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Proteasome inhibitors induce osteoclast survival by activating the Akt pathway

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ABSTRACT

Osteoclasts rapidly undergo spontaneous apoptosis when deprived of survival factors. Regulation of osteoclast survival is important to treat bone-related diseases, such as osteoporosis. In this study, we found that the proteasome inhibitors, MG132 and ALLN, significantly inhibited osteoclast apoptosis induced by etoposide, as well as under conditions of survival factor deprivation. MG132 and ALLN inhibited the release of cytochrome *c* from mitochondria into the cytosol in the absence of survival factors and suppressed the cleavage of pro-caspase-9 and -3 to its active forms induced by etoposide. In addition, MG132 and ALLN enhanced the phosphorylation of Akt and ERK in osteoclasts. However, MG132 and ALLN did not inhibit the cleavage of caspase-9 and -3 in the presence of the phosphatidylinositol 3-kinase (PI-3K) inhibitor, LY294002, while the inhibitory effect of MG132 and ALLN were intact in presence of the MEK1/2 inhibitor, U0126. LY294002 inhibited the survival of osteoclasts induced by MG132 and ALLN. Taken together, our results have demonstrated that proteasome inhibitors suppressed osteoclast apoptosis under conditions of survival factors deprivation through activation of the PI-3K/Akt pathway.

Bone erosion during chronic inflammation, such as rheumatoid arthritis and periodontitis, and osteoporosis after menopause, is thought to result from an imbalance between bone formation and resorption and is caused by increasing inflammatory cytokines and systemic alterations, such as estrogen deficiency [1,2]. Osteoclasts, which are bone-resorbing multinucleated cells, arise from cells of the monocyte–macrophage lineage. These cells undergo differentiation to tartrate-resistant acid phosphatase (TRAP)-positive cells, fuse to form multinucleated cells, and are activated to resorb bone, and in turn, undergo spontaneous apoptosis [3,4]. The receptor activator of NF-κB ligand (RANKL), identified as member of the tumor necrosis factor (TNF) superfamily, plays an important role in osteoclast differentiation, function, and survival by binding its receptor, RANK [5]. RANKL and RANK binding recruits TNF receptor-associated factor (TRAF) 6, and in turn, mediates transcription factors, such as NF-κB, PU.1, *Mi* transcription factor (MITF), c-Fos, and nuclear factor of activated T cells (NFAT) c1 [6,7].

Apoptosis is an essential event of programmed cell death to physiologic homeostasis in organisms. Apoptotic signaling can be divided into two main pathways: the extrinsic pathway and the intrinsic pathway [8,9]. These pathways are critically involved in the activation of caspases, which ultimately leads to apoptosis. In the mitochondria-dependent apoptosis pathway, the release of cytochrome *c* from mitochondria causes the formation of an apoptosome, containing dATP and apoptotic proteinase-activating factor-1 (Apaf-1), and leads to the recruitment and activation of pro-caspase-9. Active caspase-9 then cleaves pro-caspase-3 at Asp^{175} and activates caspase-3, which in turn cleaves various cellular proteins, such as fodrin, poly (ADP-ribose) polymerase (PARP), and DNA fragmentation factor-45 (DEF-45) [10–12].

Bone-resorbing osteoclasts have a short life span and rapidly undergo spontaneous apoptosis when deprived of survival factors, such as RANKL, M-CSF, and TNF-α, which can induce phosphatidylinositol 3-kinase (PI-3K)/Akt and ERK activation, both of which play an important role in osteoclast survival [13–15]. Recently, Akiyama et al. [16] reported that Bim, a BH-3 only protein of the Bcl-2 family, is greatly increased during osteoclast apoptosis under conditions of survival factors deprivation, and activating ERK inhibits the protein level of Bim during osteoclast apoptosis by activating the ubiquitin–proteasome degradation pathway.

The ubiquitin–proteasome pathway is a critical process for the regulation of cellular homeostasis, such as degradation of abnormal proteins, metabolic adaptation, cell cycle, and apoptosis [17].

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Deregulation of the ubiquitin–proteasome pathway can lead to diverse physiologic disorders, including cancer, autoimmune diseases, and neurodegenerative disorders, which have been reported to be critically involved in apoptosis [18,19]. Proteasome inhibitors containing MG132, lactacystin, and ALLN have been shown to be potent inhibitors of NF-κB and are considered therapeutic agents for inflammation. In addition, proteasome inhibitors, such as lactacystin, may have a dual effect on cell survival [20–22]. We therefore investigated the effect of proteasome inhibitors on osteoclast survival. In this study, our results have shown that proteasome inhibitors greatly induce osteoclast survival, suppress cytochrome *c* release into the cytosol, and inhibit caspase activity.

