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Dynein Light Chain LC8 Inhibits Osteoclast Differentiation and Prevents Bone Loss in Mice

Hyeryeon Kim,* Seungha Hyeon,* Hojin Kim,* Yoohee Yang,* Ji Young Huh,* Doo Ri Park,* Hyojung Lee,* Dong-Hyun Seo,† Han-Sung Kim,‡ Soo Young Lee,* and Woojin Jeong*

NF-κB is one of the key transcription factors activated by receptor activator of NF-κB ligand (RANKL) during osteoclast differentiation. The 8-kDa dynein L chain (LC8) was previously identified as a novel NF-κB regulator. However, its physiological role as an NF-κB inhibitor remains elusive. In this study, we showed the inhibitory role of LC8 in RANKL-induced osteoclastogenesis and signaling pathways and its protective role in osteoelastic animal models. LC8 suppressed RANKL-induced osteoclast differentiation, actin ring formation, and osteoclastic bone resorption. LC8 inhibited RANKL-induced phosphorylation and subsequent degradation of IκBα, the expression of c-Fos, and the consequent activation of NFATc1, which is a pivotal determinant of osteoclastogenesis. LC8 also inhibited RANKL-induced activation of JNK and ERK. LC8-transgenic mice exhibited a mild osteopetrotic phenotype. Moreover, LC8 inhibited inflammation-induced bone erosion and protected against ovariectomy-induced bone loss in mice. Thus, our results suggest that LC8 inhibits osteoclast differentiation by regulating NF-κB and MAPK pathways and provide the molecular basis of a new strategy for treating osteoporosis and other bone diseases. The Journal of Immunology, 2013, 190: 000–000.

Bone undergoes a metabolic remodeling process that depends on a balance between the breakdown (resorption) by osteoclasts and the synthesis (formation) of bone by osteoblasts (1). Most bone diseases such as osteoporosis and rheumatoid arthritis are caused by excessive osteoclastic activity, which leads to an imbalance between resorption and formation during bone remodeling that favors resorption (2). Thus, the regulatory mechanisms of osteoclast differentiation have significant clinical implications.

Receptor activator of NF-κB ligand (RANKL) (3), also known as TNF-related activation-induced cytokine (4), osteoclast differentiation factor (5), and osteoprotegerin ligand (6), is an essential molecule for osteoclast differentiation. Specifically, RANKL is expressed in osteoblasts, activated T cells, and stromal cells (7) and stimulates morphologic changes, cell survival, and osteolytic activity in osteoclasts, in cooperation with M-CSF (8). The binding of RANKL to its receptor, RANK, induces the recruitment of TNFR-associated factor (TRAF) family proteins such as TRAF6, which activates the NF-κB and MAPK pathways, including JNK, ERK, and p38 (9, 10).

TRAF6-activated NF-κB induces the initial expression of NFATc1, a key determinant of osteoclast differentiation. RANKL also induces the expression of c-Fos, which is involved in the robust induction of NFATc1 as a component of the AP-1 transcription factor family (11). NFATc1 cooperates with AP-1, PU.1 transcription factor, and microphthalmia-associated transcription factor to induce expression of several osteoclast-specific genes, such as cathepsin K, tartrate-resistant acid phosphatase (TRAP), calcitonin receptor, and osteoclast-associated receptor (9, 10). Previous studies have shown that c-Fos and NFATc1 play critical roles in osteoclast differentiation and bone resorption and that NFATc1 is the downstream target of c-Fos during osteoclastogenesis: c-Fos–knockout mice were deficient in osteoclasts and exhibited an osteopetrotic phenotype (12), but ectopic expression of NFATc1 in c-Fos–deficient cells restored the transcription of osteoclastogenesis-associated genes and resumed osteoclast formation and bone resorption (13).

