



## Research article

# Korean Red Ginseng inhibits apoptosis in neuroblastoma cells via estrogen receptor $\beta$ -mediated phosphatidylinositol-3 kinase/Akt signaling



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## ABSTRACT

**Background:** Ginseng has been shown to exert antistress effects both *in vitro* and *in vivo*. However, the effects of ginseng on stress in brain cells are not well understood. This study investigated how Korean Red Ginseng (KRG) controls hydrogen peroxide-induced apoptosis via regulation of phosphatidylinositol-3 kinase (PI3K)/Akt and estrogen receptor (ER)- $\beta$  signaling.

**Methods:** Human neuroblastoma SK-N-SH cells were pretreated with KRG and subsequently exposed to H<sub>2</sub>O<sub>2</sub>. The ability of KRG to inhibit oxidative stress-induced apoptosis was assessed in MTT cytotoxicity assays. Apoptotic protein expression was examined by Western blot analysis. The roles of ER- $\beta$ , PI3K, and p-Akt signaling in KRG regulation of apoptosis were studied using small interfering RNAs and/or target antagonists.

**Results:** Pretreating SK-N-SH cells with KRG decreased expression of the proapoptotic proteins p-p53 and caspase-3, but increased expression of the antiapoptotic protein BCL2. KRG pretreatment was also associated with increased ER- $\beta$ , PI3K, and p-Akt expression. Conversely, ER- $\beta$  inhibition with small interfering RNA or inhibitor treatment increased p-p53 and caspase-3 levels, but decreased BCL2, PI3K, and p-Akt expression. Moreover, inhibition of PI3K/Akt signaling diminished p-p53 and caspase-3 levels, but increased BCL2 expression.

**Conclusion:** Collectively, the data indicate that KRG represses oxidative stress-induced apoptosis by enhancing PI3K/Akt signaling via upregulation of ER- $\beta$  expression.

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## 1. Introduction

The  $\alpha$  and  $\beta$  estrogen receptors (ERs) regulate various brain functions in an estradiol-dependent manner. Signaling pathways elicited via ER- $\alpha$  and ER- $\beta$  activation are interrelated and feedback inhibition occurs mainly by estradiol engagement of the ER- $\alpha$  receptor. Most studies involving ER- $\beta$  have focused on brain functions and behavioral patterns [1,2]. ER- $\beta$  is a member of the nuclear receptor superfamily [3]. Upon ligand binding, ER- $\beta$  regulates gene expression by binding directly to regulatory regions of target genes or by interacting with other transcription factors such as nuclear factor  $\kappa$ B, activating protein 1, and stimulating protein 1 [4]. ER- $\beta$  also controls gene expression by activating signaling pathways that

stimulate kinases such as protein kinase A, protein kinase C, and mitogen-activated protein kinase [5]. Recent studies show that ER- $\beta$  has neuroprotective, anti-inflammatory, antiproliferative, anti-oxidant, and immune-modulatory activities [2,6]. However, the effects of stress on ER- $\beta$  expression in the brain cells remain largely unknown.

ERs regulate activation of phosphatidylinositol-3 kinases (PI3Ks) by interacting with the p85 regulatory subunit of PI3K [7]. Activation of ER- $\alpha$  upregulates PI3K/Akt signaling, which in turn stimulates cell growth in breast cancer cells [8]. ER- $\beta$  also activates signaling through PI3K/Akt and improves myocardial function in female hearts following acute ischemia [9]. PI3Ks are a family of kinases that phosphorylate the 3'-OH group of

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the inositol ring in inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate [10], which can bind to and activate Akt (p-Akt). Akt activation plays a key role in cell proliferation, cell cycle progression, and apoptosis [10]; thus, PI3K/Akt signaling is important for cell survival.

*Panax ginseng* Meyer is one of the most popular herbal medicines in Korea, and has long been used in Asian countries for stimulating immunity and inhibiting various cancers [11–13]. Ginsenosides are active compounds present in ginseng that are known to have antioxidative, anti-inflammatory, and anticancer activities [14]. Ginsenoside Rb1, a known phytoestrogen, shows anti-inflammatory activity in smooth muscle cells [15] and inhibits interleukin-1 $\beta$ -induced apoptosis in human chondrocytes [16]. Ginsenoside Rg3 exerts neuroprotective, anti-inflammatory, and antioxidative effects [17,18]. Although the role of ginseng in regulating the development of cancer is well defined, the mechanism by which it protects brain cells from oxidative stress is not well understood. Recent studies have revealed that ginseng upregulates ER- $\beta$  expression *in vitro* and *in vivo* [17,19]. Previously, we reported that Korean Red Ginseng (KRG)-induced ER- $\beta$  expression inhibits oxidative stress-induced apoptosis in mouse brain and SK-N-SH neuroblastoma cells by inhibiting PADI4 expression [17]. However, the downstream signaling effector molecules of ER- $\beta$  have not been explored. Thus, the aims of this study were to identify signaling effector molecules immediately downstream of ER $\beta$  and to understand how KRG-induced ER- $\beta$  expression regulates apoptosis via PI3K/Akt signaling in oxidative stressed brain cells.

## 2. Materials and methods

### 2.1. Cell culture and KRG

Human neuroblastoma SK-N-SH cells (catalog number HTB-11; ATCC, Manassas, VA, USA) were cultured in RPMI 1640 (Lonza, Walkersville, MD, USA) media containing 10% FBS, 1% penicillin-streptomycin (10,000 U penicillin/mL, 10,000  $\mu$ g streptomycin/mL), 1mM HEPES, 1mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L bicarbonate, and 2mM L-glutamine at 37°C, and 5% CO<sub>2</sub>.

