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Indole-3-carbinol as inhibitors of glucocorticoid-induced apoptosis in osteoblastic cells through blocking ROS-mediated Nrf2 pathway

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ABSTRACT

Apoptosis of osteoblasts induced by glucocorticoid (GC) has been identified as a main cause of osteoporosis, bone loss and fractures, and the oxidative stress was found as an important contributor. Therefore, natural or synthetic agents with antioxidant activities can antagonize GCs-induced apoptosis in osteoblasts, and thus demonstrate the potential application to reverse osteoporosis. In this study, we showed that, indole-3-carbinol (I3C), a natural product found in broadly consumed plants of the *Brassica genus*, could block the cytotoxic effects of dexamethasone (Dex), and elucidated the underlying molecular mechanisms. Firstly, we showed that, I3C could effectively suppress Dex-induced cytotoxicity and apoptotic cell death in osteoblastic cells, as evidenced by the decrease in Sub-G1 cell population. Treatment of the cells with Dex resulted in activation of caspase-3/-8/-9 and subsequent cleavage of PARP, which was also effectively blocked by co-incubation of I3C. Moreover, exposure to Dex triggered a rapid onset and time-dependent superoxide overproduction in osteoblastic cells, which was effectively suppressed by addition of I3C. Excess intracellular ROS induced by Dex significantly suppressed the expression levels of Nrf2 and the downstream effectors, HO1 and NQO1, but these changes could be reversed by I3C. Knockdown of Nrf2 using siRNA silencing technique significantly reversed the protective effects of I3C against Dex-induced apoptosis and ROS generation. Taken together, I3C can reverse cytotoxicity of Dex through blocking ROS overproduction and enhancement of Nrf2 expression. This study may provide a safe and good strategy for molecular intervention of GCs-induced osteoporosis by using natural products.

1. Introduction

Osteoporosis, a prevalent health concern, is a bone disease associated with reduced bone mineral density, disordered bone architecture and increased fragility, which leads to an increased risk of fracture affecting the quality of life and life expectancy [1]. The loss of osteoblastic activity and increase in osteoclastic activity would cause osteoporosis, lower bone mineral densities, decrease in bone mass and increased fragility [2]. Osteoblasts are cells originating in the bone marrow and contributing to bone production. Although evidence suggests that many cytokines, hormones, signaling pathways and transcription factors may be involved in osteoblast differentiation, the signaling mechanisms that contribute to decreased osteoblastic differentiation in osteoporosis are not well known [3]. Both natural and synthetic glucocorticoids (e.g. prednisone, dexamethasone (Dex)) are widely used in clinic in a variety of diseases as effective anti-inflammatory drugs. However, their future clinical application is always limited by substantial adverse side effects, including osteoporosis and obesity [4]. GC-induced osteoporosis is regarded as a major cause of osteoporosis, bone loss and fractures [5]. Studies have showed that, prolonged and over-dose intake of GCs was the leading cause of secondary osteoporosis and bone fractures by increasing apoptosis of osteoblasts and osteocyte [6]. Many studies have been carried out to illustrate the molecular mechanisms which accounts for apoptosis-inducing effects of GCs, such as activation of Bim [7],

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regulation of 11β-hydroxysteroid dehydrogenase type 2 [8], suppression of cytokines and interaction with monomeric GR and AP-1 [9], activation of GSK 3β and p38 [10]. Although many studies have been conducted, the molecular mechanisms remain elusive, which impedes the prevention and treatment of this side effect.

Oxidative stress is caused by the loss of the imbalance between the free radical generation and the scavenging activities of intracellular antioxidants. Nowadays, studies have found that, oxidative stress played important roles in many chronic and degenerative diseases, including osteoporosis, cancer and neurodegenerative diseases [11]. Much research has been focused on the use of natural phytochemicals, such as those found in vegetables and fruits, for their antioxidant and functional properties [12,13]. The natural antioxidants in foods have also caused concern because of their safety and potential nutritional and therapeutic effects. Recent studies have showed that, natural phytochemicals from fruits and vegetables with antioxidant activities can antagonize GCs-induced apoptosis in osteoblasts [14,15]. Indole-3-carbinol (I3C) is a natural product found in broadly consumed plants of the Brassica genus, such as broccoli, cabbage, and cauliflower. I3C can be orally administered and clinical trials have demonstrated that I3C and derivatives are safe in humans. Previous studies have described the chemopreventive and antitumor activities of both natural and synthetic I3C derivatives, through regulation of inflammation, cell proliferation, and inhibition of tumor invasion in a variety of tumors [16–19]. Studies have showed that I3C induced G1-phase cell-cycle arrest and apoptosis in human melanoma cells by interaction with NEDD4-1 and disruption of wild-type PTEN degradation [19]. Perez-Chacon and his co-workers found that I3C induced the down-modulation of cMYC and IAP-family proteins and promoted the apoptosis of EBV lymphoma cells [17]. Interestingly, Tin et al. found that, I3C stimulated nucleostemin-MDM2 interactions to free p53 to trigger its apoptotic response in human breast cancer cells [16]. Caruso et al. also found that I3C and its derivatives preferentially targeted ERα-positive breast cancer cells to inhibit its growth through induction of oxidative stress [18]. These results have demonstrated the potential application of I3C in cancer chemoprevention and therapy, but the underlying mechanisms are poorly defined. Furthermore, due to the strong antioxidant activities of I3C, it is possible that this kind of phytochemicals could be used to reverse GCs-induced osteoporosis. Therefore, this study aimed to examine the potential application of I3C as inhibitors to antagonize GCs-induced apoptosis in osteoblasts and elucidate the underlying molecular mechanisms. Our results showed that I3C effectively reversed GCs-induced apoptosis in osteoblastic cells through inhibition of ROS overproduction and regulation of Nrf2 pathway. This study may provide a novel strategy for molecular intervention against GCs-induced osteoporosis by using natural products.