The 8-kDa dynein L chain (LC8; also known as DLC8 or DLC1) is necessary for intracellular motile processes such as mitosis and vesicular transport as an essential component of microtubule-based motor dynein (14, 15). In addition, LC8 binds to and regulates the biological functions of many proteins (16–23). In a previous study, we identified LC8 as a potential substrate of 14-kDa human thioredoxin-related protein (TRP14), a disulfide reductase that inhibits TNF-α–induced NF-κB activation by suppressing the phosphorylation of IκBα (24). More recently, we showed that LC8 inhibits IκBα phosphorylation by IκB kinase (IKK) via its redox-dependent interaction with IκBα and that TRP14 regulates this inhibitory activity by facilitating the reduction of LC8 (25, 26).

Several genetic studies have shown that the NF-κB plays a crucial role in osteoclast differentiation. NF-κB p50 and p52 double-
knockout mice (27) and IKB-deficient mice (28) had severe osteoporosis due to the inhibition of osteoclast formation. In addition, dominant-negative IκB proteins (29) and NF-κB inhibitors (30) were shown to block osteoclastogenesis. Moreover, previous studies have shown that LC8 inhibits NF-κB activation by potent NF-κB stimulators, including TNF-α, LPS, and IL-1β (25, 26). Therefore, we sought to determine whether LC8 inhibits RANKL-induced NF-κB activation and subsequent osteoclast differentiation and bone resorption using transgenic (Tg) mice with ectopic expression of LC8. An inhibitory role of LC8 in osteoclast differentiation and bone resorption might provide the molecular basis of a new strategy for treating osteoporosis and arthritic bone diseases.

Materials and Methods

Reagents and Abs

Human recombinant RANKL and M-CSF proteins were purchased from PeproTech and R&D Systems, respectively. A rabbit polyclonal Ab against β-actin was purchased from Abcam. Rabbit polyclonal Ab against phospho-IκBα, phospho-JNK, phospho-ERK, and phospho-p38 was purchased from Cell Signaling Technology. Rabbit polyclonal Abs against IκBα, JNK1, p38, c-Fos, and LC8, a goat polyclonal Ab against cyclooxygenase-2 (COX-2), and mouse mAbs against tubulin, ERK2, and NFATc1 were purchased from Santa Cruz Biotechnology. LPS (from Escherichia coli 0127:B8) was purchased from Sigma-Aldrich.

Construction of LC8 expression vectors

The DNA sequence encoding hemagglutinin (HA)-tagged LC8 was amplified via the PCR using the pCGN-LC8 vector (25, 26) as a template with a forward primer (5′-CG GAA TTC ATG GCT TCT AGC TAT CCT TAT GAC G-3′) containing both an EcoRI site (underlined) and the initiation codon (bold) and a reverse primer (5′-GG GGT ACC TTA ACC AGATTT GAA CAG AAG GAT G-3′) containing both a Kpnl site (underlined) and the stop codon (bold). The PCR product was purified, digested with restriction enzymes EcoRI and Kpnl, and cloned into the corresponding sites of the pCBA-M vector. The resulting plasmid, pCBA-HA-LC8, was used to generate Tg mice with overexpression of LC8.

Generation and genotyping of LC8 Tg mice

pCBA-HA-LC8 was digested with the restriction enzymes PvuI, SalI, and PstI, and the resulting 2.6-kb DNA fragment containing the LC8 gene under the control of a chicken β-actin promoter and a CMV enhancer was purified from agarose gel. The linear DNA fragment was injected into the pronuclei of fertilized eggs of C57BL/6 mice. The injected embryos were implanted into the uterus of pseudopregnant C57BL/6 mice to generate LC8 Tg mice. The genotypes of all of the mice were determined via PCR analysis using the tail DNA (500 ng) and the following primer pair: 5′-CCT ACA GCT CCT GGG CAA CG-3′ and 5′-GAG CCA GAG CAT TGG CCA AC-3′. PCR was performed at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s for a total of 32 cycles, and the resulting products were analyzed via 1.5% agarose gel electrophoresis to identify the 455-bp DNA.

