Acta Biochim Biophys Sin, 2016, 48(8), 750–755
doi: 10.1093/abbs/gmw049
Advance Access Publication Date: 13 May 2016
Original Article

Original Article

Ginsenoside Rh2 inhibits proliferation and induces apoptosis in human leukemia cells via TNF-α signaling pathway

Jingjia Huang, Kunjian Peng, Linghao Wang, Bin Wen, Lin Zhou, Tiao Luo, Min Su, Jijia Li, and Zhiyong Luo*

Molecular Biology Research Center, State Key Laboratory of Medical Genetics, School of Life Sciences, Xiangya School of Medicine, Central South University, Changsha 410008, China

*Correspondence address. Tel/Fax: +86-731-84805025; E-mail: luozhiyong@csu.edu.cn

Received 5 March 2016; Accepted 20 April 2016

Abstract

Ginsenoside Rh2, a triterpene saponin extracted from *Panax ginseng*, exhibits pharmacological activity against multiple cancers. However, the anticancer mechanism of ginsenoside Rh2 is unclear. In this study, we found that ginsenoside Rh2 effectively inhibits growth and induces apoptosis of HL-60 cells. Using microarray technology, we found that tumor necrosis factor-α (TNF-α) is clearly up-regulated. Furthermore, anti-TNF-α antibody relieved the Rh2-induced HL-60 cell apoptosis via suppression of caspase-8, caspase-9, and caspase-3 activation. In addition, TNF-α up-regulation was also observed in other Rh2-treated cancer cell lines. These results demonstrate that TNF-α plays a key role in ginsenoside Rh2-induced cell apoptosis.

Key words: ginsenoside Rh2, apoptosis, tumor necrosis factor-α, microarray

Introduction

Panax ginseng C. A. meyer, a medicinal herb, has been used for >2000 years in East Asia. Ginsenosides are one of the major active constituents extracted from ginseng [1]. The ginsenosides, which consist of triterpene aglycones, are classified into three major categories: panaxidiols, panaxatriols, and oleanolic acid derivatives [2]. Recently, ginsenosides and their derivatives have been reported to show antioxidant [3,4], anti-inflammatory [5–7], anti-obesity [6,8], angiogenic [3,9,10], and anticancer activities [11–14]. The ginsenoside Rh2 (**Fig. 1A**) is a rare panaxidiol with potential therapeutic effects against breast cancer [15], leukemia [16], prostate cancer [13], hepatocellular carcinoma [11,17], glioblastoma [18], pancreatic cancer [19], and lung cancer [20,21]. In addition, ginsenoside Rh2 was found to induce anticancer activities via apoptosis or cell cycle inhibition of the cancer cells [16]. However, the precise molecular mechanisms of inhibition are unclear.

Tumor necrosis factor (TNF) was identified as a cytotoxic product of immune cells causing lysis of tumor cells and as a therapeutic target in cancer therapy [22]. TNF induces apoptosis through two distinct caspase-8 activation pathways via interaction with TNF receptors [23,24]. TNF also activates the transcription of nuclear factor kappa B (NF-κB), which inhibits apoptosis via suppression of caspase-8 activation [23]. Interestingly, NF-κB is down-regulated and caspase-8 is activated following treatment with ginsenoside Rh2, which suggests the induction of apoptosis [17,25].

In this study, the effects of ginsenoside Rh2 on mRNA levels in human leukemia HL-60 cells were investigated using microarray technology, and the significance of TNF-α in ginsenoside Rh2-induced apoptosis was identified. TNF-α overexpression was also demonstrated in human lung adenocarcinoma A549, human erythroleukemia K562, and human breast cancer MCF-7 cells.