KRG extract was manufactured by Korea Ginseng Corporation (Seoul, Korea) by steaming and drying 6-year-old roots from *Panax ginseng* Meyer and analyzed as described previously [17]. The ginsenoside content of KRG extracts used in this study was: Rg1 0.71 mg/g, Re 0.93 mg/g, Rf 1.21 mg/g, Rh1 0.78 mg/g, Rg2(s) 1.92 mg/g, Rg2(r) 1.29 mg/g, Rb1 4.62 mg/g, Rc 2.41 mg/g, Rb2 1.83 mg/g, Rd 0.89 mg/g, Rg3(s) 2.14 mg/g, and Rg3(r) 0.91 mg/g.

### 2.2. Inhibitor treatments

Specific inhibitors of ER- $\beta$  (PHTPP; catalog number sc-204191) and Akt (inhibitor VIII; catalog number sc-2002048) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The PI3K-specific inhibitor LY294002 (catalog number L9908) was purchased from Sigma–Aldrich (St Louis, MO, USA). SK-N-SH cells were treated with KRG extract for 48 h and subsequently treated with 5 $\mu$ M PHTPP [20], 80 $\mu$ M LY294002 [21], or 50 $\mu$ M Akt inhibitor VIII for 5 h.

### 2.3. RNA interference studies

ER- $\beta$  expression in SK-N-SH cells was silenced by transfecting cells with 50nM ER- $\beta$  small interfering RNA (siER- $\beta$ ; Santa Cruz Biotechnology, Inc), using the TransIT-TKO transfection reagent (Mirus, Madison, USA) as described [17].

### 2.4. MTT assays

The effects of KRG treatment on cell viability were determined by MTT assays to assess mitochondrial function [22]. SK-N-SH cells were seeded in 96 well-plate and incubated with KRG (1mg/mL) for 48 h and subsequently treated with 0.5mM H<sub>2</sub>O<sub>2</sub> for 2 h. Next, RPMI medium containing MTT dye (2 mg/mL) was added to cell cultures, and plates were incubated for 1 h at 37°C with 5% CO<sub>2</sub>. Supernatants were then removed, 150  $\mu$ l of dimethyl sulfoxide was added to wells for 15 min to solubilize liberated formazan, and absorbance was read at 540 nm with a plate reader. Experiments were performed in triplicate.

### 2.5. Western blot analysis

Cells were washed with phosphate-buffered saline (PBS), harvested, and collected by centrifugation. Cell pellets were lysed in radioimmunoprecipitation assay buffer containing 50mM Tris-Cl pH 7.4, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 150mM NaCl, 1mM ethylenediaminetetra-acetic acid, 1mM phenylmethylsulfonyl fluoride, and 1 $\times$  protease inhibitor cocktail. Protein concentrations in samples were determined by Bradford assays, and 30–40  $\mu$ g of protein from each sample were resolved on 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. Samples were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA), which were blocked on a shaker at room temperature for 2–3 h in Tris-buffered saline with 0.1% Tween-20 (T-TBS) containing 7% skim milk. Membranes were then washed three times with T-TBS and incubated overnight with primary antibodies at 4°C. Primary antibodies recognizing human ER- $\beta$  (sc-53494), bcl-2 (sc-7382), p-p53 (sc-101762), PI3K-p110 (sc-7189), Akt (sc-8312), and p-Akt (sc-7985-R) were purchased from Santa Cruz Biotechnology, Inc. Primary antibodies recognizing  $\beta$ -actin and anti-caspase-3 were obtained from Sigma–Aldrich and Cell Signaling Technology (Beverly, MA, USA), respectively. Subsequently, membranes were washed 4 times with T-TBS and incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (Sigma–Aldrich). Membranes were washed in T-TBS and proteins of interest were detected using the Power Optic-ECL Western blotting Detection reagent (Animal Genetics Inc., Gyeonggi-do, Korea).