Materials and methods

Preparation of osteoclasts. Osteoclasts were obtained by co-culture of bone marrow cells (BMCs) and osteoblasts. Briefly, osteoblasts were isolated from calvariae of newborn mice by using 0.1% collagenase (Wako, Osaka, Japan) and 0.2% dispase (Boehringer Mannheim, Ingelheim am Rhein, Germany) digestion. BMCs were obtained from long bones by flushing α-minimum essential medium (α-MEM; Welgene, Daegu, Korea) containing antibiotics. Osteoblasts and BMCs were cultured on a collagen-coated 90-mm dish with 1α,25-dihydroxyvutamin D_3 ($VitD_3$) and prostaglandin E_2 (PGE_2). After 6 days, the co-cultured cells were detached by digesting the collagen with 0.1% collagenase and then seeded on 6- or 48-well plates. Osteoblasts were detached with trypsin/EDTA treatment and the adherent cells were used as osteoclasts.

Survival assay. Osteoclasts were isolated as described above. The cells were treated for 6 h with proteasome inhibitors, etoposide, or both. The remaining cells were fixed with 3.7% formalin, permeabilized with 0.1% Triton X-100, and stained with TRAP solution. TRAP-positive cells containing five or more nuclei were counted as surviving osteoclasts.

Western blotting. Cells were washed with ice-cold PBS and then lysed in lysis buffer (50 mM Tris–Cl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM sodium fluoride, 1 mM sodium vandadate, 1% deoxycholate) in the presence of protease inhibitors. Equal amounts of cell lysates were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ, USA). The membrane was incubated overnight at 4 °C with appropriate primary antibodies, followed by three times for 10 min with TBS-T, and then incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h. The immune complex was visualized by using enhanced chemiluminescence (ECL).

Isolation of mitochondria and cytosol fraction. Cells were washed with ice-cold PBS, suspended in buffer A (150 mM NaCl, 50 mM Tris–Cl, and 100 μM EDTA) containing protease inhibitors, and disrupted by three passages through a 25-gauge needle. Cell debris and nuclei were removed by centrifugation at 2000 rpm for 10 min. The supernatants were then centrifuged at 100,000 rpm for 60 min. The supernatants were saved as a cytosolic fraction. The resulting pellet was resuspended with buffer A containing protease inhibitors and 0.1% SDS, centrifuged at 14,000 rpm for 20 min, and the supernatants were saved as the mitochondrial fraction.

Caspase-3 activity assay. Caspase-3 activity assay was determined using the caspase-3 colorimetric assay kit (R&D Systems), according to the manufacturer's suggestions. Osteoclasts were treated with MG132 or ALLN for 6 h. Cells were lysed in a cell lysis buffer (R&D Systems). Cell lysates were added to the colorimetric caspase-3 substrate DEVE-pNA and were incubated for 2 h. Caspase activity was measured at 405 nm in a microtiter plate reader.

Nuclear and actin ring staining. Cells were fixed with 3.7% formalin, permeabilized with 0.1% Triton X-100, and stained with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) or rhodamine-conjugated phalloidin to visualize nuclei or F-actin, respectively. Fluorescent images were obtained under a Zeiss Axiovert 200 fluorescent microscope (Carel Zeiss, Jena, Germany).

Statistical analysis. All quantitative data were performed 3–5 times and expressed as means ± S.E. Statistical analysis was analyzed using Student's *t* test, and $p < 0.05$ was considered as statistically significant.

Results

Proteasome inhibitors suppress apoptosis in osteoclasts

To examine whether a proteasome inhibitor is involved in the survival of osteoclasts, we examined the effect of proteasome inhibitors, such as MG132 and ALLN, on the survival of osteoclasts. MG132 and ALLN induced osteoclast survival in the absence of survival factors and prevented apoptosis, despite the presence of etoposide (Fig. 1A). Furthermore, MG132 and ALLN significantly induced osteoclast survival in a dose-dependent manner (Fig. 1B and C). To confirm this result, morphologic analysis of apoptotic cells was determined using DAPI staining. Nuclear condensation during apoptosis was inhibited in osteoclasts treated with MG132 and ALLN. Also, MG132 and ALLN prevented actin ring disruption during apoptosis (Fig. 1D). Together, these results suggest that proteasome inhibitors significantly induce the survival of osteoclasts.

Effect of proteasome inhibitors on caspase activity during osteoclast apoptosis

To examine the involvement of apoptogenic factors, such as cytochrome *c* and caspase in the prevention of osteoclast apoptosis by proteasome inhibitors, we first investigated the extent of cytochrome *c* release from mitochondria. Proteasome inhibitors suppressed the release of cytochrome *c* from mitochondria (Fig. 2A and B). Next, to determine whether inhibition of caspase activity was involved in the survival of osteoclasts treated with proteasome inhibitors, osteoclasts were treated for 6 h with MG132 or ALLN in the presence or absence of etoposide. MG132 and ALLN greatly inhibited the cleavage of pro-caspase-9 and -3 during apoptosis and also suppressed activation of caspsae-9 and -3 induced by etoposide (Fig. 2C and D). To further examine the effect of proteasome inhibitors on caspase-3 activity, caspase activity was determined using DEVD-pNA as a substrate. Consistent with the Western blotting results, caspase-3 activity was significantly decreased in osteoclasts treated with proteasome inhibitors (Fig. 2E and F). These results suggest that proteasome inhibitors-mediated inhibition of apoptogenic factors, such as cytochrome *c*, caspase-9, and -3, is involved in the survival of osteoclasts.