2. Materials and methods

2.1. Cell culture, drug treatment, determination cell viability and cell proliferation

MC3T3-E1 cells were obtained from RIKEN cell bank (Tsukuba, Japan), and maintained in α-MEM medium supplemented with 10% fetal bovine serum (GIBCO BRL, Carlsbad, CA, USA). They were cultured in a humid incubator at 37 °C (95% O_2 and 5% CO_2). For examination of the protective effects of I3C on MC3T3-E1 cells, the cells were pre-treated with various concentrations of I3C for 2 h, and Dex was added into the culture medium, and incubated for 24 h. The viability of the cells after different treatments was determined by MTT enzyme activity assay [20]. The effects of Dex and I3C on the cell proliferation were determined using the 5-bromo-2-deoxyuridine (BrdU) incorporation cell proliferation assay kit (Roche, Indianapolis, IN, USA).

2.2. Flow cytometric analysis

The effects of Dex and I3C on the cell cycle distribution were examined by DNA flow cytometric analysis with propidium iodide staining. Briefly, the cells after different treatments of Dex and I3C were fixed in 75% ethanol at −20 °C overnight, and stained with propidium iodide for 2 h in darkness, and then analyzed on a Coulter Epics XL flow cytometer (Coulter, Germany).

2.3. Examinations of caspase activation and PARP cleavage

The activation of caspases by Dex and the protective effects of I3C were evaluated by enzymatic activity assay [21]. The level of PARP cleavage in cells after treatments was examined using PathScan® Cleaved PARP (Asp214) Sandwich ELISA Kit following the manufacturer's instructions.

2.4. Determination of ROS overproduction

Intracellular ROS generation was examined by dihydroethidium (DHE) fluorometric assay to detect the superoxide overproduction as previously described [20].

2.5. Gene silencing of Nrf2 by using siRNA technology

The silencing of Nrf2 in MC3T3-E1 cells was carried out by using Nrf2 siRNA assay kit (Santa Cruz) according to the manufacturer's instructions. The expression level of Nrf2 and the downstream proteins were examined by Western blot analysis.

2.6. Western blot analysis

MC3T3-E1 cells after different treatments were harvested and collected as cell pellets. The cellular protein was extracted by using cell lysis buffer (Cell Signaling Technology, Inc.), and the protein concentration was determined by bicinchoninic acid protein assay kit (Sigma–Aldrich, USA). The effects of Dex and I3C on the expression levels of Nrf2, HO1 and NQO1, were examined by Western blot analysis as previously described [22].

2.7. Statistical analysis

In this study, all the experiments were conducted at least in triplicate and the results were expressed as mean ± SD. Significance in the difference among different treatments was analyzed by one-way ANOVA analysis at $P < 0.05$ (*) or $P < 0.01$ (**) level.

3. Results

3.1. I3C blocks Dex-induced cytotoxicity and apoptotic cell death in osteoblasts

MC3T3-E1 osteoblastic cell line, which exhibits the ability to differentiate into osteoblasts and osteocytes and form calcified bone tissue *in vitro*, was chosen as a cell model to simulate GCs-induced osteoporosis. From the results of MTT assay, after 24-h treatment, Dex (0.25–8.0 μM) significantly inhibited the MC3T3-E1 cell growth in a dose-dependent manner (Fig. S1). To evaluate the safety for future clinical use of I3C, we also examined the cytotoxic effects of this compound on MC3T3-E1 cells. As shown in Fig. S2, I3C at concentrations less than 40 μM showed no cytotoxicity against MC3T3-E1 cells, with slight growth inhibition

