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A Bacterial Metabolite, Compound K, Induces Programmed Necrosis in MCF-7 Cells *via* GSK3β

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Ginsenosides, the major active component of ginseng, are traditionally used to treat various diseases, including cancer, inflammation, and obesity. Among these, compound K (CK), an intestinal bacterial metabolite of the ginsenosides Rb_1, Rb_2, and Rc from *Bacteroides* JY-6, is reported to inhibit cancer cell growth by inducing cell-cycle arrest or cell death, including apoptosis and necrosis. However, the precise effect of CK on breast cancer cells remains unclear. MCF-7 cells were treated with CK (0–70 μM) for 24 or 48 h. Cell proliferation and death were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and flow cytometry assays, respectively. Changes in downstream signaling molecules involved in cell death, including glycogen synthase kinase 3β (GSK3β), GSK3β, β-catenin, and cyclin D1, were analyzed by western blot assay. To block GSK3β signaling, MCF-7 cells were pretreated with GSK3β inhibitors 1 h prior to CK treatment. Cell death and the expression of β-catenin and cyclin D1 were then examined. CK dose- and time-dependently inhibited MCF-7 cell proliferation. Interestingly, CK induced programmed necrosis, but not apoptosis, *via* the GSK3β signaling pathway in MCF-7 cells. CK inhibited GSK3β phosphorylation, thereby suppressing the expression of β-catenin and cyclin D1. Our results suggest that CK induces programmed necrosis in MCF-7 breast cancer cells *via* the GSK3β signaling pathway.

Keywords: Compound K, ginsenoside, programmed necrosis, breast cancer cells, MCF-7, GSK3β

Introduction

Ginsenosides, active components of ginseng, have various biological effects including antidiabetic, antimutagenic, anti-inflammatory, antioxidant, and anticancer activities [5, 12]. Moreover, ginsenosides regulate the immune activity *via* modulation of cell survival or differentiation [24, 34]. Several researchers have shown that some ginsenosides cannot be absorbed in their native form through the intestinal barrier, due to their hydrophilicity. Instead, protopanaxadiol (PD) ginsenosides, including Rb_1, Rb_2, and Rc, are metabolized into an absorbable form in the intestinal tract [1, 3]. Compound K (CK), also known as IH-901 or M1, is the final bacterial metabolite of PD ginsenosides in the intestine [23, 30]. Moreover, ginsenosides are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol

(compound K, CK) by intestinal bacteria, namely *Bacteroides* sp., *Eubacterium* sp., and *Bifidobacterium* sp. [3] after the oral administration of which is absorbed into the blood [1]. CK is produced as a metabolic by-product after the sequential bioconverting of Rb1, Rd, and F2 in each step by β-glucosidase secreted by *Leuconostoc citreum* LH1 [36]. Interest in CK has increased owing to its anticancer effects against various cancer cell lines, including hepatoma, gastric carcinoma, and lung carcinoma cells [14, 27]. However, the mechanisms of CK-induced cancer cell death remain unclear.

There are three types of cell death: apoptosis, autophagy, and necrosis [7]. Apoptosis is characterized by DNA fragmentation, cellular shrinkage, and chromatin condensation [13]. In contrast, autophagy is characterized by activation of class III phosphatidylinositol 3-phosphate kinase (PI3K), generation of reactive oxygen species (ROS), and the formation of autophagosomes [8, 17]. Necrosis is generally considered to be an accidental event that occurs without the activation of signal transduction. Intriguingly, recent data suggest that necrosis could occur through programmed signaling, termed programmed necrosis [28, 29].

Glycogen synthase kinase 3β (GSK3β) is associated with programmed cell death. GSK3β promotes intrinsic apoptosis mediated by mitochondria, and inhibits extrinsic apoptosis induced by death receptors [4]. In contrast, suppression of GSK3β induces necrosis without Bax activation, which is primarily involved in apoptosis [38]. Recently, data showed that GSK3β may be associated with both autophagy [35] and programmed necrosis [16]. Together, these reports suggest that GSK3β could interact with different signaling molecules, depending on the type of cell death [26].

In the present study, we investigated the anticancer effects of CK in MCF-7 breast cancer cells by evaluating cell death and the related signaling pathways that are involved. We found that CK induces programed necrosis *via* GSK3β-mediated signaling.

