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Elevated Expression of CaMKIIγ During Osteoclastogenesis and Its Functional Implications

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Abstract Ca^{2+}/calmodulin signaling has been recognized recently as a major regulator in osteoclastogenesis. Efforts have ensued to identify the downstream targets of this signaling pathway in the context of regulating osteoclastogenesis. The calcineurin-NFAT pathway has thus been identified as one such target. In this article, we describe the discovery of another novel downstream target, CaMKIIγ. We also demonstrate that CaMKIIγ is the sole known CaMK expressed in significant amounts in osteoclasts and their precursors. Other known CaMKs such as CaMKIV and CaMKIIα, β, δ, were not detectable, and CaMKI was only expressed at a negligible level. Furthermore, the expression of CaMKIIγ was tightly correlated with the osteoclastogenic process, with a peak level on Day 3 of cell culturing. Osteoclastogenesis is halted by treatment with the CaMKIIγ inhibitor, KN93, independently from apoptosis, with the IC_{50} for osteoclastogenesis matching that for blocking CaMKIIγ function. Collectively, these data indicate that CaMKIIγ may be a significant regulator of osteoclastogenesis. J. Cell. Biochem. 101: 1038–1045, 2007.

Key words: bone; osteoclast; differentiation; calmodulin kinase

Ca^{2+}/calmodulin (CaM) signaling is important in living organisms and regulates various biological processes such as muscle contraction, fertilization, cell proliferation, vesicular fusion, and apoptosis [reviewed by Hoeflich and Ikura, 2002]. Recent studies from our laboratory and from other investigators have implicated Ca^{2+}/CaM signaling as having an important role in osteoclastogenesis [Takayanagi et al., 2002; Boyle et al., 2003; Zhang et al., 2003, 2005; Hirotani et al., 2004; Koga et al., 2004]. The most direct evidence derives from studies with CaM antagonists. CaM antagonists inhibit osteoclast formation in vitro and this effect is reversed by overexpression of CaM [Zhang et al., 2003]. Furthermore, trifluperazine (TFP), a CaM antagonist, reduces ovariectomy-induced osteoclastogenesis, which leads to a recovery of bone loss in the ovariectomized mice [Zhang et al., 2003]. In agreement with these findings, recent reports have shown that RANKL, a key factor in osteoclastogenesis, evokes Ca^{2+} oscillations in preosteoclasts [Takayanagi et al., 2002; Koga et al., 2004], which should activate CaM. Other studies have begun to identify which of the 40 different pathways initiated by Ca^{2+}/CaM signaling might be responsible for the underlying effects on osteoclastogenesis. Notably, calcineurin, a major downstream target of Ca^{2+}/CaM signaling, has been recognized as a key regulator via activating NFAT, a crucial transcription factor in osteoclastogenesis [Takayanagi et al., 2002; also see reviews by Boyle et al., 2003; Hirotani

Abbreviations used: BMMs, bone marrow monocytes; CaM, calmodulin; CaMK, CaM-dependent kinase; CaMKII, CaMK type II; CNS, central nervous system; FBS, fetal bovine serum; KN93, 2-[N-(2-hydroxyethyl]-N-(4-methoxyenzenesulfonyl]-amino-N-(4-chlorocinnamy)-N-methylbenzylamine; MOC, mouse osteoclast; NFAT, nuclear factor of activated T-cells; RANKL, receptor activator of NFκB ligand; TFP, trifluperazine; TRAP, tartrate resistant acid phosphatase; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling; 18S, 18 S rRNA; Z-VAD-FMK, caspase inhibitor I [sequence: ZVAD(OMe)-FMK].

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et al., 2004; Ikeda et al., 2004; Koga et al., 2004; Day et al., 2005; Zhang et al., 2005; Zhu et al., 2005]; while the role of calcineurin-NFAT pathway in osteoclastogenesis is now widely accepted, our present data provide yet another mechanism, strongly suggesting a role for CaM-dependent kinase II (CaMKII), another major downstream target of Ca^{2+}/CaM signaling, in osteoclastogenesis.