Proteasome inhibitors activate Akt and ERK phosphorylation

The PI-3 K/Akt pathway has been shown to prevent cell apoptosis in various cell types [14]. Both Akt and ERK pathways play a critical role in osteoclast survival [15]. Thus, to examine whether protreasome inhibitors mediate Akt or ERK phosphorylation, osteoclasts were stimulated with proteasome inhibitors for various times. MG132 and ALLN induced the phosphorylation of Akt and ERK (Fig. 3A and B). These findings raise the possibility that MG132- and ALLN-mediated Akt and ERK phosphorylation may involve osteoclast survival induced by MG132 and ALLN.

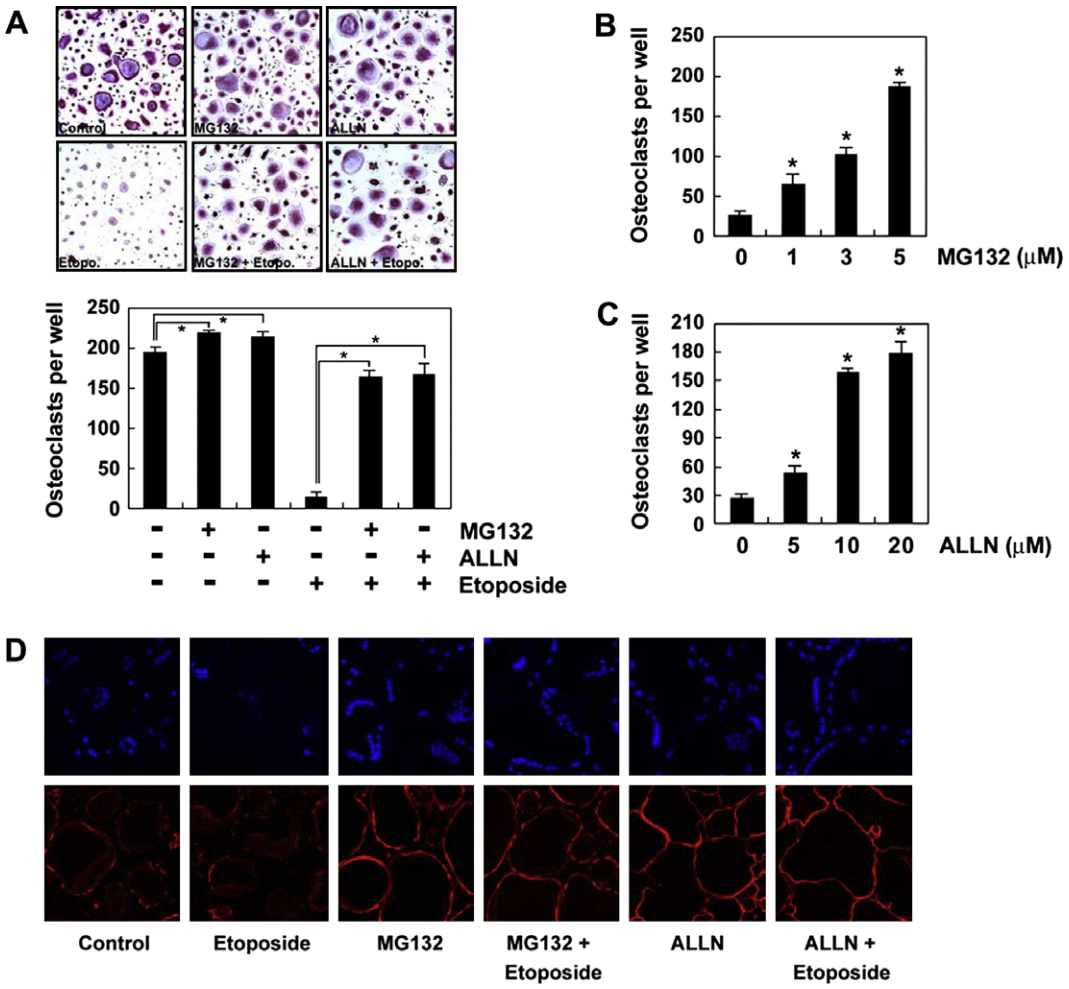

Fig. 1. Effect of proteasome inhibitors on osteoclast apoptosis. (A) Osteoclasts were treated for 6 h with MG132 (5 μM), ALLN (20 μM), or etoposide (100 μM). The cells were fixed, permeabilized, stained with TRAP solution, and photographed under light microscopy (top). TRAP-positive cells containing five or more nuclei were counted as osteoclasts. Asterisks indicate statistical difference ($p < 0.01$). Osteoclasts were treated for 6 h with increasing concentrations of MG132 (B) or ALLN (C). Asterisks indicate statistical differences from the control ($p < 0.01$). Osteoclasts were counted as in (A). Osteoclasts were stimulated as in (A). The cells were fixed, permeabilized, stained with DAPI (D) or rhodamine-conjugated phalloidin (E), and photographed under a fluorescent microscope.