Materials and Methods

Materials

Ginsenoside CK was purified as previously described [18]. One hundred micromole of CK was dissolved in dimethyl sulfoxide (DMSO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 3-MA were purchased from Sigma-Aldrich (St. Louis, MO, USA). AnnexinV-FITC was purchased from BD Biosciences (San Jose, CA, USA) and GSK3β inhibitor VIII was purchased from Calbiochem (San Diego, CA, USA). Antibodies against phospho-GSK3β (Upstate Biotechnology, Lake Placid, NY, USA), GSK3β, β-catenin, cyclin D1 (Cell Signaling Technology, Danvers, MA, USA), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used.

Cell Proliferation Assay

MCF-7 cells were purchased from the American Type Culture Collection and maintained in DMEM with 10% fetal bovine serum and 1% antibiotics (Invitrogen, Grand Island, NY, USA) at 37°C in a humidified incubator with 5% CO_2. The cells were seeded in a 96-well plate at a density of 5×10^3 cells/well and incubated for 24 h. Then, the cells were treated with CK (0, 10, 30, 50, and 70 μM) for the indicated time period. In order to block autophagy, 3-MA (1 mM) was added for 1 h prior to CK (70 μM) treatment. Then, MTT solution (5 mg/ml) was added to each well at a final concentration of 0.5 mg/ml for 3 h at 37°C in a humidified incubator with 5% CO_2. The supernatant was removed and MTT formazan was dissolved with DMSO. The optical density of each well was measured at 570 nm by a microplate reader (Molecular Device, Sunnyvale, CA, USA).

Cell Cycle Analysis

For the detection of cell cycle, MCF-7 cells were seeded in a 6-well plate at a density of 3×10^5 cells/well and incubated for 24 h in a humidified incubator with 5% CO_2 at 37°C. The cells were treated with CK (0, 10, 30, 50, and 70 μM) for the indicated time period. Then, the cells were trypsinized, washed twice in phosphate-buffered saline (PBS), and fixed with 70% ethanol at 4°C overnight. After centrifugation (800 ×*g*, for 10 min at 4°C), the supernatant was discarded. The cells were stained with PI solution (0.05% Triton-X 100, 10 μg/ml RNase, and 10 μg/ml PI in PBS). Then, the DNA content was measured by flow cytometry (FACSCalibur) with Cell Quest software (BD Biosciences, Sparks, MD, USA). All flow cytometric data were analyzed by Flowjo software (Tree Star, San Carlos, CA, USA).

Annexin V/PI Staining Assay

MCF-7 was seeded in a 6-well plate at a density of 3×10^5 cells/well and incubated for 24 or 48 h at 37°C in a humidified incubator with 5% CO_2. The cells were treated with CK (0, 10, 30, 50, and 70 μM) for the indicated time periods. For blocking programmd necrosis, GSK3β inhibitor VIII (GSK3βi, 20 μM) was pretreated for 1 h before CK (70 μM) treatment. Floating and attached cells were collected, washed twice with PBS, and stained with Annexin V-FITC and PI as described by the manufacturer's instructions (BD Biosciences, Sparks, MD, USA). The samples were detected by flow cytometry with Cell Quest software (BD Biosciences, Sparks, MD, USA). All flow cytometric data were analyzed by Flowjo software (Tree Star).

Western Blot Analysis

MCF-7 cells were seeded in a 35 mm dish at a density of 3×10^5 cells/dish in a humidified incubator at 5% CO_2 and 37°C. The cells were treated with CK (0, 10, 30, 50, and 70 μM) for the indicated time periods. The cells were washed twice with cold PBS. Then, the cells were lysed with cold RIPA lysis buffer containing 150 mM

NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 7.4), and protease inhibitor cocktail (Roche, Mannheim, Germany) and incubated on ice for 30 min. After centrifugation (12,000 ×*g* for 10 min at 4°C), soluble proteins were obtained and the concentration of protein was determined by Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). Protein samples (20 μg) were separated by 10~12% SDS-PAGE and transferred to a polyvinylidene difluoride transfer membrane (Amersham Bioscience, Piscataway, NY, USA). The membrane was blocked with 5% skim milk in TBST (0.1 M Tris, 0.9% NaCl, and 0.1% Tween 20) at room temperature for 1 h. The membrane was washed twice with PBS and incubated with the appropriate primary antibody at 4°C overnight. After washing with PBS three times, the membrane was incubated for 1 h with a horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG or HRP-conjugated goat anti-rabbit IgG secondary antibody (SantaCruz Biotechnology) for 1 h at room temperature. Expected protein bands were visualized with the enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK).