CaMKII responds to stimulation by many hormones, neurotransmitters, cytokines, and other signaling molecules, and is implicated in the regulation of ion channels/receptors, neurotransmitter synthesis and release, gene transcription, cytoskeletal organization, and calcium homeostasis [Braun and Schulman, 1995; Soderling et al., 2001]. The multifunctionality of CaMKII is reflected in the diversity of both its substrates and its gene products. There are four CaMKII genes (α, β, γ, and δ) found on separate chromosomes in humans, each with its own tissue prevalence: α and β are predominantly in the brain, while δ and γ are found in peripheral tissues [Tobimatsu and Fujisawa, 1989; Edman et al., 1994; Urquidi and Ashcroft, 1997; Bayer et al., 1999; Lorenz et al., 2002; Zhang and Brown, 2004]. CaMKII is a family of widely studied molecules with diverse functions; however, no other reports to date have implicated any of its member in osteoclastogenesis. CaMKIIα is perhaps the most well-known member in the family, and has been labeled "the memory molecule" due to its highest concentration in hippocampus and its central role in neuronal memory mechanism [Silva, 2003]. Unexpectedly, CaMKIIα (+/−) mutant mice, a model used in memory study [Frankland et al., 2001], exhibit a phenotype of low TRAP (tartrate resistant acid phosphatase) activity in their tibia and femurs [Zhang et al., 2005]. However, this decrement was not associated with any change in bone mineral density or body size in these mice (L.Z., unpublished data). This puzzling finding led us to investigate whether CaMKIIα and/or other subtype(s) of CaMK are expressed in osteoclasts and their precursors, and if present, how they are related to osteoclastogenesis.

MATERIALS AND METHODS

Cell Culture and Usage of Chemical Inhibitors

Receptor activator of NFκB ligand (RANKL) was prepared and used to generate osteoclasts as described previously [Zhang et al., 2003]. Briefly, bone marrow monocytes (BMMs) were isolated from the whole marrow of 4- to 8-week-old C57/BL/6 mice (Charles River Laboratories, Wilmington, MA) and cultured overnight in α-MEM (plus penicillin and streptomycin) supplemented with 10% heat inactivated fetal bovine serum (FBS) (HyClone, Logan, UT) and 10 ng/ml M-CSF (R&D, Minneapolis, MN). The non-adherent cells were collected and subjected to Ficoll-Hypaque gradient purification. Cells at the gradient interface were collected and cultured on 24- or 6-well tissue culture plates in α-MEM with heat-inactivated FBS (10%), RANKL (50 ng/ml), and M-CSF (10 ng/ml). Culture media and indicated supplement(s) were changed every 2 days for a total of 4 days. For inhibiting calmodulin signaling, 0.5–3 μM of TFP (Sigma, St. Louis, MO) were applied on the Day 3 of cell culturing for 24 h as described previously [Zhang et al., 2003]; while 0.5–2 μM KN93 (EMD Biosciences, San Diego, CA) were used in a similar fashion to inhibit CaMKII. In the case of Z-VAD-FMK (EMD Biosciences) used, an hour of pretreatment with 75 μM of Z-VAD-FMK on the Day 3 of cell culturing was followed by a 24 h treatment of either TFP or KN93.

RT-PCR and Semi-Quantitative PCR

To detect mRNA for each isoform of CaMKII, we used primers published previously by Lorenz et al. [2002]. The forward primers are following: α, 5′-CAATATCGTCCGACTCCATG-3′; β, 5′-GCTGCTCACAGAGACCTCAAG-3′; γ, 5′-CATCCACCAGCATGACATCG-3′; and δ, 5′-CCTAAATGGCATAGTTCAC-3′. The reverse primers are: α, 5′-CATCTGGTGACAGTGTAGC-3′; β, 5′-CCAGATGTCCACAGGTTTGC-3′; γ, 5′-CTTTCCTCAAGACCTCAGG-3′; and δ, 5′-GGATCTTTACGTAGGACTTC-3′. The PCR protocol was as follows [also see the report by Lorenz et al., 2002]: An initial heating for 5 min at 94°C, followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 57°C, and extension for 30 s at 72°C, with a final extension for 5 min at 72°C. The PCR products were separated in 4.0% agarose gels and visualized with ethidium bromide stain. For internal control, 18 S rRNA was used: forward primer (5′-CGCCGCTAGAGGTGAAATTCT-3′) and reverse primer (5′-CGAACCTCCGACTTTCGTTCT-3′).

