Biochemical and Biophysical Research Communications 460 (2015) 157–163

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dexamethasone-induced apoptosis of osteocytic and osteoblastic cells is mediated by TAK1 activation

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ARTICLE INFO

Article history:
Received 23 February 2015
Available online 6 March 2015

Keywords:
Dexamethasone
Osteonecrosis
TAK1
Pyk2-JNK signaling
Apoptosis

ABSTRACT

Increased apoptosis of osteoblasts and osteocytes is the main mechanism of glucocorticoid (GC)-induced osteonecrosis. In the current study, we investigated whether dexamethasone (Dex)-induced osteoblastic and osteocytic cell apoptosis is mediated through activation of transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1), and whether TAK1 inhibition could promote survival opposing the deleterious effects of Dex. We found that TAK1 was activated by Dex in both osteocytic MLO-Y4 and osteoblastic OB-6 cells, which was prevented by two known anti-oxidants N-acetylcysteine (NAC) and ebselen. TAK1 inhibitors, including LYTAK1 and 5Z-7-oxozeaenol (57-OZ), inhibited Dex-induced apoptosis of MLO-Y4 and OB-6 cells. Meanwhile shRNA-mediated knockdown of TAK1 also suppressed Dex-induced damages to MLO-Y4 and OB-6 cells. On the other hand, exogenously over-expressing TAK1 enhanced Dex-induced MLO-Y4 and OB-6 cell apoptosis. At the molecular level, we found that TAK1 mediated Dex-induced pro-apoptotic Pyk2-JNK activation. Inhibition or silencing of TAK1 almost abolished Pyk2-JNK phosphorylations by Dex in MLO-Y4 and OB-6 cells. TAK1 over-expression, on the other hand, increased Dex's activity on Pyk2-JNK phosphorylations in above cells. We conclude that part of the pro-apoptotic actions of Dex on osteoblastic and osteocytic cells are mediated through TAK1 activation, and that inhibition of TAK1 might protect from GC-induced damages to osteoblasts and osteocytes.

1. Introduction

For patients with rheumatoid arthritis or many other chronic autoimmune or inflammatory diseases, glucocorticoid (GC) is routinely prescribed [1,2]. However, long-term or excess GC usage will lead to non-trauma osteonecrosis, which would cause bone fractures if not treated properly [2,3]. Histological studies revealed consistent bone reduction and increased bone resorption in these patients [1–3]. Although the link between excess GC usage and bone loss has been well-established, the underlying mechanisms are not fully understood [4,5]. It is now known that reduced number of osteoblast/osteoclast precursors and increased apoptosis of osteoblasts/osteocytes are the direct actions of GC [4,6].

Induction of apoptosis of osteoblasts and osteocytes is the main cause of GC-induced osteonecrosis [3–5]. In both osteoblasts and osteocytes, GC-induced apoptosis is due to direct hormonal effects [3,7–9], and could be reversed by overexpressing of beta-hydroxysteroid dehydrogenase type 2, the enzyme that inactivates GC [10]. *In vitro* tissue-culture studies as well as animal studies have revealed that pro-apoptotic actions of GC are regulated through the classical GC receptor, and are triggered by rapid activation of proline-rich tyrosine kinase 2 (Pyk2)-c-Jun N-terminal kinase (JNK) signaling in a reactive oxygen species (ROS)-dependent manner [7,9,11], although the detailed signaling mechanisms are not fully understood.

TAK1, or transforming growth factor-β (TGF-β)-activated kinase 1, is a well-known mitogen-activated protein kinase kinase kinase (MAP3K), which mediates JNK activation under various stress conditions [12]. In the current study, we investigated whether dexamethasone (Dex)-induced osteoblastic and osteocytic apoptosis is due to activation of TAK1, and whether inhibition of TAK1 could promote survival opposing the deleterious effects of Dex. Our results revealed that TAK1 activation is required for Dex-induced apoptosis in both osteocytic MLO-Y4 and osteoblastic OB-6 cells, probably through mediating Pyk2-JNK signaling.

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http://dx.doi.org/10.1016/j.bbrc.2015.02.161
0006-291X/

2. Material and methods

2.1. Materials

Tissue culture media and Hank's balanced salt solution (HBSS) were purchased from Gibco BRL (Grand Island, NY). Fetal bovine serum (FBS) and calf serum (CS) were from Hyclone Laboratories (Logan, UT). Dex, 5Z-7-oxozeaenol (57-OZ), N-acetylcysteine (NAC) and ebselen were purchased from Sigma Chemicals (St. Louis, MO). Anti-TAK1, tubulin and JNK1/2 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies recognizing phosphorylated- (p-)TAK1 (Thr-184/187), p-Pyk2 (Tyr-402), Pyk2, p-JNK1/2 (Thr-183/Tyr-185) were purchased form Cell Signaling Technology (Danvers, MA). LYTAK1 was obtained from Lilly Research Laboratories (Indianapolis, IN).