Statistical Analysis

All data were expressed as means ± standard deviation (SD). Statistical significance was analyzed by Student's *t*-test or one-way ANOVA using Prism ver. 4.0 (GraphPad Software, Inc., San Diego, CA, USA). The significance of differences was considered statistically significant at $p < 0.05$.

Results

CK Inhibits the Proliferation of MCF-7 in a Time- and Dose-Dependent Manner

To examine the anticancer effects of CK, MCF-7 cells were treated with CK (0, 10, 30, 50, and 70 μM) and cell proliferation was assessed by MTT assay. CK significantly ($p < 0.05–0.01$) inhibited the proliferation at 50 and 70 μM for 24 and 48 h (Figs. 1A and 1B). CK at 50 and 70 μM resulted in 21% and 59% inhibition at 24 h, and 35% and 88% at 48 h, respectively, compared with the control group. These results suggest that CK has an inhibitory effect on the proliferation in time- and dose-dependent manner.

Apoptosis and Autophagy Are Not Induced by CK in MCF-7 Cells

To examine whether CK induces apoptosis of MCF-7 cells, we measured the peak of sub G1 using flow cytometry after PI staining. The cells treated with CK showed a similar percentage of the sub G1 compared with that of the control (Figs. 2A and 2B). To further validate this result, we examined the expression pattern of PARP-1, which is known to be cleaved in apoptotic cells [33]. As a result, PARP-1 was not cleaved in cells treated with CK, suggesting that CK did not induce apoptosis in MCF-7 cells. It was further noted that neither sub G1 nor PARP-1 expression was different at 24 and 48 h compared with the control (data not shown).

Autophagy occurs with degradation and recycling of cellular components and it is often associated with cellular stress and eventual cell death. To investigate autophagy, the cells were treated with CK (70 μM) alone or together with 3-MA (an autophagy inhibitor). The cells treated with CK alone showed 39% of proliferation (*i.e.*, 61% inhibition) at 24 h, and the cells pretreated with 1 mM of 3-MA at 1 h prior to the administration of CK showed 34% of proliferation (*i.e.*, 66% inhibition) when compared with those of control (Fig. 2C). Taken together, these results suggest that CK induced neither apoptosis nor autophagy in MCF-7 cells.

Inhibition of GSK3β Suppressed the Programmed Necrosis in MCF-7 Cells Treated with CK

Next, in order to further investigate the type of cell death responsible for the inhibition of MCF-7 proliferation, the cells treated with CK were examined by using Annexin V

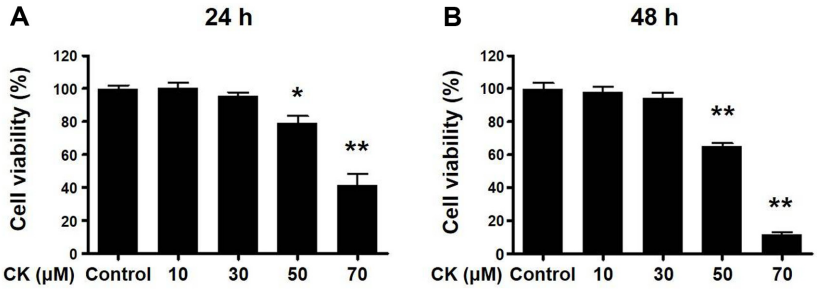

Fig. 1. Inhibitory effect of CK on the proliferation of MCF-7 cells.

MCF-7 cells were seeded in a 96-well plate at a density of 5×10^3 cells/well and treated with CK (0, 10, 30, 50, and 70 μM). The proliferation was measured by MTT assay after (**A**) 24 h and (**B**) 48 h. The data are expressed as the mean ± SD from triplicate experiments. * $p < 0.05$, ** $p < 0.01$. Experimental results are the representative of three independent experiments.