Preparation of bone marrow–derived macrophages

Bone marrow–derived macrophages (BMMs) were prepared as osteoclast precursor cells from the femurs and tibiae of 4–8-wk-old C57BL/6 male mice as described below. Bone marrow cells were flushed from the bone marrow cavity using anti-MEM supplemented with 10% FBS and 20 ng/ml M-CSF for 3–5 d. After osteoclast differentiation, the cells were washed twice with 1× PBS, fixed for 10 min with 4% paraformaldehyde, and stained for TRAP using a leukocyte acid phosphatase cytochemistry kit (Sigma-Aldrich) according to

the manufacturer's instructions. TRAP-positive multinucleated cells (TRAP+ MNCs) containing three or more nuclei were counted as osteoclasts under a light microscope.

Actin-ring staining

BMMs were fixed with 3.7% formaldehyde solution in PBS, permeabilized with 0.1% Triton X-100, and incubated with Alexa Fluor 488-phalloidin (Invitrogen) for 20 min. After being washed with PBS, the cells were incubated with DAPI (Roche) for 2 min and then photographed under a fluorescence microscope.

Bone resorption assay

BMMs were plated onto dentine discs (Immunodiagnostic Systems) and treated with 20 ng/ml M-CSF and 100 ng/ml RANKL for 7 d. The cells were completely removed from the dentine discs via abrasion with a cotton tip, and the dentine discs were stained with hematoxylin. Photographs of resorption pits were taken under a light microscope at ×40 original magnification, and their area was measured via Image-Pro Plus 4.5 software (Media Cybernetics).

TRAP activity assay

BMMs were loaded onto a 12-well plate and lysed in 100 mM sodium acetate buffer (pH 5.2) containing 10 mM sodium tartrate, 1% Triton X-100, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. After centrifugation, the protein concentration of the supernatant was determined using Bradford reagent, and TRAP activity was assessed via a standard assay that uses p-nitrophenyl phosphate as a substrate. The 120-μl reaction mixture containing 100 mM sodium acetate (pH 5.2), 10 mM sodium tartrate, 1 mg/ml p-nitrophenyl phosphate, and 2 μg total protein was incubated at 37°C for 2 h, and the reaction was stopped by adding 80 μl 1 N NaOH. The amount of p-nitrophenol liberated during the reaction was determined by measuring the absorbance at 405 nm in a 96-well plate. TRAP activity was expressed as the specific TRAP activity (405 nm/min/mg protein).

Calvarial injection and histologic analysis

The LC8 Tg mice were injected with a 28-gauge needle with 15 mg/kg body weight of LPS or PBS, both in a 40-μl volume, at a point on the middle of the skull in the space between the s.c. tissue and the peristeum. All animal study protocols were approved by the Animal Care Committee of the Ewha Laboratory Animal Genomics Center. The mice were killed in a CO2 chamber 7 d after the injection. The calvariae were dissected and fixed with 4% paraformaldehyde at room temperature overnight. The specimens were washed with PBS and decalcified with 0.5 M EDTA for 5 d. Then, the specimens were embedded in low-melting paraffin, cut into 4-μm sections, and stained for TRAP.

Ovariectomy and micro-computed tomography imaging

Ovariectomy (O VX) and micro-computed tomography (μCT) were carried out as previously described (31). Female 8-wk-old wild-type and LC8 Tg mice underwent either sham operation or O VX. At least four mice in each group were examined. The mice were killed 4 wk after surgery, and the femurs were evaluated using μCT.

Statistical analysis

Data are presented as the means ± SD. Unless otherwise stated, all experiments were repeated at least two times, and the results from one representative experiment are reported. Statistical significance was calculated using the Student t test, and p values <0.05 were considered statistically significant.

