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Overexpression of prohibitin-1 inhibits RANKL-induced activation of p38-Elk-1-SRE signaling axis blocking MKK6 activity

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ABSTRACT

Prohibitin-1 (PHB) regulates diverse cellular processes by controlling several signaling pathways. In this study, we investigated the functional involvement of PHB in osteoclast differentiation. PHB expression was time-dependently increased by RANKL in BMMs. However, the retroviral over-expression of PHB strongly inhibited the expression of c-Fos and NFATc1, and activation of p38-Elk-1-SRE signaling pathway. Anti-osteoclastogenic action of PHB was significantly inhibited by constitutively active forms of MKK6, but not Elk-1. Collectively, PHB negatively regulates the formation of mature osteoclasts via inhibition of MKK6 activity that affects the activation of the p38-Elk-1 signaling axis required for the expression of c-Fos and NFATc1.

1. Introduction

Prohibitin-1 (PHB, also known as PHB1) is highly conserved evolutionarily, virtually identical across species, and widely expressed in most cell types. PHB regulates diverse cellular processes by controlling several signaling pathways, and recent data supports its therapeutic potential in various diseases, including cancer, inflammatory bowel disease, diabetes, and obesity [1,2]. Most studies have focused on the functional role of PHB as an inhibitor of cell proliferation, and several studies recently suggested its involvement in cell differentiation. For examples, PHB was increased during ovarian development, and its increased expression has been reported to correlate with granulosa cell differentiaition [3,4]. The role of PHB in spermiogenesis has been recently suggested [5], and it also suggested to be a marker of the differentiation of cancer cells [6]. Furthermore, the upregulation of PHB during adipogenesis of mouse 3T3-L1 preadipocytes suggested its role as the potential targets for obesity therapies; in mouse 3T3-L1 preadipocytes, the knockdown of PHB reduced the expression of adipogenic markers [7], and conversely, its overexpression was sufficient to induce adipogenesis [8].

Mature multinucleated osteoclasts are differentiated and functionalized by the fusion of macrophage precursor cells in a response to two cytokines: osteoblast-secreted macrophage-colony stimulating factor (M-CSF) and osteoblast-expressed receptor activator of nuclear factor-κB ligand (RANKL) [9]. The binding of M-CSF to its receptor, M-CSFR, on the cellular surface of bone marrow mononuclear macrophage/monocyte-lineage hematopoietic precursors induces surface expression of RANK (a receptor of RANKL), and then the binding of RANKL to RANK triggers activation of signaling

Abbreviations: PHB, Prohibitin-1; M-CSF, macrophage-colony stimulating factor; RANKL, receptor activator of nuclear factor-κB ligand; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; NFATc1, nuclear factor of activated T cells; FBS, fetal bovine serum; BMCs, bone marrow cells; BMMs, bone marrow-derived macrophages; SDS, sodium dodecyl sulfate; PVDF, polyvinylidenedifluoride; TRAP, tartrate-resistant acid phosphatase; MNC, multinucleated osteoclasts.

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molecules such as mitogen-activated protein kinases (MAPKs) for the expression of genes required for osteoclast differentiation [10]. The control of osteoclast-specific genes are regulated by activation of transcription factors, such as nuclear factor-κB (NF-κB), and the activator protein-1 family members including c-Fos and nuclear factor of activated T cells (NFATc1). c-Fos is an essential factor for induction of NFATc1, a master transcription factor regulating osteoclast-specific genes [11,12]. Specifically, excessive bone resorption by overactivated osteoclasts is involved in several bone loss-associated disorders such as osteoporosis, rheumatoid arthritis, osteosarcoma, and cancer bone metastasis [13–15]. Therefore, understanding the molecular mechanisms by which the cellular components control the osteoclastogenesis is helpful to develop the therapeutic strategies to overcome bone loss-related disorders [16,17].

With regards to the therapeutic role of PHB, it has been identified as a strong binding protein for anti-resorptive compounds to inhibit osteoclast differentiaton, but no evidence yet indicates the functional involvement of PHB in the process of osteoclast differentiation [18]. Therefore, in the present study, we investigated the expression of PHB during osteoclast differentiaton, its functional involvement via its over-expression, and its role in osteoclast differention.

2. Materials and methods

2.1. Reagents and antibodies

Penicillin, streptomycin, α-minimum essential medium (α-MEM), and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Human soluble RANKL and M-CSF were obtained from PeproTech EC Ltd. (London, UK). Antibodies against NFATc1, c-Fos, lamin B, and PHB were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against phospho (p)-JNK, p-ERK, p-p38, p-Elk, ERK, JNK, p38, and Elk were obtained from Cell Signaling Technology Inc. (Beverly, MA, USA). Antibody against mye-epitope was purchased from Abcam. Anti-actin antibody was obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Osteoclast cell culture, co-culture system, and TRAP staining

All experiments were carried out as described in a previous study with modifications [19].

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR), western blot analysis, and bone pit formation

RT-PCR Table 1, western blot analysis, and bone pit formation were performed as described previously [19].

