J Appl Biol Chem (2014) 57(1), 17–22
http://dx.doi.org/10.3839/jabc.2014.003

Online ISSN 2234-7941
Print ISSN 1976-0442

Original Article: Biochemistry/Molecular Biology

Mechanism for Antioxidant Activity of *Nardostachys chinensis* root Extract

Jee-In Heo · Jeong-Hyeon Kim · Jeong-Min Lee · Sung Chan Kim · Jae-Bong Park · Jaebong Kim · Jae-Yong Lee*

Received: 14 March 2013 / Accepted: 22 August 2013 / Published Online: 31 March 2014

Abstract *Nardostachys chinensis* (*N. chinensis*) has been used in traditional medicine as a sedative and analgesic. It has been reported that *N. chinensis* extract has an antioxidant activity. However, the mechanism has not been elucidated. In this study, we showed that FOXO3a was activated by *N. chinensis* extract. FOXO3a is a transcriptional factor that involved in cell cycle arrest, DNA repair, apoptosis, and detoxification of reactive oxygen spices (ROS). Protein level of FOXO3a was increased by *N. chinensis* extract whereas phospho-FOXO3a (Thr 32) was not changed. Promoter activities of target genes of FOXO3a such as MnSOD, p27, and GADD45 were increased by *N. chinensis* extract. Among target genes, protein level of MnSOD was increased by *N. chinensis* extract, and this leads to removal of ROS level in human embryonic fibroblast (HEF) cells. These results suggested that *N. chinensis* extract has an antioxidant activity by upregulation of MnSOD through FOXO3a activation.

Keywords antioxidant · FOXO3a · MnSOD · *Nardostachys chinensis* · reactive oxygen species

Introduction

Reactive oxygen species (ROS) are generated as a side product during mitochondrial respiration and by NADPH oxidase. ROS was also produced by environmental stress such as UV and ionizing radiation. While ROS plays an important role in various cellular processes, it also causes damage of nucleic acids, proteins, and lipid. This is referred to as oxidative stress which has been known to be a major cause of cancer and aging (Circu and Aw, 2010; Ray et al., 2012). Antioxidant is one of the defense mechanisms against oxidative stress. Superoxide dismutase, glutathione peroxidase, and catalase are enzymatic antioxidant. Non-enzymatic antioxidant include ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione, carotenoids, and flavonoids (Valko et al., 2007).

Nardostachys chinensis (*N. chinensis*) belongs to family Valerianaceae and has been used as a sedative and analgesic. *N. chinensis* contains ursolic acid, nardosinone, pinoresinol, desoxo-narchinol A, kanshone B, epoxyconiferyl alcohol, debilon, 4a,5-dimethyl-1,3-dioxo-1,2,3,4,4a,5,6,7-octahydronaphthalene, p-coumaric acid, isoferulic acid, narchinol B, and narchinol C (Hwang et al., 2012). It has been reported that nardosinone enhanced neurite outgrowth from PC12D cells (Li et al., 2003a; Li et al., 2003b). *N. chinensis* induced granulocytic differentiation in human leukemia HL-60 cells (Yoon et al., 2006). *N. chinensis* has also known to have an antioxidant activity and anti-inflammatory activity (Baek et al., 2009).

FOXO protein is a transcriptional factor that promotes cell-cycle arrest, DNA repair, apoptosis, and detoxification of ROS through transcriptional activation of target genes by binding to the consensus binding motif TTGTTTAC (Weidinger et al., 2008; Monsalve and Olmos, 2011). FOXO consists of four members: FOXO1 (FKHR), FOXO3a (FKHRL1), FOXO4 (AFX), and FOXO6. FOXO regulates proliferation through expression of p27, cell cycle inhibitor (Dijkers et al., 2000). FOXO protects cells from oxidative damage by transcriptional regulation of manganese superoxide dismutase (MnSOD) (Kops et al., 2002) and catalase (Tan et al., 2008). FOXO also protects cells from DNA damage by increasing DNA repair through up-regulation of GADD45 mRNA and protein (Tran et al., 2002). FOXO regulates apoptosis through directly upregulation of pro-apoptotic Bcl-2 family member Bim