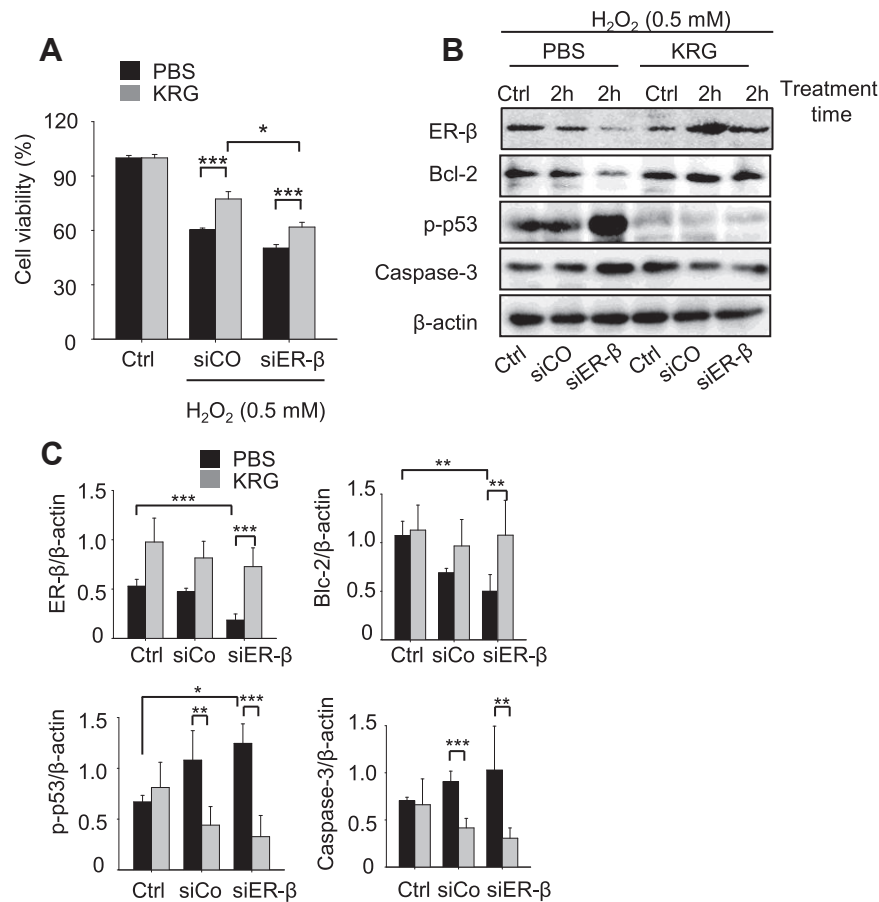
### 2.6. Statistical analysis

Statistical differences between group medians from three independent experiments were analyzed by analysis of variance. Differences were considered statistically significant in cases where  $p < 0.05$ .

## 3. Results

### 3.1. Inhibition of ER- $\beta$ -mediated inhibition of apoptotic signaling during oxidative stress

Previously, we showed that ER- $\beta$  expression is inhibited by oxidative stress and upregulated following exposure to KRG [17]. ER- $\beta$  is an upstream regulator of apoptosis [23,24]. Here, we examined whether KRG inhibits oxidative stress-induced apoptosis via ER- $\beta$  upregulation (Fig. 1). ER- $\beta$  expression was blocked by transfecting SK-N-SH cells with siER- $\beta$  prior to treating cells with 0.5mM H<sub>2</sub>O<sub>2</sub> to cause oxidative stress. Cytotoxicity was examined by MTT assays, and expression of apoptotic markers such as p-p53, caspase-3, and BCL2 were determined. Results showed that oxidative stress reduces SK-N-SH cell viability, that KRG pretreatment protects against oxidative stress-induced cytotoxicity, and that the



**Fig. 1.** Estrogen receptor- $\beta$  small interfering RNA inhibits apoptosis in SK-N-SH cells resulting from oxidative stress. SK-N-SH cells were transfected for 24 h with 40nM estrogen receptor- $\beta$  small interfering RNA. Following transfection, cells were incubated with Korean Red Ginseng extract (1 mg/mL) for 48 h and then with 0.5mM H<sub>2</sub>O<sub>2</sub> for 2 h. (A) Cell viabilities were determined by MTT assays. (B) Cell lysates were used to estimate protein expression levels by Western blot analysis. (C) Quantification of at least three independent experimental results from (B). Statistical significances were determined by analysis of variance. Data shown are representative of three independent experiments. \* $p$  < 0.05. \*\* $p$  < 0.01. \*\*\* $p$  < 0.001.

protective effects of KRG are reversed by silencing ER- $\beta$  expression (Fig. 1A). Expression of the antiapoptotic protein BCL2 was also suppressed by siER- $\beta$  transfection (Fig. 1B, 1C). By contrast, expression of proapoptotic factors such as p-p53 and caspase-3 were enhanced by siER- $\beta$  transfection. However, KRG-treatment upregulated BCL2 expression and downregulated expression of p-p53 and caspase-3 (Fig. 1B, 1C), indicating that KRG protects against apoptosis induced by oxidative stress.

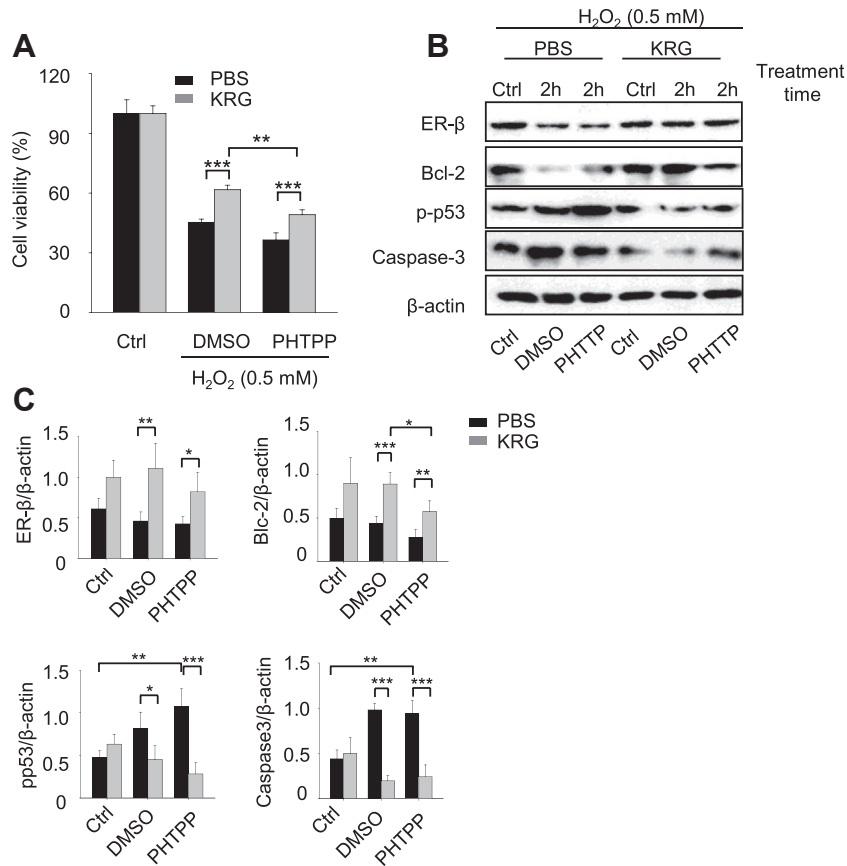
To confirm these observations, we studied the effects of an ER- $\beta$  antagonist (PHTTP) on cell viability and expression of apoptotic markers in oxidatively stressed brain cells. The ER- $\beta$  inhibitor consistently reduced cell viability during oxidative stress, compared with dimethyl sulfoxide-treated control cells (Fig. 2A). Moreover, ER- $\beta$  inhibitor treatment decreased BCL2 expression but increased p-p53 and caspase-3 levels (Fig. 2B, 2C). These results suggest that KRG prevents oxidative stress-induced apoptosis by inhibiting ER- $\beta$ -dependent apoptotic signaling.