2.2. Cell cultures

As reported [13], osteocytic MLO-Y4 cells, obtained from the Cell Bank of CAS Shanghai Institute of Biological Science, were cultured on collagen-coated surfaces in α-modified essential medium (α-MEM) supplemented with 5% FBS and 5% CS and incubated in 5% CO_2 incubator at 37 °C. OB-6 osteoblastic cells, also purchased from the Cell Bank of CAS Shanghai Institute of Biological Science, were maintained and differentiated as described [9,14].

2.3. Trypan blue staining

As described, MLO-Y4/*OB-6* cell death after applied treatment was tested by trypan blue staining assay [9]. Cells that excluded the dye were considered alive, and stained cells were considered dead. Data is reported as the percentage of dead cells [9].

2.4. Histone-DNA ELISA for detection of cell apoptosis

The Cell Apoptosis Detection Enzyme-Linked Immunosorbent Assay (ELISA) Kit was utilized for testing apoptosis in above cells following indicated treatment according to the protocol provided [15]. Briefly, the cells were lysed and the cell lysates were overlaid and incubated in microtiter plate modules coated with anti-histone antibody. Samples were then incubated with anti-DNA peroxidase followed by color development with ABTS substrate. The absorbance of the samples were determined with a microplate reader (Tecan, Durham, NC) at 405 nm.

2.5. Western blot analysis

After treatment, cells were lysed in lysis buffer [50 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.5% Triton X-100, 2.5 mM sodium orthovanadate, 10 μL/mL protease inhibitor cocktail] by incubating for 20 min at 4 °C. The protein concentration was determined with the Bio-Rad assay system (Hercules, CA). Total proteins were fractionated by SDS-PAGE and transferred onto PVDF membranes for Western blots as described earlier [16]. The immunoreactive proteins after incubation with appropriately labeled secondary antibody were detected with the enhanced chemiluminescence (ECL) detection kit (Amersham, Buckinghamshire, UK). Band intensity was quantified by ImageJ software (NIH) after normalization to the corresponding loading control.

2.6. ShRNA knockdown of TAK1

Three pSUPER-puro-retro vectors enconding non-overlapping TAK1 small hairpin RNA (shRNA) sequences (5′-GACACACAT GACCAATAACAA-3′ (TAK1 shRNA-1) [17], 5′-GAGGAAAGCGTTTATTG-TATT-3′ (TAK1-shRNA-2) [18] and 5′-CCCAATGGCTTATCTTACATT-3′ (TAK1-shRNA-3) [18]) were from Dr. Youhong Jiang's group [19]. The TAK1 plasmid or the plasmid containing scramble control shRNA ("Ctrl shRNA") were then transfected into HEK-293 cells with plasmids encoding viral packaging proteins VSVG and Hit-60 (Promega) [20] using the Lipofectamine 2000 (Invitrogen, Shanghai, China) reagent with corresponding procedure. The virus-containing supernatants were collected after 48 h of transfection and filtered through a 0.45 μm filter (Corning, Shanghai, China), and were then added to MLO-Y4 or