Materials and Methods

Ginsenoside Rh2 treatment and cell culture

The ginsenoside Rh2 (analytical grade with 98.7% purity) was purchased from the College of Chemistry, Jilin University

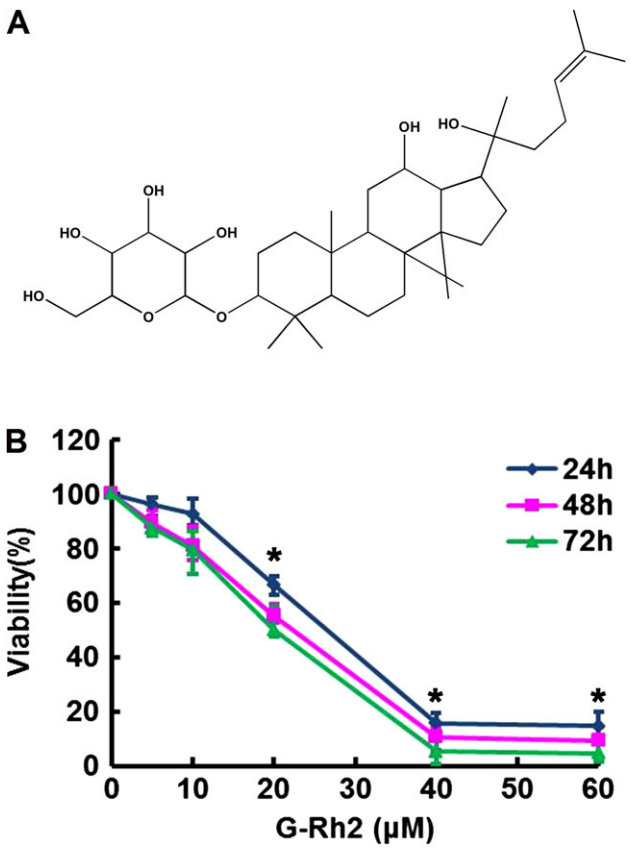

Figure 1. Effects of ginsenoside Rh2 on the viability of HL-60 cells (A) The chemical structure of ginsenoside Rh2. (B) Cells were treated with ginsenoside Rh2 for the indicated times and concentrations. Data represent the mean ± SD of three independent experiments. *$P < 0.05$ compared with control.

(Changchun, China), dissolved in dimethyl sulphoxide at 50 mM and stored at −20°C until use. The human promyelocytic leukemia cell line HL-60, the human erythroleukemic cell line K562, the human lung adenocarcinoma cell line A549, and the human breast cancer cell line MCF-7 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. All the cells were maintained at 37°C with 5% CO_2.

Cell viability assay

The viability of HL-60 cells was evaluated by methylthiazolyldiphenyltetrazolium bromide (MTT) assay. Approximately 4000 cells in 200 μl medium were seeded in each well in 96-well plates. When cells adhered to the plate, cells were incubated in medium supplemented with different concentrations of ginsenoside Rh2 or dimethyl sulphoxide (DMSO) only (as control). After 48 h, the cells were further incubated with MTT solution (0.5 mg/ml) for an additional 4 h at 37°C. The absorbance of the samples was measured at 570 nm.

Cell cycle analysis by flow cytometry

The cells were trypsinized and harvested by centrifugation at 1600 *g* for 5 min. After being washed with phosphate buffer saline (PBS), the cells were fixed with 70% ethanol overnight. The fixed cells were washed with PBS again and stained with propidium iodide (PI) solution containing 50 μg/ml PI, 100 μg/ml RNase, and 0.2% TritonX-100 for 30 min at 37°C in the dark. Finally, cells were analyzed using FACScan (Becton-Dickinson FACScanto II, San Jose, USA).

DNA ladder assay

HL-60 cells were seeded at 2×10^6 cells/ml and treated with different concentrations of ginsenoside Rh2 for different durations. All the cells were harvested by centrifugation (1600 *g* for 5 min) and washed twice using pre-cold PBS.

DNA was extracted using DNA Ladder extraction kit with Spin Column (Beyotime Institute of Biotechnology, Haimen, China), and separated by 1% agarose gel electrophoresis. DNA ladders were visualized using a Bio-Rad GelDoc XR System (Bio-Rad, Hercules, USA).