### 3.2. ER- $\beta$ inhibition suppresses Akt/PI3K signaling in oxidatively stressed brain cells

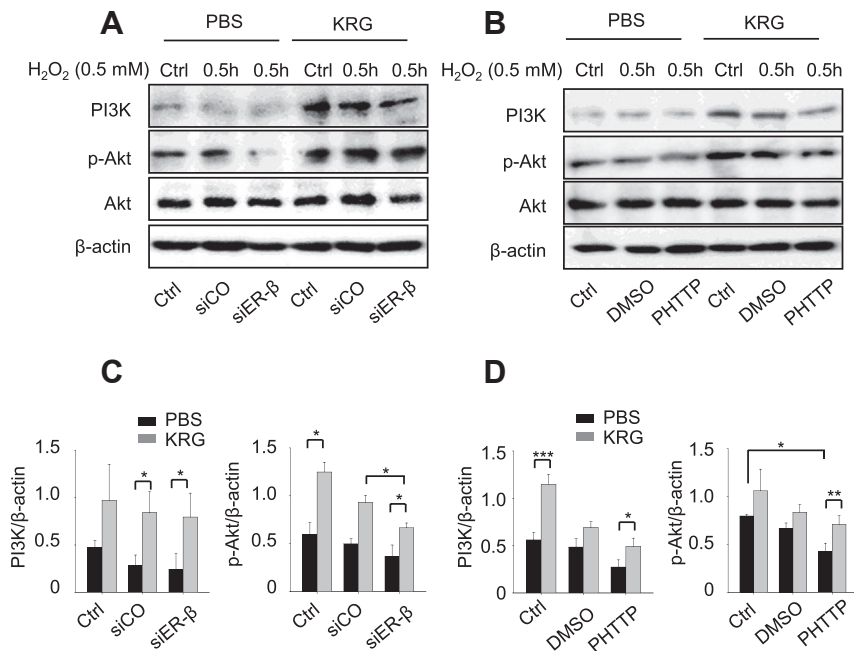
Akt plays important roles in cell survival and apoptosis [25,26] and blocks apoptosis by inhibiting caspase-3 expression and enhancing BCL2 expression [26,27]. Thus, it was hypothesized that ER- $\beta$  regulates Akt activation to promote inhibition of apoptosis in oxidatively stressed brain cells. To test this hypothesis, ER- $\beta$

expression was silenced by transfecting cells with siER- $\beta$  and the effect of ER- $\beta$  downmodulation on Akt expression was determined. As expected, siER- $\beta$  transfection reduced p-Akt levels but not total Akt levels. By contrast, KRG pretreatment increased p-Akt expression, thus enhancing cell survival under conditions of oxidative stress (Fig. 3A, 3B). Moreover, treatment with the ER- $\beta$  inhibitor PHTTP decreased p-Akt levels marginally, whereas KRG treatment increased basal p-Akt levels significantly without increasing Akt levels (Fig. 3C, 3D).

Because PI3K is an upstream regulator of Akt, ER- $\beta$ -dependent Akt activation (p-Akt) may be in part mediated by PI3K upregulation. To test this possibility, the effect of siER- $\beta$  silencing on PI3K levels during oxidative stress was determined by Western blot analysis. The results show that oxidative stress, but not siER- $\beta$  transfection, decreases PI3K levels compared to negative controls (Fig. 3A, 3B). However, KRG treatment significantly upregulated PI3K expression compared to the PBS group. Neither oxidative stress nor siER- $\beta$  transfection decreased PI3K levels back to the normal nonstressed control level (Fig. 3A, 3B). Consistently, treatment with the ER- $\beta$  inhibitor PHTTP resulted in a moderate although nonsignificant decrease in PI3K expression levels. However, KRG treatment upregulated PI3K levels compared to the PBS group (Fig. 3C, 3D). These results suggest that KRG upregulates ER- $\beta$ -mediated PI3K/Akt signaling in brain cells even under conditions of oxidative stress, which normally diminishes PI3K/Akt signaling.



**Fig. 2.** The estrogen receptor- $\beta$  inhibitor PHTPP inhibits apoptosis in SK-N-SH cells resulting from oxidative stress. SK-N-SH cells were incubated sequentially with Korean Red Ginseng extract (1 mg/mL) for 48 h, 5  $\mu$ M PHTPP for 5 h, and 0.5 mM H<sub>2</sub>O<sub>2</sub> for 2 h. (A) Cell viabilities were determined by MTT assays. (B) Cell lysates were used to estimate protein expression levels by Western blot analysis. (C) Quantification of at least three independent experimental results from (B). Statistical significances were determined by analysis of variance. Data shown are representative of three independent experiments. \* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .



**Fig. 3.** Functional estrogen receptor (ER)- $\beta$  inhibition suppresses phosphatidylinositol-3 kinase/Akt signaling in SK-N-SH cells resulting from oxidative stress. (A) SK-N-SH cells were transfected with 40 nM ER- $\beta$  small interfering RNA for 24 h and then incubated with 1 mg/mL of Korean Red Ginseng extract for 48 h. (B) SK-N-SH cells were incubated with 1 mg/mL of Korean Red Ginseng extract for 48 h and then exposed to the ER- $\beta$  inhibitor PHTPP (5  $\mu$ M) for 5 h. Following the indicated treatments, cells from (A) and (B) were incubated with H<sub>2</sub>O<sub>2</sub> for 30 min, and Western blots were performed to estimate gene expression levels in cell lysates. (C, D) Quantification of at least three independent experimental results from (A, B). Statistical significances were determined by analysis of variance. Data shown are representative of three independent experiments. \* $p < 0.05$ . \*\*\* $p < 0.001$ .