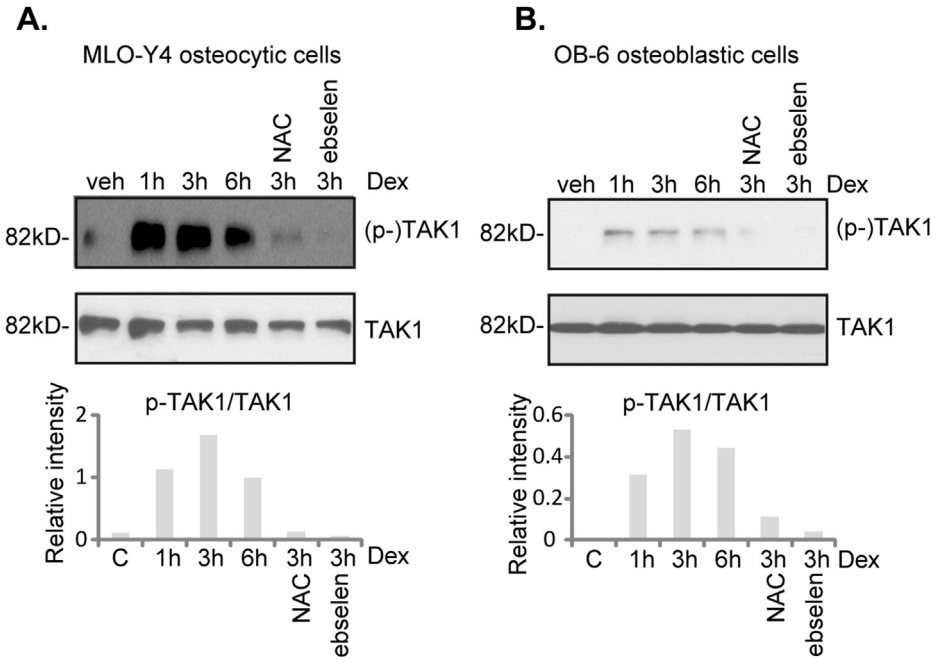

Fig. 1. Dexamethasone activates TAK1 in osteocytic and osteoblastic cells. MLO-Y4 osteocytic cells or OB-6 osteoblastic cells, pretreated with or without anti-oxidant ebselen (10 μM) or NAC (500 μM), were stimulated with dexamethasone (Dex, 1 μM) or vehicle (veh, 0.1% DMSO) for indicated time, p-and regular TAK1 were assayed by Western blots (A and B). TAK1 phosphorylation was quantified (A and B). Experiments in this and all following figures were repeated three times, with consistent results obtained.

OB-6 cells, in the presence of polybrene (Sigma). Afterwards, the cells were selected by puromycin (0.5 μg/mL). Expressions of TAK1 and tubulin (the equal loading) were tested by Western blots.

2.7. TAK1 overexpression

As described, TAK1-expressing plasmid, a gift from Dr. Lai Jiang [21], was constructed by inserting human TAK1 cDNA (purchased from Kaiji Biotech, Shanghai, China) into the pcDNA3 (Invitrogen, Shanghai, China) expressing vector. The plasmid was amplified (using JM 109 bacteria), extracted, and purified by Plasmid Midi Kit (Invitrogen, Shanghai, China). TAK1-expressing plasmid or the empty vector (0.25 μg/mL each) was transfected with Lipofectamine 2000. All the procedures were performed according to the manufacturer's instructions. Afterwards, expressions of TAK1 and tubulin (the equal loading) were tested by Western blots in transfected cells.

2.8. Statistical analysis

Data were expressed as means ± standard deviation (SD). Comparisons across more than two groups involved use of one-way ANOVA and then Student–Newman–Keuls test (SPSS 16.0, Chicago, IL). Means were considered significantly different at $p < 0.05$.

3. Results

3.1. Dexamethasone activates TAK1 in osteocytic and osteoblastic cells, prevented by anti-oxidants

Herein, we are set to understand the potential functions of TAK1 in GC-induced apoptosis of osteocytic and osteoblastic cells. We first examined the potential of dexamethasone (Dex) on TAK1 activation in MLO-Y4 osteocytic cells and OB-6 osteoblastic cells. In each cell type, Dex time-dependently induced TAK1 activation, evidenced by TAK1 phosphorylation at Thr-184/187 (Fig. 1A and B). Notably, pre-treatment with the anti-oxidant NAC or ebselen dramatically inhibited Dex-induced TAK1 activation in above bone cells (Fig. 1A and B). These results showed that Dex activated TAK1 in osteocytic and osteoblastic cells, and ROS production might be involved in the process.

3.2. Dexamethasone-induced apoptosis of osteocytic and osteoblastic cells is inhibited by TAK1 inhibitors

Similar to other studies reported [9,11], we showed that Dex induced apoptosis in MLO-Y4 osteocytic cells and OB-6 osteoblastic cells, evidenced by the histone-bound DNA ELISA assay (Fig. 2A and B). Further, Dex increased the percentage of MLO-Y4 and OB-6 cells exhibiting trypan blue uptake (Fig. 2C and D), another sign of apoptotic cell death induced by GC, as shown by other studies [7–9]. Significantly, pre-treatment with the TAK1 inhibitors, LYTAK1 [18,19] and 57-OZ, or the anti-oxidant NAC, suppressed Dex-induced apoptosis of either cell type, although the inhibitory effect of above inhibitors was not complete (Fig. 2A–D). Thus, these results suggest that TAK1 activation is important for Dex-induced apoptosis of osteocytic and osteoblastic cells.

3.3. Dexamethasone-induced apoptosis of osteocytic and osteoblastic cells is inhibited by TAK1 shRNA-knockdown, but is enhanced by TAK1 over-expression

To further study the role of TAK1 in Dex-induced apoptosis of osteocytic and osteoblastic cells, shRNA method was applied. As