J.-I. Heo · J. H. Kim · J.-M. Lee · S. C. Kim · J.-B. Park · J. Kim · J.-Y. Lee
Department of Biochemistry, Hallym University Medical School, Chuncheon, Gangwon-do 200-702, Republic of Korea

J.-I. Heo · J.-Y. Lee
Institute of Natural Medicine, Hallym University Medical School, Chuncheon, Gangwon-do 200-702, Republic of Korea

J.-I. Heo · J. H. Kim · J.-M. Lee · S. C. Kim · J.-B. Park · J. Kim · J.-Y. Lee
Institute of Cell Differentiation and Aging, Hallym University Medical School, Chuncheon, Gangwon-do 200-702, Republic of Korea

*Corresponding author (J.-Y. Lee: jyolee@hallym.ac.kr)

(Sunters et al., 2003).

Although it has been reported that *N. chinensis* extract had an antioxidant activity, the mechanism has not been elucidated. The present study showed that of *N. chinensis* extract has antioxidant activity through increased expression of MnSOD by FOXO activation in human embryonic fibroblast (HEF) cells.

Materials and Methods

Cell culture. A primary HEF cells were established from an abortus at Hallym University Hospital (Kim et al., 2005). HEF cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) (Bio-Whitaker) at 37°C in a humidified atmosphere containing 5% CO_2. U2OS cells were stably transfected with FHRE-GFP vector (three copies of forkhead responsive element) (Heo et al., 2013). U2OS-FHRE-GFP cell lines were cultured in DMEM containing 10% FBS and G418 (0.8 mg/mL).

Preparation of *N. chinensis* extract. *N. chinensis* was purchased from Dae Kwang Herb Medicine Co., Ltd., Korea and the voucher specimen (No. RIC-1517) was deposited at the center for efficacy assessment and development of functional foods and drugs (Regional Innovation Center Hallym University, Korea). Dried and chopped roots of *N. chinensis* (500g) mixed with 3 liter of 70% ethanol in a 5,000 mL round bottom flask fitted with a cooling condenser. The extraction was performed at 70°C for 6 h. The extracts were combined and concentrated under reduced pressure with a Model EYELA N-1000 rotary evaporator (Tokyo Rikakikai, Japan). 98.5 g (19.7%) of the crude extract was produced and used for biological activity test.

High Content Screening. U2OS-FHRE-GFP stable cells were seeded in 96-well plate. After 24 h, cells were treated with *N. Chinesis* extract for 24 h. Cells were fixed using 3.7% formaldehyde for 10 min and washing with 1× PBS 3 times. GFP intensity was measured by high content screening (ArrayScan V, Cellomics, USA).

Western blot analysis. Cells were harvested and lysed in lysis buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 5 mM EDTA, 10% glycerol, and 1% NP-40 containing a mixture of protease inhibitors). 40 μg of protein was separated on a sodium dodecyl sulfate polyacrylamide gel and then transferred to immune-blot PVDF membrane. The membranes were incubated with primary antibodies. The membranes were then incubated with the secondary antibody conjugated with anti-mouse or anti-rabbit IgG-horseradish peroxidases. Protein detection was performed using the ECL system (Amersharm Biosciences, USA). The anti-FOXO3a and the anti-phospho-FOXO3a (T32) antibody were purchased from Cell Signaling Technology (Essex, USA). The anti-MnSOD antibody was purchased from BD PharMingen (USA). The anti-actin antibody was purchased from Sigma (USA). Horseradish peroxidase-coupled anti-rabbit and anti-mouse were obtained from Pierce Technology Corporate (Meridian RD, USA).

Luciferase assay. The HEF cells were seeded in 24-well plate and cultivated for 24 h. Cells then were transfected with plasmid DNA using Lipofectamine LTX (Invitrogen, USA) and incubated for 24 h. Cells were treated with *N. chinensis* extract for 24 h. Luciferase activity of cell extract was measured using a commercial kit, Luciferase Assay Reagent (Promega, USA). β-Galactosidase activity was also measured to correct for the transfection efficiency.