For semi-quantitative PCR, equal amounts of cDNA from the same source of reverse transcribed products were used for PCR with different pairs of primers corresponding to respective genes. Then 5 μl of PCR products were collected at different cycle points from each sample and the PCR products were separated in 4.0% agarose gels and visualized with ethidium bromide stain. The relative abundance of a particular mRNA is thus determined by the cycle number required for its earliest detection: the smaller the number, the more abundant of the corresponding mRNA. For internal control, 18 S rRNA was used as described above.

Western Analysis

Total cell lysate proteins (25 μg) were separated by routine SDS–PAGE and then transferred to PVDF membranes [Zhang et al., 2003]. Specific antibodies against various CaMKII isoforms (α, β, γ, δ), CaMKI, and CaMKIV from Santa Cruz (Santa Cruz, CA), and used as described by the manufacturers.

Terminal Deoxynucleotidyl Transferase-Mediated Deoxyuridine Triphosphate Nick End-Labeling (TUNEL) Staining

For in situ detection of nuclear DNA fragmentation, TUNEL staining was performed following our previous protocol [Zhang et al., 1995, 2003].

TRAP Staining

For cell culture experiments, cells were fixed and then stained for the presence of TRAP using a commercial kit (Sigma).

TRAP Activity Assay in Cell Lysates

Cell lysates were obtained, then TRAP activity was assayed using a commercial kit (Sigma). Results are expressed as optical density (OD) readings at 405 nm.

Statistical Analysis

In the studies of the effects of CaMK antagonist on osteoclastogenesis in vitro, we compared the OD values at 405 nm and osteoclast numbers between the drug treatment groups ($n = 9$) and the control ($n = 9$) using one-way Anova followed by the Tukey test (SigmaStat, Jandel Corporation). A P-value of less than 0.05 was deemed statistically significant.

RESULTS

Characterization of CaMK Family Expression in Osteoclasts and Their Precursors

CaMKIIα is not detectable in osteoclasts and their precursors. Since our previous findings had clearly shown that CaMKIIα (+/−) mutant mice had a marked decrement in TRAP-positive cells [Zhang et al., 2005], we have first addressed the hypothesis that CaMKIIα is expressed in osteoclast precursors and thus could be regulating osteoclastogenesis. Surprisingly, CaMKIIα was not detectable in mouse preosteoclasts in our initial RT-PCR experiments with 35 amplifying cycles, conditions under which CaMKIIγ was readily detectable (Fig. 1A). In addition, CaMKIIβ and CaMKIIδ were also undetectable (Fig. 1A). As other investigators claim that CaMKIIα is present in osteoclasts, based on positive detection after prolonged PCR with 40 amplifying cycles (Dr. Jay McDonald, personal communication), semi-quantitative RT-PCR experiments were carried out to compare the gene expression of CaMKIIγ and CaMKIIα in fully differentiated mouse osteoclasts. In agreement with the findings from our initial experiments, CaMKIIγ was easily detected at around 28 cycles of amplification (Fig. 1B). However, when CaMKIIα primers were used, there were only three faint bands appearing after 40 cycles of amplification in the osteoclasts in contrast to the easy detection of brain CaMKIIα at 30 cycles using the same pair of primers (Fig. 1B). Although the results could suggest that CaMKIIα is expressed at low levels in the osteoclasts, the faint CaMKIIα bands could be a result of non-specific amplification, which is known to occur during prolonged PCR with 40 or more cycles [Kramer and Coen, 2001]. To further resolve the issue of whether functional CaMKIIα is present in osteoclasts, Western blotting was carried out to investigate the expression of CaMKIIα protein in mouse osteoclasts. The absence of CaMKIIα in mouse osteoclasts contrasted markedly with the readily detectable expression of CaMKIIα in mouse hippocampus (Fig. 2A), which is in good agreement of our PCR data (Fig. 1). In addition, CaMKIIα protein was not detectable at any stage of mouse osteoblast differentiation (data not shown). Taken together, our data demonstrate that CaMKIIα is not expressed in osteoclasts or their precursors.