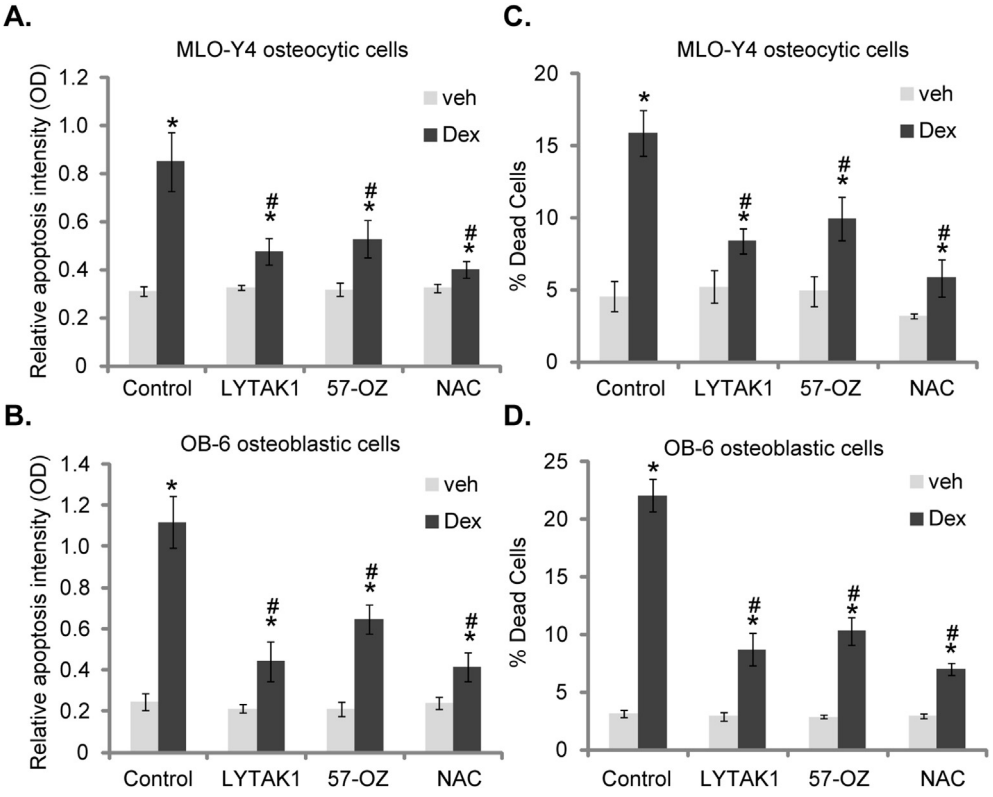

Fig. 2. TAK1 inhibitors suppress dexamethasone-induced apoptosis of osteocytic and osteoblastic cells. MLO-Y4 or OB-6 cells were pretreated with LYTAK1 (10 μM), 5Z-7-oxozeaenol (57-OZ, 10 μM) or NAC (500 μM) for 1 h, followed by Dex (1 μM) or vehicle (veh, 0.1% DMSO) stimulation for 24 h, apoptosis of each cell type was tested by Histone-DNA ELISA assay (A and B) or the trypan blue uptake assay (C and D). Bars represent the means ± SD of N = 5 independent wells. *$p < 0.05$ vs. the corresponding veh-treated cells, #$p < 0.05$ vs. Dex only treated group (one-way ANOVA).