### 3.3. Inhibition of PI3K/Akt signaling blocks apoptotic signaling during oxidative stress

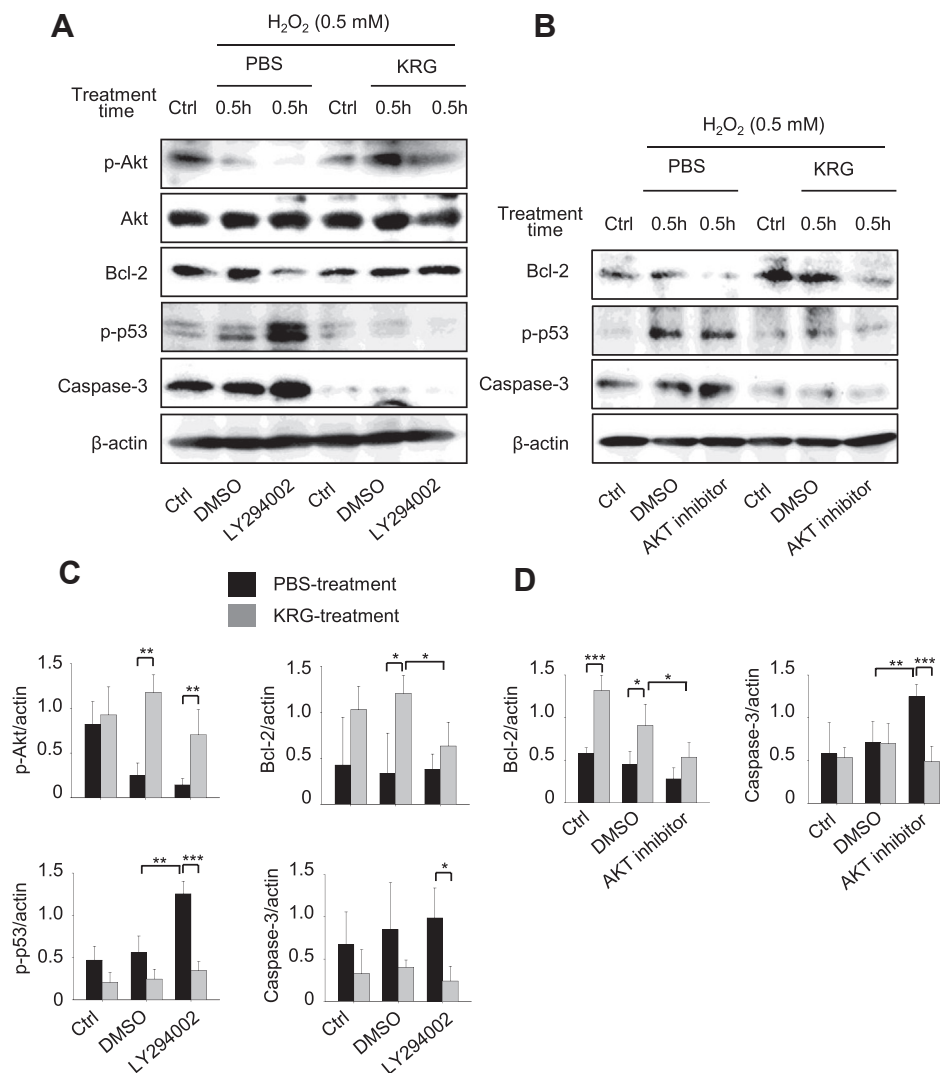
To examine further whether the PI3K/Akt signaling pathway is important in the apoptosis of oxidative stressed brain cells, SK-N-SH cells were treated with the PI3K inhibitor LY294002, and the expression levels of apoptotic markers were determined. Oxidative stress repressed p-Akt levels compared with nonstressed control cells, but induced p-p53 expression (Fig. 4A). In addition, treatment with the PI3K inhibitor LY294002 significantly lowered p-Akt levels in both PBS- and KRG-treated groups compared to control cells, but KRG treatment significantly increased p-Akt expression compared to the PBS-treated group. However, total Akt levels were unaffected by PI3K inhibition (Fig. 4A, B). These results suggest that oxidative stress inhibits p-Akt expression but that KRG reverses such inhibition and increases cells survival. Furthermore, PI3K inhibition inhibited BCL2 expression, but induced p-p53 and caspase-3 expression. However, KRG treatment reversed this result, and increased BCL2 level but decreased p-p53 and caspase-3 levels were observed (Fig. 4A, 4B), indicating that KRG protects the brain

cells from apoptosis from oxidative stress via upregulation of PI3K signals.

To confirm these results, Akt inhibitor VIII was used to inhibit Akt activity, and apoptosis marker expressions were checked in oxidative stressed brain cells. Results show that Akt inhibition downregulates BCL2 expression, but upregulates caspase-3 expression compared to solvent controls (Fig. 4C, 4D). However, KRG treatment resulted in increased BCL2 expression and decreased caspase-3 and p-p53 levels (Fig. 4C, 4D). These results suggest that KRG inhibits apoptosis via upregulation of PI3K/Akt signals in oxidative stressed brain cells.