Microarray hybridization

The total RNA isolated from HL-60 cells was treated with 25 μM Rh2 for 48 h. DMSO was used as the control. A cDNA template was synthesized to generate Biotin-16-UTP-labeled cRNA target using TrueLabeling-AMP Linear RNA Amplification Kit (SuperArray Bioscience Corporation, Frederick, USA). Microarray analysis was performed using the Oligo GEGEArray® Human Cancer Microarray OHS-802, containing 480 genes (SuperArray Bioscience Corporation). Two hours of pre-hybridization and overnight hybridization were performed in a hybridization oven using labeled cRNA at 60°C. Microarray was washed using a low-stringency washing buffer (2 × saline-sodium citrate (SSC), 1% sodium dodecyl sulfate (SDS)) and high-stringency washing buffer (0.1 × SSC, 0.5% SDS). A Chemiluminescent detection kit (SuperArray Bioscience Corporation) was used to detect chemiluminescent grayscale according to the manufacturer's protocol. All microarray images were saved as electronic grayscale files (16 bit) in TIFF format and were analyzed by GEArray Expression Analysis Suite online software.

RT-PCR analysis

To confirm the expression levels of *TNF*-α gene, reverse transcription-polymerase chain reaction was performed using SuperScript II reverse transcriptase (Invitrogen, Madison, USA) according to the manufacturer's protocol. The total RNA of HL-60 cells was reverse transcribed and stored at −80°C. The primers for PCR were as follows: *GAPDH* forward: 5′-GTCGGAGTCAACGGATTTGG-3′ and *GAPDH* reverse: 5′-ACACCCATGACGAACATGGG-3′; and *TNF-α* forward: 5′-TGAGCACTGAAAGCATGATC-3′ and *TNF-α* reverse: 5′-TCACAGGGCAATGACCCAAA-3′.

The amplification system included a total volume of 25 μl comprising 3 μl of cDNA, 0.4 μM primers, 0.2 mM dNTP, and 0.3 μl of *Taq* DNA polymerase (5 U). PCR was conducted under the following conditions: 94°C for 5 min, 38 cycles of 30 s at 94°C for denaturation, 30 s at 56°C for annealing, and 60 s at 72°C for elongation, and finally 5 min at 72°C. The PCR products were analyzed by 1% agarose gel electrophoresis.

Western blot analysis

The antibody against TNF-α was obtained from Santa Cruz Biotechnology (Santa Cruz, USA). The anti-GAPDH antibodies were purchased from Cell Signaling Technology (Danvers, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit IgG were purchased from Bioworld Technology (Louis Park,

USA). The cells were lysed by radio-immunoprecipitation assay buffer containing 50 mM Tris, 150 mM NaCl, 1% TritonX-100, 1 mM PMSF, 1% sodium deoxycholate, 0.1% SDS, 2 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 1 mM EDTA, 1 mM Na_3VO4, and 0.5 μg/ml leupeptin. After centrifugation, the protein concentration in the supernatant was measured using the Pierce bicinchoninic acid (BCA) protein assay kit (Rockford, USA). Then, the lysates were boiled in Laemmli sample buffer and separated on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, followed by transferring to polyvinylidene fluoride membrane. The membrane was blocked in 5% nonfat-dried milk blocking solution for 2 h, and incubated with primary antibody overnight at 4°C. After incubation, the membranes were washed four times with PBS containing 0.1% Tween-20, and incubated with HRP-conjugated secondary antibody for 2 h at room temperature.

Caspases-3, -8, -9 activity analysis

Caspases-3, -8, -9 activity was measured using caspases-3, -8, -9 Colorimetric Assay Kit (KeyGen BioTECH, Nanjing, China). HL-60 cells were harvested by centrifugation and washed twice with ice-cold PBS. The lysis buffer (1% NP-40, 20 mM EDTA, and 50 mM Tris-HCl, pH 7.5) was added into the cell pellet, and the reaction was performed on ice for 20 min. After centrifugation (12,000 *g*) for 10 min, the protein concentration in the supernatant was measured using a BCA kit. After using lysis buffer to dilute different protein samples to same concentration, 50 μl of supernatant was mixed with 50 μl of 2 × reaction buffer (Caspases-3, -8, -9 Colorimetric Assay Kit). The caspase-3, caspase-8, and caspase-9 substrates were added, respectively, into the mixture. The OD_{400} values were measured using a spectrophotometer and the caspases-3, -8, and -9 activities were evaluated as follows: activity = the OD_{400} of the experiment group/the OD_{400} of the control group.