2.4. Constructs and retrovirus preparation

The PHB, Elk-1, and MKK6 genes were prepared by RT-PCR using RNA from BMMs. The primer sequences were as follows: PHB forward (BamHI), 5′-GATCGGATCCATGGCTGCCAAGTGTTTGA-3′; PHB-c-myc reverse (XhoI), 5′-GGACCTCGAGTCAATGCAGGTCCTCCTCTGAGATCAGCTTCTGCTCCTCCTGGGGAAGCTGGAGAAGC-3′; Elk-1 forward (BamHI), 5′-GATCGGATCCATGGACCCATCTGTGACGCT-3′; Elk-1 reverse (XhoI), 5′-GGACCTCGAGTCATGGCTTCTGGGGCCCTG-3′; MKK6 forward (BamHI), 5′- GTACGGATCCATGTCTCAGTCGAAAGGCAA-3′; MKK6 reverse (XhoI), 5′-GACGCTCGAGGTCCCCAAGTATCAGTTTTA-3′. PHB-Flag, Elk-1, and MKK6 were subcloned into pcDNA3.1 (Invitrogen, Inc) at the BamHI and XhoI restriction sites. PHB-Flag was subcloned into pcDNA3.1 with a Flag tag at the 3′ end. Dominant-negative MKK6 (DN-MKK6) was constructed by replacing Ser-207 and Thr-211 with Ala residues and Ser-207 and Thr-211 with Glu in consitutively active MKK6 (CA-MKK6) using PCR-based overlap extension mutagenesis. PHB-c-myc was cloned into the retroviral vector pMX-puro (Cell Biolabs, San Diego, CA, USA). Retroviral packaging was performed by transfecting the packaging plasmids into Plat-E cells using Lipofectamine 2000 (Invitrogen). The viral supernatant was collected from the culture media 48 h after transfection. BMMs were incubated with viral supernatant in the presence of polybrene (8 ng/ml). After infection, BMMs were cultured overnight, detached using StemPro® Accutase® Cell Dissociation Reagent (Invitrogen), and cultured with M-CSF (30 ng/ml) and puromycin (2 μg/ml) for 2 days. Puromycin-resistant BMMs were induced to differentiate in the presence of M-CSF (30 ng/ml) and RANKL (100 ng/ml) for 5 days.

Table 1
Primer sequences used in this study.

Target gene	Forward (5′–3′)	Reverse (5′–3′)
PHB	CCTCATCCCTTGGGTACAGA	AGCTCTCGCTGGGTAATCAA
c-Fos	CTGGTGCAGCCCACTCTGGTC	CTTTCAGCAGATTGGCAATCTC
NFATc1	CTTCAGCTGGAGGACACCCC	GGAAGACCAGCCTCACCCTG
TRAP	ACTTCCCCAGCCCTTACTAC	TCAGCACATAGCCCACACCG
OSCAR	GAACACCAGAGGCTATGACTGTTC	CCGTGGAGCTGAGGAAAAGGTTG
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCT GTA

2.5. Luciferase assay

Luciferase reporter plasmid was constructed by cloning c-Fos-luc vector into pGL3 basic (Promega, Madison, WI, USA) with the

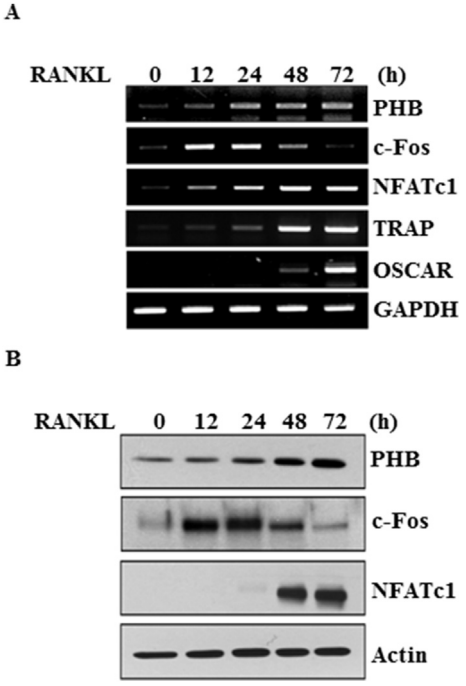

Fig. 1. M-CSF and RANKL increased PHB expression during osteoclastogenesis. BMMs were cultured for 3 days with M-CSF and stimulated with RANKL for the indicated times. (A) Semiquantitative RT-PCR showed a slight increase in PHB mRNA expression during osteoclast differentiation in BMMs. (B) Western blot analysis showed an increase in PHB protein during osteoclastogenesis in BMMs.

