

The Functional Involvement of Rac on Bone and Tooth Formation: Characteristic of N17Rac Transgenic Mice

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Introduction

Since, Mundy G et al showed that statin, inhibitor of cholesterol (Ch) biosynthesis pathway, enhanced bone formation in rodents, the role of this pathway in hard tissues is actively observed. It is also known that Ch biosynthesis pathway produces some essential metabolites that are used as substrates for prenylation of small G-proteins such as Ras and Rho. We previously reported that Toxin B (Rho inhibitor) mimics the effects of statin on osteoblastic function and the dominant negative of Rac (N17Rac), one of Rho family proteins, stimulates osteoblastic bone formation in vitro. In this study, we tried to generate N17Rac transgenic mice (Tg) by the overexpression of N17Rac in osteoblast-specific manner and characterized the phenotype to examine the role of Rac in bone metabolism.

Materials and Methods

DNA construct which connected type1 collagen promoter with N17Rac was prepared to express N17Rac in osteoblast-specific manner in vivo and injected into a fertile egg to generate Tg. The expression level of N17Rac was analyzed by Northern blot. Bone mineral density (BMD) was measured by pQCT and the bone metabolism on the surface of cancellous bone was estimated by bone histomorphometry. Distribution of mRNA of osteoblastic specific proteins such as type I collagen (Col), osteopontin (Op) and osteocalcin (Oc) assessed in situ hybridization. To assess the spontaneous healing on fracture, we used a model of tibial fracture fixed with a semi-rigid intramedullary pin.

Results

The skeleton of Tg appeared normal at birth and grew up above 12-weeks old normally in appearance. There was no difference in between Tg and Wt at 4-weeks old but BMD of Tg significantly decreased at 10-weeks old in comparison with Wt. According to osteopenia, there were some mice broken incisor tooth. In situ hybridization, distribution patterns of osteoblast differentiation factors such as Col, Op and Oc in Tg resembled to those of Wt but the expression contents of these factors increased in Tg by Northern blot analysis. In bone histomorphometry, the parameters of bone formation such as osteoid surface and mineralization surface also increased in Tg. The spontaneous healing on fracture of Tg declined against Wt. In histological evaluation of tooth thin section, Tg disappeared odontoblastic layer and lamella of pre-dentin and lamella of dentin was studded with odontoblastic cells.

Discussion

The inhibition of Rac function generated osteopenic mice which enhanced osteoblastic bone formation. The disturbance of healing on fracture and disappearance of odontoblastic layer may indicate the dysfunction of bone remodeling. There is no distinct relationship between anti-osteopenic effect of statin and inhibition of Rac function but these results suggest the presence of stimulating signal on osteoblast differentiation in the lower pathway of Rac.