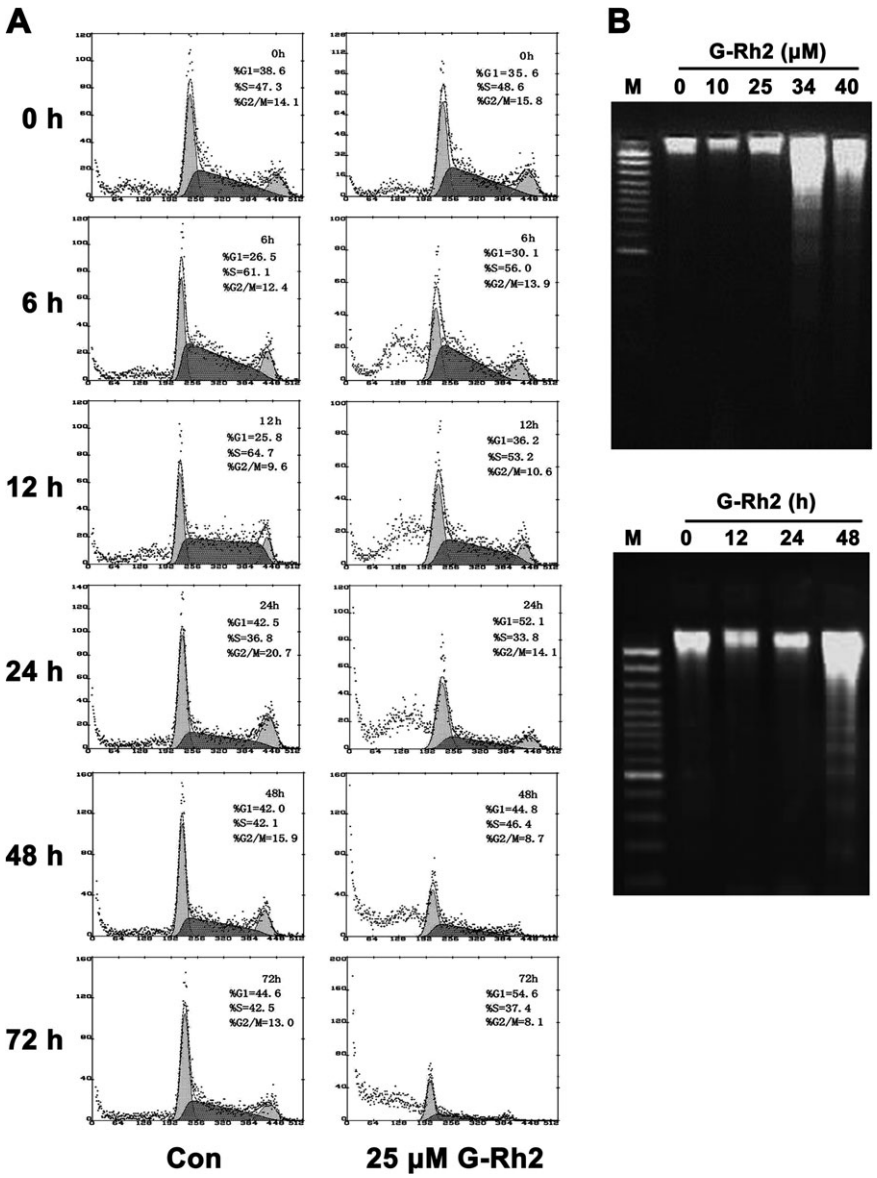

Figure 2. Effects of ginsenoside Rh2 on growth and apoptosis in HL-60 cells (A) Cell cycle analysis of HL-60 cells treated with 25 μM ginsenoside Rh2 from 0 to 72 h compared with DMSO. (B) Rh2-induced apoptosis was assessed by DNA ladder assay in HL-60 cells treated with different concentrations of ginsenoside Rh2 for 6 h and with 25 μM ginsenoside Rh2 for different times.

