学 位 論 文 の 要 旨

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学 位 論 文 名　Role of Osteoglycin in the Linkage Between Muscle and Bone

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論 文 内 容 の 要 旨

INTRODUCTION

The interaction between muscle tissues and bone metabolism is incompletely understood. Recent studies indicate that muscle tissues play some important roles through interactions between muscle tissues and bone metabolism, and that muscle tissues can produce local growth factors which have anabolic effects upon bone tissues. We therefore hypothesized that there might be some humoral factors that are produced in muscle tissues and affect bone in an anabolic fashion. Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disorder characterized by progressive heterotopic ossification in skeletal muscle. We hypothesized that this disease might provide some clue to link muscle tissues to bone. We therefore performed comparative DNA microarray analysis using ALK2 (R206H), which is found in patients with FOP, and eventually identified osteoglycin (OGN). The present study was designed to investigate the role of OGN in the linkage between muscle and bone.

MATERIALS AND METHODS

We used mouse osteoblastic MC3T3-E1 cells, mouse calvarial osteoblasts from 2- to 4-day-old ICR mice, and mouse myoblastic C2C12 cells in this study. Each vector was transfected into MC3T3-E1 or C2C12 cells with Lipofectamine for transient or stable cell lines. Western blot and real-time PCR were used to analyze the amounts of protein and mRNA, respectively. For Western blot analysis, proteins lysed from cells were transferred to

polyvinylidene difluoride membranes, which then were immunoblotted with each primary antibody. The antigen-antibody complexes were visualized using secondary antibodies and an enhanced chemiluminescence detection system (LAS-4000IR). For real-time PCR quantification, cDNA was synthesized using the SuperScript ^{TM} First-Strand Synthesis System and spesicific mRNA was quantified using a 7500 Real-time PCR system with SYBR Premix Ex Taq^{TM} II kits. In microarray analysis, total RNA was extracted from C2C12 cells that were stably transfected with empty vector or ALK2 (R206H), and hybridization samples were prepared. The Affymetrix Murine Genome 430 2.0 set was used to compare gene expression. Mineralization of MC3T3-E1 cells was determined in 6-well plates using Alizarin Red staining. For quantitation, cells stained with Alizarin Red were destained with ethylpyridinium chloride, then the extracted stain was transferred to a 96-well plate, and the absorbance at 562 nm was measured using a microplate reader. In luciferase assay, cells were transfected using Lipofectamine with 3 μg of reporter plasmid (3GC-Lux or 3TP-Lux) together with the empty vector only or OGN. Cells were lysed, and the luciferase activity was measured. All experiments were repeated at least three times. Data are expressed as mean ± S.E. Statistical analysis was performed using analysis of variance. A P value <0.05 was considered significant.

RESULTS AND DISCUSSION

We performed comparative DNA microarray analysis between stable empty vector- and ALK2 (R206H)-transfected mouse myoblastic C2C12 cells. We hypothesized that the expression of muscle-derived bone-anabolic factors might be suppressed by the conversion of muscle tissues into bone, because those factors could be predominantly expressed in muscle tissues, compared with their expressions in bone, and also because their systemic effects could be more important than their local effects within bone tissues. OGN was identified as one of the 25 genes whose expressions were decreased <1/4 by ALK2 (R206H) expression in these cells. Although there were several bone-related factors among them, OGN and FAM5C (family with sequence similarity 5, member C) enhanced the osteoblast phenotype. We, therefore, investigated the function of OGN and FAM5C as bone anabolic factors. We have already shown the role of FAM5C in the linkage between muscle and bone (reference), and the function of OGN was investigated in this study.

OGN is the seventh member of the small leucine-rich proteoglycans and it was initially isolated from bovine bone as an inducer of matrix mineralization. In this study, recombinant OGN as well as stable overexpression of OGN significantly enhanced the levels of alkaline phosphatase (ALP), type 1 collagen (Col1), β-catenin and osteocalcin (OCN) as well as mineralization in mouse osteoblastic cells (MC3T3-E1 and mouse primary osteoblasts). A reduction in endogenous OGN level by siRNA showed the opposite effects in these cells. On the

other hand, OGN suppressed the levels of ALP and OCN mRNA induced by bone morphogenetic protein (BMP)-2 in C2C12 cells. These findings indicate that OGN is a factor that suppresses osteoblastic differentiation of premature osteoblasts and enhances osteoblasts phenotype and mineralization in well differentiated osteoblasts. Moreover, conditioned medium from OGN-overexpressed and OGN-suppressed myoblastic cells enhanced and decreased the levels of ALP and OCN, respectively in osteoblastic cells. These effects were parallel to the effects of OGN overexpression and repression in osteoblasts. OGN was detected in human serum or culture supernatant from mouse myoblasts and myotubes. These findings indicate that OGN, produced in muscle, exhibits bone anabolic activity in osteoblasts.

Previous studies indicate that OGN interacts with transforming growth factor (TGF)-β and plays a role in regulating the collagen fibrillogenesis. In the present study, OGN enhanced the levels of phosphorylated extracellular signal-regulated kinase (ERK) 1/2, TGF-β-induced transcriptional activity, and Col1 mRNA in MC3T3-E1 cells. ERK1/2 inhibitor antagonized the levels of Col1 mRNA enhanced by OGN, although an inhibitor of endogenous TGF-β did not affect it. These findings indicate that OGN enhances the levels of Col1 mRNA partly through ERK1/2 in osteoblasts. Although further studies are necessary to clarify the details of the relationship between OGN and collagen in bone, OGN may enhance bone strength through alterations of the structure and quality of bone matrix proteins including collagens. Humoral bone anabolic factors, produced in muscle tissues, may be important as target molecules for the treatment and prevention of osteoporosis as well as for the bone metabolic index-related muscle and exercise therapy.

CONCLUSION

OGN was selected as a molecule that is down-regulated by ALK2 (R206H)-transfected myoblastic cells. The level of OGN as well as the effects of the conditioned medium from OGN-modulated myoblastic cells was positively correlated with osteoblast phenotype and mineralization in osteoblastic cells, although it seemed to reduce osteoblast differentiation in osteoblasts at the early differentiation stage and myoblasts. OGN may be a crucial humoral bone anabolic factor that is produced in muscle.