MTT assay. 5×10^3 HEF cells were seeded in 24-well plate and cultivated for 24 h. Cells were then treated with *N. chinensis* extract for 24 h. MTT solution was incubated for 4 h, and the absorbance of each well was measured at 570 nm.

Measurement of reactive oxygen species. Cells were incubated with 30 μM DCDHF-DA for 30 min at 37°C. Cells were washed twice with PBS, detached with trypsin. DCF fluorescence intensities of 10,000 cells were measured by flow cytometry (FACSCalibur, BD, USA).

Statistical Analysis. Results were analyzed using GraphPad Prism 4 (Ver. 4.03, GraphPad Software, USA). Standard deviation and comparison of results have been performed by paired student's t-test.

Results

***N. chinensis* extract (10 μg/mL) activates FOXO3a.** U2OS-FHRE-GFP cells were prepared by stable transfection of U2OS cells with FHRE-GFP vector which has three FOXO responsive elements (Heo et al., 2013). GFP expression was increased by FOXO activation in these cells. To investigate whether *N. chinensis* extract activates FOXO3a, U2OS-FHRE-GFP cells were seeded in 96-well plates and *N. chinensis* extract (10 μg/mL) was treated for 24 h. And then GFP expression level was measured by HCS. Expression of GFP was increased by *N. chinensis* extract (Fig. 1A). To test the cytotoxic effect of *N. chinensis* extract, HEF cells were treated with *N. chinensis* extract for 24 h and cell viability was measured by MTT assay. Cell viability was unaffected by these concentrations of treatment (Fig. 1B).

Protein level of FOXO3a was increased by *N. chinensis* extract. Because FOXO3a was activated by *N. chinensis* extract, we measured the protein level of FOXO3a. HEF cells were treated with *N. chinensis* extract (10 and 20 μg/mL) for 24 h and western blot analysis was performed. The protein level of FOXO3a was increased more than 5.5 folds by *N. chinensis* extract (Fig. 2). Akt phosphorylates FOXO3a at three residues (Thr 32, Ser253, and Ser315). Because this phosphorylation of FOXO3a leads to nuclear exclusion, FOXO3a activity was inhibited by this phosphorylation (Brunet et al., 1999). The level of phosphorylated FOXO3a (T32) was not changed by *N. chinensis* extract. This result showed that protein level of FOXO3a was increased by *N. chinensis* extract regardless of Akt signaling.

Promoter activities of FOXO3a target genes are increased by *N. chinensis* extract. MnSOD, p27, and GADD45 are transcriptional target gene of FOXO3a. To investigate whether the target genes of FOXO3a were activated by *N. chinensis* extract, HEF cells were transfected with reporter plasmid DNA for 24 h, and then treated