observed at 80 μM. Therefore, the concentrations lower than 40 μM were used to screen their protective effects against Dex-induced cytotoxicity. Interestingly, as shown in Fig. 1A, I3C (5–20 μM) significantly and dose-dependently inhibited the cytotoxic effect of Dex on MC3T3-E1 cells. Consistently, the examination of cell proliferation revealed that, I3C effectively inhibited the Dex-induced suppression of MC3T3-E1 cell proliferation in a dose-dependent fashion (Fig. 1B). As apoptosis has been identified as a major mode of cell death in GCs-induced osteoporosis, we also examined the Dex-induced changes in cell cycle distribution of MC3T3-E1 cells. As shown in Fig. 1C, 1 μM Dex effectively induced an increase in Sub-G1 apoptotic peak from 0.4% (control) to 33.2%, with no significant changes in G0/G1, S and G2/M phases observed. These findings confirm that apoptosis plays an essential part in the cytotoxic action of Dex on MC3T3-E1 cells. Interestingly, addition of I3C at 10 μM dramatically decreased the apoptotic peak to 5.6%, demonstrating the novel protection of I3C. Besides, the I3C alone showed no effects on the cell cycle distribution of MC3T3-E1 cells, illustrating its safety property in future use. Considering all, the outcome suggests that I3C could block Dex-induced cytotoxicity and apoptotic cell death in MC3T3-E1 cells.

3.2. Dex-induced caspase activation and PARP cleavage and protection by I3C

Caspase family members have been identified as central regulators of cell apoptosis [23], while the cleavage of poly (ADP-ribose) polymerase (PARP) is now considered to be a biochemical hallmark of cells undergoing apoptosis. Therefore, we also examined the involvement of these two cellular events in the interaction of Dex and I3C in MC3T3-E1 cells. As shown in Fig. 2, treatments of the cells with 1 μM Dex resulted in steep increase in the activities of caspase-3, -8 and 9 to 185%, 119% and 174% respectively. The activation of caspase-9 and caspase-8 indicates the initiation of mitochondria-mediated and death receptor-mediated apoptotic pathways by Dex. Interestingly, the addition of I3C (5–20 μM) significantly and dose-dependently inhibited the activation of caspase-3, -8 and 9 induced by Dex. For instance, after co-treatment with 20 μM I3C, the activities of caspase-3, -8 and 9 were blocked to 116%, 102% and 119%, respectively. Moreover, exposure of cells to Dex resulted in PARP cleavage to 190% of control (Fig. 2D). As expected, it was suppressed to 160%, 134% and 116% respectively after addition of 5–20 μM I3C. Collectively, these results indicate that I3C is a potent inhibitor of Dex-induced apoptosis in osteoblastic cells.

3.3. ROS overproduction and inhibition of Nrf2 pathways induced by Dex and the protective effects of I3C

As ROS has been found involved in the apoptosis of osteoblastic cells induced by GCs, agents with antioxidant activities could be possible to antagonize this effect [14,15]. Therefore, in this study, we have examined the intracellular levels of superoxide production induced by Dex using DHE fluorescence assay in order to examine the protective effects of I3C. As shown in Fig. 3A, treatment of the cells with 1 μM Dex resulted in a rapid onset and time-dependent increase in superoxide production. As expected, addition of I3C at 10 and 20 μM effectively inhibited the superoxide production induced by Dex. These results suggest that I3C could protect osteoblastic cells from Dex-induced cytotoxicity through blocking ROS overproduction.

Further studies have also been carried out to examine the downstream effects of Dex-induced ROS overproduction on the osteoblastic cells. Nrf2 signaling pathway has been identified as one of the major regulators of cytoprotective responses to endogenous and exogenous stresses caused by ROS and electrophiles [24]. Our previous study also demonstrated that upregulation of Nrf2 was an important action mechanism for the protective effects of sulforaphane against GCs-induced osteoporosis [22]. Consequently, we examined the effects of Dex on the expression level of Nrf2 and the downstream effectors by Western blot analysis. As indicated in Fig. 3B, treatment of the cells with 1 μM Dex greatly suppressed the expression of Nrf2, and down-regulated its downstream effectors,