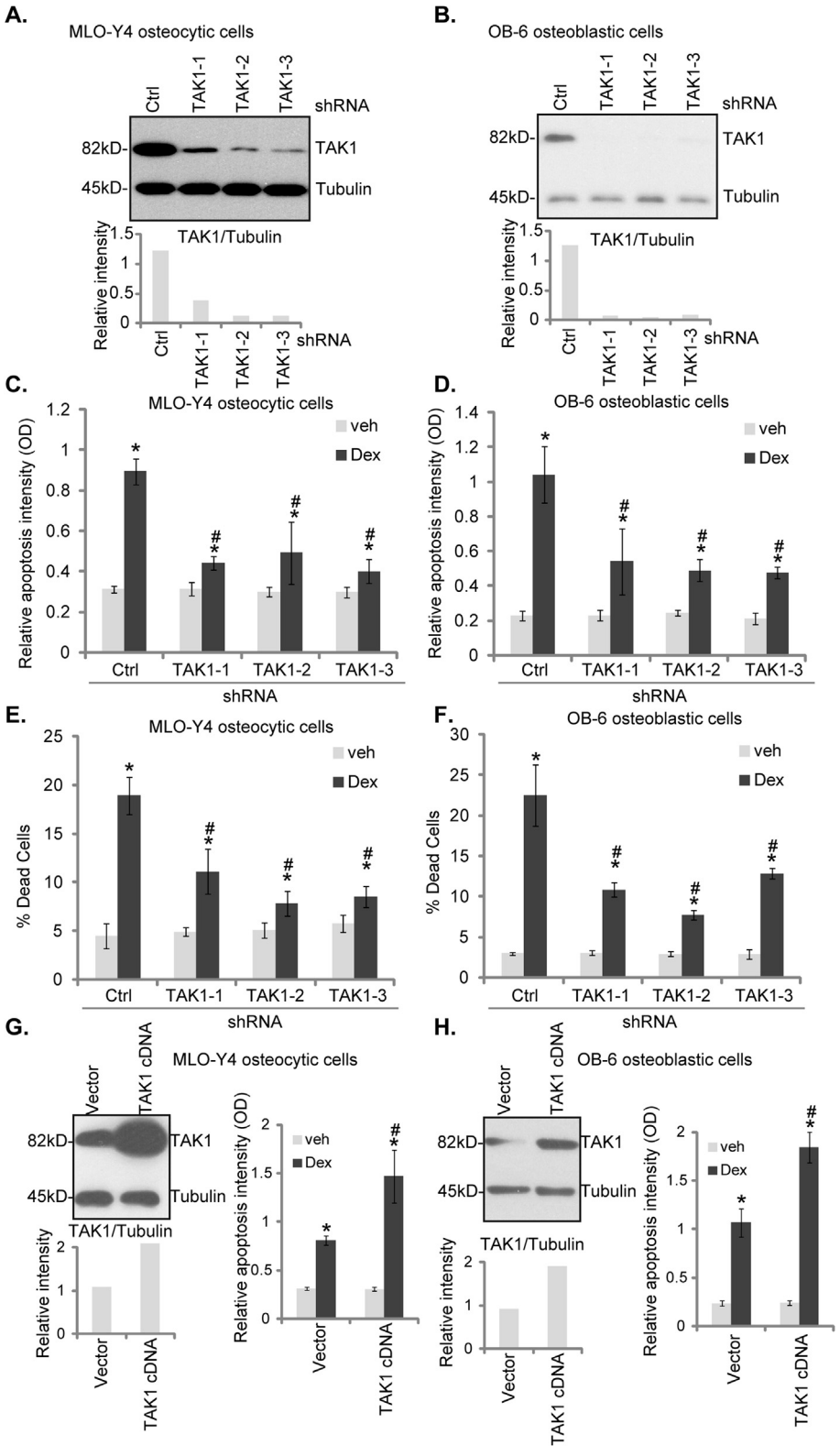

Fig. 3. Manipulating TAK1 expression level changes the sensitivity of dexamethasone in osteocytic and osteoblastic cells. Expression of TAK1 and tubulin (the loading control) in MLO-Y4 (A) or OB-6 (B) cells infected with scramble control (Ctrl) or indicated TAK1-shRNA containing virus. TAK1 expression was quantified. Above MLO-Y4 or OB-6 cells were treated with Dex (1 μM) or vehicle (veh, 0.1% DMSO) for 24 h, cell apoptosis was analyzed by Histone-DNA ELISA assay (C and D) or the trypan blue uptake assay (E and F). MLO-Y4 cells or OB-6 cells expressing wile-type TAK1 cDNA or the empty vector (pcDNA3) were treated with Dex (1 μM) or vehicle (veh, 0.1% DMSO) for 24 h, cell apoptosis was analyzed by histone-DNA ELISA assay (G and H, right panels), expression level of TAK1 and tubulin was also tested (G and H, left panels). TAK1 expression was quantified (G and H, left panels). Bars represent the means ± SD of N = 5 independent wells. *$p < 0.05$ vs. the corresponding veh-treated cells, #$p < 0.05$ vs. Dex treatment group with Ctrl shRNA (C–F) or vector control (G and H) (one-way ANOVA).