Table 1. Cell cycle distribution of HL-60 cells treated with 25 μM of ginsenoside Rh2

Time of treatment (h)	G0/G1 (%)		S (%)		G2/M (%)	
	Control	25 μM G-Rh2	Control	25 μM G-Rh2	Control	25 μM G-Rh2
0	38.6 ± 1.2	35.6 ± 1.5	47.3 ± 2.1	48.6 ± 2.8	14.1 ± 1.5	15.8 ± 2.8
6	26.5 ± 1.5	30.1 ± 2.2	61.1 ± 2.3	56.0 ± 2.7	12.4 ± 1.7	13.9 ± 2.4
12	25.8 ± 2.3	36.2 ± 3.2*	64.7 ± 2.5	53.2 ± 2.8	9.6 ± 1.9	10.6 ± 2.3
24	42.5 ± 2.6	52.1 ± 2.1*	36.8 ± 1.4	33.8 ± 2.9	20.7 ± 1.2	14.1 ± 2.6
48	42.0 ± 3.1	44.8 ± 1.9	42.1 ± 1.3	46.4 ± 3.1	15.9 ± 1.5	8.7 ± 1.9
72	44.6 ± 2.8	54.6 ± 1.7*	42.5 ± 1.8	37.4 ± 3.6	13.0 ± 1.4	8.1 ± 1.5

*Significant difference between 25 μM G-Rh2 treated and control in G0/G1 phase.

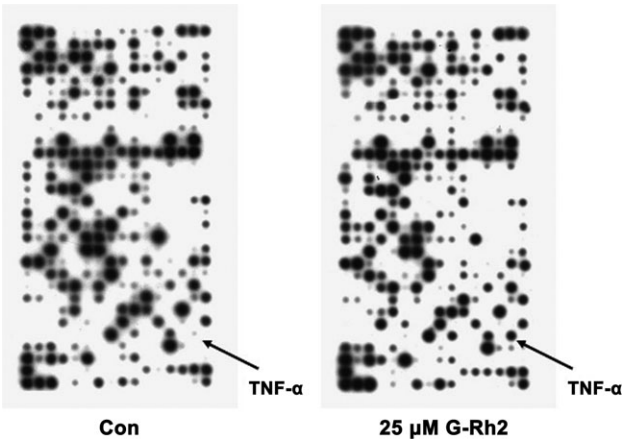

Figure 3. Microarray analysis after treatment with 25 μM ginsenoside Rh2 for 48 h Seventy-six significant genes were identified. *TNF-α* gene was the most significantly up-regulated gene.

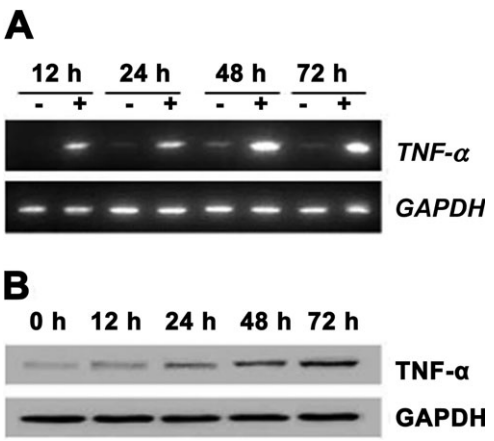

Figure 4. Verification of TNF-α expression levels (A) RT-PCR analysis of HL-60 cells treated with 25 μM ginsenoside Rh2 for different times. (B) Western blot analysis of HL-60 cells treated with different concentrations of Rh2.

Neutralization assay

Goat anti-human TNF-α polyclonal antibody was obtained from PeproTech (Rocky Hill, USA). HL-60 cells were seeded at 2000 per well in 96-well plate and incubated with appropriate concentrations of ginsenoside Rh2 and TNF-α antibody for 48 h. The optimum concentration of TNF-α antibody was determined by cell viability assay.

The HL-60 cells were seeded at 5×10^5 per well in a 12-well plate and incubated with optimum concentrations of ginsenoside Rh2 and TNF-α antibody for 48 h. All the cells were harvested for analysis of DNA fragmentation.

Statistical analysis

The data are expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using SPSS 18.0 software (SPSS Inc. Chicago, USA). Student's *t*-test was used between two groups and the differences were considered significant when P-value < 0.05.

Results

Ginsenoside Rh2 inhibits HL-60 cell line viability

Using the MTT assay, the HL-60 cell viability after the treatment with ginsenoside Rh2 was evaluated. HL-60 cells were incubated for 24, 48, and 72 h with various concentrations of ginsenoside Rh2. Ginsenoside Rh2 inhibited HL-60 cell proliferation in a dose-dependent manner. The IC_{50} value of ginsenoside Rh2 treatment for 48 h was 25 μM (**Fig. 1B**).