Proteasome inhibitors induce osteoclast survival via the Akt pathway

Next, to investigate whether proteasome inhibitors-induced Akt or ERK activation was involved in proteasome inhibitor-mediated inhibition of caspase-9 and -3, osteoclasts were treated with proteasome inhibitors in the presence of the PI-3K inhibitor, LY294002. The inhibitory effect of proteasome inhibitors on caspase-9 and -3 activation was suppressed in osteoclasts treated with LY294002 (Fig. 4A and B). However, the MEK inhibitor, U0126, had no effect on MG132 and ALLN-induced caspase inhibition (Fig. 4C and D). LY294002 treatment abrogated the survival of osteoclasts induced by MG132 and ALLN (Fig. 4E). These results suggest that MG132 and ALLN regulate osteoclast survival through activation of the PI-3K/Akt pathway.

Discussion

In the present study, we showed that MG132 and ALLN suppress osteoclast apoptosis by inhibiting mitochondrial cytochrome *c* release, and preventing the activation of caspase-9 and -3 in the absence of survival factors (Figs. 1 and 2). In addition, we observed that MG132 and ALLN mediate the phosphorylation of Akt and ERK (Fig. 3). These results are consistent with those previously reported, which showed that MG132 activates the phosphorylation of Akt and ERK [23–25], both of which play an important role in osteoclast survival [18]. It has been reported that TNF-α and IL-1 enhance the survival of osteoclasts by activating PI-3K/Akt and ERK signaling pathways [15,26]. The mammalian target of rapamycin (mTOR), regulated by the PI-3K pathway, plays an essential role in stimulating osteoclast survival by suppressing Bim expression [27]. Furthermore, ERK signaling has been shown to contribute to the survival of osteoclasts by inducing a proteasome-dependent Bim protein degradation pathway [16]. These results strongly suggest that proteasome inhibitors mediate Akt and ERK phosphorylation that may lead to osteoclast survival.

PI-3K/Akt signaling pathway is a critical survival pathway in various cell types and activates cell survival through phosphorylation of the pro-apoptotic proteins, Bad and Bax, to its inactive form [28,29]. It has also been shown that Akt promotes the expression of anti-apoptotic proteins, such as inhibitors of apoptosis (IAP) and FLIP, through activation of the NF-κB pathway [30,31]. MG132 and ALLN, are proteasome inhibitors that have frequently been used as potent inhibitors of NF-κB. However, proteasome inhibitors affect other signaling pathways, as well as the NF-κB pathway [32,33]. To determine whether new protein synthesis induced by MG132 and ALLN was involved on the survival of osteoclasts, we determined the effect of the protein synthesis inhibitor, cycloheximide, which did not change osteoclast survival induced by MG132