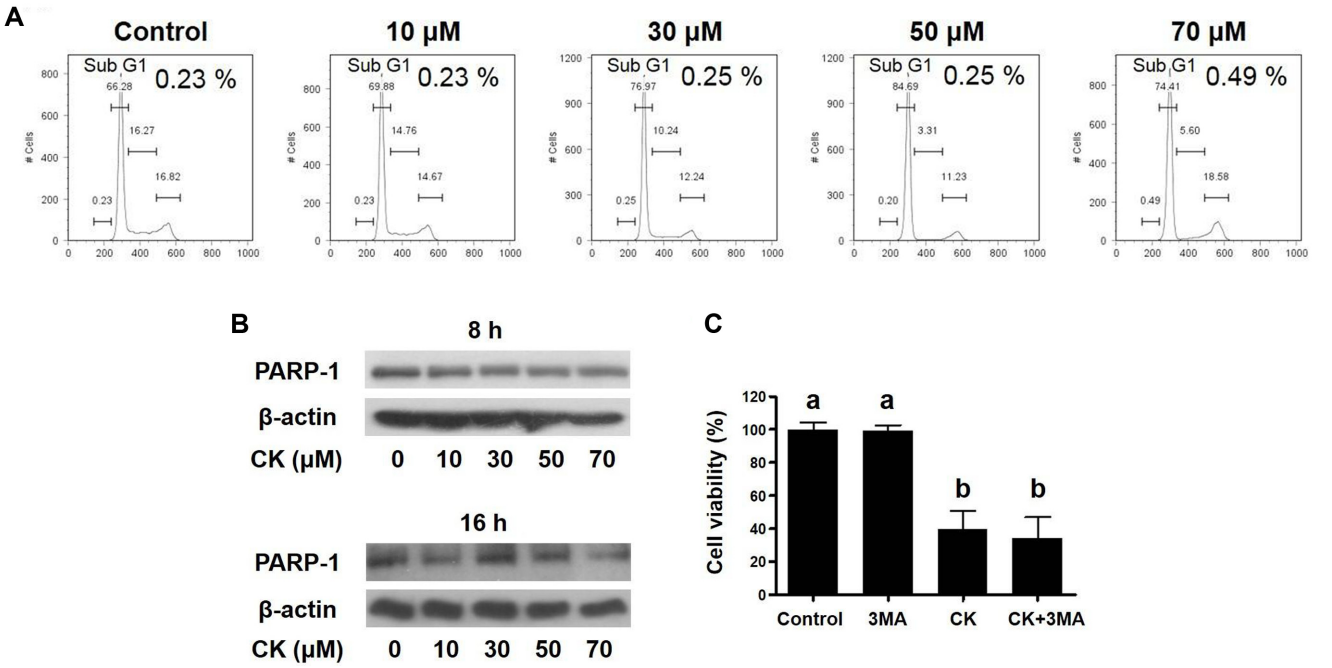

Fig. 2. CK induced neither apoptosis nor autophagy in MCF-7 cells.
(**A**) MCF-7 cells were treated with CK (0, 10, 30, 50, and 70 μM) for 24 h, and then stained with PI, and apoptosis was detected by using flow cytometry. (**B**) The cells were treated with 70 μM CK for 8 or 16 h. Then, the expression of PARP-1 level was examined by western blot assay. (**C**) MCF-7 cells were treated with 1 mM 3-MA, an autophagy inhibitor, 1 h prior to the treatment of CK (70 μM) for 24 h. The proliferation was measured by MTT assay. The data, expressed as the mean ± SD of three separate experiments, were analyzed by one-way ANOVA. Means with different superscripts are significantly different at $p < 0.05$.

and PI. Interestingly, necrosis (Annexin V^-/PI^+ region) was increased in CK-treated cells when compared with the control (Fig. 3A), suggesting that the reduced cell proliferation in the cells treated with CK is attributed to necrosis.

There is some evidence suggesting that GSK3β is involved in cell fate, including its survival, necrosis, and programmed necrosis [15, 16]. To examine whether CK-induced cell death was mediated *via* GSK3β, the cells were pretreated with GSK3β inhibitor VIII (GSK3βi) followed by CK treatment, and necrosis was examined by using flow cytometry. The results showed that necrosis in the cells pretreated with GSK3βi and then treated with CK were decreased when compared with the cells treated with CK alone (Fig. 3B). These results strongly suggested that GSK3β is involved in the CK-mediated cell death of MCF-7 cells.

CK Induces Programmed Necrosis *via* GSK3β in MCF-7 Cells

To further investigate the exact cause of CK-induced cell death in MCF-7, the cells were treated with CK and the expression of phospho-GSK3β, β-catenin, and cyclin D1 was examined by western blot assay. Phosphorylation of GSK3β and the expression of β-catenin and cyclin D1 were all decreased by CK treatment (Fig. 4A). As expected, the expression of β-catenin and cyclin D1 in the cells pretreated with GSK3βi was higher than that of cells treated with CK alone (Fig. 4B). Taken together, CK induced programmed necrosis of MCF-7 cells *via* GSK3β.