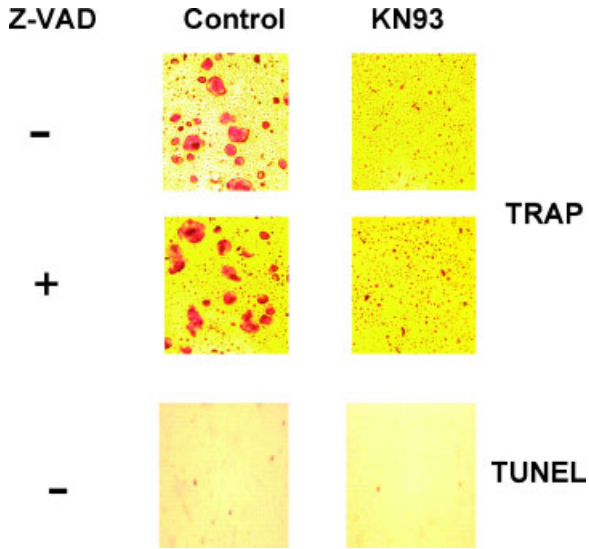

Fig. 1. Detection of gene expression of CaMKIIγ but not that of CaMKIIα or other CaMKII isoforms in osteoclasts and their precursors. **A**: Gene expression of CaMKII isoforms in mouse preosteoclasts: RNA was purified from mouse preosteoclasts on Day 3 of cell culturing and then RT-PCR was performed. PCR products of 35 cycles were separated in 4% Argarose gel and visualized under UV light after staining with ethidium bromide. **B**: Semi-quantitative measurement of gene expression of CaMKIIγ and CaMKIIα in mouse osteoclasts: RNA was purified either from mouse brain cortex homogenates or from fully differentiated mouse osteoclasts (MOC) on Day 6 of cell culturing, and then reverse-transcribed into cDNA. Equal amounts of cDNA were used for PCR using the indicated primers. Then 5 μl of PCR products were collected at different cycle points (as indicated by the numbers on the top) from each sample and were separated in 4% Argarose gel, and visualized as described above. 18S: 18 S rRNA.

Expression of CaMKIIγ and its relationship to the osteoclastogenic process. To futher explore the potential biological relevance of the CAMKII isoforms, Western blotting was carried out to investigate expression of various CaMK proteins, during mouse osteoclasts differentiation.

Treatment of mouse preosteoclasts for 3 days with M-CSF and RANKL elicited a marked increase in CaMKIIγ (Fig. 2B). The expression level of CaMKIIγ varied during the process of osteoclastogenesis: it was barely detectable at the non-differentiated early stage, increased markedly with a peak on Day 3 of culturing, and then declined but was still elevated at Day 6. CaMKI was detectable albeit at a very low level