Ginsenoside Rh2 blocks HL-60 cell cycle at G1 phase and induces HL-60 cell apoptosis

The cell cycle profiles after ginsenoside Rh2 treatment were determined using flow cytometry. Compared with control, HL-60 cells were blocked and arrested at G1 phase with increased treatment time (**Fig. 2A** and **Table 1**).

In order to determine the apoptosis in HL-60 cells, DNA ladder assay was used. Results showed that ginsenoside Rh2-induced HL-60 cell apoptosis in a dose- and time-dependent manner (**Fig. 2B**).

Microarray analysis following treatment with ginsenoside Rh2

High-throughput screening results of different genes from 14,000 cDNA fragments after treatment with 25 μM ginsenoside Rh2 are shown in **Supplementary Fig. S1** and **Supplementary Table S1**. The Human Cancer Microarray, which contains 480 relative cancer genes, was used to corroborate our findings. After microarray hybridization, 76 significant genes (ratio ≥2 or ≤0.5, flags is P, S/N > 2) were identified from the 480 relative cancer genes (**Fig. 3**). Sixteen genes were found to be up-regulated (**Supplementary Table S2**) and 60 genes down-regulatated in the ginsenoside Rh2-treated HL-60 cells (**Supplementary Table S3**). Compared with control, *TNF-α* mRNA was up-regulated by about 9 folds. In addition, the *TNF-α* mRNA level showed the maximum growth in both microarrays.

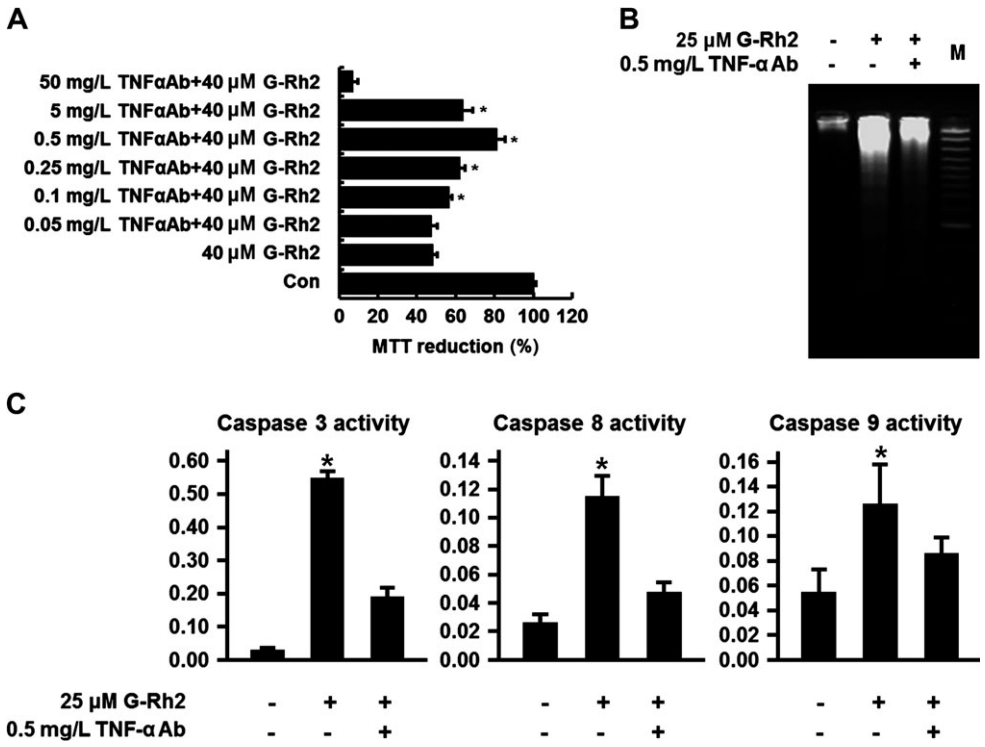

Figure 5. The effects of TNF-α neutralizing antibody on Rh2-induced apoptosis in HL-60 cells (A) Viability of HL-60 cells treated with 40 μM Rh2 and various concentrations of TNF-α neutralizing antibody measured by MTT assay. The data represent the mean ± SD of three independent experiments. *$P < 0.5$ vs. control group. (B) Rh2-induced apoptosis after treatment with TNF-α neutralizing antibody was assessed by DNA ladder assay. (C) The caspases-3, -8, and -9 activities in HL-60 cells were analyzed after treatment with various concentrations of Rh2 and TNF-α neutralizing antibody.