Results

LC8 inhibits RANKL-induced osteoclast differentiation

To assess the role of LC8 in RANKL-induced osteoclast differentiation, LC8 Tg mice were generated by injecting a linear DNA fragment containing the LC8 gene under the control of a chicken β-actin promoter and a CMV enhancer (Fig. 1A) into the pronuclei of fertilized eggs of C57BL/6 mice. Three lines (18, 79, and 83) of LC8 Tg mice were identified via genotype analysis of the offspring of surrogate mothers implanted with injected embryos (Fig. 1B). HA-tagged LC8 was expressed in all of the tested tissues of the LC8 Tg mice (line 83) (Fig. 1C).

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In BMMs of LC8 Tg mice line 83, HA-tagged LC8 was expressed at a higher rate than endogenous LC8 (Fig. 2A). When RANKL induced the differentiation of the BMMs to osteoclasts in the presence of M-CSF, the formation of TRAP+ MNCs was significantly lower in LC8-overexpressing BMMs than in wild-type BMMs (Fig. 2B, 2C). In addition, tests involving another LC8 Tg mouse line 18 yielded similar results (data not shown). These results suggest that LC8 inhibits RANKL-induced osteoclast differentiation of primary osteoclast precursors.

**LC8 inhibits RANKL-induced actin-ring formation and bone resorption**

Mature osteoclasts have a sealing zone that consists of a ring of F-actin that is required for bone resorption and have clear margins (32). Therefore, we next determined whether LC8 reduces actin-ring formation and bone resorption. Wild-type BMMs formed actin rings with clear margins in the presence of RANKL, but actin-ring formation was either reduced or absent from LC8-overexpressing BMMs (Fig. 3A). To determine whether the inhibition of actin-ring formation by LC8 affects the resorption ability of osteoclasts, we induced differentiation of BMMs into osteoclasts in dentine discs in the presence of RANKL. Consistent with its inhibitory effect on actin-ring formation, LC8 overexpression markedly reduced the formation of resorption pits (Fig. 3B). These results suggest that LC8 inhibits actin-ring formation and bone resorption.

**LC8 inhibits RANKL-induced NF-κB activation**

To determine whether LC8 inhibits osteoclastogenesis by suppressing NF-κB activation, we assessed the effects of LC8 overexpression on RANKL-induced NF-κB activation in BMMs. In response to potent NF-κB activators such as TNF-α, IκBα is phosphorylated at residues Ser32 and Ser36 by IKK and subsequently degraded by the ubiquitin–proteasome system (33).
vious studies showed that LC8 blocked IκBα phosphorylation by IKK in TNF-α-treated cells (25). Therefore, we examined the effect of LC8 on IκBα phosphorylation and degradation in BMMs stimulated with RANKL. The level of serine phosphorylation of IκBα in LC8-overexpressing BMMs was considerably lower than that in wild-type BMMs, and this effect was accompanied by a decrease in the rate of IκBα degradation (Fig. 4A, 4B). We also determined the effect of LC8 on the induction of the expression of COX-2, one of the NF-κB target genes. Consistently, LC8 overexpression markedly inhibited the induction of COX-2 expression in RANKL-stimulated BMMs (Fig. 4C). These data indicate that LC8 inhibits RANKL-induced NF-κB activation by blocking IκBα phosphorylation by IKK as it does in TNF-α signaling.

LC8 suppresses RANKL-induced c-Fos and NFATc1 expression

NF-κB has been known to be activated upstream of c-Fos/NFATc1 during RANKL-induced osteoclast differentiation. Thus, we examined the effect of LC8 overexpression on the expression of c-Fos and NFATc1 in BMMs stimulated with RANKL. RANKL-induced c-Fos expression reached the maximum level after 8 h and was sustained until 24 h in wild-type BMMs, whereas it was markedly decreased in LC8-overexpressing BMMs (Fig. 5A). RANKL robustly induced NFATc1 expression in wild-type BMMs, but this induction was almost abrogated in LC8-overexpressing BMMs (Fig. 5B). Also, a TRAP activity assay revealed that the expression of TRAP, an NFATc1 target gene, was considerably lower in LC8-overexpressing BMMs than in wild-type BMMs (Fig. 5C).