Discussion

CK is known to exert anticancer effects [14]. However, its mechanism of action remains unclear. The aim of the study was to evaluate the anticancer effects of CK in the human breast cancer cell line MCF-7, focusing particularly on programmed necrosis and its associated signaling pathways. We investigated whether CK (i) has anticancer activity in MCF-7 cells and (ii) induces cell death *via* GSK3β. Our results showed that CK dose- and time-dependently inhibited MCF-7 cell proliferation and reduced GSK3β phosphorylation.

CK has been suggested to induce apoptosis and autophagy in human cancer cells [20, 21]. Nevertheless, our study revealed that CK did not suppress MCF-7 cell proliferation *via* these pathways. It is important to note that the previous

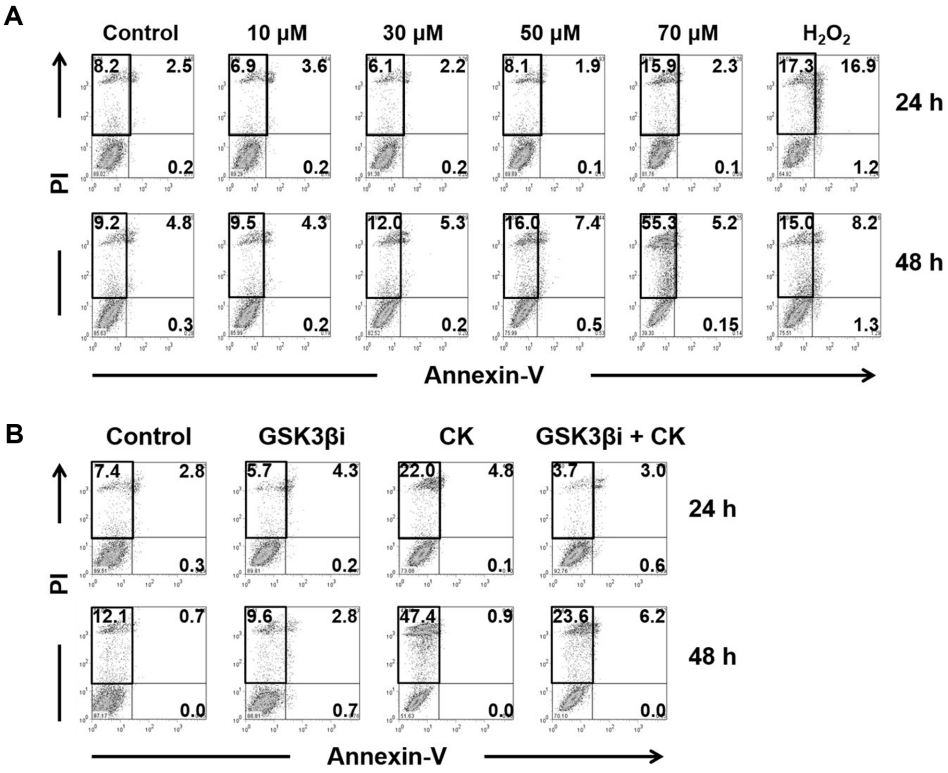

Fig. 3. Reduction of CK-induced necrosis in MCF-7 cells by GSK3β inhibitor VIII.
(**A**) MCF-7 cells were treated with CK (0, 10, 30, 50, and 70 μM) for 24 or 48 h. The cells were stained with Annexin V and PI. Apoptosis and necrosis were measured in the cells by flow cytometry. (**B**) MCF-7 cells were pretreated with GSK3βi at 1 h before CK treatment (70 μM) for 24 or 48 h. Then, the cells were stained with Annexin V and PI and measured by flow cytometry. The consistency of all results were confirmed more than three times.

