ATTENUATION OF BONE MASS AND INCREASE OF OSTEOCLAST FORMATION IN DECOY RECEPTOR 3 TRANSGENIC MICE

INTRODUCTION: Decoy receptor 3 (DcR3), a soluble receptor for FasL, LIGHT and TLI-A, induces osteoclast formation from monocytes, macrophages and bone stromal marrow cells. However, the function of DcR3 on bone formation remains largely unknown. To investigated the function of DcR3 in bone formation in vivo, transgenic mice over-expressing DcR3 were generated. Bone mineral density (BMD) and bone mineral content (BMC) of total body were significantly lower in DcR3 transgenic mice compared with wild-type controls. The number of osteoclast increased in DcR3 transgenic mice. These results indicate that DcR3 may play an important role in osteoporosis or other bone diseases.

METHODS: The DcR3 heterozygous transgenic mice is generated from a BALB/c background for at least six generations. The coding sequence of human DcR3 is subcloned into pPGK-Neo-bpA to regulate the expression of DcR3 ubiquitously under the control of PGK promoter. DcR3 transgenic mice were screened by PCR using the primers pGKP (sense; 5'-GCCAATTGCAGGCTTCTGTC-3') and DcR3-207 (antisense; 5'-TAGTGGGTTTCTGCAC-3'), which amplify a 350 bp fragment from the pPGK-DcR3 transgene. The DcR3 transgene-negative litters were used as wild-type mice.

Local administration of DcR3 into tibia in young rats: Male Sprague-Dawley rats weighing 75-90 grams were used. Implantation of a cannula (22 gauge) in young rats weighing 75 to 90 g and the rats were sacrificed later on Day-14. Bone mineral density (BMD) and the bone mineral content (BMC) of the tibia were measured with a dual-energy X-ray absorptiometer (DXA). The tibiae were then fixed in 10% ice-cold formaldehyde for 48 h at 4°C for bone histomorphometry analysis. Serial sections (5 μm) of the tibia were cut longitudinally and stained with Mayer’s hematoxylin–eosin solution. The bone volume of the secondary spongiosa was measured.

Osteoclast generation: Bone marrow cells were prepared by removing femurs and tibiae of adult mice and flushing the bone marrow cavity with a MEM which was supplemented with 20 mM HEPES, 10% heat-inactivated FBS, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml). The non-adherent cells (hematopoietic cells) were collected after 24 hr and used as osteoclast precursors. Cells were seeded at 1×10⁶ cells/well in 24-well plates in the presence of human recombinant soluble RANKL (50 ng/ml) and M-CSF (20 ng/ml). The culture medium was replaced every 3 days. Osteoclast formation was measured by TRAP staining on Day-2, 4, 6 and 8, respectively. In brief, adherent cells were fixed with 10% formaldehyde in PBS for 3 min and then stained with Naphthol AS-MX phosphate and tartrate solution for 1 hr at 37°C. Osteoclast-like cells in each well were scored by counting the number of TRAP-positive and multinucleated cells containing more than three nuclei.

RESULT: To understand the function of DcR3 in bone remodeling in vivo, transgenic mice over-expressing DcR3 systemically were generated. The adult mice were used and the age, sex and body weight of transgenic mice were matched to the controls. BMD and BMC of total body, as assessed by dual-energy X-ray absorptiometry, were significantly lower (11.9 and 17.1%, respectively) in DcR3 transgenic mice compared with wild-type controls. Bone histomorphometric analysis in tibia showed that DcR3 transgenic mice had a 55.7% decrease in trabecular bone volume compared with wild-type mice (Fig. 1). In addition, we also counted the number of osteoclasts in the region of primary spongiosa. It was found that the number of osteoclasts significantly increased in the DcR3 transgenic mice compared with wild-type control (Fig. 1). To simply look at the effect of exogenous application of DcR3 on trabecular bone, local injection of DcR3 into tibia in young rats was used. DcR3 (30 μg/ml, 10 μl, once/day) was locally administered into tibia for 7 consecutive days via an implantation of a needle cannula (22 gauge) in young rats weighing 75 to 90 g and the rats were sacrificed later on Day-14. The vehicle was injected into contralateral side for comparison. Compared with the vehicle-injected side (Fig. 2A; arrow shows the hole of injection site), DcR3 significantly decreased bone volume of the secondary spongiosa (Fig. 2A). Trabecular bone in the secondary spongiosa decreased by 31.5 % after local administration of DcR3.

DISCUSSION: We demonstrated that the decoy receptor for TNF family cytokines, DcR3, is a novel effector molecule to enhance the formation of osteoclasts and their resorption activity. DcR3 thus has a hitherto unrecognized role in the regulation of bone mass and bone turnover. The novel function of DcR3 demonstrated in this study indicates a possible new role of DcR3 in osteolytic bone metastases of cancer cells, and will be helpful in developing better strategies for the treatment of cancer metastasis in bone.

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