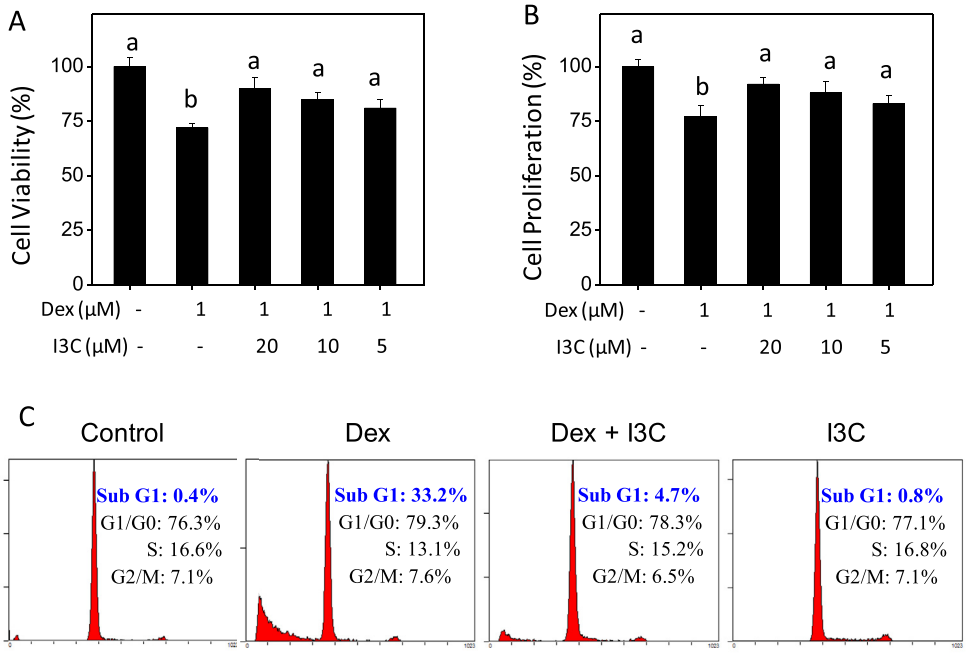

Fig. 1. Protective effects of I3C on MC3T3-E1 cells against Dex-induced cytotoxicity and apoptosis. Cells were pretreated with I3C for 2 h and then cultured in combination with 1 μM Dex for 24 h. (A) Examination of cell viability by MTT assay. (B) Examination of cell proliferation by BudU incorporation assay. (C) Change in cell cycle distribution as examined by flow cytometric analysis. Cells were pretreated with 10 μM I3C for 2 h and then cultured in combination with 1 μM Dex for 24 h.

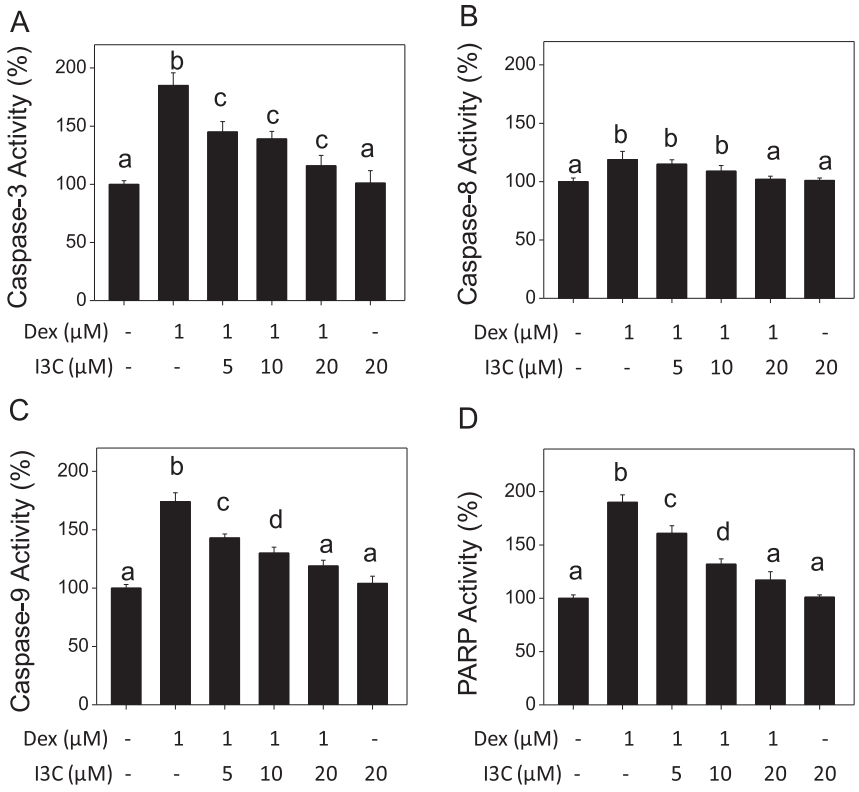

Fig. 2. I3C suppresses Dex-induced caspase activation (A, B and C) and PARP cleavage (D) in MC3T3-E1 cells. Cells were pretreated with I3C for 2 h and then cultured in combination with 1 μM Dex for 24 h. Data are presented as means ± SD from three independent experiments. Bars with different characters are statistically different at $P < 0.05$ level.