## 4. Discussion

Brain myelin sheaths contain relatively large amounts of iron and lipids and have high rates of oxidative metabolism with limited antioxidant capacity. Thus, myelin sheaths are highly susceptible to oxidative damage [28–30]. Moreover, oxidative stress due to free radicals has been implicated as a major pathological mechanism of brain disorders, such as Parkinson's disease, Alzheimer's disease,



**Fig. 4.** Inhibition of phosphatidylinositol-3 kinase/Akt signaling counteracts apoptosis in oxidative stressed brain cells. (A, B) SK-N-SH cells were incubated with 1 mg/mL of Korean Red Ginseng extract for 48 h. Subsequently, cells were exposed for 6 h to 80  $\mu$ M of phosphatidylinositol-3 kinase inhibitor LY294002 (A) or 50  $\mu$ M of Akt inhibitor VIII (B). Cells were then treated with H<sub>2</sub>O<sub>2</sub> for 30 min. Cell lysates were subject to Western blot analysis to estimate protein levels of interest following treatment. (C, D) Quantification of at least three independent experimental results from (A, B). Statistical significances were determined by analysis of variance. Data shown are representative of three independent experiments. \* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .

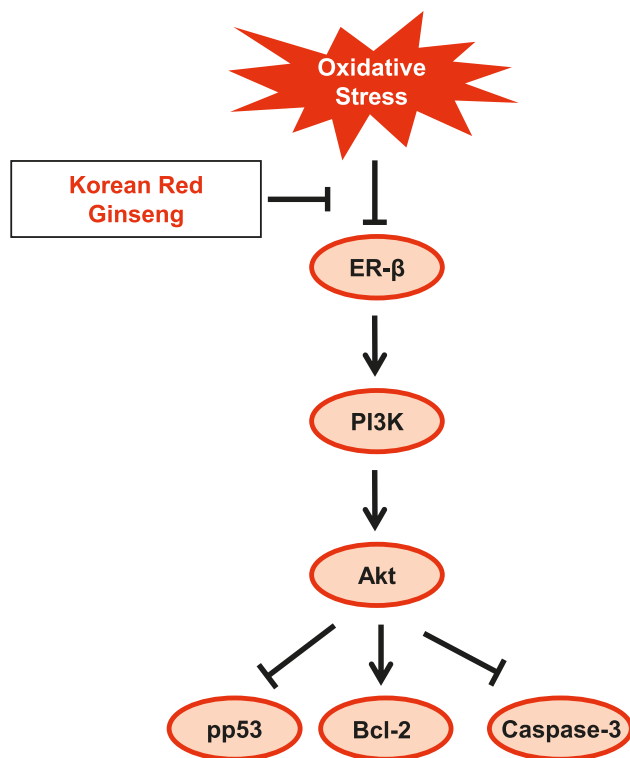


and brain trauma [31,32]. In the brain, stress stimulates secretion of glucocorticoids, which augments the extracellular accumulation of glutamate in the hippocampus. Because glutamate can induce neuronal excitotoxicity and leads to TNF- $\alpha$  release, such an outcome would activate iNOS and COX2 induction and generate free radicals, which can mutate DNA, oxidize proteins and lipids, and finally results in neural degeneration and cell death [33]. Therefore, development of a potential candidate for various degenerative disorders in the brain is required.

The PI3K/Akt pathway plays an important role in neural survival pathways [34]. PI3K is an upstream antiapoptotic effector in the growth factor signaling pathway. Akt is also a key antiapoptotic effector of cellular growth factors [35]. PI3K activation by growth factors leads to Akt activation, which is an important player in survival pathway [36]. Some studies have shown that Akt suppresses apoptosis signaling via BCL2 induction [27], and p-p53 inhibition through MDM2 activation [37]. Previously, KRG was shown to upregulate PI3K/Akt signaling and to inhibit apoptosis via the regulation of BCL2 and caspase-3 expression, thus protecting endothelial cells from starvation [38]. Moreover, *Panax notoginseng* saponins inhibit ischemia-induced apoptosis by stimulating PI3K/Akt signaling in cardiomyocytes [39]. However, the mechanism by which KRG activates PI3K/Akt signal via ER- $\beta$  under oxidative stress in brain cells has been unclear until now. In this study, we demonstrated that KRG increases PI3K/Akt signaling via upregulation of ER- $\beta$ , thus inhibiting apoptosis through p-p53 and caspase-3 downregulation and BCL2 induction in oxidatively stressed brain cells.

Excitotoxicity is the pathological process caused by neurotransmitter glutamate such as *N*-methyl-D-aspartate (NMDA) and kainic acid [40]. These excitotoxins bind to glutamate receptor and result in increase of intracellular  $Ca^{2+}$ . Subsequently, overload of intracellular  $Ca^{2+}$  stimulates activation of enzymes comprising calpains, which are the ubiquitously expressed family of  $Ca^{2+}$ -dependent proteases [40]; thus these enzymes can damage cellular structures such as cytoskeleton, and are important for apoptosis and necrosis. Estrogen induced ER- $\alpha$  inhibited excitotoxicity via downregulating calpain expression [41]. In addition, ER- $\beta$  play an important role in estrogenic neuroprotection against NMDA-induced excitotoxicity [42]. Red ginseng extract was reported to have neuroprotective activity against kainic acid-induced excitotoxicity *in vitro* and *in vivo* by inhibition of ROS level [40]. Moreover, ginsenoside Rg<sub>3</sub> exhibited neuroprotection against homocysteine-induced excitotoxicity via inhibition of homocysteine-mediated NMDA receptor activation [43]. Our results showed that KRG increases ER- $\beta$  expression and provides ER- $\beta$  mediated-neuroprotection. Taken together, KRG-induced ER- $\beta$  seems to play some role in protection against excitotoxicity. However, further studies are necessary for elucidation of the underlying mechanism.

