Osteocyte-specific Cas knockout mice exhibit decreased bone mass through increased osteoclastic bone resorption

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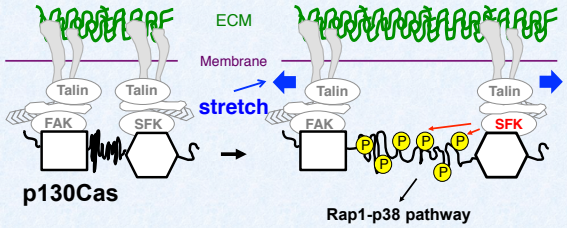
Introduction

The skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. Osteoporosis, which is fostered by advancing age, is the most common clinical disorder affecting bones. Although it has been postulated that osteocytes play an important role in sensing mechanical load in bone tissues, detailed molecular mechanisms of how osteocytes regulate bone metabolism remain largely unclear.

The adaptor molecule p130Cas (Crk-associated substrate, hereafter referred to as Cas), which is phosphorylated at focal adhesions upon extracellular matrix engagement, is involved in various cellular processes including migration, survival, transformation, and invasion. It is composed of multiple functional domains, including the amino-terminal SH3 domain (CasSH3), the central substrate domain (CasSD) and the carboxy-terminal Src-binding domain (CasSBD). CasSH3 interacts with various proteins including focal adhesion kinase (FAK). CasSD comprises fifteen YxxP motifs which are the major sites of Src family kinase-mediated tyrosine phosphorylation. By stretching detergent-insoluble cytoskeletal complexes as well as single molecules in vitro, we identified that Cas can function as an ion channel-independent initiator of intracellular signaling cascades through force-dependent changes in the cytoskeleton network. However, it remains unclear whether Cas acts as a mechano-sensor in vivo.

Here, we report the interesting findings that the bone loss in osteocyte-specific *Cas* knockout mice was caused by increased bone-resorbing activity and that osteoclasts are the primary effector cells controlled by the Cas-mediated regulatory function of osteocytes.



ECM: extracellular matrix, FAK: focal adhesion kinase, SFK: Src-family kinase

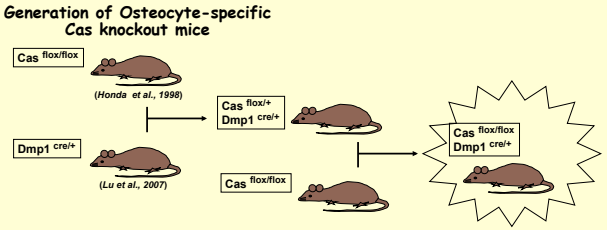
Methods

Generation of Cas conditional knockout mice

Osteocyte-specific *Cas* conditional knockout (cKO) mice were generated by mating $Cas^{flox/flox}$ mice with *Dentin matrix protein 1 (Dmp1)-Cre* transgenic mice, in which the Cre recombinase gene was specifically expressed in osteocytes. The resulting $Dmp1\text{-}Cre^{+/-};Cas^{flox/flox}$ mice (referred to herein as *Cas* cKO mice) were born alive at predicted Mendelian frequencies.

Analysis of bone phenotype

Radiography was performed using a high-resolution soft X-ray system (Softex). Microcomputed tomography (μCT) scanning was performed using a ScanXmate-L090 Scanner (Comscan Techno). Three-dimensional microstructural image data were reconstructed, and structural indices were calculated using TRI/3D-BON software (RATOC). Bone histomorphometric analyses were performed using Osteomeasure software.



Results

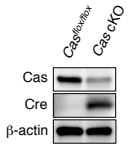


Figure 1. Osteocyte-*specific Cas* knockout.
Western blotting of Cas and Cre recombinase in *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates using β-actin as an internal control. Cas expression was markedly reduced in osteocytes of *Cas* cKO mice.