HO1 and NQO1. In contrast, I3C alone effectively increased the expression levels of Nrf2, HO1 and NQO1. Therefore, co-incubation of the cells with I3C significantly recovered the inhibitory effects of Dex on Nrf2, HO1 and NQO1. Collectively, these results suggest that I3C could block Dex-induced superoxide overproduction through activation of Nrf2 pathway.

3.4. Critical role of Nrf2 pathway in the interaction of Dex and I3C

To make further confirmation for the contribution of Nrf2 pathway to GCs-induced apoptosis, Nrf2 siRNA silencing technique was used to examine its effects on the apoptosis-inducing ability of Dex and the protection of I3C. As shown in Fig. 4B, Nrf2 siRNA effectively down-regulated the expression of Nrf2 and its downstream effectors, HO1 and NQO1, demonstrating the efficacy of this technique to silence Nrf2. Interestingly, knockdown of Nrf2 substantially reduced the protein level of Nrf2 elevated by I3C, and also suppressed the up-regulation of NQO1 and HO1 by I3C in Dex-treated cells. In addition, we examined the effects of Nrf2 knockdown on Dex-induced cell apoptosis. As indicated in Fig. 4B and C, I3C effectively suppressed the Dex-induced apoptosis and superoxide production. In contrast, Nrf2 knockdown significantly blocked the protective effects I3C and thus recovered the cytotoxicity of Dex. For instance, addition of I3C reversed the apoptotic cell death induced by Dex from 30% to 5.6%. However, Nrf2 knockdown increased the apoptosis to 24%. Consistently, I3C blocked the Dex-

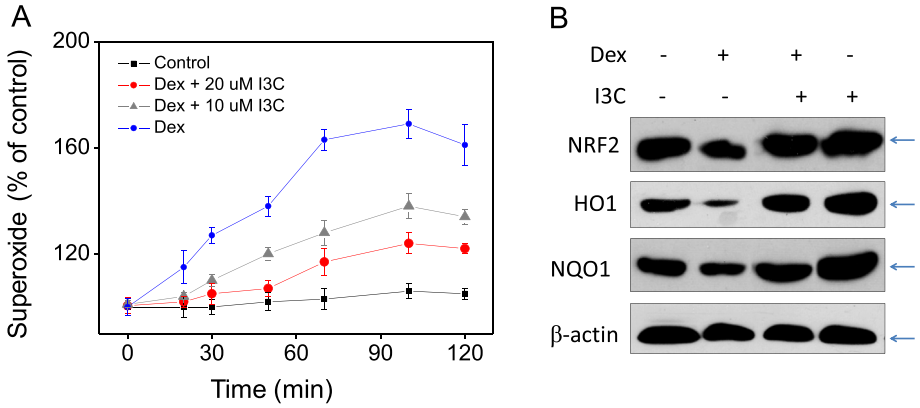

Fig. 3. Dex induces superoxide overproduction and down-regulation of Nrf2 pathway, and the effects of I3C. (A) The cells loaded with DHE fluorescence probe were treated with Dex and I3C for various periods of time and then examined by fluorescence intensity. (B) Examination of the expression levels of Nrf2 and downstream effectors, HO1 and NQP1. The cells were pretreated with 10 μM I3C for 2 h and then cultured in combination with 1 μM Dex for 24 h. The protein expression levels were analyzed by Western blot analysis. Experiments were repeated three times with similar results.