LC8 inhibits RANKL-induced JNK and ERK activation

RANKL also induces the activation of MAPKs including JNK, p38, and ERK, which are known to play crucial roles in osteoclastogenesis (34). Therefore, we sought to determine whether RANKL-induced activation of these MAPKs was affected by LC8 overexpression. MAPKs are activated by dual phosphorylation of threonine and tyrosine residues (Thr183 and Tyr185 for JNK; Thr180 and Tyr182 for p38; and Thr202 and Tyr204 for ERK) within a Thr-X-Tyr motif. Immunoblot analysis with Abs against the dually phosphorylated proteins revealed that the level of RANKL-induced JNK and ERK phosphorylation in LC8-overexpressing BMMs was significantly lower than that in wild-type cells (Fig. 6). However, the extent of p38 phosphorylation was only minimally affected by LC8 overexpression.

LC8 inhibits inflammation- and OVX-induced bone loss

To assess the possibility of using LC8 to treat bone diseases that result from excessive osteoclastic activity, we injected LPS into the calvariae of LC8 Tg mice and examined the effects of LC8 on pathologic osteoclast formation and bone erosion during inflammation. The extent of both bone erosion and the formation of TRAP+ MNCs was significantly lower in LC8 Tg mice than in wild-type mice (Fig. 7A, 7B), which suggests that LC8 inhibits inflammation-induced bone destruction.

In addition, LC8 Tg mice were subjected to an OVX-induced model of postmenopausal osteoporosis. The bone volume, trabecular bone number, and bone mineral density were significantly reduced by OVX in wild-type mice, but such a reduction was observed to a much lesser extent in LC8 Tg mice (Fig. 7C, 7D), suggesting that LC8 inhibits pathologic bone destruction.

**FIGURE 4.** Inhibition of RANKL-induced NF-κB activation by LC8 overexpression. BMMs were exposed to RANKL (100 ng/ml) in the presence of M-CSF (20 ng/ml) for the indicated times. (A) Cell lysates were subjected to immunoblot analysis with Abs against phospho-IκBα, IκBα, LC8, or β-actin. (B) The chemiluminescence signals for phospho-IκBα and IκBα were quantified and normalized according to those of β-actin. (C) The abundance of COX-2, LC8, and β-actin in cell lysates was determined via immunoblot analysis using their specific Abs.

**FIGURE 5.** Suppression of RANKL-induced expression of c-Fos and NFATc1 by LC8 overexpression. BMMs were incubated with M-CSF (20 ng/ml) and RANKL (100 ng/ml) for the indicated times. The abundance of c-Fos (A) and NFATc1 (B) in cell lysates was determined via immunoblot analysis using their specific Abs. (C) The sp. act. of TRAP was determined as described in Materials and Methods. All values represent means ± SD. n = 3. *p < 0.05, **p < 0.005.
In this study, we showed that LC8 inhibits osteoclast differentiation and bone resorption of primary osteoclast precursor cells after RANKL stimulation and that it prevents inflammation- and OVX-induced bone destruction in mice. During the RANKL-induced osteoclast differentiation, LC8 inhibited the phosphorylation and subsequent degradation of IκBα and, consequently, NF-κB activation. Therefore, LC8 likely regulates RANKL-induced NF-κB activation via the same mechanism that it does in TNF-α signaling (25, 26): it inhibits NF-κB activation by interacting with IκBα and consequently preventing IκBα phosphorylation by IKK.

The NF-κB pathway can contribute to RANKL-induced osteoclast differentiation via the expression of NF-κB-dependent genes such as COX-2, c-Fos, and NFATc1. NF-κB-dependent COX-2 expression plays a key role in RANKL-induced osteoclast differentiation by inducing PGE2 production (35). The RANKL-responsive region of the COX-2 promoter contains an NF-κB site. Blockade of COX-2 by celecoxib inhibits osteoclast differentiation of osteoclast precursor cells, and this inhibition can be rescued by adding exogenous PGE2.