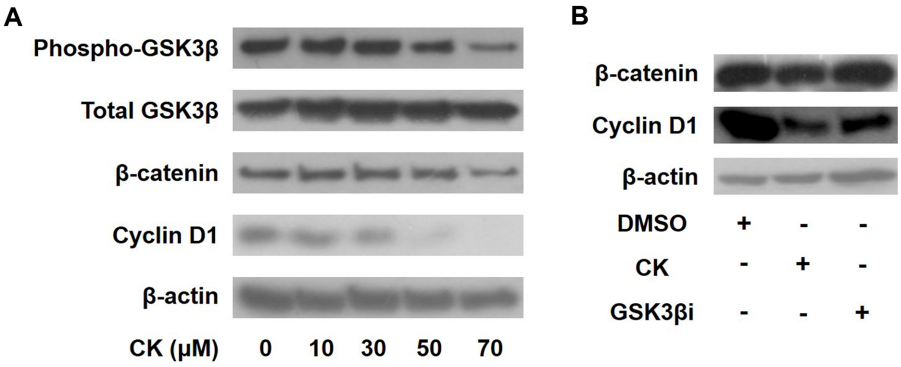

Fig. 4. Induction of programmed necrosis in MCF-7 cells treated with CK *via* GSK3β.
(**A**) MCF-7 cells were treated with CK (0, 10, 30, 50, and 70 μM) for 16 h, and the expression levels of phospho-GSK3β, β-catenin, and cyclin D1 were measured by western blot assay. (**B**) MCF-7 cells were pretreated with GSK3βi before CK treatment (70 μM) for 6 h in β-catenin or 12 h in cyclin D1, respectively. The expression of β-catenin and cyclin D1 was measured by western blot assay. β-Actin was used as a loading control. The consistency of all results was confirmed more than three times.

report suggesting apoptotic MCF-7 cell death following CK treatment used DNA fragmentation and cell viability assays, which are limited in defining the type of cell death.

Moreover, CK has shown inhibition activity against various cancers, not only breast cancer. CK induced the apoptosis of colorectal cancer through the inhibition of

histone deacetylase activity [19] and the down-regulation of cdc2 and cdc25A that arrested the G1 phase cell cycle [41]. For lung cancer, CK was effective for apoptosis by improving p53 expression [25]. Nasopharyngeal carcinoma was inhibited by CK through apoptosis-inducing factor activation [22].

In the steady state, PARP-1 plays a homeostatic protective and regulatory role, as it is associated with the DNA repair process [11, 32]. During apoptosis, PARP-1 is cleaved by caspases, which leads to the inactivation of poly(ADP-ribosyl)ation [33]. However, PARP-1 levels are increased in programmed necrosis [37]. Indeed, cleavage of PARP-1 was not observed, whereas its expression was increased in the present study. It has also been reported that PARP-1-dependent programmed necrosis relies on AIF [2]. Our results indicated that CK induces high AIF expression levels (data not shown), suggesting that CK induces programmed necrosis.

GSK3β, a serine threonine kinase, phosphorylates a number of proteins with various cellular functions, thereby regulating metabolism, structure, transcription, and gene expression [10]. GSK3β has numerous substrates, including metabolic and signaling proteins, structural proteins, and transcription factors [10]. Among them, β-catenin, cyclin D1, and c-myc are the oncogenic proteins. β-Catenin is phosphorylated by GSK3β in the Wnt signaling pathway [40]. Furthermore, Wnt/β-catenin signaling is involved in the development of cancer [39] by enhancing proliferation [31]. A number of oncogenic proteins are degraded *via* GSK3β-mediated phosphorylation [6, 9, 40], indicating that GSK3β also acts as a tumor suppressor. Moreover, GSK3β is involved in regulating various functions, including cell survival and death [15]. In our study, CK treatment induced GSK3β dephosphorylation, and reduced the expression of β-catenin and cyclin D1, suggesting that CK is associated with GSK3β-mediated programmed necrosis.

The present study demonstrated that CK induced programmed necrosis *via* the dephosphorylation and activation of GSK3β, which coincided with the decreased expression of β-catenin and cyclin D1. Understanding the involvement and the precise role of GSK3β signaling could become important for the development of therapeutic strategies to improve the efficacy of anticancer agents. Therefore, the present study provides a potential target for future investigation in breast cancer therapy.