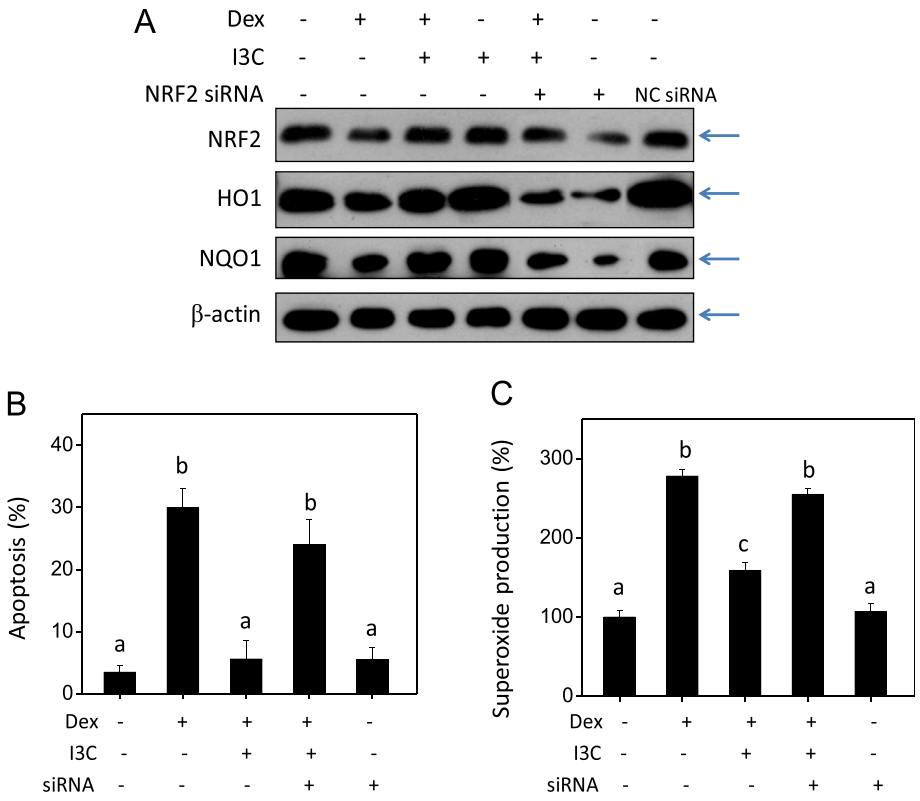

Fig. 4. Knockdown of Nrf2 blocks the cytoprotective effects of I3C on Dex-induced apoptosis and ROS overproduction. (A) Knockdown of Nrf2 and its effects on the expression levels of HO1 and NQP1. (B) Effects of Nrf2 knockdown on the cytoprotective effects of I3C on Dex-induced apoptosis. Cells were pretreated with 10 μM I3C for 2 h and then cultured in combination with 1 μM Dex for 24 h. (C) Effects of Nrf2 knockdown on the cytoprotective effects of I3C on Dex-induced apoptosis. Cells were co-treated with 10 μM I3C and 1 μM Dex for 100 min and then examined by DHE fluorescence. Data are presented as means ± SD from three independent experiments. Bars with different characters are statistically different at $P < 0.05$ level.

induced superoxide overproduction from 278% of control to 159%, which was recovered to 255% of control. Collectively, these results suggest that, ROS-mediated down-regulation of Nrf2 is an important mechanism accounting for the GCs-induced cytotoxicity in osteoblastic cells, and I3C can reverse Dex-cytotoxicity through blocking ROS overproduction and enhancement of Nrf2 expression.

4. Discussion

At present, the positive correlation between oxidative stress and osteoporotic status has been shown in numerous studies. For example, decreased bone mineral density was believed to be associated with higher oxidative stress index values and total plasma oxidant status in osteoporotic patients [25]. Activities of Catalase and glutathione peroxidase were found to be decreased in postmenopausal women with osteoporosis [26]. Studies in the mouse model provides a paradigm changing from “estrogen-centric” account of the pathogenesis of evolutional osteoporosis to one, in which age-related mechanisms intrinsic to bone and oxidative stress are protagonists [27]. Therefore, natural and synthetic agents with antioxidant activities could be possible to antagonize oxidative stress-related diseases, including osteoporosis. GCs are widely used for their unsurpassed anti-inflammatory and immunomodulatory effects, but their therapeutic application is greatly limited by the serious side effects due to high dose and long time use, such as osteoporosis, diabetes, and obesity. Nowadays, GC-induced osteoporosis has been regarded as a leading cause of osteoporosis, bone loss as well as fractures, and the oxidative stress was found as an important contributor [5]. Recent studies have showed that, natural phytochemicals from fruits and vegetables with antioxidant activities can antagonize GCs-induced apoptosis in osteoblasts [14,15,28,29]. Our previous study also demonstrated that sulforaphane could effectively suppress GCs-induced apoptosis in osteoblastic cells [22]. In this study, we showed that, I3C, a natural product found in broadly consumed plants of the *Brassica genus*, could be used as inhibitors of GCs-induced apoptosis in osteoblastic cells through blocking ROS overproduction and inhibition of the downstream signaling pathways.

Firstly, we showed that, I3C could effectively block Dex-induced cytotoxicity and apoptotic cell death in osteoblastic cells. Due to the important roles of caspase family members and PARP in cell apoptosis [23], we also examined the involvement of these two cellular events in the interaction of Dex and I3C in MC3T3-E1 cells. As expected, I3C acted as a potent inhibitor of Dex-induced apoptosis in osteoblastic cells by blocking of caspase activation and PARP cleavage. As ROS has been found involved in the apoptosis of osteoblastic cells induced by GCs, agents with antioxidant activities could be possible to antagonize this effect [14,15,29]. Consistently, we found that, Dex induced a rapid onset and time-dependent superoxide overproduction in osteoblastic cells, which was effectively suppressed by addition of I3C. These results suggest that I3C could protect osteoblastic cells from Dex-induced cytotoxicity through blocking ROS overproduction.