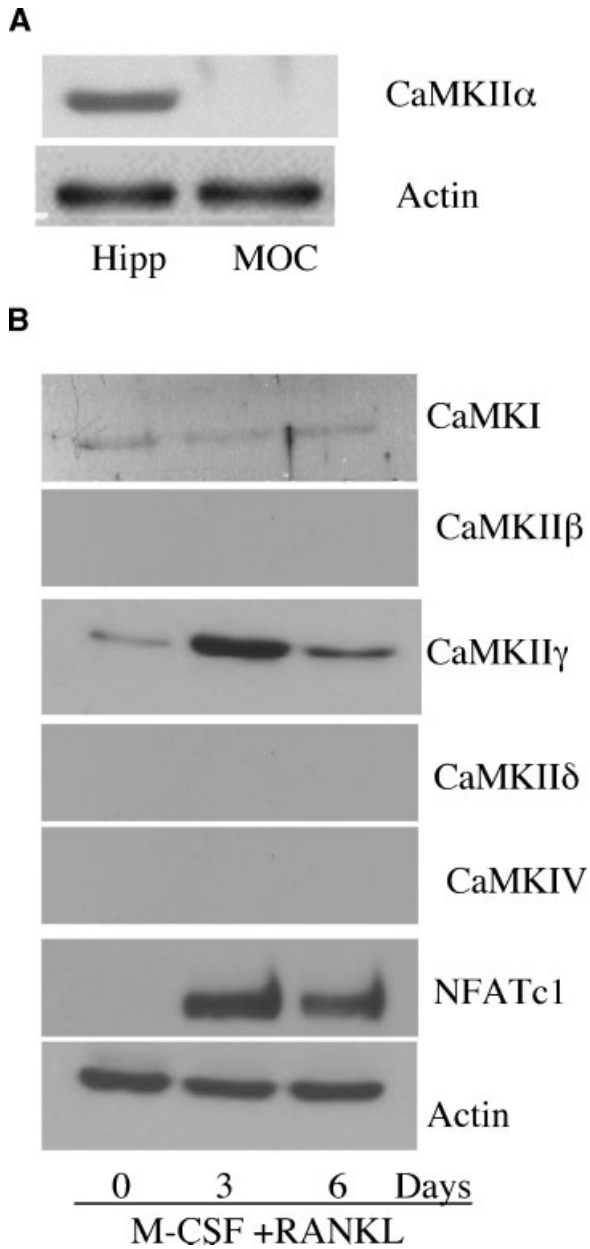

Fig. 2. Detection of protein expression of CaMKIIγ and CaMKI but neither that of CaMKIIα nor other CaMK in osteoclasts and their precursors. **A**: Detecting CaMKIIα in mouse hippocampus but not in mouse osteoclasts (MOC) by Western blotting. Twenty micrograms proteins from either mouse hippocampus homogenates or from cell lysates of fully differentiated mouse osteoclasts on Day 6 of cell culturing, were separated in SDS–PAGE, and blotted with antibodies against CaMKIIα or actin. **B**: Characterization of CaMK family expression during osteoclastogenesis by Western blotting. Purified mouse bone marrow monocytes were cultured in the presence of M-CSF and RANKL for the time indicated and then cells were lysed. Twenty micrograms of protein from each sample were separated in SDS–PAGE, and blotted with antibodies against the proteins specified, and then followed by a re-blotting with anti-actin. NFAT, nuclear factor of activated T-cells.

that did not change during the osteoclastogenic process. CaMKIIβ, CaMKIIδ, and CaMKIV were not detected in untreated preosteoclasts or after 3- or 6-day treatment with MCSF and RANKL. These three isoforms were also undetectable in both non-differentiated precursors and fully differentiated mouse osteoclasts by RT-PCR (not shown). The M-CSF and RANKL treatment also elicited the expected increase in NFATc1 expression (Fig. 2B).

Functional Study of CaMKIIγ in Preosteoclasts: KN93 Inhibits Osteoclastogenesis Without Involving Apoptosis

As CaMKIIγ was the sole CaMK expressed in significant amounts in osteoclasts and their precursors, KN93, a general CaMK inhibitor, was an appropriate tool for determining the function of CaMKIIγ in osteoclasts and preosteoclasts. It was found previously that Day 3 of cell culturing is the time point at which the inhibitory effects of calmodulin antagonists are readily detectable [Zhang et al., 2003]. Thus, the same methods were used to test the involvement of CaMKIIγ: the cells were treated with KN93 on Day 3 of culturing and osteoclastogenesis assays were performed 24 h later. KN93 inhibited osteoclast formation with an IC_{50} of ~1 μM (80% maximum inhibition obtained at 2 μM) as determined by assaying TRAP activity in cell lysates (Fig. 3, top panel). However, an even greater reduction was observed when osteoclast numbers were counted, with an IC_{50} for KN93 of ~0.3 μM (over 90% inhibition achieved at 1 μM, Fig. 3, middle panel), which matches its IC_{50} (≅370 nM) in blocking CaMKII [Sumi et al., 1991]. The discrepancy between counting osteoclast number and measuring TRAP activity may be due to the existence of TRAP activity in the mononuclear preosteoclasts, which confers a high background in the TRAP assay. As reported previously, TFP, a general calmodulin inhibitor, also inhibited osteoclastogenesis with an IC_{50} about 1 μM, based on counts of osteoclast number (Fig. 3, lower panel).