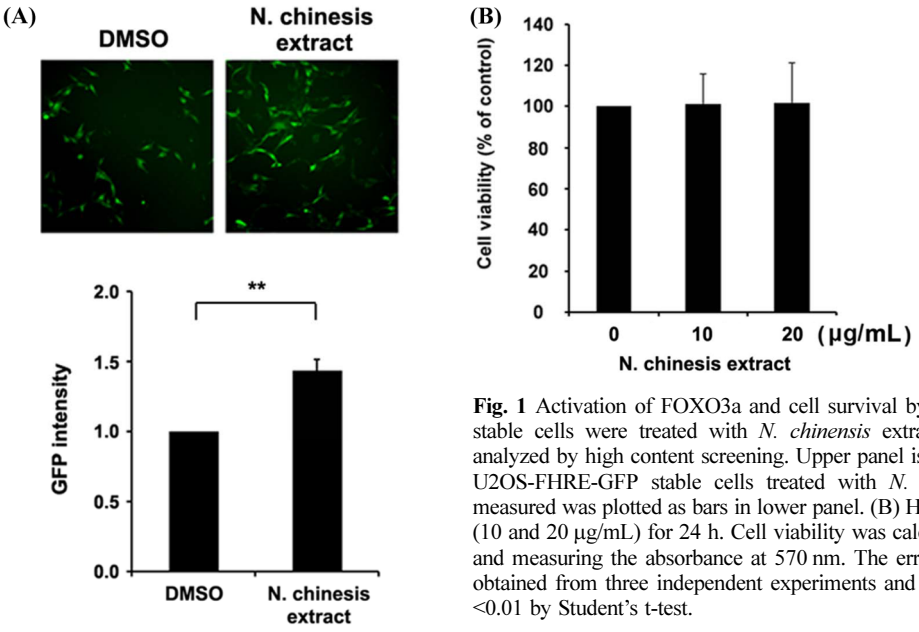

Fig. 1 Activation of FOXO3a and cell survival by *N. chinensis* extract. (A) U2OS-FHRE-GFP stable cells were treated with *N. chinensis* extract (10 μg/mL) for 24 h. GFP intensity was analyzed by high content screening. Upper panel is the photographs showing the fluorescence of U2OS-FHRE-GFP stable cells treated with *N. chinensis* extract and fluorescence intensity measured was plotted as bars in lower panel. (B) HEF cells were treated with *N. chinensis* extract (10 and 20 μg/mL) for 24 h. Cell viability was calculated by incubation in MTT solution for 4 h and measuring the absorbance at 570 nm. The error bars indicate standard deviations of results obtained from three independent experiments and the statistical significance is indicated as $^{**}p < 0.01$ by Student's t-test.

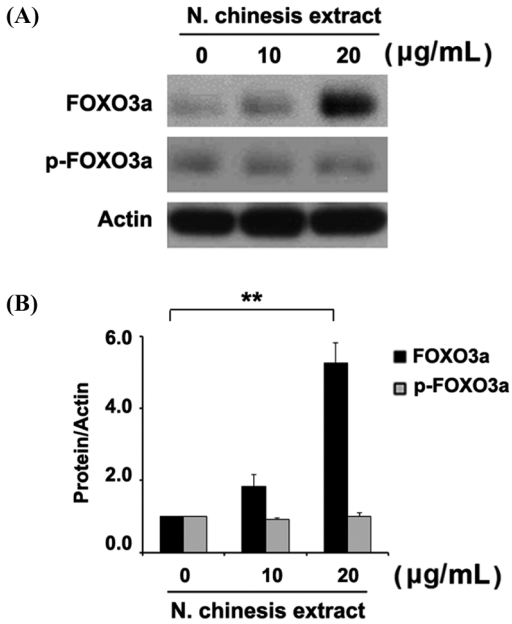

Fig. 2 Increase of FOXO3a protein level by *N. chinensis* extract. (A) HEF cells were treated with *N. chinensis* extract for 24 h. The protein levels of FOXO3a, p-FOXO3a (Thr32), and actin were measured by western blot analysis. (B) Band intensities were quantified by densitometry of Fig. 2A and the results were plotted as bars. The error bars indicate standard deviations of results obtained from three independent experiments and the statistical significance is indicated as $^{**}p < 0.01$ by Student's t-test.

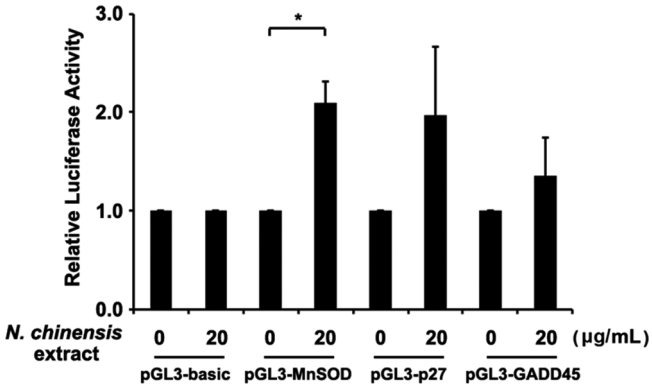

Fig. 3 Increase of promoter activities of FOXO3a target genes by *N. chinensis* extract. HEF cells were cotransfected with indicated reporter plasmid and pCMV-β-gal plasmid for 24 h. Transfected cells were treated with *N. chinensis* extract for 24 h. Luciferase activities and β-galactosidase activities were measured. β-Galactosidase activity was used to correct transfection efficiency. The error bars indicate standard deviations of results obtained from five independent experiments and the statistical significance is indicated as $^{*}p < 0.05$ by Student's t-test.

with *N. chinensis* extract for 24 h. Luciferase activity of MnSOD, p27 and GADD45 reporter genes appeared to be increased by *N. chinensis* extract, although there is no statistical significance for p27 anad GADD45 (Fig. 3). Activated FOXO3a by *N. chinensis* activates particularly MnSOD promoter rather than p27 and GADD45 promoter. These result suggested that *N. chinensis* may be involved in detoxification of ROS through activation of FOXO3a and MnSOD.