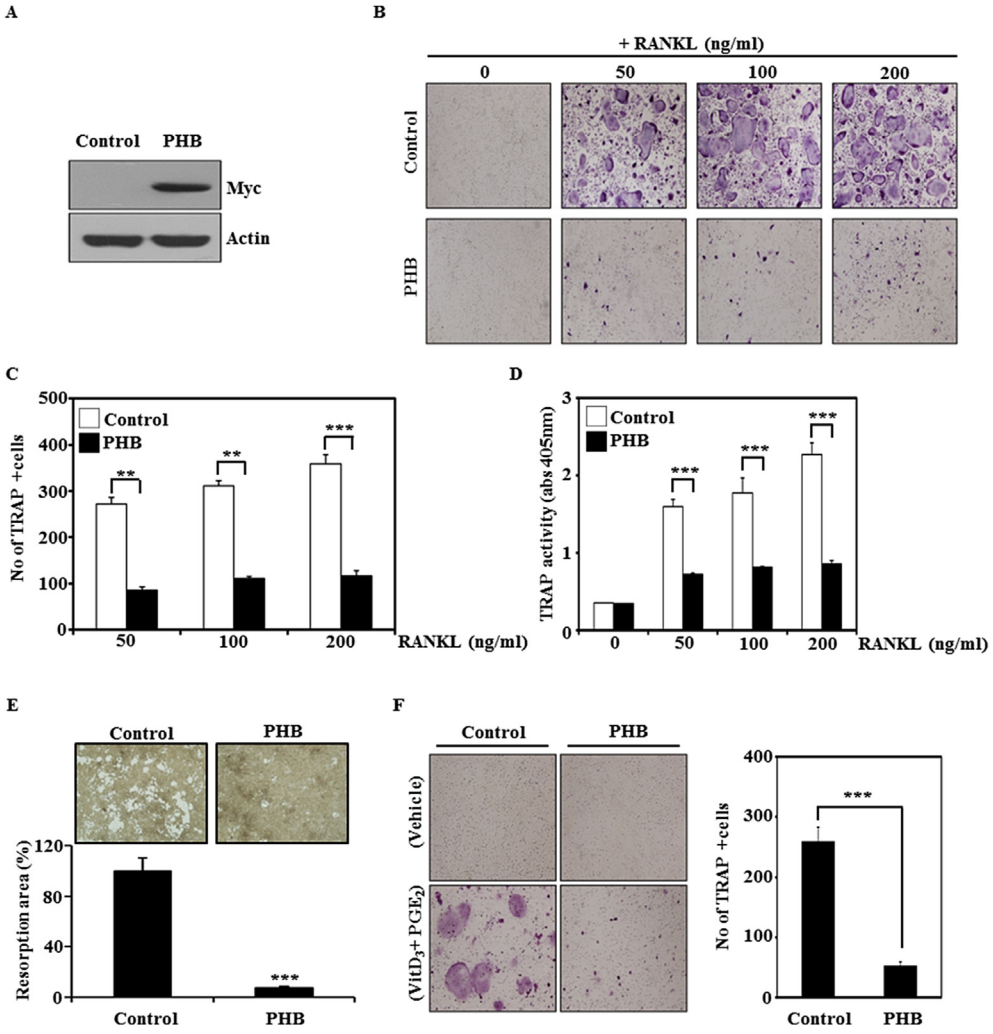

Fig. 2. Over-expression of PHB suppressed RANKL-induced osteoclastogenesis. (A) BMMs were transduced with retroviruses harboring PHB or pMX-IRES-PURO (control), selected with puromycin (2 μg/ml) for 2 days, and cultured for 2 days in the presence of M-CSF. The protein levels were analyzed by western blot. (B) Infected BMMs were cultured for 5 days in the presence of RANKL (0, 50, 100, or 200 ng/ml) and M-CSF (30 ng/ml). Cells were fixed in 3.7% formalin, permeabilized with 0.1% Triton X-100, and stained with TRAP solution. Mature TRAP-positive multinucleated osteoclasts (MNCs) were photographed under a light microscope. Magnification × 100. (C) and (D) TRAP-positive MNCs and the TRAP activity in osteoclasts generated from control BMMs (white column) or PHB-induced BMMs (black column) were counted and measured. $^{**}p < 0.01$, $^{***}p < 0.001$ versus control. (E) Mature osteoclasts derived from infected BMMs were cultured on BioCoat Osteologic MultiTest plates for 6 days. Cells were removed and photographed under a light micoscope. The bone resorption area was quantified using the ImageJ program. $^{***}p < 0.001$ versus control. (F) Infected BMMs were cocultured with primary osteoblasts for 6 days and then fixed and stained for TRAP. Magnification × 100. TRAP-positive MNCs were counted. $^{***}p < 0.001$ versus control.

0.5 kb promoter region of the c-Fos gene, and the 2 × serum response element (2 × SRE) was constructed by inserting two copies of the SRE sequence (CCATATTAGG) into the pGL3 basic vector. Full-length human RANK cDNA was amplified from human leukocyte cDNA (Clontech, Palo Alto, CA, USA) and cloned into the HindIII-EcoRI site of pcDNA3.1. To measure luciferase activity, human embryonic kidney HEK293T cells were plated at a density of 5×10^4 cells/well on a 48-well plate in triplicate for 1 day. A total of 500 ng of plasmid DNA was mixed with Lipofectamine 2000 (Invitrogen) and transfected into the HEK293T cells following the manufacturer's protocol. The amount of transfected DNA was consistently 500 ng by adding empty vector DNA when necessary. After 48 h the transfected cells were lysed with lysis buffer (Promega, Madison, WI, USA) and the luciferase activity measured using the dual-luciferase assay system (Promega) and Wallac EnVision microplate reader (Perkin Elmer). The luciferase activity was normalized to renilla luciferase activity for each sample and the values represented with standard deviations for triplicate samples.

