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Extended Abstract

Osteocyte-specific Cas knockout mice exhibit decreased bone mass through increased osteoclastic bone resorption Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Japan

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Abstract

The skeleton could be a metabolically active organ that undergoes continuous remodeling throughout life. Osteoporosis, which is fostered by advancing age, is that the most typical clinical disorder affecting bones. Although it's been postulated that osteocytes play a crucial 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 stated as Cas), which is phosphorylated at focal adhesions upon extracellular matrix engagement, is involved in various cellular processes including migration, survival, transformation, and invasion. additionally, we reported that Cas binds to the cytoskeletons in an exceedingly stretch-dependent manner. this means that Cas can function as an initiator of intracellular signaling cascades through force-dependent changes within the cytoskeleton network. to research the role of Cas in bone metabolism, we generated osteocyte-specific Cas conditional knockout (cKO) mice by mating Casflox/flox mice with Dentin matrix protein 1 (Dmp1)-Cre transgenic mice, within which the Cre recombinase gene was specifically expressed in osteocytes. The resulting Dmp1Cre+/–; Casflox/flox mice (referred to herein as Cas cKO mice) exhibited a big decrease in bone volume, as determined by μCT analysis. Histomorphometric analysis of Cas cKO mice revealed a big increase within the eroded surface/bone surface ratio, osteoclast surface, and osteoclast number. Furthermore, the expression levels of RANKL genes were significantly increased within the osteocyte fractions derived from Cas cKO mice. Collectively, these findings suggest that the bone loss in Cas cKO mice was caused by increased osteoclatstic bone resorption. Under physiologic conditions, interactions between cells present within the bone remodeling compartment (BRC) lead to a balanced and matched remodeling of bone, a lifelong process to blame for bone damage repair and mineral homeostasis. one in all the functions of the osteocyte network is to detect microdamage and trigger its repair ; consequently, osteocytes have a prominent role within the initiation of bone remodeling at selected sites of the skeleton. Increased osteocyte apoptosis has been shown to be induced in vivo in areas of microdamage in several pathological conditions related to enhanced bone resorption like overload, bone disuse, glucocorticoid administration or estrogen deficiency suggesting that apoptosis is linked to targeted bone resorption . per this notion, exclusive induction of apoptosis of osteocytes is sufficient to extend resorption and ends up in bone loss. it's been proposed that apoptotic osteocytes trigger a cascade of signals initiated within the endosteum that contains the liner cells, which lift from the bone surface to create the cover that encloses the BRC. Osteocytes are now considered a significant source in bone of the pro-osteoclastogenic cytokine RANKL, a master stimulator of osteoclastogenesis. Indeed, osteocytes express higher levels of RANKL than osteoblasts and bone marrow stromal cells, and mice lacking the RANKL gene in osteocytes exhibit an osteopetrotic phenotype characterized by reduced bone resorption. However, it's still under debate whether the osteocytic membrane-bound or the soluble RANKL form is to blame for osteocyte-mediated bone resorption. Recent data suggest that osteocytic RANKL is predominantly provided as a membrane-bound form to osteoclast precursors through osteocyte cytoplasmatic projections, whereas the contribution of soluble RANKL to osteoclastogenesis is minor . Thus, direct cell-to-cell

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contact between membrane-bound RANKL expressing osteocytes and osteoclast precursors appears to be required to initiate osteoclast development. Osteocytes also secrete the anti-osteoclastogenic cytokine OPG, a soluble decoy receptor that competes with RANKL for its receptor tumor necrosis factor receptor superfamily, member 11a, NFKB activator (RANK) on osteoclast precursors. OPG secretion is regulated by the Wnt/β-catenin pathway and mice lacking β-catenin in osteocytes exhibit increased osteoclast numbers and bone resorption RANKL and M-CSF are two major factors that contribute to osteoclast differentiation and maturation. Recent evidence suggests that osteocytes are a further source of secreted M-CSF in bone . Moreover, M-CSF expression is increased by PTH receptor signaling in osteocytes and exhibits similar pattern of expression to RANKL expression . Further, deletion of the distal control region of the RANKL gene regulated by PTH (DCR) eliminates the power of the PTH receptor/cAMP pathway to extend not only RANKL expression, needless to say, but also M-CSF expression, demonstrating that M-CSF regulation is secondary to RANKL regulation . This evidence is that the first in vivo demonstration of M-CSF regulation by RANKL and is in keeping with an earlier in vitro study showing that RANKL augmented M-CSF production in pre-osteoclastic cells. The regulation of M-CSF by RANKL appears counter-intuitive, since M-CSF increases the expression of the RANKL receptor RANK in osteoclast precursors, suggesting that M-CSF is required for RANKL signaling. However, deletion of the DCR only reverses the PTH-induced M-CSF expression demonstrating that basal levels of M-CSF are sufficient to induce RANK expression and to permit the initial action of PTH-stimulated osteocytic RANKL on bone resorption. RANKL/RANK signaling successively could induce higher M-CSF expression in a very feedback loop, stimulating osteoclastogenesis. Further, M-CSF is expressed not only in osteoclast precursors but also in osteocytes suggesting that RANKL (either membrane-bound or soluble form) could upregulate M-CSF organic phenomenon in osteoclast precursors or in osteocytes. Future studies are required to see the cellular source of M-CSF in osteocyte-driven resorption induced by PTH receptor activation. Besides the strong evidence demonstrating that osteocytes support osteoclast development, novel findings suggest that osteocytes, as osteoclasts, may also remove mineralized matrix by remodeling their perilacunar/canalicular matrix. The expression of genes known to be utilized by osteoclasts to get rid of bone, including tartrate-resistant acid phosphatase (TRAP) and cathepsin K, carbonic anhydrase 2 and matrix metalloproteinases is elevated in osteocytes from lactating mice. Furthermore, recent work demonstrated that global deletion of the calcitonin receptor ends up in increased osteocytic, but not osteoclastic, osteolysis during lactation, providing the primary evidence for a physiological role of the calcitonin receptor to safeguard the maternal skeleton from bone loss during lactation by direct actions on osteocytes . Although the physiologic implications of those observations remain to be determined, this data suggests that, as previously described for phosphate metabolism, osteocytes also contribute to calcium mobilization and mineral homeostasis through perilacunar remodelling.

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