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Cell fusion in osteoclasts plays a critical role in controlling bone mass and osteoblastic activity

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ABSTRACT

The balance between osteoclast and osteoblast activity is central for maintaining the integrity of bone homeostasis. Here we show that mice lacking dendritic cell specific transmembrane protein (DC-STAMP), an essential molecule for osteoclast cell–cell fusion, exhibited impaired bone resorption and upregulation of bone formation by osteoblasts, which do not express DC-STAMP, which led to increased bone mass. On the contrary, DC-STAMP over-expressing transgenic (DC-STAMP-Tg) mice under the control of an actin promoter showed significantly accelerated cell–cell fusion of osteoclasts and bone resorption, with decreased osteoblastic activity and bone mass. Bone resorption and formation are known to be regulated in a coupled manner, whereas DC-STAMP regulates bone homeostasis in an un-coupled manner. Thus our results indicate that inhibition of a single molecule provides both decreased osteoclast activity and increased bone formation by osteoblasts, thereby increasing bone mass in an un-coupled and a tissue specific manner.

Bone homeostasis is maintained through continuous remodeling by osteoclasts, bone resorbing cells, and osteoblasts, bone forming cells. Bone resorption by osteoclasts reduces bone volume, however, osteoclast activity stimulates bone formation by osteoblasts, and is required for appropriate bone formation [1]. Defects of osteoclast activity result in abnormal bone formation as in osteopetrosis [2], while increased bone resorption relative to bone formation induces osteoporosis [3]. Therefore, the balance between osteoclast and osteoblast activity is crucial for maintaining bone volume and the integrity of bone homeostasis [4]. Osteoblastic activity occurs in parallel with osteoclastic activity, and the balance between osteoclasts and osteoblasts is called 'coupling' [5]. However, over-activation of osteoclasts decreases bone mass, although osteoblastic activity is also over-activated [3]. Thus, a target molecule for 'un-coupling' which would inhibit osteoclast bone resorption but stimulate bone formation by osteoblasts is sought as it is believed it would increase bone mass.

Osteoclasts are multinuclear giant cells derived from hematopoietic stem cells or monocyte/macrophage progenitor cells [6]. The multinucleation of osteoclasts is induced by a cell–cell fusion of mononuclear osteoclasts. Various molecules have been identified that are involved in the cell–cell fusion of osteoclasts or macrophage giant cells [7–9], and we identified dendritic cell specific transmembrane protein (DC-STAMP), a seven transmembrane protein, as an essential molecule for the cell–cell fusion of osteoclasts and macrophage giant cells [10,11]. DC-STAMP-deficient mice showed a complete lack of cell–cell fusion in osteoclasts as well as in macrophage giant cells [10,11]. Interestingly, the bone resorbing activity of DC-STAMP-deficient mononuclear osteoclasts was lower than that of wild-type multinucleated osteoclasts, even though DC-STAMP-deficient osteoclasts did not show any defects related to the expression of cathespin K, a terminal differentiation marker of osteoclasts, or the enzymatic activity of TRAP [10]. This suggests that DC-STAMP regulates osteoclast cell–cell fusion rather than differentiation, and that through the multinucleation of osteoclasts, DC-STAMP enhances the activity of bone resorption.

In the present study, we generated DC-STAMP transgenic (DC-STAMP-Tg) mice under the control of an actin promoter. The cell–cell fusion of osteoclasts was stimulated in the osteoclasts de-

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rived from the DC-STAMP-Tg mice. Interestingly, accelerated bone resorption, decreased bone mass, and reduced osteoblast activity were seen in the DC-STAMP-Tg mice. Meanwhile, reduced bone resorption by osteoclasts, and increased osteoblast activity and bone mass were observed in DC-STAMP-deficient mice, suggesting that DC-STAMP regulates the function of osteoblasts, which do not express DC-STAMP, in an un-coupled manner. DC-STAMP expression was detected ubiquitously in various tissues in DC-STAMP-Tg mice, but ectopic cell–cell fusion was not evident, and cell–cell fusion defects in non-osteoclasts are not observed in DC-STAMP-deficient mice, suggesting that the cell–cell fusion of osteoclasts through DC-STAMP was regulated in a cell type specific manner. This will provide new insight into bone homeostasis by regulating both osteoclast and osteoblast activity in an un-coupled and tissue specific manner.

Materials and methods

Generation of DC-STAMP transgenic (DC-STAMP-Tg) mice. Animals were cared for in accordance with the guidelines of Keio University School of Medicine. The mouse *DC-STAMP* open reading frame beginning with a suitable Kozak sequence (*DC-STAMP* fragment) followed by a FLAG sequence was PCR amplified. A *DC-STAMP-FLAG* fragment was inserted between the chicken *actin* (CAG) promoter and bovine poly A sequence in the pBS vector to yield *CAG-DC-STAMP-polyA*. Transgenic mice were generated in a Slc:BDF1 background by transgene microinjection (Japan SLC, Shizuoka, Japan), and injected embryos were implanted into pseudopregnant Slc:ICR females. The presence of the transgene was evaluated by PCR for mouse tail DNA using the following primer set: forward primer: 5′-GAATTCATGAGGCTCTGGACCTTGGGCACCAGT-3′, reverse primer: 5′-GTCGACTTATTTGTCGTCATCATCCTTATAGTC-3′. The DC-STAMP-Tg mice were backcrossed with C57/Black-background and used for analysis.

In vitro osteoclastogenesis assay. Bone marrow mononuclear cells were isolated from DC-STAMP-deficient, DC-STAMP-Tg, and wild-type mice and osteoclastogenesis was performed as previously described [12,13].