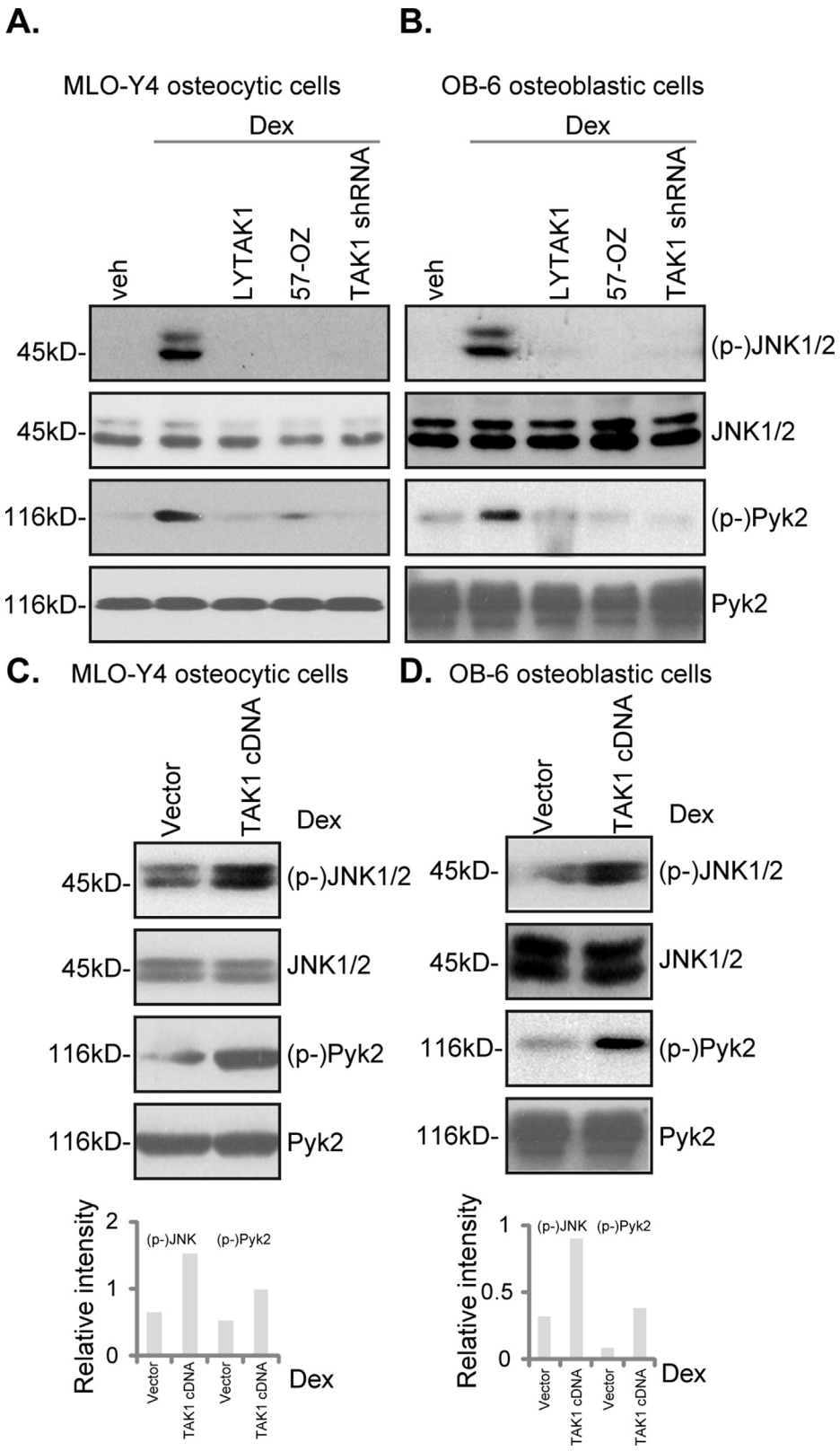

Fig. 4. TAK1 mediates dexamethasone-induced Pyk2-JNK activation in osteocytic and osteoblastic cells. MLO-Y4 (A) or OB-6 cells (B) were pretreated with LYTAK1 (10 μM, for 1 h), 5Z-7-oxozeaenol (57-OZ, 10 μM, for 1 h), or infected with TAK1 shRNA ("−3"), followed by Dex (1 μM) or vehicle (veh, 0.1% DMSO) stimulation for 3 h, p- and regular JNK1/2 and Pyk2 were assayed by Western blots using applied antibodies. MLO-Y4 (C) or OB-6 cells (D) expressing TAK1 cDNA or the empty vector (pcDNA3) were treated with Dex (1 μM) for 3 h, p- and regular JNK1/2 and Pyk2 were assayed by Western blots. JNK1/2 and Pyk2 phosphorylations were quantified.

described, three non-overlapping shRNAs targeting different sequence of TAK1 cDNA were applied. All the shRNAs applied dramatically inhibited TAK1 expression in both MLO-Y4 and OB-6 cells (Fig. 3A and B). Notably, Dex-induced apoptosis, tested by histone-DNA ELISA assay (Fig. 3C and D) and trypan blue uptake assay (Fig. 3E and F), were significantly inhibited in osteocytic or osteoblastic cells expressing TAK1 shRNAs (Fig 3C–F). On the other hand, exogenously over-expressing TAK1 (Fig. 3G and H, left panels) enhanced Dex-induced MLO-Y4 or OB-6 cell apoptosis (Fig. 3G and H, right panels). Together, these results further confirmed the important role of TAK1 in Dex-induced osteocytic and osteoblastic cell apoptosis.

3.4. TAK1 mediates dexamethasone-induced Pyk2-JNK activation in osteocytic and osteoblastic cells

At last, we studied the signaling mechanism of TAK1's involvement in Dex-induced osteocytic and osteoblastic cell apoptosis. Previous studies have showed that Pyk2-JNK activation by Dex is an important mediator of osteocytic and osteoblastic cell apoptosis [9,11,22,23]. We thus tested TAK1's role in Dex-induced Pyk2-JNK activation. As shown in Fig. 4A and B, LYTAK1 and 57-OZ, the TAK1 inhibitors, dramatically inhibited Dex-induced Pyk2 and JNK1/2 phosphorylations in both MLO-Y4 (Fig. 4A) or OB-6 (Fig. 4B) cells. Likewise, TAK1 shRNA ("−3") also prevented Pyk2-JNK1/2 activations by Dex (Fig. 4A and B). Reversely, enhanced JNK and Pyk2 phosphorylations were achieved in MLO-Y4 (Fig. 4C) or OB-6 (Fig. 4D) cells over-expressing TAK1. Together, these results indicate that TAK1 is an important mediator of Pyk2-JNK activation by Dex in osteocytic and osteoblastic cells.

