, activating canonical Wnt-β-catenin signal, and stimulating IGF-I production.

However, little is known about PTH effect on bone morphogenetic proteins (BMPs), strong mediators for bone formation. BMP-2, BMP-4, and BMP-7, which accelerate bone formation and fracture repair, play critical roles in osteoblastic differentiation as well as bone formation and could be good candidates for mediating the osteogenic signalings of PTH. On the contrary, statin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, increased bone formation when injected subcutaneously over the calvaria of mice and increased

cancellous bone volume when orally administered to rats, via increased expression of BMP-2. Growing evidence suggest that BMP-2 expression is regulated by the mevalonate pathway and Rho-associated protein kinase (ROK) activity. This study is performed to examine if PTH affects BMP-2 expression and to clarify its involvement of the mevalonate pathway.

MATERIALS AND METHODS

Osteoblastic MC3T3-E1 cells were treated with human PTH-(1-34) to determine BMP-2 mRNA expression levels by real-time PCR and to measure the ROK activity by the kinase assay. MC3T3-E1 cells, a clonal osteoblastic cell line isolated from calvariae of late stage mouse embryo, were cultured in α-MEM (containing 50μg/ml of ascorbic acid) with 10% fetal bovine serum and 1% penicillin-streptomycin in 5% CO_2 at 37°C. Total RNA was isolated using Trisol reagent, and first-strand cDNA was synthesized using oligo-dT primer. The double-stranded DNA-specific dye SYBR Green I was incorporated into the PCR buffer to allow for quantitative detection of the PCR product. Real-time PCR was performed using the sense and antisense primers designed based on published cDNA sequences. After the cell lysates were centrifused, supernatants were collected for assessing Rho kinase activities based on ELISA method. Each value was normalized to the protein concentration.

RESULTS AND DISCUSSION

The expression of BMP-2 mRNA was increased in a time dependent manner up to 6 hours and was decreased thereafter. Incubation with 10^{-9} and 10^{-8}M of hPTH-(1-34) for 6 hours induced significant upregulation of BMP-2 mRNA levels in MC3T3-E1 cells, compared with the control treated with vehicle alone (100nM of acetic acid). Next, ROK activity in whole cell lysate was measured to address whether or not PTH would

affect the ROK activity. We observed that 10 minutes treatment of MC3T3-E1 cells with 10^{-8}M hPTH-(1-34) significantly decreased the ROK activity compared with the vehicle-treated control.

Next, we determined mevalonate kinase (MVK) mRNA expression by real-time PCR, which is located upstream of ROK. MVK mRNA level was significantly decreased by 3 hours treatment of the cells with 10^{-8}M hPTH-(1-34) compared with the control. Significant inhibition of MVK mRNA expression was also observed at 6 and 12 hours after PTH treatment. These findings indicate that PTH might rapidly suppress the MVK activity.

Finally, we investigated whether or not addition of geranylgeranyl pyrophosphate (GGPP), downstream of MVK, or mevalonate, upstream of MVK, would be able to reverse the PTH-induced BMP-2 upregulation in MC3T3-E1 cells. PTH-induced upregulation of BMP-2 mRNA was inhibited by overnight pretreatment with 5μM GGPP but not with 1mM mevalonate, indicating that PTH directly interacts with MVK, but not with its upstream molecules.

Taken together, these findings suggest that BMP-2 mRNA expression was upregulated by PTH mediated by suppression of the mevalonate pathway followed by ROK inhibition.

CONCLUSION

We for the first time demonstrated that PTH stimulated BMP-2 mRNA expression via the mevalonate pathway and ROK in osteoblastic MC3T3-E1 cells. This might be one of the mechanisms by which PTH can accelerate bone formation.