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References

1. Akao T, Kida H, Kanaoka M, Hattori M, Kobashi K. 1998. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. *J. Pharm. Pharmacol.* **50:** 1155-1160.
2. Artus C, Boujrad H, Bouharrour A, Brunelle MN, Hoos S, Yuste VJ, *et al.* 2010. AIF promotes chromatinolysis and caspase-independent programmed necrosis by interacting with histone H2AX. *EMBO J.* **29:** 1585-1599.
3. Bae EA, Choo MK, Park EK, Park SY, Shin HY, Kim DH. 2002. Metabolism of ginsenoside R(c) by human intestinal bacteria and its related antiallergic activity. *Biol. Pharm. Bull.* **25:** 743-747.
4. Beurel E, Jope RS. 2006. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. *Prog. Neurobiol.* **79:** 173-189.
5. Choi KH, Min JY, Ganesan P, Bae IH, Kwak HS. 2015. Physicochemical and sensory properties of red ginseng extracts or red ginseng hydrolyzates-added Asiago cheese during ripening. *Asian Australas J. Anim. Sci.* **28:** 120-126.
6. Diehl JA, Cheng M, Roussel MF, Sherr CJ. 1998. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* **12:** 3499-3511.
7. Edinger AL, Thompson CB. 2004. Death by design: apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.* **16:** 663-669.
8. Eom JM, Seo MJ, Baek JY, Chu H, Han SH, Min TS, *et al.* 2010. Alpha-eleostearic acid induces autophagy-dependent cell death through targeting AKT/mTOR and ERK1/2 signal together with the generation of reactive oxygen species. *Biochem. Biophys. Res. Commun.* **391:** 903-908.
9. Gregory MA, Qi Y, Hann SR. 2003. Phosphorylation by glycogen synthase kinase-3 controls *c-myc* proteolysis and subnuclear localization. *J. Biol. Chem.* **278:** 51606-51612.
10. Grimes CA, Jope RS. 2001. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog. Neurobiol.* **65:** 391-426.
11. Haince JF, Rouleau M, Hendzel MJ, Masson JY, Poirier GG. 2005. Targeting poly(ADP-ribosyl)ation: a promising approach in cancer therapy. *Trends Mol. Med.* **11:** 456-463.
12. Hao HP, Zheng X, Wang GJ. 2014. Insights into drug discovery from natural medicines using reverse pharmacokinetics. *Trends Pharmacol. Sci.* **35:** 168-177.
13. Hengartner MO. 2000. The biochemistry of apoptosis. *Nature* **407:** 770-776.
14. Hu C, Song G, Zhang B, Liu Z, Chen R, Zhang H, Hu T. 2011. Intestinal metabolite compound K of panaxoside inhibits