2.6. Statistical analysis

All quantitative values are presented as mean ± SD. Each experiment was performed three to five times, and the results from one representative experiment are shown. Statistical differences were analyzed using the Student's *t* test. A value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. PHB is induced during osteoclast differentiation

The expression of PHB during osteoclast differentiation was analyzed in BMMs. When the BMMs differentiated into osteoclasts in the presence of M-CSF and RANKL, PHB mRNA expression was time-dependently induced during osteoclast differentiation (Fig. 1A). This is the first report to show the RANKL-mediated induction of PHB during osteoclastogenesis, and moreover, its expression level was higher in the late stage of osteoclast

differentiation. Osteoclast differentiation in BMMs was confirmed by evaluating the mRNA expression of molecules related to osteoclast differentiation, including c-Fos, NFATc1, TRAP, and OSCAR. TRAP and OSCAR are target molecules of c-Fos and/or NFATc1 in osteoclasts [11,12,21]. The induced expression of PHB during the differentiation of BMMs into osteoclasts was also confirmed by Western blot analysis (Fig. 1B). The spatial induction of transcription factors c-Fos and NFATc1 in the process of osteoclast differentiation was also confirmed in a previous study [20,22]. Relatively higher expression level of PHB due to its time-dependent increase by RANKL suggested that it might be functionally involved in the late stage of osteoclast differentiation.

3.2. PHB negatively regulates osteoclast differentiation

To examine the functional role of PHB in the regulation of osteoclastogenesis, a retrovirus-based gain-of-function experiment was carried out. The over-expression of PHB by retroviral infection in BMMs was confirmed by Western blot analysis (Fig. 2A) and its effect on osteoclast differentiation evaluated by TRAP staining. Interestingly, the formation of RANKL-induced TRAP-positive MNCs was strongly inhibited by the over-expression of PHB in BMMs (Fig. 2). The number of TRAP-positive MNCs and TRAP activity was also significantly inhibited by the over-expression of PHB (Fig. 2C and D). The inhibitory effect of PHB over-expression on osteoclast differentiation was not overcome by increasing the dosage of RANKL. Since the over-expression of PHB in BMMs strongly inhibited the formation of the RANKL-induced TRAP-positive multinuclear cells, the spacious resorbed area by osteoclasts maturated after the normal process of osteoclast differentiation was not observed in the condition of PHB over-expression (Fig. 2E). The over-expression of PHB also significantly reduced the formation of TRAP-positive osteoclasts in a co-culture model with primary calvarial osteoblasts in the presence of $VitD_3$ and PGE_2 (Fig. 2F). These results suggested that PHB is time-dependently induced during osteoclast differentiation but negatively regulates the formation of multinucleated osteoclasts. In other words, PHB could be time-dependently induced by RANKL via the activation of signaling molecules and transcription factors required for osteoclast differentiation, but its strong induction negatively regulates the RANKL-induced osteoclastogenesis.

3.3. PHB inhibits RANKL-induced expression of c-Fos and NFATc1 and the activation of p38

Anti-osteoclastogenic mechanism of PHB was further investigated. The expression of c-Fos, an AP-1 transcription factor, is induced by RANKL in the early stage of osteoclast differentiation and is essential for RANKL-mediated induction of NFATc1, the master regulator of osteoclast differentiation [21,23,24]. As the most distal transcription factor of osteoclast differentiation, NFATc1 regulates osteoclast-related genes during osteoclast differentiation [12,25,26]. Because the anti-osetoclastogenic action of PHB might be due to its potential to inhibit the expression of c-Fos and/or NFATc1, the effect of PHB on the RANKL-induced expression of these transcription factors was evaluated by Western blot analysis. As shown in Fig. 3A, the RANKL-induced expression of c-Fos and NFATc1 was attenuated by the over-expression of PHB. The induction of NFATc1 in the late stage of osteoclast differentiation was completely inhibited by the over-expression of PHB. Normally, RANKL strongly induces the expression and nuclear translocation of NFATc1 to autoamplify and continuously regulate osteoclast-specific genes during differentiation [12,27], but even in response to RANKL, NFATc1 was not detected in either the cytosolic or nuclear fractions of BMMs over-expressing PHB (Fig. 3B), which might result in the down-regulation of c-Fos by PHB in the early stage of osteoclast differentiation.

To gain insight into the mechanism by which PHB negatively regulates osteoclastogenesis, the effect of PHB on the RANKL-mediated activation of early signaling molecules, such as MAP kinases, was evaluated. The RANKL-mediated activation of MAP kinases regulates the expression and/or activation of c-Fos and NFATc1 [28–30]. PHB over-expression strongly inhibited the RANKL-induced phosphorylation of p38, but not JNK and ERK (Fig. 3C). These results suggest that PHB exerts anti-osteoclastogenic activity via the p38 signaling pathway.