We next investigated whether apoptosis was involved in the inhibitory effects of KN93 on osteoclastogenesis. Z-VAD-FMK, a broad caspase inhibitor [Zhang et al., 2003], provided no protection against the inhibitory effect of 1 μM KN93 (Figs. 4, 5). We also performed a TUNEL assay after 24 h treatment with 1 μM KN93 and found no increase of TUNEL-positive cells (Fig. 4). We further quantified the effects of

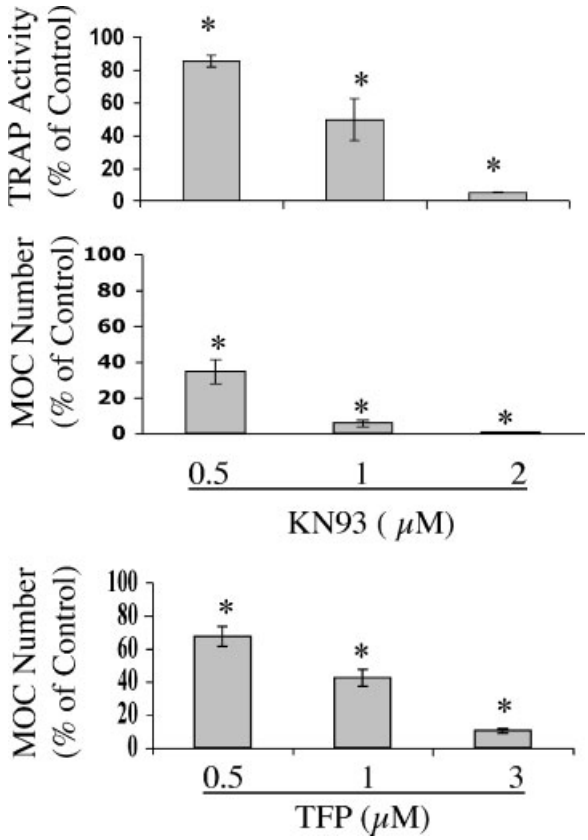

Fig. 3. KN93 inhibits osteoclastogenesis. Purified mouse bone marrow monocytes were cultured in the presence of M-CSF and RANKL. Different concentrations of KN93 or TFP were added into culture as indicated on Day 3 of cell culturing, for 24 h. Cell lysates were measured for TRAP activity (KN93 treatment shown in the **upper panel**). In parallel culture, cells were fixed and TRAP stained, and then, mouse osteoclasts (MOC) were counted (KN93 treatment shown in the **middle panel**, TFP treatment in the **lower panel**). N = 9. *$P < 0.05$, compared to control.

KN93 and TFP on osteoclastogenesis in the presence and absence of Z-VAD-FMK by counting osteoclasts number (Fig. 5). The IC_{50} of TFP on osteoclastogenesis remained about 1 μM regardless the presence or absence of Z-VAD-FMK. KN93 also maintained an IC_{50} of 0.3 μM on osteoclastogenesis in the presence of Z-VAD-FMK.

DISCUSSION

This is the first report to identify CaMKIIγ as the sole osteoclast CaMK elevated during osteoclastogenesis. Data presented in this article demonstrate, by Western blotting (Fig. 2), that CaMKIIγ, but not CaMKIIα, CaMKIIβ, CaMKIIδ, or CaMKIV are expressed in osteoclasts