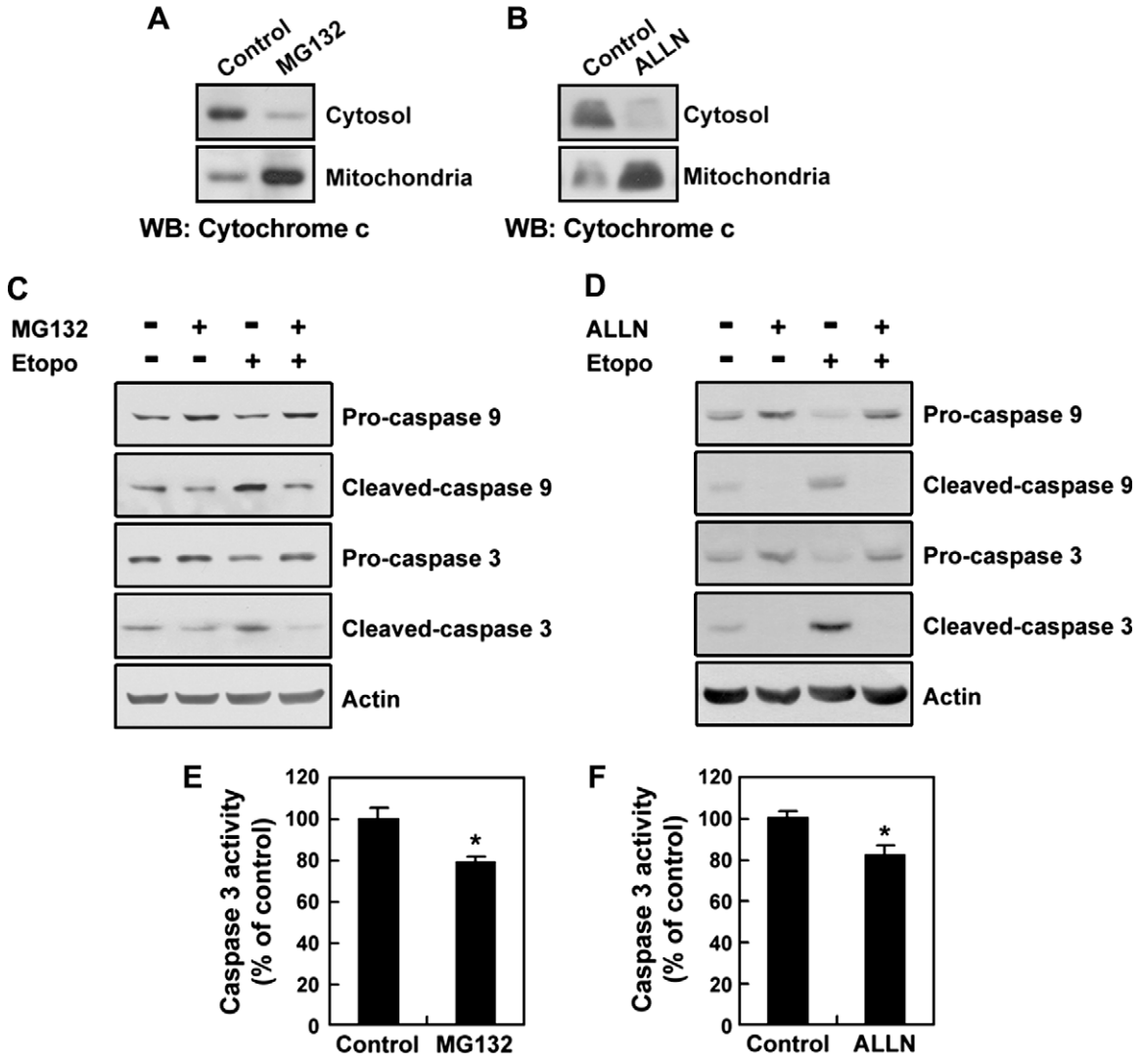

Fig. 2. Proteasome inhibitors suppress cytochrome *c*-dependent caspase activity. Osteoclasts were stimulated for 3 h with MG132 (5 μM) (A) or ALLN (20 μM) (B). The mitochondrial and cytosolic fractions were analyzed by Western blotting with an antibody against cytochrome *c*. Osteoclasts were treated for 6 h with MG132 (5 μM) (C) or ALLN (20 μM) (D) in the presence or absence of etoposide (100 μM). The cell lysates were analyzed for cleavage of caspase-9 and -3 by Western blotting with the indicated antibodies. Osteoclasts were incubated with MG132 (5 μM) (E) or ALLN (20 μM) (F) for 6 h. Caspase-3 activity was determined with the caspase-3 substrate (DEVD-pNA). Asterisks indicate statistical differences from the control ($p < 0.01$).

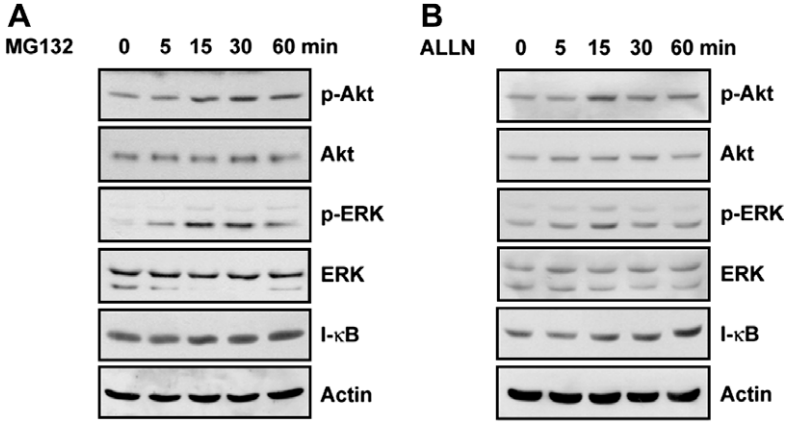

Fig. 3. Proteasome inhibitors stimulate the phosphorylation of Akt and ERK (A) Osteoclasts were treated with MG132 (5 μM) for the indicated time. The cells were lysed, and the cell lysates were analyzed by Western blotting with the indicated antibodies. (B) Osteoclasts were treated with ALLN (20 μM) for the indicated time. The cell lysates were analyzed by Western blotting as described above.