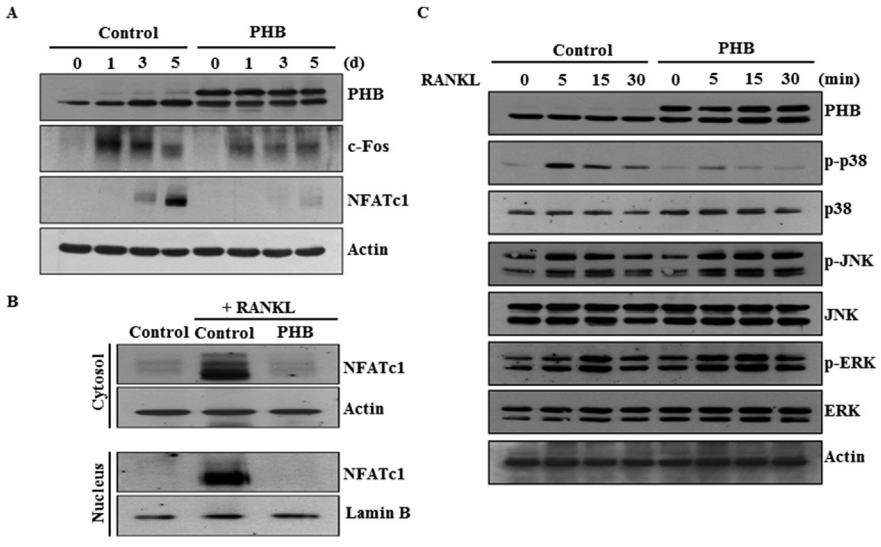

Fig. 3. Over-expression of PHB attenuates osteoclastogenesis by suppressing c-Fos and NFATc1 expression via p38 inactivation. BMMs were transduced with pMX-IRES-PURO (control) or PHB retrovirus and cultured with M-CSF and RANKL for the indicated times. (A) The cells were harvested at each time point and the lysates anlayzed by Western blotting using antibodies specific for PHB, c-Fos, NFATc1, and actin. (B) Cytosolic and nuclear fractions were harvested from cells cultured for 2 days and subjected to SDS-PAGE and Western blot analysis to detect NFATc1. Antibodies specific for actin and lamin B were used to normalize the cytosolic and nuclear extracts, respectively. (C) The BMMs were stimulated with RANKL (100 ng/ml) for the indicated times. Whole cells lysates were analyzed by immunoblotting with specific antibodies as indicated.

3.4. PHB inhibits activation of the MKK6-p38-Elk-1-c-Fos SRE signaling axis

The inactivation of p38 by PHB might affect the down-regulation of c-Fos and/or NFATc1 gene expression. We evaluated the effect of PHB on the activation of p38 downstream molecules to elucidate its mode of action in detail. Among the several candidates for study, we focused on the involvement of Elk-1, as it is a downstream effecter of p38 that directly regulates the expression of c-Fos through the SRE in its promoter region [31–34]. In addition, ERK recruitment to SRE promoter complexes has been shown to correlate with its binding to Elk-1 [35]. In this study, RANKL induced the activation of p38 and Elk-1 in HEK293T cells transfected with both RANK and Elk-1, but phosphorylation was strongly inhibited by the over-expression of PHB (Fig. 4A). Elk-1-mediated activation of the SRE was significantly induced by RANKL stimulation, but its induction was dose-dependently inhibited by PHB (Fig. 4B). These results suggested that overexpression of PHB could inhibit the RANKL-induced activation of SRE via the p38-Elk-1 signaling pathway.

We also investigated the possible involvement of MAP kinase kinase 6 (MKK6, also called stress-activated protein kinase kinase-3, SAPKK-3), an upstream molecule of the p38-Elk-1 signaling pathway that is required for activating the c-Fos promoter for the anti-osteoclastogenic action of PHB [36,37]. As shown in Fig. 4C, the SRE-luciferase activity assay revealed that both mature and constitutively active forms of MKK6 significantly activated the SRE, but its activation was significantly inhibited by PHB in a dose-dependent manner. The dominant negative form of MKK6 did not activate the SRE. Furthermore, MKK6-induced phosphorylation of Elk-1 was inhibited by PHB in a dose-dependent manner. These results led to the hypothesis that PHB reduces the activity of SRE by