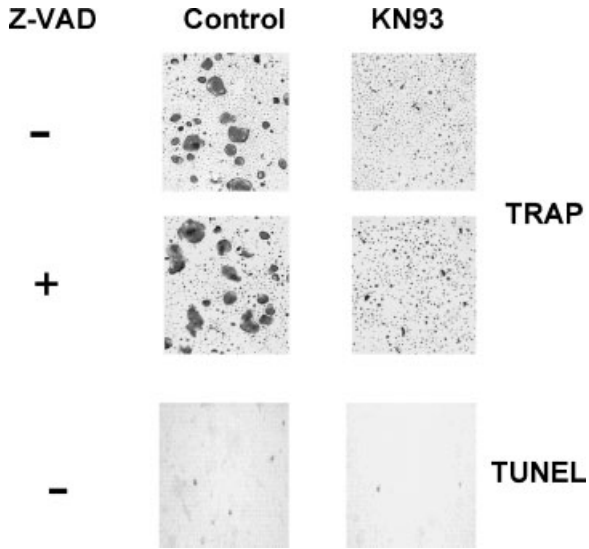

Fig. 4. Assess the involvement of Apoptosis in the inhibitory process of KN93 on osteoclastogenesis. On Day 3 of mouse bone marrow monocytes culturing in the presence of M-CSF and RANKL, with or without an hour of pretreatment with Z-VAD (Z-VAD-FMK, 75 μM), cells were treated with 1 μM KN93 for additional 24 h. Then cells were fixed, TRAP stained or TUNEL stained. Reddish color indicates TRAP positive, while brownish color TUNEL positive. An example of six (for TRAP) or three (for TUNEL) independent experiments is shown. Magnification: 40×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and their precursors. RT-PCR results (Fig. 1) cast doubt on the presence of CaMKIIα mRNA in osteoclasts, since the high number of PCR cycles required to elicit expression of a band could have been amplifying a non-specific product. Since our data indicate that CaMKIIα is not expressed locally in osteoclasts, the observation that CaMKIIα (+/−) mutant mice exhibited a phenotype of marked decrement of TRAP-positive cells in their bones [Zhang et al., 2005] suggests that this results from an indirect effect on bone from Ca/CaM signaling in another tissue. It is plausible that a central nervous system (CNS) mechanism might be involved in the mutant phenotype as CaMKIIα is the predominant isoform of CaMKII in the brain, which is an important organ in regulating bone metabolism and the development of bone cells [Amling et al., 2001; Takeda et al., 2002]. Alternatively, CaMKIIα may participate in the regulation of osteoclastogenesis via modulating the synthesis of osteoclastogenic factors in osteoblasts, as a recent report indicated that CaMKIIα does exist in osteoblasts, and involve in osteoblast differentiation and bone formation [Zayzafoon et al., 2004]. Beyond osteoclastogenesis, it is still not understood why the decrement of TRAP-positive cells in these mutant mice has no effect on bone mineral density and the animal body size (L.Z., unpublished data).

Besides CaMKIIγ, CaMKI is the only other CaMK detectable in osteoclasts and their precursors (Fig. 2A). Although treatment with M-CSF and RANKL increased CaMKIIγ, it did not affect CaMKI. Thus, because of its very low expression level and lack of correlation with the osteoclastogenesis process, it seems unlikely that CaMKI has a significant role in osteoclastogenesis. With all other CaMKs being undetectable in osteoclasts and precursor cells, CaMKIIγ remains the only one with abundant expression that correlates particularly well with the osteoclastogenic process. Most importantly, the peak level of CaMKIIγ expression is on Day 3 of culturing, a critical time-point for osteoclast precursors to start to undergo cell fusion, which may implicate a mechanistic involvement of CaMKIIγ in osteoclastogenesis. The potential importance of this is underscored by the fact that this is a also a time-point at which KN93 and CaM antagonists inhibit osteoclastogenesis [see Fig. 3 and also Zhang et al., 2003], a strong indication of the involvement of CaMKIIγ in osteoclastogenesis. Interestingly, Day 3 is also a peak time-point for expression of another important osteoclastogenesis regulator, NFAT, which has been reported by others [Takayanagi et al., 2002; Hirotani et al., 2004; Ikeda et al., 2004; Koga et al., 2004; Zhu et al., 2005] and confirmed by us (Fig. 2). It remains to be determined whether these two signaling molecules interact or independently regulate different aspects of osteoclastogenesis.

As CaMKIIγ is the sole known CaMK expressed at a significant level in osteoclasts and their precursors, KN93, an otherwise general CaMK inhibitor, becomes a selective tool for dissecting the function of CaMKIIγ in osteoclasts and their precursors. This is further supported by the pharmacological study in which KN93 concentration-dependently inhibited osteoclastogenesis with an IC_{50} that matches its IC_{50} in blocking CaMKIIγ function (Fig. 3). Thus, these data collectively indicate that CaMKIIγ is a positive regulator of osteoclastogenesis.