4. Discussions

Excess GC-induced apoptosis of osteoblasts and osteocytes is one main reason of the reduced bone formation, increased bone fragility, leading to osteonecrosis [2,4,5,10]. Thus, understanding the mechanisms by which GC induces apoptosis of the bone cells is vital for the development of possible intervention therapies [2,4,5,10]. Published literature have shown that GC (i.e. Dex) actions involve binding to the glucocorticoid receptor (GR), causing it nuclear translocation, followed by cis- or trans-interactions with DNA, and thereby promoting or inhibiting gene transcriptions [1,4,5,10].

At the meantime, GC exerts actions independently of gene transcription alterations, but through regulating of the activity of multiple intracellular kinases [11,22,23]. Earlier studies have demonstrated that ROS-mediated Pyk2-JNK activation might be the key signaling for GC-mediated apoptosis of osteocytes and osteoblasts [9,11]. In the current study, we investigated the involvement of TAK1 in the process. Our results showed that TAK1 was activated in Dex-stimulated osteoblastic and osteocytic cells. TAK1 inhibitors and TAK1 shRNAs prevented the pro-apoptotic actions of GC on tested bone cells, while TAK1 over-expression increased the sensitivity of Dex in the same cells. Thus, activation of TAK1 might be the key mediator of GC-induced apoptosis in both osteoblastic and osteocytic cells.

It has been shown that Pyk2 acts as the upstream regulator of JNK in response to Dex [7]. The precise molecular mechanisms by which Dex activates Pyk2 leading to JNK activation and cell apoptosis are not yet understood [7]. In this report, we provided evidence to support that TAK1 might be the upstream kinase of Pyk2-JNK signaling by Dex. Inhibition or downregulation of TAK1 suppressed Dex-induced Pyk2-JNK activation and subsequent osteoblastic and osteocytic cell apoptosis. While over-expression of TAK1 enhanced Dex-induced Pyk2-JNK phosphorylations as well as apoptosis of tested bone cells. Significantly, anti-oxidants (NAC and ebselen) inhibited Dex-induced TAK1 activation and osteoblastic/osteocytic cell apoptosis, these same antioxidants were also reported to block Pyk2-JNK activation by Dex [9,11]. Together, these results indicate that Dex induces ROS production in osteoblastic and osteocytic cells, causing TAK1 activation, which serves as the upstream signal for Pyk2-JNK signaling activation, leading to osteoblastic and osteocytic cell apoptosis.

Together, we describe herein a novel signaling triggered by Dex in osteocytic and osteoblastic cells. It involves activation of TAK1 leading to subsequent Pyk2-JNK signaling activation and cell apoptosis. Interference with this pathway may prove beneficial for counteracting the adverse skeletal impact of GC.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgments

This study is supported in part by the National Natural Science Foundation of China (Grant No: 81200586)

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.161.