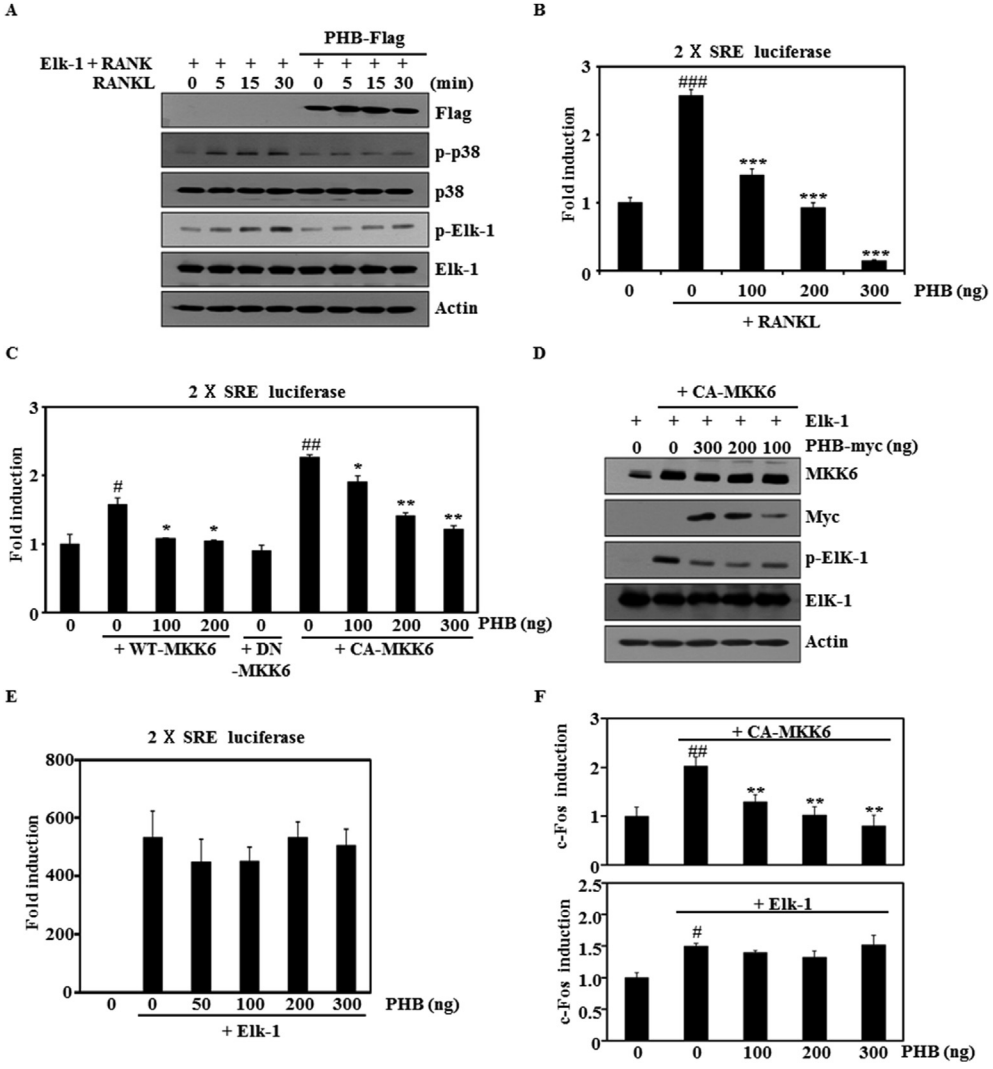

Fig. 4. PHB attenuated RANKL-induced Elk/c-Fos expression via the inhibition of p38 activity. (A) HEK293T cells were co-transfected with empty vector (200 ng/ml) containing Elk-1 (100 ng/ml) and RANK (100 ng/ml) or PHB-flag (200 ng/ml). After 48 h of transfection, the cells were stimulated with hRANKL (500 ng/ml) for the indicated time points. Cell lysates were analyzed by Western blot with the specific antibodies indicated. (B) A 2 × SRE luciferase reporter was co-transfected with RANK (100 ng/ml) and increasing amounts of PHB (0, 100, 200, 300), as well as pGL4 renilla-luciferase (20 ng). After 6 h, transfected cells were stimulated by RANKL for 48 h and the luciferase activity measured. Activity was expressed as fold induction compared to the activity of 2 × SRE luciferase alone. pGL4 renilla-luciferase activity was used to normalize the transfection efficiency and luciferase activity. $^{###}p < 0.001$ versus negative control, $^{***}p < 0.001$ versus positive control. (C) and (E) A 2 × SRE luciferase reporter was co-transfected with MKK6 (WT, DN, or CA) or Elk-1 (100 ng/ml) and PHB plasmid as indicated. After 48 h the luciferase activity was measured. $^{#}p < 0.05$, $^{##}p < 0.01$ versus negative control; $^{*}p < 0.05$, $^{**}p < 0.01$ versus positive control. (D) HEK293T cells were co-transfected with CA-MKK6 (100 ng/ml), Elk-1 (100 ng/ml), and various amounts of PHB plasmid as indicated. Transfected cells were cultured for 48 h and the lysates anlayzed by Western blotting using antibodies specific for MKK6, Myc, p-Elk-1, Elk-1, and actin. (F) The 0.5-kb c-Fos promoter-luciferase construct was co-transfected with CA-MKK6 or Elk-1 with various amounts of PHB. After 48 h the luciferase activity was measured. $^{#}p < 0.05$, $^{##}p < 0.01$ versus negative control; $^{**}p < 0.01$ versus positive control.

inhibiting the activity of MKK6, which further affects activation of the p38-Elk-1 signaling axis.

In addition, no direct inhibition of Elk-1-SRE signaling by PHB was observed (Fig. 4E). This data suggested that PHB could exhibit its anti-osteoclastogenic activity via inhibiting the upstream molecule of Elk-1, MKK6. To clarify our hypothesis, the effect of PHB on the MKK6- or Elk-1-induced activation of the c-Fos promoter was evaluated. As shown in Fig. 4F, PHB significantly inhibited MKK6-induced, but not Elk-1-induced, activation of the c-Fos promoter, suggesting that the signaling downstream of Elk-1 is not affected by PHB.