Ginsenosides are structurally similar to glucocorticoids or estrogens. In agreement, ginsenosides Re and Rg1 are functional ligands of the glucocorticoid receptor, whereas ginsenosides Rb1 and Rh1 are functional ligands of the ER [44]. Ginseng was also shown to activate ER in breast cancer cells *in vitro* but not *in vivo* [19]. Previously, we found that the ER- $\alpha$  expression was not affected *in vitro* by oxidative stress nor by KRG treatment, thus ER $\alpha$  would not be predicted to play a major role in oxidative stress in the brain [17]. In this study, KRG extract was shown to inhibit oxidative stress-induced apoptosis in neuroblastoma cells. This apoptosis inhibition is mediated by ER- $\beta$  upregulation via the PI3K/Akt signaling pathway. The upregulation of PI3K/Akt signaling inhibits apoptotic signals by decreasing p-p53 and caspase-3 expression, but increasing BCL2 expression. Therefore, KRG protects brain cells from oxidative stress-induced cell death. Collectively, these data suggest that activation of ER- $\beta$  by KRG inhibits apoptosis in oxidatively stressed brain cells (Fig. 5).



**Fig. 5.** Upregulation of estrogen receptor (ER)- $\beta$  by Korean Red Ginseng extract inhibits apoptosis in oxidatively stressed brain cells. Oxidative stress suppresses ER- $\beta$ , which is required for the activation of phosphatidylinositol-3 kinase (PI3K)/Akt signals. Activation of PI3K/Akt signals induces antiapoptotic factors or represses proapoptotic factors. Korean Red Ginseng extract induces ER- $\beta$ , leading to the upregulation of PI3K/Akt signals, thereby inhibiting apoptosis in the brain.

### Conflicts of interest

All authors have no conflicts of interest to declare.

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### References

- Handa RJ, Ogawa S, Wang JM, Herbison AE. Roles for oestrogen receptor  $\beta$  in adult brain function. *J Neuroendocrinol* 2012;24:160–73.
- Weiser MJ, Foradori CD, Handa RJ. Estrogen receptor beta in the brain: from form to function. *Brain Res Rev* 2008;57:309–20.
- Pettersson K, Gustafsson JÅ. Role of estrogen receptor beta in estrogen action. *Annu Rev Physiol* 2001;63:165–92.
- Chaudhary SC, Singh T, Talwelkar SS, Srivastava RK, Arumugam A, Weng Z, Elmets CA, Afaq F, Kopelovich L, Athar M. Erb-041, an estrogen receptor beta agonist inhibits skin photocarcinogenesis in SKH-1 hairless mice by down-regulating WNT signaling pathway. *Cancer Prev Res (Phila)* 2013;7:186–98.
- Zhao C, Dahlman-Wright K, Gustafsson JÅ. Estrogen signaling via estrogen receptor  $\beta$ . *J Biol Chem* 2010;285:39575–9.
- Islander U, Erlandsson MC, Hasséus B, Jonsson CA, Ohlsson C, Gustafsson JÅ, Dahlgren U, Carlsten H. Influence of oestrogen receptor  $\alpha$  and  $\beta$  on the immune system in aged female mice. *Immunology* 2003;110:149–57.
- Mannella P, Brinton R. Estrogen receptor protein interaction with phosphatidylinositol 3-kinase leads to activation of phosphorylated Akt and extracellular signal-regulated kinase 1/2 in the same population of cortical neurons: a unified mechanism of estrogen action. *J Neurosci* 2006;26:9439–47.
- Lee YR, Park J, Yu HN, Kim JS, Youn HJ, Jung SH. Up-regulation of PI3K/Akt signaling by 17 $\beta$ -estradiol through activation of estrogen receptor- $\alpha$ , but not estrogen receptor- $\beta$ , and stimulates cell growth in breast cancer cells. *Biochem Biophys Res Commun* 2005;336:1221–6.