Further studies were also carried out to examine the downstream effects of Dex-induced ROS overproduction on the osteoblastic cells. Nrf2 signaling pathway has been identified as one of the major regulators of cytoprotective responses to endogenous and exogenous stresses caused by ROS and electrophiles [24]. Previous studies have demonstrated the important roles of Nrf2 signaling in the action of GCs [30,31]. Our previous study also demonstrated that upregulation of Nrf2 was an important action mechanism for the protective effects of sulforaphane against GCs-

induced osteoporosis [22]. Hence, we examined the effects of Dex on the expression level of Nrf2 and the downstream effectors by Western blot analysis. As expected, treatment of cells with Dex effectively suppressed the expression levels of Nrf2 and the downstream effectors, HO1 and NQO1. To further verify the role of Nrf2 pathway to GCs-induced apoptosis, Nrf2 siRNA silencing technique was used to examine its effects on the apoptosis-inducing ability of Dex and the protection of I3C. The results revealed that, knockdown of Nrf2 significantly reversed the protective effects of I3C against Dex-induced apoptosis and ROS generation. These results suggest that, ROS-mediated down-regulation of Nrf2 is an important mechanism accounting for the GCs-induced cytotoxicity in osteoblastic cells, and I3C can reverse Dex-cytotoxicity through blocking ROS overproduction and enhancement of Nrf2 expression. This study may provide a safe and good strategy for molecular intervention of GCs-induced osteoporosis by using natural products.

In summary, the present study demonstrates the protective effects of I3C against Dex-induced cytotoxicity, and elucidates the underlying molecular mechanisms. Specifically, treatments of the cells with I3C attenuated Dex-induced caspase activation, PARP cleavage, activation of Nrf2 pathway and down-regulation of downstream effectors through regulation of ROS generation. This study may provide a safe and good strategy for molecular intervention of GCs-induced osteoporosis by using natural products.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.049.