In conclusion, this study is the first to report the functional involvement of PHB in osteoclast differentiation. Although it is not clear so far that RANKL-mediated induction of PHB is controlled by c-Fos and/or NFATc1 during osteoclastogenesis, PHB was time-dependently induced during osteoclast differentiation, and its overexpression in precursors inhibited the RANKL-induced formation of mature osteoclasts via inhibiting MKK6 activation that subsequently affects the activation of the p38-Elk-1 signaling axis required for the expression of c-Fos and NFATc1. In further studies, the effect of PHB on the mitochondrial function, gene expression and apoptosis in preosteoclasts and/or mature osteoclasts would be investigated for further elucidating its anti-osteoclastogenic activity [4,38].

Conflict of interest

The authors declare no competing financial interest.

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Transparency document

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References

[1] S. Mishra, S.R. Ande, B.L. Nyomba, The role of prohibitin in cell signaling, FEBS J. 277 (2010) 3937–3946.
[2] A.L. Theiss, S.V. Sitaraman, The role and therapeutic potential of prohibitin in disease, Biochim. Biophys. Acta 1813 (2011) 1137–1143.
[3] W.E. Thompson, J.M. Powell, J.A. Whittaker, R. Sridaran, K.H. Thomas, Immunolocalization and expression of prohibitin, a mitochondrial associated protein within the rat ovaries, Anat. Rec. 256 (1999) 40–48.
[4] W.E. Thompson, A. Branch, J.A. Whittaker, D. Lyn, M. Zilberstein, K.E. Mayo, et al., Characterization of prohibitin in a newly established rat ovarian granulosa cell line, Endocrinology 142 (2001) 4076–4085.
[5] H.T. Mao, D.H. Wang, Z. Lan, H. Zhou, W.X. Yang, Gene expression profiles of prohibitin in testes of Octopus tankahkeei (ot-phb) revealing its possible role during spermiogenesis, Mol. Biol. Rep. 39 (2012) 5519–5528.
[6] X. Kang, L. Zhang, J. Sun, Z. Ni, Y. Ma, X. Chen, et al., Prohibitin: a potential biomarker for tissue-based detection of gastric cancer, J. Gastroenterol. 43 (2008) 618–625.
[7] D. Liu, Y. Lin, T. Kang, B. Huang, W. Xu, M. Garcia-Barrio, et al., Mitochondrial dysfunction and adipogenic reduction by prohibitin silencing in 3T3-L1 cells, PLoS One 7 (2012) e34315.
[8] S.R. Ande, Z. Xu, Y. Gu, S. Mishra, Prohibitin has an important role in adipocyte differentiation, Int, J. Obes. (Lond.) 36 (2012) 1236–1244.
[9] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342.
[10] Z.H. Lee, H.H. Kim, Signal transduction by receptor activator of nuclear factor kappa B in osteoclasts, Biochem. Biophys. Res. Commun. 305 (2003) 211–214.
[11] A.E. Grigoriadis, Z.Q. Wang, M.G. Cecchini, W. Hofstetter, R. Felix, H.A. Fleisch, et al., c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling, Science 266 (1994) 443–448.
[12] H. Takayanagi, S. Kim, T. Koga, H. Nishina, M. Isshiki, H. Yoshida, et al., Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts, Dev. Cell. 3 (2002) 889–901.
[13] T. Akiyama, C.R. Dass, P.F. Choong, Novel therapeutic strategy for osteosarcoma targeting osteoclast differentiation, bone-resorbing activity, and apoptosis pathway, Mol. Cancer Ther. 7 (2008) 3461–3469.
[14] M.L. Broadhead, J.C. Clark, C.R. Dass, P.F. Choong, D.E. Myers, Therapeutic targeting of osteoclast function and pathways, Expert Opin. Ther. Targets 15 (2011) 169–181.
[15] J. Kikuta, M. Ishii, Osteoclast migration, differentiation and function: novel therapeutic targets for rheumatic diseases, Rheumatol. (Oxf.) 52 (2013) 226–234.
[16] G.A. Rodan, T.J. Martin, Therapeutic approaches to bone diseases, Science 289 (2000) 1508–1514.
[17] S.L. Teitelbaum, Bone resorption by osteoclasts, Science 289 (2000) 1504–1508.
[18] S.Y. Chang, S.J. Bae, M.Y. Lee, S.H. Baek, S. Chang, S.H. Kim, Chemical affinity matrix-based identification of prohibitin as a binding protein to anti-resorptive sulfonyl amidine compounds, Bioorg. Med. Chem. Lett. 21 (2011) 727–729.
[19] S.W. Choi, J.T. Yeon, K.I. Park, C.H. Lee, B.S. Youn, J. Oh, et al., VapB as a regulator of osteoclastogenesis via modulation of PLCā2-$Ca^{(2+)}$-NFAT signaling, FEBS Lett. 586 (2012) 263–269.