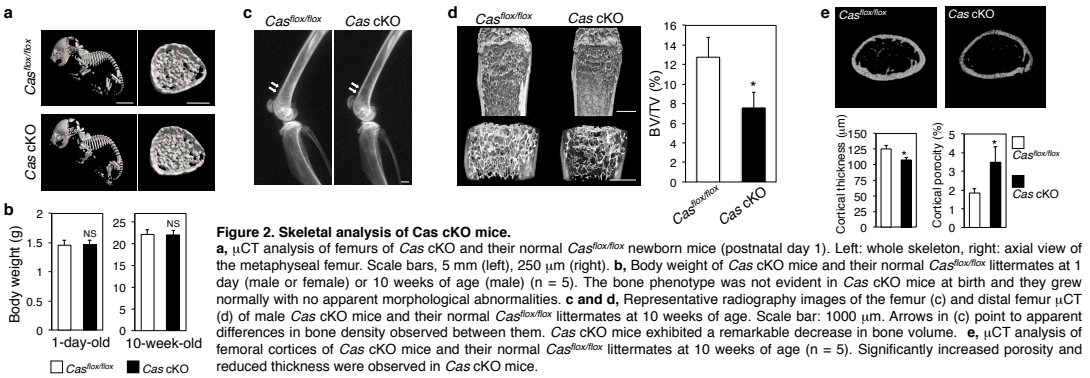


Figure 2. Skeletal analysis of Cas cKO mice.
a, μCT analysis of femurs of *Cas* cKO and their normal $Cas^{flox/flox}$ newborn mice (postnatal day 1). Left: whole skeleton, right: axial view of the metaphyseal femur. Scale bars, 5 mm (left), 250 μm (right). **b,** Body weight of *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates at 1 day (male or female) or 10 weeks of age (male) (n = 5). The bone phenotype was not evident in *Cas* cKO mice at birth and they grew normally with no apparent morphological abnormalities. **c and d,** Representative radiography images of the femur (c) and distal femur μCT (d) of male *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates at 10 weeks of age. Scale bar: 1000 μm. Arrows in (c) point to apparent differences in bone density observed between them. *Cas* cKO mice exhibited a remarkable decrease in bone volume. **e,** μCT analysis of femoral cortices of *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates at 10 weeks of age (n = 5). Significantly increased porosity and reduced thickness were observed in *Cas* cKO mice.

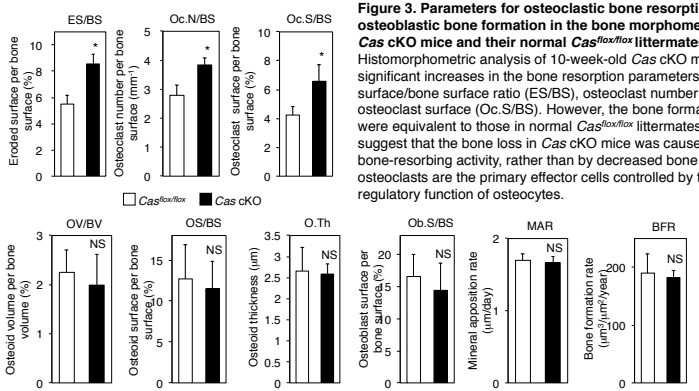


Figure 3. Parameters for osteoclastic bone resorption and osteoblastic bone formation in the bone morphometric analysis of *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates.
Histomorphometric analysis of 10-week-old *Cas* cKO mice revealed significant increases in the bone resorption parameters, such as eroded surface/bone surface ratio (ES/BS), osteoclast number (Oc.N/BS), and osteoclast surface (Oc.S/BS). However, the bone formation parameters were equivalent to those in normal $Cas^{flox/flox}$ littermates. These findings suggest that the bone loss in *Cas* cKO mice was caused by increased bone-resorbing activity, rather than by decreased bone formation, and that osteoclasts are the primary effector cells controlled by the Cas-mediated regulatory function of osteocytes.

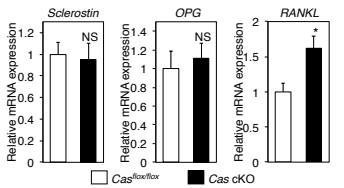


Figure 4. The mRNA expression levels of *Sost, OPG, and RANKL* genes in the osteocyte fractions derived the femurs and tibiae of 10-week-old male *Cas* cKO mice and their normal $Cas^{flox/flox}$ littermates.
Real-time RT-PCR analysis showed increased expression level of RANKL despite there being no significant alteration of OPG expression in *Cas* cKO mice, indicating that the RANKL/OPG ratio favored bone resorption in *Cas* cKO mice as compared with the control mice. This is consistent with our histomorphometric analysis that *Cas* cKO mice exhibited a significant increase in parameters of osteoclastic bone resorption.

Conclusion

In this study, we report the interesting finding that histomorphometric analysis of *Cas* cKO mice revealed significant increases in the bone resorption parameters. In addition, osteocyte-specific *Cas* deficiency increased RANKL expression without affecting the expression levels of OPG or sclerostin, indicating that osteoclasts are the primary effector cells controlled by the Cas-mediated regulatory function of osteocytes. These observations point to what we believe to be a novel link between mechanosensor Cas and RANKL expression in osteocytes. Further investigation of Cas-mediated regulation of RANKL expression in osteocytes will give us new insights into the molecular mechanism regulating bone homeostasis.