- [9] Wang M, Wang Y, Weil B, Abarbanell A, Herrmann J, Tan J, Kelly M, Meldrum DR. Estrogen receptor β mediates increased activation of PI3K/Akt signaling and improved myocardial function in female hearts following acute ischemia. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R972–8.
- [10] Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 2004;30:193–204.
- [11] Lee SH, Jung BH, Kim SY, Lee EH, Chung BC. The antistress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress. *Pharmacol Res* 2006;54:46–9.
- [12] Cho SO, Lim JW, Kim H. Red ginseng extract inhibits the expression of MCP-1 and iNOS in *Helicobacter pylori*-infected gastric epithelial cells by suppressing the activation of NADPH oxidase and Jak2/Stat3. *J Ethnopharmacol* 2013;150:761–4.
- [13] Park SE, Park C, Kim SH, Hossain MA, Kim MY, Chung HY, Son WS, Kim GY, Choi YH, Kim ND. Korean Red Ginseng extract induces apoptosis and decreases telomerase activity in human leukemia cells. *J Ethnopharmacol* 2009;121:304–12.
- [14] Lü JM, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. *Curr Vasc Pharmacol* 2009;7:293–302.
- [15] Li QY, Chen L, Fu WH, Li ZD, Wang B, Shi XJ, Zhong MK. Ginsenoside Rb1 inhibits proliferation and inflammatory responses in rat aortic smooth muscle cells. *J Agric Food Chem* 2011;59:6312–8.
- [16] Cheng W, Wu D, Zuo Q, Wang Z, Fan W. Ginsenoside Rb1 prevents interleukin-1 beta induced inflammation and apoptosis in human articular chondrocytes. *Int Orthop* 2013;37:2065–70.
- [17] Kim EH, Kim IH, Lee MJ, Thach Nguyen C, Ha JA, Lee SC, Choi S, Choi KT, Pyo S, Rhee DK. Anti-oxidative stress effect of red ginseng in the brain is mediated by peptidyl arginine deiminase type IV (PADI4) repression via estrogen receptor (ER) β up-regulation. *J Ethnopharmacol* 2013;148:474–85.
- [18] Shin YM, Jung HJ, Choi WY, Lim CJ. Antioxidative, anti-inflammatory, and matrix metalloproteinase inhibitory activities of 20(S)-ginsenoside Rg3 in cultured mammalian cell lines. *Mol Biol Rep* 2013;40:269–79.
- [19] Shim MK, Lee YJ. Estrogen receptor is activated by Korean red ginseng *in vitro* but not *in vivo*. *J Ginseng Res* 2012;36:169–75.
- [20] Li QY, Chen L, Zhu YH, Zhang M, Wang YP, Wang MW. Involvement of estrogen receptor-β in farrerol inhibition of rat thoracic aorta vascular smooth muscle cell proliferation. *Acta Pharmacol Sin* 2011;32:433–40.
- [21] Kang J, Rychahou PG, Ishola TA, Mourrot JM, Evers BM, Chung DH. N-myc is a novel regulator of PI3K-mediated VEGF expression in neuroblastoma. *Oncogene* 2008;27:3999–4007.
- [22] Musser DA, Oseroff AR. The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following *in vitro* photodynamic therapy with Photofrin II. *Photochem Photobiol* 1994;59:621–6.
- [23] Helguero LA, Faulds MH, Gustafsson JA, Haldosén LA. Estrogen receptors alpha (ERalpha) and beta (ERbeta) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11. *Oncogene* 2005;24:6605–16.
- [24] Zhang G, Yanamala N, Lathrop KL, Zhang L, Klein-Seetharaman J, Srinivas H. Ligand-independent antiapoptotic function of estrogen receptor-beta in lung cancer cells. *Mol Endocrinol* 2010;24:1737–47.
- [25] Kim AH, Khursigara G, Sun X, Franke TF, Chao MV. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. *Mol Cell Biol* 2001;21:893–901.
- [26] Zhang HM, Rao JN, Guo X, Liu L, Zou T, Turner DJ, Wang JY. Akt kinase activation blocks apoptosis in intestinal epithelial cells by inhibiting caspase-3 after polyamine depletion. *J Biol Chem* 2004;279:22539–47.
- [27] Pugazhenthii S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE-B. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. *J Biol Chem* 2000;275:10761–6.
- [28] Choi BH. Oxygen, antioxidants and brain dysfunction. *Yonsei Med J* 1993;34:1–10.
- [29] Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. *Proc Soc Exp Biol Med* 1999;222:236–45.
- [30] Herbert V, Shaw S, Jayatilleke E, Stopler-Kasdan T. Most free-radical injury is iron-related: it is promoted by iron, hemin, holoferritin and vitamin C, and inhibited by desferoxamine and apoferritin. *Stem Cells* 1994;12:289–303.
- [31] Basso M, Giraud S, Corpillo D, Bergamasco B, Lopiano L, Fasano M. Proteome analysis of human substantia nigra in Parkinson's disease. *Proteomics* 2004;4:3943–52.
- [32] McGrath LT, McGleenon BM, Brennan S, McColl D, McLroy S, Passmore AP. Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. *Q J Med* 2001;94:485–90.
- [33] Madrigal J, García-Bueno B, Caso J, Pérez-Nieves B, Leza J. Stress-induced oxidative changes in brain. *CNS Neurol Disord Drug Targets* 2006;5:561–8.
- [34] Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME. Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 1997;275:661–5.
- [35] Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 1997;91:231–41.
- [36] Franke TF, Hornik CP, Segev L, Shostak GA, Sugimoto C. PI3K/Akt and apoptosis: size matters. *Oncogene* 2003;22:8983–98.
- [37] Gottlieb T, Leal J, Seger R, Taya Y, Oren M. Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis. *Oncogene* 2002;21:1299–303.
- [38] Kim Y, Kim J, Kwon H, Lee D, Won M, Kwon Y, Kim Y. Korean Red Ginseng protects endothelial cells from serum-deprived apoptosis by regulating Bcl-2 family protein dynamics and caspase S-nitrosylation. *J Ginseng Res* 2013;37:413–24.
- [39] Chen S, Liu J, Liu X, Fu Y, Zhang M, Lin Q, Zhu J, Mai L, Shan Z, Yu X, et al. *Panax notoginseng* saponins inhibit ischemia-induced apoptosis by activating PI3K/Akt pathway in cardiomyocytes. *J Ethnopharmacol* 2011;137:263–70.
- [40] Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica* 2009;30:379–87.
- [41] Gamberdinger M, Manthey D, Behl C. Oestrogen receptor subtype-specific repression of calpain expression and calpain enzymatic activity in neuronal cells – implications for neuroprotection against Ca-mediated excitotoxicity. *J Neurochem* 2006;97:57–68.
- [42] Aguirre C, Jayaraman A, Pike C, Baudry M. Progesterone inhibits estrogen-mediated neuroprotection against excitotoxicity by down-regulating estrogen receptor-β. *J Neurochem* 2010;115:1277–87.
- [43] Kim JH, Cho SY, Lee JH, Jeong SM, Yoon IS, Lee BH, Lee JH, Pyo MK, Lee SM, Chung JM, et al. Neuroprotective effects of ginsenoside Rg3 against homocysteine-induced excitotoxicity in rat hippocampus. *Brain Res* 2007;1136:190–9.
- [44] Cho J, Park W, Lee S, Ahn W, Lee Y. Ginsenoside-Rb1 from *Panax ginseng* C.A. Meyer activates estrogen receptor-alpha and -beta, independent of ligand binding. *J Clin Endocrinol Metab* 2004;89:3510–5.