[20] J.T. Yeon, S.W. Choi, K.I. Park, M.K. Choi, J.J. Kim, B.S. Youn, et al., Glutaredoxin2 isoform b (Glrx2b) promotes RANKL-induced osteoclastogenesisthrough activation of the p38-MAPK signaling pathway, BMB Rep. 45 (2012) 171–176.
[21] K. Matsuo, D.L. Galson, C. Zhao, L. Peng, C. Laplace, K.Z. Wang, et al., Nuclear factor of activated T-cells (NFAT)rescues osteoclastogenesis in precursors lacking c-Fos, J. Biol. Chem. 279 (2004) 26475–26480.
[22] H. Huang, E.J. Chang, J. Ryu, Z.H. Lee, Y. Lee, H.H. Kim, Induction of c-Fos and NFATc1 during RANKL-stimulated osteoclast differentiation is mediated by the p38signaling pathway, Biochem. Biophys. Res. Commun. 351 (2006) 99–105.
[23] N. Ishida, K. Hayashi, M. Hoshijima, T. Ogawa, S. Koga, Y. Miyatake, et al., Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator, J. Biol. Chem. 277 (2002) 41147–41156.
[24] M. Asagiri, H. Takayanagi, The molecular understanding of osteoclast differentiation, Bone 40 (2007) 251–264.
[25] T.N. Crotti, M. Flannery, N.C. Walsh, J.D. Fleming, S.R. Goldring, K.P. McHugh, NFATc1regulation of the human beta3 integrin promoter in osteoclast differentiation, Gene 372 (2006) 92–102.
[26] M. Matsumoto, M. Kogawa, S. Wada, H. Takayanagi, M. Tsujimoto, S. Katayama, et al., Essential role of p38 mitogen-activated protein kinase in cathepsin K gene expression during osteoclastogenesis through association of NFATc1 and PU.1, J. Biol. Chem. 279 (2004) 45969–45979.
[27] M. Asagiri, K. Sato, T. Usami, S. Ochi, H. Nishina, H. Yoshida, et al., Autoamplification of NFATc1 expression determines its essential role in bone homeostasis, J. Exp. Med. 202 (2005) 1261–1269.
[28] S.E. Lee, W.J. Chung, H.B. Kwak, C.H. Chung, K.B. Kwack, Z.H. Lee, et al., Tumor necrosis factor-alpha supports the survival of osteoclasts through the activation of Akt and ERK, J. Biol. Chem. 276 (2001) 49343–49349.
[29] J.P. David, K. Sabapathy, O. Hoffmann, M.H. Idarraga, E.F. Wagner, JNK1 modulates osteoclastogenesis through both c-Jun phosphorylation-dependent and -independent mechanisms, J. Cell. Sci. 115 (2002) 4317–4325.
[30] X. Li, N. Udagawa, K. Itoh, K. Suda, Y. Murase, T. Nishihara, et al., p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function, Endocrinology 143 (2002) 3105–3113.
[31] N. Benbernou, S. Esnault, F. Galibert, Activation of SRE and AP1 by olfactory receptors via the MAPK and Rho dependent pathways, Cell. Signal 25 (2013) 1486–1497.
[32] J. van Riggelen, G. Buchwalter, U. Soto, J. De-Castro Arce, H. zurHausen, B. Wasylyk, et al., Loss of net as repressor leads to constitutive increased c-fos transcription in cervical cancer cells, J. Biol. Chem. 280 (2005) 3286–3294.
[33] A.N. Kukushkin, M.V. Abramova, S.B. Svetlikova, Z.A. Darieva, T.V. Pospelova, V.A. Pospelov, Downregulation of c-fos gene transcription in cells transformed by E1A and cHa-ras oncogenes: a role of sustained activation of MAP/ERK kinase cascade and of inactive chromatin structure at c-fos promoter, Oncogene 21 (2002) 719–730.
[34] T.V. Pospelova, A.V. Medvedev, A.N. Kukushkin, S.B. Svetlikova, A.J. van der Eb, J.C. Dorsman, et al., E1A + cHa-ras transformed rat embryo fibroblast cells are characterized by high and constitutive DNA binding activities of AP-1 dimers with significantly altered composition, Gene Expr. 8 (1999) 19–32.
[35] H.M. Zhang, L. Li, N. Papadopoulou, G. Hodgson, E. Evans, M. Galbraith, et al., Mitogen-induced recruitment of ERK and MSK to SRE promoter complexes by ternary complex factor Elk-1, Nucleic Acids Res. 36 (2008) 2594–2607.
[36] J. Raingeaud, A.J. Whitmarsh, T. Barrett, B. Dérijard, R.J. Davis, MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway, Mol. Cell. Biol. 16 (1996) 1247–1255.
[37] I. Ferreiro, M. Barragan, A. Gubern, E. Ballestar, M. Joaquin, F. Posas, The p38 SAPK is recruited to chromatin via its interaction with transcription factors, J. Biol. Chem. 285 (2010) 31819–31828.
[38] S. Mishra, L.C. Murphy, L.J. Murphy, The Prohibitins: emerging roles in diverse functions, J. Cell. Mol. Med. 10 (2006) 353–363.