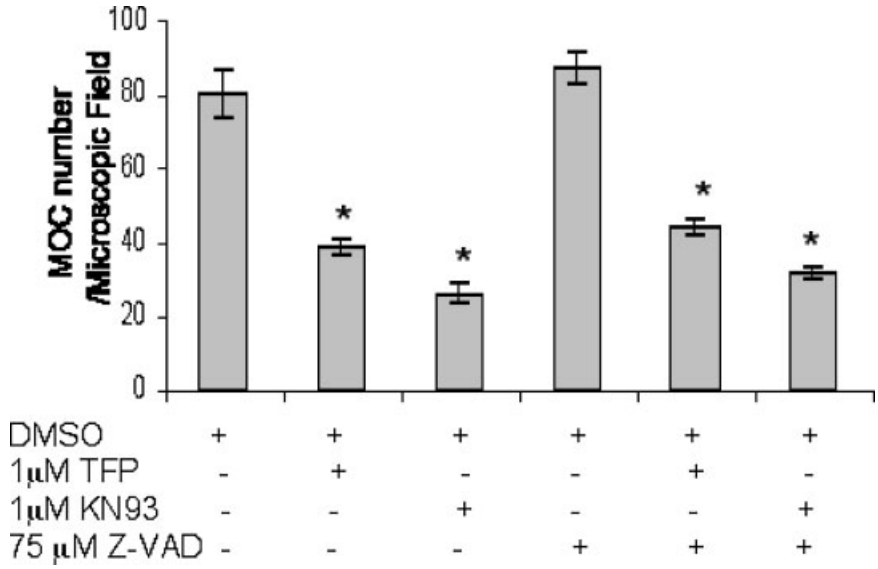

Fig. 5. Quantify the effects of KN93 and TFP on osteoclastogenesis in the presence or absence of Z-VAD-FMK. On Day 3 of mouse bone marrow monocytes culturing in the presence of M-CSF and RANKL, with or without an hour of pretreatment with Z-VAD (as in Fig. 4), cells were treated with 1 μM KN93 or 1 μM TFP for additional 24 h. Then cells were fixed, TRAP stained. Osteoclasts (TRAP positive with three or more nuclei) were counted and expressed as means ± SEM. (*$P < 0.05$, comparing with control; n = 6).

To begin evaluating the molecular mechanisms by which CaMKIIγ regulates osteoclastogenesis, the first question we have dealt with is the involvement of apoptosis. One hypothesis to explain the observed inhibitory effect of the CaMKIIγ antagonist is that the decrement in osteoclast number may be due to a selective killing of newly formed large osteoclasts. Our data suggest that this is not the mechanism. A general caspase inhibitor, Z-VAD-FMK [Zhang et al., 2003], provided no protection against the inhibitory effect of KN93 on osteoclastogenesis (Fig. 3C). In addition, KN93 treatment did not induce an increase of TUNEL-positive cells (Fig. 4). Thus, apoptosis is not responsible for the inhibitory effects of KN93 on osteoclastogenesis. Since RANKL is a key factor in osteoclastogenesis [Boyle et al., 2003], we are currently testing the hypothesis that CaMKIIγ modulates RANK signaling, as the underlying mechanism for its role in the regulation of osteoclastogenesis.

In summary, we have found that: (1) CaMKIIγ is the sole, known CaMK expressed at significant amounts in osteoclasts and their precursors; (2) CaMKIIγ expression is markedly increased during osteoclastogenesis; (3) the CaMK specific inhibitor KN93 inhibits osteoclastogenesis, without eliciting apoptosis. Therefore, it may be CaMKIIγ, but not CaMKIIα and other known CaMK that is responsible for the regulation of osteoclastogenesis. These novel findings indicate that CaMKIIγ may represent a new, unique target for the rational design of pharmacological intervention in osteoporosis and other aging associated bone-wasting diseases. This notion is further supported by recent DNA array data obtained from a bone-wasting animal model: Onyia et al. [2005] reported a 1.5-fold increase of CaMKII in the distal femoral metaphysis of rats treated with a continuous PTH infusion. This is highly significant, given the fact a mixed cellular population was used in their sample preparation, which may be the reason why they also only detected a 1.5-fold increase of cathepsin K, a well-known molecular marker of osteoclasts [Onyia et al., 2005]. In conclusion, the current studies identify CaMKIIγ as a critical signaling molecule in osteoclastogenesis and a potential target for anti-resorptive therapy.

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