References

[1] R.S. Weinstein, Clinical practice. Glucocorticoid-induced bone disease, N. Engl. J. Med. 365 (2011) 62–70.
[2] D.W. Dempster, Bone histomorphometry in glucocorticoid-induced osteoporosis, J. Bone Min. Res. 4 (1989) 137–141.
[3] R.L. Jilka, R.S. Weinstein, T. Bellido, P. Roberson, A.M. Parfitt, S.C. Manolagas, Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone, J. Clin. Invest. 104 (1999) 439–446.
[4] M.A. Kerachian, C. Seguin, E.J. Harvey, Glucocorticoids in osteonecrosis of the femoral head: a new understanding of the mechanisms of action, J. Steroid Biochem. Mol. Biol. 114 (2009) 121–128.
[5] M.A. Mont, L.C. Jones, D.S. Hungerford, Nontraumatic osteonecrosis of the femoral head: ten years later, J. Bone Jt. Surg. Am. 88 (2006) 1117–1132.
[6] K.H. Koo, R. Kim, Y.S. Kim, I.O. Ahn, S.H. Cho, H.R. Song, Y.S. Park, H. Kim, G.J. Wang, Risk period for developing osteonecrosis of the femoral head in patients on steroid treatment, Clin. Rheumatol. 21 (2002) 299–303.
[7] L.I. Plotkin, S.C. Manolagas, T. Bellido, Glucocorticoids induce osteocyte apoptosis by blocking focal adhesion kinase-mediated survival. Evidence for inside-out signaling leading to anoikis, J. Biol. Chem. 282 (2007) 24120–24130.
[8] L.I. Plotkin, R.S. Weinstein, A.M. Parfitt, P.K. Roberson, S.C. Manolagas, T. Bellido, Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin, J. Clin. Invest. 104 (1999) 1363–1374.
[9] A.Y. Sato, X. Tu, K.A. McAndrews, L.I. Plotkin, T. Bellido, Prevention of glucocorticoid induced-apoptosis of osteoblasts and osteocytes by protecting against endoplasmic reticulum (ER) stress in vitro and in vivo in female mice, Bone 73C (2015) 60–68.
[10] C.A. O'Brien, D. Jia, L.I. Plotkin, T. Bellido, C.C. Powers, S.A. Stewart, S.C. Manolagas, R.S. Weinstein, Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength, Endocrinology 145 (2004) 1835–1841.
[11] M. Almeida, L. Han, E. Ambrogini, R.S. Weinstein, S.C. Manolagas, Glucocorticoids and tumor necrosis factor alpha increase oxidative stress and suppress Wnt protein signaling in osteoblasts, J. Biol. Chem. 286 (2011) 44326–44335.
[12] H. Sakurai, Targeting of TAK1 in inflammatory disorders and cancer, Trends Pharmacol. Sci. 33 (2012) 522–530.
[13] B. Cheng, S. Zhao, J. Luo, E. Sprague, L.F. Bonewald, J.X. Jiang, Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells, J. Bone Min. Res. 16 (2001) 249–259.
[14] L.L. Zhu, S. Zaidi, Y. Peng, H. Zhou, B.S. Moonga, A. Blesius, I. Dupin-Roger, M. Zaidi, L. Sun, Induction of a program gene expression during osteoblast differentiation with strontium ranelate, Biochem. Biophys. Res. Commun. 355 (2007) 307–311.
[15] C.H. Wu, C. Cao, J.H. Kim, C.H. Hsu, H.J. Wanebo, W.D. Bowen, J. Xu, J. Marshall, Trojan-horse nanotube on-command intracellular drug delivery, Nano Lett. 12 (2012) 5475–5480.
[16] M.B. Chen, M.X. Wei, J.Y. Han, X.Y. Wu, C. Li, J. Wang, W. Shen, P.H. Lu, MicroRNA-451 regulates AMPK/mTORC1 signaling and fascin1 expression in HT-29 colorectal cancer, Cell. Signal 26 (2014) 102–109.

[17] M. Yu, D.T. Ting, S.L. Stott, B.S. Wittner, F. Ozsolak, S. Paul, J.C. Ciciliano, M.E. Smas, D. Winokur, A.J. Gilman, M.J. Ulman, K. Xega, G. Contino, B. Alagesan, B.W. Brannigan, P.M. Milos, D.P. Ryan, L.V. Sequist, N. Bardeesy, S. Ramaswamy, M. Toner, S. Maheswaran, D.A. Haber, RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis, Nature 487 (2012) 510–513.
[18] D. Melisi, Q. Xia, G. Paradiso, J. Ling, T. Moccia, C. Carbone, A. Budillon, J.L. Abbruzzese, P.J. Chiao, Modulation of pancreatic cancer chemoresistance by inhibition of TAK1, J. Natl. Cancer Inst. 103 (2011) 1190–1204.
[19] F. Meng, Y. Li, X. Tian, L. Fu, Y. Yin, C. Sui, P. Ma, Y. Jiang, Identification of TGF-beta-activated kinase 1 as a possible novel target for renal cell carcinoma intervention, Biochem. Biophys. Res. Commun. 453 (2014) 106–111.
[20] A. Zecevic, H. Menard, V. Gurel, E. Hagan, R. DeCaro, A. Zhitkovich, WRN helicase promotes repair of DNA double-strand breaks caused by aberrant mismatch repair of chromium-DNA adducts, Cell Cycle 8 (2009) 2769–2778.
[21] Z. Chen, X. Shen, F. Shen, W. Zhong, H. Wu, S. Liu, J. Lai, TAK1 activates AMPK-dependent cell death pathway in hydrogen peroxide-treated cardiomyocytes, inhibited by heat shock protein-70, Mol. Cell. Biochem. 377 (2013) 35–44.
[22] A. Druilhe, S. Letuve, M. Pretolani, Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action, Apoptosis 8 (2003) 481–495.
[23] D. Chauhan, P. Pandey, A. Ogata, G. Teoh, S. Treon, M. Urashima, S. Kharbanda, K.C. Anderson, Dexamethasone induces apoptosis of multiple myeloma cells in a JNK/SAP kinase independent mechanism, Oncogene 15 (1997) 837–843.