NF-κB induces the initial expression of NFATc1, which is a master regulator of osteoclastogenesis both in vitro and in vivo (9). c-Fos, a major component of transcription factor AP-1, is involved in the robust induction of NFATc1 expression (11). Mice lacking c-Fos developed osteopetrosis caused by the inhibition of osteoclast formation (36). RANKL did not induce c-Fos or NFATc1 expression in NF-κB p50/p52 double-knockout osteoclast precursor cells (37), indicating that NF-κB is activated upstream of c-Fos/NFATc1 during RANKL-mediated osteoclast differentiation. Because no consensus NF-κB binding sites are located on the c-Fos promoter, NF-κB seems to indirectly activate c-Fos, as previously described in a study of embryonic fibroblasts (38).

NFATc1 rescued RANKL-induced osteoclastogenesis in osteoclast precursor cells lacking c-Fos (13), indicating that NFATc1 is activated downstream of c-Fos. Collectively, RANKL-induced osteoclastogenesis may be controlled by a hierarchical transcriptional regulation in which NF-κB activation is the first event and leads to c-Fos activation followed by robust induction of NFATc1. In the current study, LC8 profoundly suppressed not only the expression of COX-2, an NF-κB target gene, but also the induction of the expression of c-Fos and NFATc1 during RANKL-induced osteoclast differentiation. Therefore, LC8 likely prevents osteoclast formation in the early stages of osteoclast precursor differentiation by inhibiting the activation of the NF-κB pathway, although LC8 might directly regulate c-Fos and NFATc1.

**FIGURE 6.** Inhibition of RANKL-induced MAPK activation by LC8 overexpression. (A) BMMs were incubated with M-CSF (20 ng/ml) and RANKL (100 ng/ml) for the indicated times, after which cell lysates were subjected to immunoblot analysis with Abs against phospho-JNK and JNK1, phospho-ERK and ERK2, or phospho-p38 and p38 (top and bottom of each pair of images, respectively). (B) The chemiluminescence signals for phospho-JNK, phospho-ERK, and phospho-p38 were quantified and normalized according to those of JNK1, ERK2, and p38, respectively.

**FIGURE 7.** Inhibition of LPS- and OVX-induced bone destruction by LC8 overexpression. (A) LPS (15 mg/kg body weight) or PBS, each in a 40-μl volume, was injected into the space between the s.c. tissue and the periosteum of the skulls of mice. Seven days after the injection, the mouse calvariae were fixed, decalcified, embedded in paraffin, and stained with TRAP. Scale bars, 100 μm. (B) Fold differences of bone cavity (left panel) and the number of TRAP+ osteoclast (right panel) were quantified. Data represent mean ± SD. n = 3. *p < 0.01, **p < 0.005. (C) Wild-type (WT) and LC8 Tg mice underwent either sham operation or OVX. Four weeks after surgery, the femurs were examined via μCT, and their three-dimensional reconstructions are shown. (D) Histograms represent the three-dimensional trabecular structural parameters in the femurs: bone volume fraction (BV/TV), trabecular number (Tb.N), and bone mineral density (BMD). Data represent mean ± SD. n = 4. *p < 0.05.
Given that RANKL stimulates not only the NF-κB pathway but also the MAPK pathway, including JNK, ERK, and p38 (39), we sought to examine whether LC8 regulates RANKL-induced MAPK activation. Though a previous report has shown that cAMP inhibits p38 activation via CREB-induced LC8, which interferes with association between MAPK kinase 3/6 and p38 (40), LC8 marginally inhibited p38 activation during osteoclast differentiation, whereas it significantly inhibited activation of JNK and ERK. In the MAPK pathway, activated JNK and ERK can directly phosphorylate c-Jun and c-Fos, respectively (41), which are major components of transcription factor AP-1, suggesting that LC8 may also contribute to osteoclastogenesis by regulating AP-1 activity. LC8 has known to bind to a conserved (K/R)XTQT amino acid sequence motif in a wide variety of target proteins (42). However, the failure to find a (K/R)XTQT motif in JNK1/2/3 and ERK1/2, and their upstream kinases MAPK kinase 4/7 and MEK1/2, suggest that LC8 may indirectly regulate JNK and ERK activities.