Transparency document

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References

[1] W.L. Hsu, C.Y. Chen, J.Y. Tsauo, R.S. Yang, Balance control in elderly people with osteoporosis, J. Formos. Med. Assoc. 113 (2014) 334–339.
[2] I.R. Reid, Anti-resorptive therapies for osteoporosis, Semin. Cell. Dev. Biol. 19 (2008) 473–478.
[3] S.N. Salam, R. Eastell, A. Khwaja, Fragility fractures and osteoporosis in CKD: pathophysiology and diagnostic methods, Am. J. Kidney Dis. 63 (2014) 1049–1059.
[4] M.J. Seibel, M.S. Cooper, H. Zhou, Glucocorticoid-induced osteoporosis: mechanisms, management, and future perspectives, Lancet Diabetes Endocrinol. 1 (2013) 59–70.
[5] T.C. Brennan-Speranza, H. Henneicke, S.J. Gasparini, K.I. Blankenstein, U. Heinevetter, V.C. Cogger, D. Svistounov, Y. Zhang, G.J. Cooney, F. Buttgereit, C.R. Dunstan, C. Gundberg, H. Zhou, M.J. Seibel, Osteoblasts mediate the adverse effects of glucocorticoids on fuel metabolism, J. Clin. Invest 122 (2012) 4172–4189.
[6] R.S. Weinstein, Clinical practice. Glucocorticoid-induced bone disease, N. Engl. J. Med. 365 (2011) 62–70.
[7] B. Espina, M. Liang, R.G. Russell, P.A. Hulley, Regulation of bim in glucocorticoid-mediated osteoblast apoptosis, J. Cell. Physiol. 215 (2008) 488–496.
[8] 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.
[9] A. Rauch, S. Seitz, U. Baschant, A.F. Schilling, A. Illing, B. Stride, M. Kirilov, V. Mandic, A. Takacz, R. Schmidt-Ullrich, S. Ostermay, T. Schinke, R. Spanbroek, M.M. Zaiss, P.E. Angel, U.H. Lerner, J.P. David, H.M. Reichardt, M. Amling, G. Schutz, J.P. Tuckermann, Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor, Cell. Metab. 11 (2010) 517–531.
[10] S.I. Yun, H.Y. Yoon, S.Y. Jeong, Y.S. Chung, Glucocorticoid induces apoptosis of osteoblast cells through the activation of glycogen synthase kinase 3beta, J. Bone Min. Metab. 27 (2009) 140–148.
[11] C.C. Benz, C. Yau, Ageing, oxidative stress and cancer: paradigms in parallax, Nat. Rev. Cancer 8 (2008) 875–879.
[12] C. Borek, Aging and antioxidants. Fruits and vegetables are powerful armor, Adv. Nurse Pract. 14 (2006) 35–38.
[13] A. Kubo, T.R. Levin, G. Block, G.J. Rumore, C.P. Quesenberry Jr., P. Buffler, D.A. Corley, Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus, Am. J. Gastroenterol. 103 (2008) 1614–1623 quiz 1624.
[14] Y. Wang, W.L. Wang, W.L. Xie, L.Z. Li, J. Sun, W.J. Sun, H.Y. Gong, Puerarin stimulates proliferation and differentiation and protects against cell death in human osteoblastic MG-63 cells via ER-dependent MEK/ERK and PI3K/Akt activation, Phytomedicine 20 (2013) 787–796.
[15] X.T. Yan, S.H. Lee, W. Li, Y.N. Sun, S.Y. Yang, H.D. Jang, Y.H. Kim, Evaluation of the antioxidant and anti-osteoporosis activities of chemical constituents of the fruits of *Prunus mume*, Food Chem. 156 (2014) 408–415.
[16] A.S. Tin, A.H. Park, S.N. Sundar, G.L. Firestone, Essential role of the cancer stem/progenitor cell marker nucleostemin for indole-3-carbinol anti-proliferative responsiveness in human breast cancer cells, BMC Biol. 12 (2014) 72.
[17] G. Perez-Chacon, C. de Los Rios, J.M. Zapata, Indole-3-carbinol induces cMYC and IAP-family downmodulation and promotes apoptosis of Epstein-Barr virus (EBV)-positive but not of EBV-negative Burkitt's lymphoma cell lines, Pharmacol. Res. 89 (2014) 46–56.
[18] J.A. Caruso, R. Campana, C. Wei, C.H. Su, A.M. Hanks, W.G. Bornmann, K. Keyomarsi, Indole-3-carbinol and its N-alkoxy derivatives preferentially target ERalpha-positive breast cancer cells, Cell. Cycle 13 (2014) 2587–2599.
[19] I. Aronchik, A. Kundu, J.G. Quirit, G.L. Firestone, The antiproliferative response of indole-3-carbinol in human melanoma cells is triggered by an interaction with NEDD4-1 and disruption of wild-type PTEN degradation, Mol. Cancer Res. 12 (2014) 1621–1634.
[20] C. Fan, J. Chen, Y. Wang, Y.S. Wong, Y. Zhang, W. Zheng, W. Cao, T. Chen, Selenocystine potentiates cancer cell apoptosis induced by 5-fluorouracil by triggering reactive oxygen species-mediated DNA damage and inactivation of the ERK pathway, Free Radic. Biol. Med. 65 (2013) 305–316.
[21] J. Su, H. Lai, J. Chen, L. Li, Y.S. Wong, T. Chen, X. Li, Natural borneol, a monoterpenoid compound, potentiates selenocystine-induced apoptosis in human hepatocellular carcinoma cells by enhancement of cellular uptake and activation of ROS-mediated DNA damage, PLoS One 8 (2013) e63502.
[22] H. Lin, B. Wei, G. Li, J. Zheng, J. Sun, J. Chu, R. Zeng, Y. Niu, Sulforaphane reverses glucocorticoid-induced apoptosis in osteoblastic cells through regulation of the Nrf2 pathway, Drug Des. Devel Ther. 8 (2014) 973–982.
[23] D.R. McIlwain, T. Berger, T.W. Mak, Caspase functions in cell death and disease, Cold Spring Harb. Perspect. Biol. 5 (2013) a008656.
[24] E. Kansanen, S.M. Kuosmanen, H. Leinonen, A.L. Levonen, The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer, Redox Biol. 1 (2013) 45–49.
[25] O.F. Sendur, Y. Turan, E. Tastaban, M. Serter, Antioxidant status in patients with osteoporosis: a controlled study, Jt. Bone Spine 76 (2009) 514–518.
[26] S. Ozgocmen, H. Kaya, E. Fadillioglu, Z. Yilmaz, Effects of calcitonin, risedronate, and raloxifene on erythrocyte antioxidant enzyme activity, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis, Arch. Med. Res. 38 (2007) 196–205.
[27] S.C. Manolagas, From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis, Endocr. Rev. 31 (2010) 266–300.
[28] E.M. Choi, G.H. Kim, Y.S. Lee, Protective effects of dehydrocostus lactone against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells, Toxicol Vitro 23 (2009) 862–867.
[29] J.K. Zhang, L. Yang, G.L. Meng, J. Fan, J.Z. Chen, Q.Z. He, S. Chen, J.Z. Fan, Z.J. Luo, J. Liu, Protective effect of tetrahydroxystilbene glucoside against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells, Eur. J. Pharmacol. 689 (2012) 31–37.
[30] A. Tarozzi, C. Angeloni, M. Malaguti, F. Morroni, S. Hrelia, P. Hrelia, Sulforaphane as a potential protective phytochemical against neurodegenerative diseases, Oxid. Med. Cell. Longev. 2013 (2013) 415078.
[31] C.A. Houghton, R.G. Fassett, J.S. Coombes, Sulforaphane: translational research from laboratory bench to clinic, Nutr. Rev. 71 (2013) 709–726.