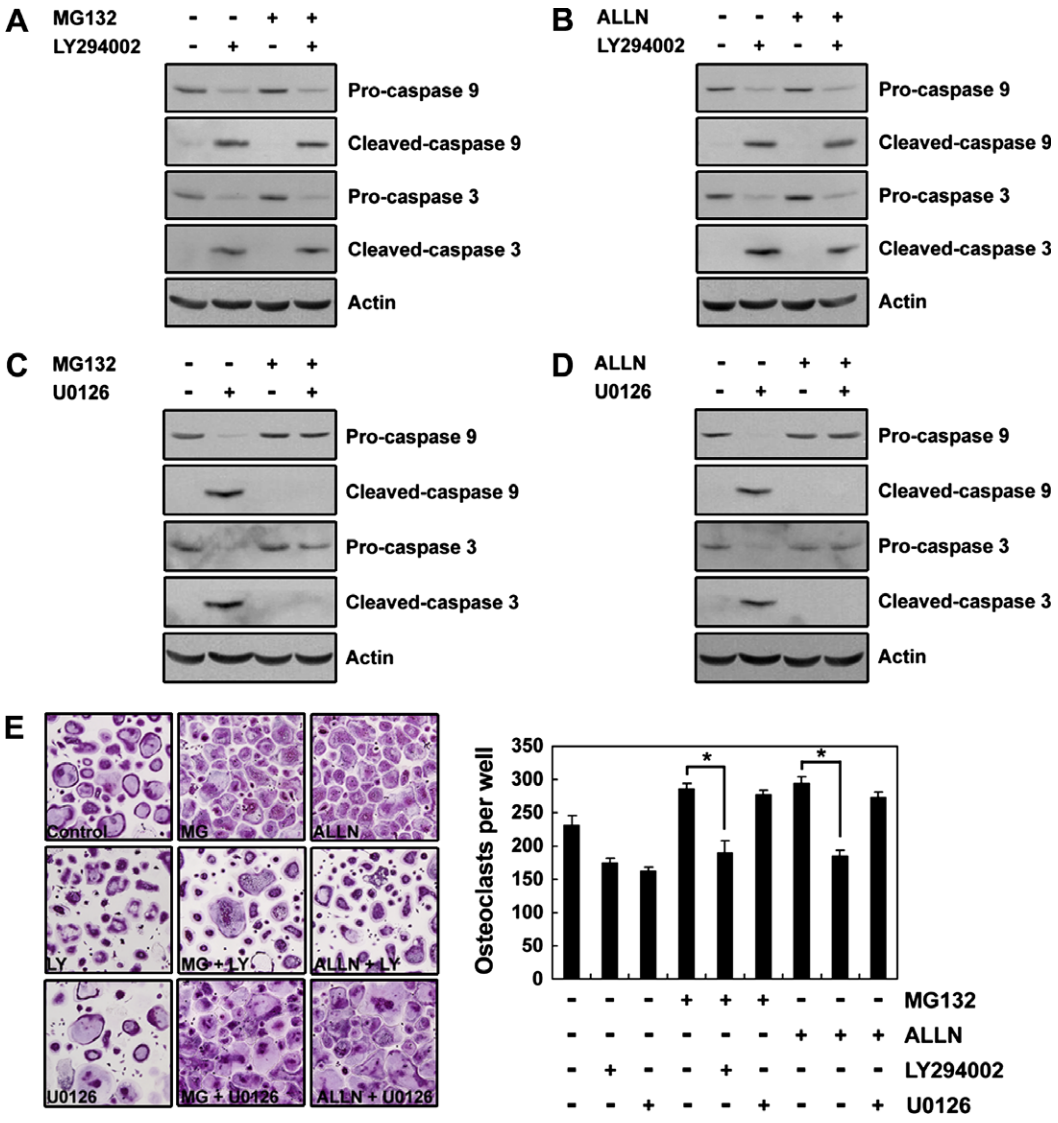

Fig. 4. Proteasome inhibitors induce osteoclast survival through activation of the Akt pathway. Osteoclasts were pretreated with LY294002 (10 μM) for 60 min and then stimulated with MG132 (5 μM) (A) or ALLN (20 μM) (B) for 6 h. The cell lysates were analyzed by Western blotting with the indicated antibodies. Osteoclasts were pretreated with U0126 (20 μM) for 60 min and then stimulated with MG132 (5 μM) (C) or ALLN (20 μM) (D) for 6 h. The cell lysates were analyzed by Western blotting as described above. (E) Osteoclasts were treated for 6 h with MG132 (5 μM) or ALLN (20 μM) in the presence or absence of LY294002 (10 μM) or U0126 (20 μM). The cells were fixed, permeabilized, stained with TRAP solution, and photographed under a light microscope (left). TRAP-positive cells were counted as described in Fig. 1A (right). Asterisks indicate statistical difference ($p < 0.01$).

and ALLN (data not shown). However, LY294002 treatment inhibited proteasome inhibitors-mediated osteoclast survival (Fig. 4). These results indicate that the PI-3K/Akt-dependent pathway induces proteasome inhibitors-mediated osteoclast survival.

Apoptosis is characterized by morphologic and biochemical features, such as chromatin condensation, cell shrinkage, and caspase activation. Release of cytochrome *c* into the cytosol leads to apoptosis and is known to result from a balance between pro-apoptotic members, such as Bak, Bax, and the BH-3 only proteins, including Bad, Bid, and Bim, and anti-apoptotic members, such as Bcl-2 and Bcl-xL [34,35]. It is well-known that caspase-9 is an initiator caspase that is considered an important trigger for the activation of effector caspases, such as caspase-3, leading to the release of cytochrome *c* from mitochondria [10,11]. Etoposide is a chemotherapeutic drug that has been widely used to promote cytochrome *c* release and cell death by increasing DNA damage, resulting duration of DNA cleavage sites [36]. Thus, we have used an etoposide to determine more precisely the role of proteasome inhibitors in osteoclast apoptosis. We showed that MG132 and ALLN greatly inhibit caspase activation and apoptosis induced by etoposide treatment (Figs. 1 and 2).