Protein level of MnSOD was increased by *N. chinensis* extract. MnSOD is a major cellular antioxidant enzyme that removes cellular ROS. MnSOD gene is a target gene of FOXO3a and has multiple FOXO biding sites in promoter. Because *N. chinensis* extract activates FOXO3a, protein level of MnSOD was measured. HEF cells were treated with *N. chinensis* extract for 24 h and western blot analysis was performed. Protein level of MnSOD was increased about 3.2 folds by 20 μg/mL of *N. chinensis* extract (Fig. 4).

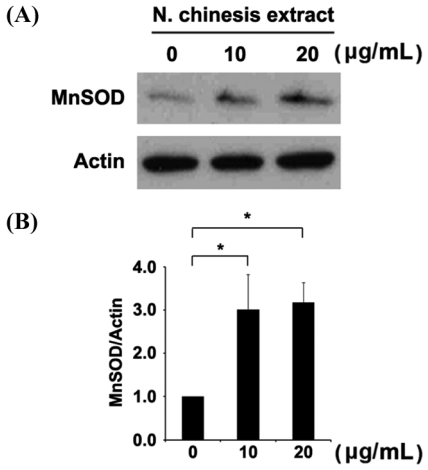

Fig. 4 Increase of MnSOD protein level by *N. chinensis* extract. (A) HEF cells were treated with *N. chinensis* extract for 24 h. Protein level of MnSOD and actin were measured by western blot analysis. (B) Band intensities of Fig. 4A were quantified by densitometry and plotted as bars The error bars indicate standard deviations of results obtained from three independent experiments and the statistical significance is indicated as $*p < 0.05$ by Student's t-test.

ROS level was decreased by *N. chinensis* extract. It has been reported that *N. chinensis* extract has antioxidant activity. To investigate that *N. chinensis* extract has antioxidant activity in HEF cells, HEF cells were treated with *N. chinensis* extract for 24 h. ROS level was measured using DCFHF-DA staining. ROS level was decreased by *N. chinensis* extract in a dose-dependent manner (Fig. 5).

Discussion

It has been reported that *N. chinensis* extract has an antioxidant activity under in vitro assays such as DPPH radical, superoxide anion and nitric oxide radical scavenging capacity (Baek et al., 2009). However, the molecular mechanism has not been verified. In this study, we demonstrated that FOXO3a was activated by *N. chinensis* extract and the protein level of MnSOD, a FOXO target gene, was increased. ROS was reduced by action of increased MnSOD in HEF cells.

ROS activates various signaling pathways including Ras, Protein tyrosine phosphatases, serine/threonine kinases such as AKT and mitogen-activated protein kinases (MAPKs), and nuclear transcription factors such as NF-κB, AP-1, p53, and HIF-1 (Valko et al., 2006). Particularly, MAP-kinase/AP-1 and NF-κB pathway affects proliferation and apoptosis of cells. ROS is known to result in DNA damage, mutations, and altered gene expressions to activate carcinogenesis. Oxidative stress can also cause various diseases such as neurodegeneration, atherosclerosis, diabetes, and aging (Ray et al., 2012).