Western blot analysis. Total lysates were collected from mouse tails or cultured cells, and western blot analysis was performed as described [14,15] using polyclonal antibodies to detect Flag and Actin (both antibodies were from Sigma).

Analysis of skeletal morphology. Bone mineral density (BMD: mg/cm^2) of 8-week-old DC-STAMP-deficient, -Tg, and wild-type mice as measured by the dual energy X-ray absorptiometry (DEXA) method using DCS-600R (Aloka Co. Ltd., Tokyo, Japan). Bone parameters were determined by bone morphometric analysis.

RT-PCR analysis. Total RNA was extracted from cultured macrophages, osteoclasts, and macrophage giant cells using an RNeasy mini kit (Qiagen GmbH, Hilden, Germany), or from tissues using a Trizole (Invitrogen). First strand cDNA was prepared and PCR was performed as previously described [10].

Real-time PCR was performed in a Thermal cycler Dice Real-Time system (Takara bio, Tokyo, Japan). The primer sets for real-time PCR were as below:

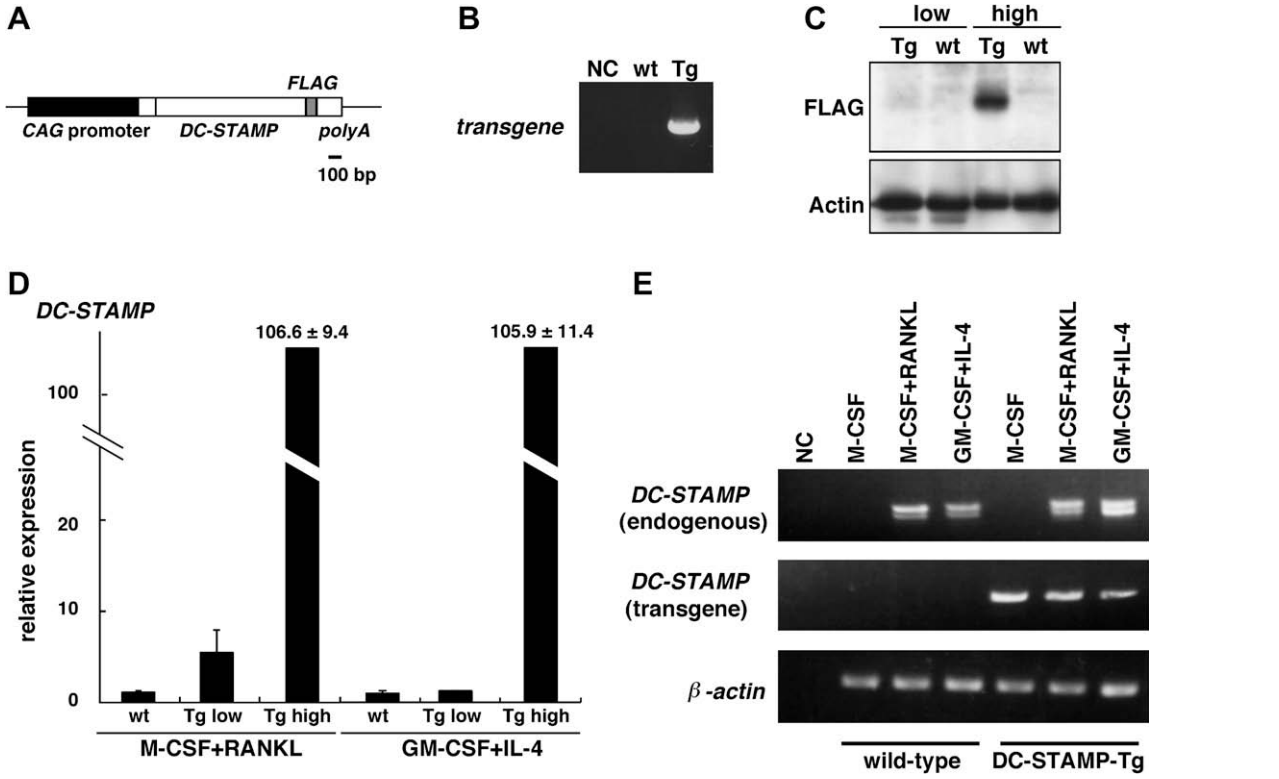

Fig. 1. Generation of DC-STAMP-Tg mice. (A) Schematic contrast of the DC-STAMP-Tg transgene. (B) Genotyping of DC-STAMP-Tg F0 founders. Mouse tail DNA was extracted from each mouse, and genomic PCR to detect the *DC-STAMP* transgene was performed. Representative data are shown. (C) BM cells were isolated from independent DC-STAMP-Tg lines, and were cultured in the presence of M-CSF (50 ng/ml). After 3 days of culture, total lysates were extracted from M-CSF-dependent adherent cells and western blot analysis for Flag and the internal control, Actin, was undertaken. The expression of DC-STAMP-Flag protein varied between Tg lines. (D) Osteoclasts were generated from DC-STAMP low and high expressing lines or wild-type mice, and real-time PCR analysis was undertaken to determine the mRNA levels of *DC-STAMP* relative to β-*actin*. Data are means ± sd of *DC-STAMP* expression relative to β-*actin* in osteoclasts (M-CSF + RANKL) or macrophage giant cells (GM-CSF + IL-4) derived from the DC-STAMP-Tg high or low expressing mice relative to those of the wild-type mice ($^*P < 0.01$). (E) Combined expression of endogenous and transduced *DC-STAMP* in DC-STAMP-Tg cells. RT-PCR analysis of endogenous and transduced *DC-STAMP*. β-*actin* expression was analyzed as an internal control.