the growth of gastric carcinoma by augmenting apoptosis *via* Bid-mediated mitochondrial pathway. *J. Cell Mol. Med.* **16:** 96-106.
15. Jacobs KM, Bhave SR, Ferraro DJ, Jaboin JJ, Hallahan DE, Thotala D. 2012. GSK-3beta: a bifunctional role in cell death pathways. *Int. J. Cell Biol.* **2012:** 930710.
16. Jiang X, Perez-Torres CJ, Thotala D, Engelbach JA, Yuan L, Cates J, *et al.* 2014. A GSK-3beta inhibitor protects against radiation necrosis in mouse brain. *Int. J. Radiat. Oncol. Biol. Phys.* **89:** 714-721.
17. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, *et al.* 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J.* **19:** 5720-5728.
18. Kang KA, Kim YW, Kim SU, Chae S, Koh YS, Kim HS, *et al.* 2005. G1 phase arrest of the cell cycle by a ginseng metabolite, compound K, in U937 human monocytic leukamia cells. *Arch. Pharm. Res.* **28:** 685-690.
19. Kang KA, Piao MJ, Kim KC, Zheng J, Yao CW, Cha JW, *et al.* 2013. Compound K, a metabolite of ginseng saponin, inhibits colorectal cancer cell growth and induces apoptosis through inhibition of histone deacetylase activity. *Int. J. Oncol.* **43:** 1907-1914.
20. Kim AD, Kang KA, Kim HS, Kim DH, Choi YH, Lee SJ, *et al.* 2013. A ginseng metabolite, compound K, induces autophagy and apoptosis *via* generation of reactive oxygen species and activation of JNK in human colon cancer cells. *Cell Death Dis.* **4:** e750.
21. Kim AD, Kang KA, Zhang R, Lim CM, Kim HS, Kim DH, *et al.* 2010. Ginseng saponin metabolite induces apoptosis in MCF-7 breast cancer cells through the modulation of AMP-activated protein kinase. *Environ. Toxicol. Pharmacol.* **30:** 134-140.
22. Law CK, Kwok HH, Poon PY, Lau CC, Jiang ZH, Tai WC, *et al.* 2014. Ginsenoside compound K induces apoptosis in nasopharyngeal carcinoma cells *via* activation of apoptosis-inducing factor. *Chin. Med.* **9:** 11.
23. Lee JY, Shin JW, Chun KS, Park KK, Chung WY, Bang YJ, *et al.* 2005. Antitumor promotional effects of a novel intestinal bacterial metabolite (IH-901) derived from the protopanaxadiol-type ginsenosides in mouse skin. *Carcinogenesis* **26:** 359-367.
24. Lee YJ, Son YM, Gu MJ, Song KD, Park SM, Song HJ, *et al.* 2015. Ginsenoside fractions regulate the action of monocytes and their differentiation into dendritic cells. *J. Ginseng Res.* **39:** 29-37.
25. Li Y, Zhou T, Ma C, Song W, Zhang J, Yu Z. 2015. Ginsenoside metabolite compound K enhances the efficacy of cisplatin in lung cancer cells. *J. Thorac. Dis.* **7:** 400-406.
26. Liu Q, Mier JW, Panka DJ. 2011. Differential modulatory effects of GSK-3beta and HDM2 on sorafenib-induced AIF nuclear translocation (programmed necrosis) in melanoma. *Mol. Cancer* **10:** 115.
27. Ming YL, Song G, Chen LH, Zheng ZZ, Chen ZY, Ouyang GL, Tong QX. 2007. Anti-proliferation and apoptosis induced by a novel intestinal metabolite of ginseng saponin in human hepatocellular carcinoma cells. *Cell Biol. Int.* **31:** 1265-1273.
28. Moubarak RS, Yuste VJ, Artus C, Bouharrour A, Greer PA, Menissier-de Murcia J, Susin SA. 2007. Sequential activation of poly(ADP-ribose) polymerase 1, calpains, and Bax is essential in apoptosis-inducing factor-mediated programmed necrosis. *Mol. Cell Biol.* **27:** 4844-4862.
29. Nicotera P, Lipton SA. 1999. Excitotoxins in neuronal apoptosis and necrosis. *J. Cereb. Blood Flow Metab.* **19:** 583-591.
30. Park EJ, Zhao YZ, Kim J, Sohn DH. 2006. A ginsenoside metabolite, 20-*O*-beta-D-glucopyranosyl-20(*S*)-protopanaxadiol, triggers apoptosis in activated rat hepatic stellate cells *via* caspase-3 activation. *Planta Med.* **72:** 1250-1253.
31. Schlange T, Matsuda Y, Lienhard S, Huber A, Hynes NE. 2007. Autocrine WNT signaling contributes to breast cancer cell proliferation *via* the canonical WNT pathway and EGFR transactivation. *Breast Cancer Res.* **9:** R63.
32. Shall S, de Murcia G. 2000. Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? *Mutat. Res.* **460:** 1-15.
33. Soldani C, Scovassi AI. 2002. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. *Apoptosis* **7:** 321-328.
34. Son YM, Kwak CW, Lee YJ, Yang DC, Park BC, Lee WK, *et al.* 2010. Ginsenoside Re enhances survival of human CD4+ T cells through regulation of autophagy. *Int. Immunopharmacol.* **10:** 626-631.
35. Wang SH, Shih YL, Kuo TC, Ko WC, Shih CM. 2009. Cadmium toxicity toward autophagy through ROS-activated GSK-3beta in mesangial cells. *Toxicol. Sci.* **108:** 124-131.
36. Yan Q, Zhou W, Shi XL, Zhou P, Ju DW, Feng MQ. 2010. Biotransformation pathways of ginsenoside Rb1 to compound K by beta-glucosidases in fungus *Paecilomyces* Bainier sp 229. *Process Biochem.* **45:** 1550-1556.
37. Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C, *et al.* 2010. Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. *Proc. Natl. Acad. Sci. USA* **107:** 12611-12616.
38. Yang J, Fan W, Xiao H, Guan C, Cao C, Shao H, *et al.* 2010. Genome shuffling method of *Bacillus subtilis*. *Sheng Wu Gong Cheng Xue Bao* **26:** 1385-1392.
39. Yeh CT, Yao CJ, Yan JL, Chuang SE, Lee LM, Chen CM, *et al.* 2011. Apoptotic cell death and inhibition of Wnt/beta-catenin signaling pathway in human colon cancer cells by an active fraction (HS7) from *Taiwanofungus camphoratus*. *Evid. Based Complement. Alternat. Med.* **2011:** 750230.
40. Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT. 1996. The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* **10:** 1443-1454.
41. Zhang Z, Du GJ, Wang CZ, Wen XD, Calway T, Li Z, *et al.* 2013. Compound K, a ginsenoside metabolite, inhibits colon cancer growth *via* multiple pathways including p53-p21 interactions. *Int. J. Mol. Sci.* **14:** 2980-2995.