Among the components of *N. chinensis* extract, nardosinone enhanced nerve growth factor (NGF)-mediated neurite outgrowth from PC12D cells (Li et al., 1999) by activation of a down-stream step of mitogen-activated protein kinase (MAPK)-dependent cascade of NGF (Li et al., 2003b). Nardosinone enhanced also dibutyryl cyclic AMP and staurosporine-induced neurite outgrowth from PC12D cells (Li et al., 2003a). Glycoside from *N. chinensis* induces neurite outgrowth, increase of AChE activity, cell cycle arrest in G1 and up-regulation of GAP-43, neuronal marker, via

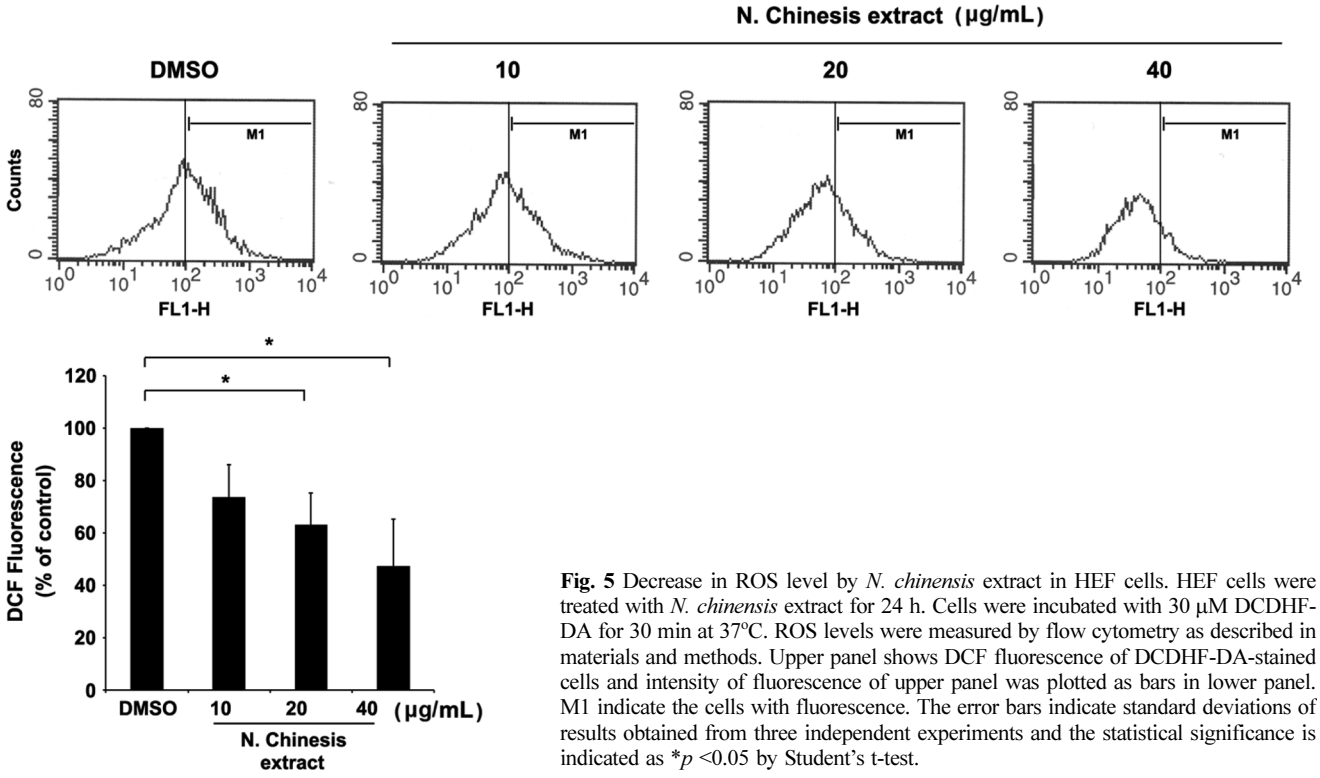

Fig. 5 Decrease in ROS level by *N. chinensis* extract in HEF cells. HEF cells were treated with *N. chinensis* extract for 24 h. Cells were incubated with 30 μM DCDHF-DA for 30 min at 37°C. ROS levels were measured by flow cytometry as described in materials and methods. Upper panel shows DCF fluorescence of DCDHF-DA-stained cells and intensity of fluorescence of upper panel was plotted as bars in lower panel. M1 indicate the cells with fluorescence. The error bars indicate standard deviations of results obtained from three independent experiments and the statistical significance is indicated as $*p < 0.05$ by Student's t-test.