Bone homeostasis is the result of a balance between bone resorption by osteoclasts and bone formation by osteoblasts. Osteoclasts are responsible for the resorption of bone, but rapidly undergo cell death when deprived of survival factors [3,4]. We examined whether proteasome inhibitors-induced osteoclast survival had an effect on bone resorption activity. Unexpectedly, although MG132 and ALLN induce osteoclast survival, bone resorption is decreased in osteoclasts treated with MG132 or ALLN (data not shown). Thus, we determined the relationship between survival of osteoclasts and bone resorption. M-CSF greatly induced the survival of osteoclasts, but did not induce bone resorption (data not shown). These results are consistent with previous reports that M-CSF had no effect on bone resorption [14]. NF-κB activation is essential for osteoclast differentiation and bone resorption and that required for induction of target genes, such as matrix metalloproteinase (MMP)-9 and carbonic anhydrase II, to promote bone resorption [37,38]. It has also been reported that

NF-κB activity has an important role in bone resorption, but has no affect on the survival of osteoclasts [14]. These results demonstrate that osteoclastic bone resorption may not be associated with increased cell survival. Taken together, we have presented new effects of proteasome inhibitors, which can regulate osteoclast survival through inhibition of cytochrome *c*-dependent caspase activation by activating the PI-3K/Akt signaling pathway.