Increasing evidence suggests that reactive oxygen species (ROS) regulate RANKL-induced osteoclast differentiation. ROS generation transiently increases in RANKL-stimulated osteoclast precursor cells via a signaling cascade involving TRAF6, Rac1, and NADPH oxidase 1 (43). The antioxidants N-acetylcysteine and ascorbate, as well as diphenylene iodonium, a NADPH oxidase inhibitor, inhibited RANKL-induced osteoclastogenesis by suppressing ROS generation (43, 44). However, the mechanism by which ROS regulate RANKL-induced osteoclast differentiation remains unknown. We recently identified the mechanism underlying the redox-dependent regulation of TNF-α–induced NF-κB activation, in which ROS oxidize LC8 to a homodimer linked with a reversible intermolecular disulfide bond that promotes the dissociation of LC8 from IκBα and allows IκBα to be phosphorylated and degraded, thereby resulting in NF-κB activation (25, 26). Therefore, LC8 is likely oxidized by RANKL-induced ROS as it is in TNF-α signaling and is thereby involved in the ROS-dependent regulation of osteoclast differentiation. LC8 was previously identified as a substrate of TRP14, a disulfide reductase (24). TRP14 is involved in the redox-dependent regulation of NF-κB by facilitating the reduction of LC8 (25, 26). Thus, future research should focus on elucidating the role of TRP14 in RANKL-induced osteoclast differentiation.

Cytosolic dynein is a large multicomponent microtubule-based motor coupled to the dynactin cofactor complex (45). Microtubule organization, intracellular vesicular trafficking, and the activity of dynein motor are critical for the activation and function of osteoclasts (46–48). LIS1 was recently identified as a microtubule regulator that interacts with both Pleckhm1, a component of late endosomal trafficking, and the dynein–dynactin motor complex in osteoclasts (47). Depletion of LIS1 dramatically inhibited the formation and bone resorption function of osteoclasts by interfering with dynein function and microtubule organization (47). Exogenous expression of dynamin, a component of the dynamin complex, disrupted the dynein–dynactin complex, leading to retardation of osteoclast formation (48). Therefore, the role of LC8 in osteoclast differentiation as an essential component of the dynein motor complex may be worthy to be studied.

Because a defect in the development of peripheral lymph nodes has been reported in mice deficient in RANKL (49) or RANK (50), it was investigated whether the development of lymph nodes is impaired in LC8 Tg mice. LC8 overexpression had no apparent effect on the formation of mesenteric lymph nodes (Supplemental Fig. 1A). Furthermore, TNF-α–induced apoptosis did not occur in BMM cells of LC8 Tg mice (Supplemental Fig. 1B), indicating that the apoptosis is not caused by the inhibition of NF-κB via LC8. LC8 Tg mice exhibited mild osteopetrotic phenotype and were resistant to OVX treatment. However, the effect of LC8 on bone phenotype could not be restricted to osteoclasts, because of its systemic expression. Thus, further study on the role of LC8 in osteoblasts may also be necessary to provide clinical implications for various bone diseases.

In conclusion, we showed that LC8 inhibits RANKL-induced osteoclast differentiation and prevents inflammation- and OVX-induced bone loss. In addition, we elucidated the molecular mechanisms by which LC8 inhibits NF-κB and MAPK activations, leading to attenuation of c-Fos and NFATc1 expression. These findings might lead to the discovery of novel approaches for preventing and treating various types of inflammatory bone destruction and postmenopausal osteoporosis.

Disclosures
The authors have no financial conflicts of interest.