MAPK-related signal cascade (Liu et al., 2005). *N. chinensis* induced granulocytic differentiation through ERK activation in human promyelocytic leukemia HL-60 cells (Yoon et al., 2006). It is unclear currently whether activation of differentiation by *N. chinensis* is related to FOXO3a activation by *N. chinensis. N. chinensis* was also known to possess an antioxidant activity and anti-inflammatory activity through suppression of NO production and the expression of iNOS and COX-2 (Baek et al., 2009). *N. chinensis* induced cell cycle arrest in G0/G1 phase in association with p21 and p27 in human lymphoma U937 cells (Kang et al., 2011). Our result suggests that antioxidant of *N. chinensis* appears to be related to FOXO3a activation of *N. chinensis* since we showed that promoter activity and protein level of MnSOD were upregulated by FOXO activation. The component that activates FOXO3a has not been verified in this work. Further detailed characterization of component of *N. chinensis* extract that activates FOXO3a will answer question.

Activity of FOXO3a is regulated by posttranslational modifications such as phosphorylation, acetylation, ubiquitination, and methylation (Huang and Tindall, 2011; Zhao et al., 2011). Akt regulates FOXO3a through phosphorylation. Akt phosphorylates FOXO3a at Thr 32, Ser 253, and Ser315. Phosphorylated FOXO3a associated with 14-3-3 proteins and leading to nuclear exclusion and inhibits FOXO3a activity (Brunet et al., 1999). Because this signaling is the most well known signaling, we examined the level of phospho-FOXO3a (T32). However, the level of phosphor-FOXO3a (T32) was not affected by *N. chinensis* extract (Fig. 2). IkB kinase physically interacts with and phosphorylates FOXO3a (Ser 644). This phosphorylation leads to inhibit the FOXO3a activity through proteolysis via ubiquitin-dependent protesome pathway (Hu et al., 2004). MST1 phosphorylates FOXO3a at Ser 207 that causes to interrupt binding with 14-3-3 proteins and promotes nuclear translocation (Lehtinen et al., 2006). The AMP-activated protein kinase phosphorylates FOXO3a at six sites (Thr179, Ser399, Ser413, Ser555, Ser588, and Ser626) and this phosphorylation leads to activation of FOXO3a transcriptional activity (Greer et al., 2007). ERK directly interacts with and phosphorylates FOXO3a at Ser 294, Ser344, and Ser 425. The phosphorylation degrades FOXO3a via an MDM2-mediated ubiquitin-proteasome pathway and eventually FOXO3a degradation leads to promote cell proliferation and tumorigenesis (Yang et al., 2008). It has been reported that FOXO3a activity is associated with tumor suppression, aging, diabetes, infertility, neurodegeneration, and immune system dysfunction (Maiese et al., 2008). Our result showed that *N. chinensis* extract did not affect Akt-mediated phosphorylation of FOXO3a (Fig. 2). However, it has not been tested whether other modifications of FOXO3a are involved in *N. chinensis* extract-induced FOXO3a activation. Further detailed characterization on the mechanism of FOXO3a activation by *N. chinensis* extract will provide better understanding of the phenomena.

Acknowledgment This work was supported partly by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2009-0094071).

References

Baek S, Choi JH, Ko SH, Lee YJ, Cha DS, Park EY et al. (2009) Antioxidant and anti-inflammatory effect of Nardostachys chinesis in IFN-ã/LPS-stimulated peritoneal macrophage. *Korean J Orient Physiol Pathol* **23**, 853–9.

Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS et al. (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857–68.

Circu ML and Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* **48**, 749–62.

Dijkers PF, Medema RH, Pals C, Banerji L, Thomas NS, Lam EW et al. (2000) Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). *Mol Cell Biol* **20**, 9138–48.

Greer EL, Oskoui PR, Banko MR, Maniar JM, Gygi MP, Gygi SP et al. (2007) The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem* **282**, 30107–19.

Heo JI, Kim JH, Lee JM, Lim SS, Kim SC, Park JB et al. (2013) Antioxidant Activity and Its Mechanism of Chelidonium majus Extract. *Korean J Medicinal Crop Sci* **21**, 136–41.

Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY et al. (2004) IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. *Cell* **117**, 225–37.