Acknowledgments

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References

[1] G.A. Rodan, T.J. Martin, Therapeutic approaches to bone diseases, Science 289 (2000) 1508–1514.
[2] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342.
[3] S.C. Manolagas, Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis, Endocr. Rev. 21 (2000) 115–137.
[4] S. Tanaka, T. Miyazaki, A. Fukuda, T. Akiyama, Y. Kadono, H. Wakeyama, S. Kono, S. Hoshikawa, M. Nakamura, Y. Ohshima, A. Hikita, I. Nakamura, K. Nakamura, Molecular mechanism of the life and death of the osteoclast, Ann. N. Y. Acad. Sci. 1068 (2006) 180–186.
[5] N. Takahashi, N. Udagawa, T. Suda, A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function, Biochem. Bipphys. Res. Commun. 256 (1999) 449–455.
[6] H. Takayanagi, S. Kim, T. Koga, H. Nishina, M. Isshiki, H. Yoshida, A. Saiura, M. Isobe, T. Yokochi, J. Inoue, E.F. Wagner, T.W. Mak, T. Kodama, T. Taniguchi, Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts, Dev. Cell 3 (2002) 889–901.
[7] H. Takayanagi, Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems, Nat. Rev. Immunol. 7 (2007) 292–304.
[8] G.S. Salvesen, V.M. Dixit, Caspases: intracellular signaling by proteolysis, Cell 91 (1997) 443–446.
[9] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479–489.
[10] X. Wang, The expanding role of mitochondria in apoptosis, Genes Dev. 15 (2001) 2922–2933.
[11] Z.B. Wang, Y.Q. Liu, Y.F. Cui, Pathways to caspase activation, Cell Biol. Int. 29 (2007) 489–496.
[12] A. Strasser, L. O'Connor, V.M. Dixit, Apoptosis signaling, Annu. Rev. Biochem. 69 (2000) 217–245.
[13] H. Glantschnig, J.E. Fisher, G. Wesolowski, G.A. Rodan, A.A. Reszkas, M-CSF, TNF-alpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase, Cell Death Differ. 10 (2003) 1165–1177.
[14] T. Miyazaki, H. Katagiri, Y. Kanegae, H. Takayanagi, Y. Sawada, A. Yamamoto, M.P. Pando, T. Asano, I.M. Verma, H. Oda, K. Nakamura, S. Tanaka, Reciprocal role of ERK and NF-kappaB pathways in survival and activation of osteoclasts, J. Cell Biol. 148 (2000) 333–342.
[15] S.E. Lee, W.J. Chung, H.B. Kwak, C.H. Chung, K.B. Kwack, Z.H. Lee, H.H. Kim, Tumor necrosis factor-alpha supports the survival of osteoclasts through the activation of Akt and ERK, J. Biol. Chem. 276 (2001) 49343–49349.
[16] T. Akiyama, P. Bouillet, T. Miyazaki, Y. Kadono, H. Chikuda, U.I. Chung, A. Fukuda, A. Hikita, H. Seto, T. Okada, T. Inaba, A. Sanjay, R. Baron, H. Kawaguchi, H. Oda, K. Nakamura, A. Strasser, S. Tanaka, Regulation of osteoclast apoptosis by ubiquitylation of proapoptotic BH3-only Bcl-2 family member Bim, EMBO J. 22 (2003) 6653–6664.
[17] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479.
[18] C.B. Thompson, Apoptosis in the pathogenesis and treatment of disease, Science 267 (1995) 1456–1462.
[19] F. Saudou, S. Finkbeiner, D. Devys, M.E. Greenberg, Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions, Cell 95 (1998) 55–66.
[20] K. Tanaka, T. Kawakami, K. Tateishi, H. Yashiroda, T. Chiba, Control of IkappaBalpha proteolysis by the ubiquitin–proteasome pathway, Biochimie 83 (2001) 351–356.
[21] J. Adams, The proteasome: a suitable antineoplastic target, Nat. Rev. Cancer 4 (2004) 349–360.
[22] D.D. Douglas, J.C. Tupper, W.A. Ricketts, C.F. Bennett, R.K. Winn, J.M. Harlan, A constitutive cytoprotective pathway protects endothelial cells from lipopolysaccharide-induced apoptosis, J. Biol. Chem. 276 (2001) 14924–14932.
[23] T. Inoue, K. Shiraki, H. Fuke, Y. Yamanaka, K. Miyashita, Y. Yamaguchi, N. Yamamoto, K. Ito, K. Sugimoto, T. Nakano, Proteasome inhibition sensitizes hepatocellular carcinoma cells to TRAIL by suppressing caspase inhibitors and AKT pathway, Anticancer Drugs 17 (2006) 261–268.
[24] H.M. Wu, H.C. Wen, W.W. Lin, Proteasome inhibitors stimulate interleukin-8 expression via Ras and apoptosis signal-regulating kinase-dependent extracellular signal-related kinase and c-Jun N-terminal kinase activation, Am. J. Respir. Cell Mol. Biol. 27 (2002) 234–243.
[25] T. Shibata, T. Imaizumi, W. Tamo, T. Matsumiya, M. Kumagai, X.F. Cui, H. Yoshida, S. Takaya, I. Fukuda, K. Satoh, Proteasome inhibitor MG-132 enhances the expression of interleukin-6 in human umbilical vein endothelial cells: Involvement of MAP/ERK kinase, Immunol. Cell Biol. 80 (2002) 226–230.
[26] Z.H. Lee, S.E. Lee, C.W. Kim, S.H. Lee, S.W. Kim, K. Kwack, K. Walsh, H.H. Kim, IL-1alpha stimulation of osteoclast survival through the PI 3-kinase/Akt and ERK pathways, J. Biochem. 132 (2002) 161–166.
[27] T. Sugatani, K.A. Hruska, Akt1/Akt2 and mammalian target of rapamycin/Bim play critical roles in osteoclast differentiation and survival, respectively, whereas Akt is dispensable for cell survival in isolated osteoclast precursors, J. Biol. Chem. 280 (2005) 3583–3589.
[28] S.R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, M.E. Green berg, Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery, Cell 91 (1997) 231–241.
[29] H. Yamaguchi, H.G. Wang, The protein kinase PKB/Akt regulates cell survival and apoptosis by inhibiting Bax conformational change, Oncogene 20 (2001) 7779–7786.
[30] T. Suhara, T. Mano, B.E. Oliveira, K. Walsh, Phosphatidylinositol 3-kinase/Akt signaling controls endothelial cell sensitivity to Fas-mediated apoptosis via regulation of FLICE-inhibitory protein (FLIP), Circ. Res. 89 (2001) 13–19.
[31] D.R. Plas, S. Talapatra, A.L. Edinger, J.C. Rathmell, C.B. Thompson, Akt and Bcl-x_L promote growth factor-independent survival through distinct effects on mitochondrial physiology, J. Biol. Chem. 276 (2001) 12041–12048.
[32] M. Lornejad-Schafer, C. Schafer, L. Richter, T. Grune, D. Haussinger, F. Schliness, Osmotic regulation of MG-132-induced MAP-kinase phosphatase MKP-1 expression in H4IIE rat hepatoma cells, Cell. Physiol. Biochem. 16 (2005) 193–206.
[33] X. Dolcet, D. Llobet, M. Encinas, J. Pallares, A. Cabero, J.A. Schoenenberger, J.X. Comella, X. Matias-Guiu, Proteasome inhibitors induce death but activate NF-κB on endometrial carcinoma cell lines and primary culture explants, J. Biol. Chem. 281 (2006) 22118–22130.
[34] T. Lindsten, A.J. Ross, A. King, W.X. Zong, J.C. Rathmell, H.A. Shiels, E. Ulrich, K.G. Waymire, P. Mahar, K. Frauwirth, Y. Chen, M. Wei, V.M. Eng, D.M. Adelman, M.C. Simon, A. Ma, J.A. Golden, G. Evan, S.J. Korsmeyer, G.R. MacGregor, C.B. Thompson, The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues, Mol. Cell 6 (2000) 1389–1399.
[35] X. Jiang, X. Wang, Cytochrome *c*-mediated apoptosis, Annu. Rev. Biochem. 73 (2004) 87–106.
[36] J. Yang, X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones, X. Wang, Prevention of Apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked, Science 275 (1997) 1129–1132.
[37] S. Kamolmatyakul, W. Chen, S. Yang, Y. Abe, R. Moroi, A.M. Ashique, Y.P. Li, IL-1alpha stimulates cathepsin K expression in osteoclasts via the tyrosine kinase-NF-kappaB pathway, J. Dent. Res. 83 (2004) 791–796.
[38] M. Bond, R.P. Fabunmi, A.H. Baker, A.C. Newby, Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: an absolute requirement for transcription factor NF-kappa B, FEBS Lett. 435 (1998) 29–34.