Huang H and Tindall DJ (2011) Regulation of FOXO protein stability via ubiquitination and proteasome degradation. *Biochim Biophys Acta* **1813**, 1961–4.

Hwang JS, Lee SA, Hong SS, Han XH, Lee C, Lee D et al. (2012) Inhibitory constituents of Nardostachys chinensis on nitric oxide production in RAW 264.7 macrophages. *Bioorg Med Chem Lett* **22**, 706–8.

Kang MS, Ju SM, Jeon BJ, Yang HM, Kim WS, and Jeon BH (2011) Nardostachys Chinensis Induces G0/G1 Phase Cell Cycle Arrest in U937 Cells. *Korean J Orient Physiol Pathol* **25**, 189–94.

Kim HS, Yeo EJ, Park SH, Park JI, Park SC, Shin JY et al. (2005) p21WAF/CIP1/SDI1 is upregulated due to increased mRNA stability during hydroxyurea-induced senescence of human fibroblasts. *Mech Ageing Dev* **126**, 1255–61.

Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ et al. (2002) Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* **419**, 316–21.

Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villen J, Becker EB et al. (2006) A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. *Cell* **125**, 987–1001.

Li P, Matsunaga K, Yamakuni T, and Ohizumi Y (2003a) Nardosinone, the first enhancer of neurite outgrowth-promoting activity of staurosporine and dibutyryl cyclic AMP in PC12D cells. *Brain Res Dev Brain Res* **145**, 177–83.

Li P, Matsunaga K, Yamamoto K, Yoshikawa R, Kawashima K, and Ohizumi Y (1999) Nardosinone, a novel enhancer of nerve growth factor in neurite outgrowth from PC12D cells. *Neurosci Lett* **273**, 53–6.

Li P, Yamakuni T, Matsunaga K, Kondo S, and Ohizumi Y (2003b) Nardosinone enhances nerve growth factor-induced neurite outgrowth in a mitogen-activated protein kinase- and protein kinase C-dependent manner in PC12D cells. *J Pharmacol Sci* **93**, 122–5.

Liu JH, Yin F, and Zheng XX (2005) Nardostachys chinensis glycoside induces characteristics of neuronal differentiation in rat pheochromocytoma PC12 cells. *Biol Pharm Bull* **28**, 768–71.

Maiese K, Chong ZZ, and Shang YC (2008) OutFOXOing disease and disability: the therapeutic potential of targeting FoxO proteins. *Trends Mol Med* **14**, 219–27.

Monsalve M and Olmos Y (2011) The complex biology of FOXO. *Curr Drug Targets* **12**, 1322–50.

Ray PD, Huang BW, and Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* **24**, 981–90.

Sunters A, Fernandez de Mattos S, Stahl M, Brosens JJ, Zoumpoulidou G, Saunders CA et al. (2003) FoxO3a transcriptional regulation of Bim

controls apoptosis in paclitaxel-treated breast cancer cell lines. *J Biol Chem* **278**, 49795–805.

Tan WQ, Wang K, Lv DY, and Li PF (2008) Foxo3a inhibits cardiomyocyte hypertrophy through transactivating catalase. *J Biol Chem* **283**, 29730–9.

Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ Jr, DiStefano PS et al. (2002) DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. *Science* **296**, 530-534.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, and Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44–84.

Valko M, Rhodes CJ, Moncol J, Izakovic M, and Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* **160**, 1–40.

Weidinger C, Krause K, Klagge A, Karger S, and Fuhrer D (2008) Forkhead box-O transcription factor: critical conductors of cancer's fate. *Endocr Relat Cancer* **15**, 917–29.

Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X et al. (2008) ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat Cell Biol* **10**, 138–48.

Yoon SH, Ju SM, Kim NS, Park SC, Park JY, Kim SH et al. (2006) Extracellular Signal-regulated Kinase (ERK) is required for Water Extract of Nardostachys chinensis-Induced Differentiation in HL-60 Cells. *Korean J Orient Physiol Pathol* **20**, 1315–20.

Zhao Y, Wang Y, and Zhu WG (2011) Applications of post-translational modifications of FoxO family proteins in biological functions. *J Mol Cell Biol* **3**, 276–82.