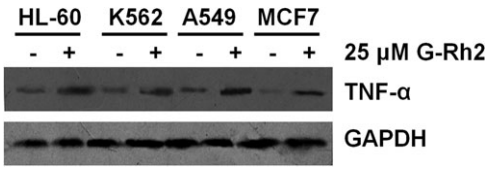

Figure 6. Western blot analysis of TNF-α After 48 h, TNF-α was detected in the HL-60, K562, A549, and MCF7 cells which were treated with 25 μM ginsenoside Rh2, and compared with DMSO controls. All these analyses were performed in triplicate. *$P < 0.05$.

TNF-α overexpression induced by ginsenoside Rh2 inhibits HL-60 cell viability

RT-PCR and western blot analysis were performed to clarify TNF-α expression. After treatment with 25 μM ginsenoside Rh2, a time-dependent up-regulation of *TNF-α* mRNA was observed in HL-60 cells (**Fig. 4A**). Western blot analysis revealed a time-dependent up-regulation of TNF-α in HL-60 cells (**Fig. 4B**).

To determine the effect of TNF-α on proliferation inhibition induced by ginsenoside Rh2, HL-60 cell viability was measured again after neutralization with different concentrations of anti-TNF-α antibody. Except at extremely high concentrations of TNF-α antibody such as 50 mg/l, the HL-60 cell survival rates were significantly restored with increasing anti-TNF-α antibody concentrations (**Fig. 5A**). DNA Ladder assay showed that DNA fragmentation induced by ginsenoside Rh2 was reduced after treatment with anti-TNF-α antibody, which suggests the inhibition of apoptosis by neutralizing TNF-α (**Fig. 5B**). In addition, the antibody suppressed the activity of cysteinyl aspartate-specific proteinases including caspase-3, caspase-8, and caspase-9, which were activated by ginsenoside Rh2 (**Fig. 5C**).

TNF-α overexpression is induced by ginsenoside Rh2 in different cell lines

In order to determine the ginsenoside Rh2-induced apoptosis in other cancer cell lines via TNF signaling, the levels of TNF-α were also determined in A549562 and MCF-7 cells. Compared with control, significant up-regulation of TNF-α was observed in the ginsenoside Rh2-treated cells (**Fig. 6**).

Discussion

Ginsenoside Rh2 is a potent anticancer compound targeting multiple cancers [11,13,15–19,26,27]. Recent reports suggested that ginsenoside Rh2-induced apoptosis in human leukemia cell via mitochondrial signaling pathways. Following the up-regulation of Bax and Bak, cytochrome c was released from mitochondria into cytoplasm and caspase 3 was activated following cleavage of pro-caspase 8 and pro-caspase 9 [26,28]. In this study, the overexpression of TNF-α in Rh2-treated HL-60 cells was examined by high-throughput screening using microarray technology. Furthermore, our results demonstrated that TNF-α plays a key role in Rh2-induced apoptosis. Moderate anti-TNF-α antibody levels attenuated Rh2-induced apoptosis, suggesting that ginsenoside Rh2-induced apoptosis via TNF signaling.

Epidermal growth factor receptor (EGFR) signaling also plays an important role in Rh2-induced inhibition of cancer cell growth. In previous studies, it was found that ginsenoside Rh2 down-regulated EGFR and recombinant EGFR reduced anticancer activity of ginsenoside Rh2 in glioblastoma [29]. Ginsenoside Rh2 inhibited the growth of glioblastoma by binding EGFR competitively against EGF, and by inactivating EGFR/PI3k/Akt/mTOR signaling. Ginsenoside Rh2 is a potent inhibitor of EGFR in cancer therapy [30]. It suggests that the

anticancer mechanisms of ginsenoside Rh2 are mediated by enhancing TNF-induced apoptosis and inhibiting EGFR survival.

In this study, two types of microarray were used to explore gene regulation in HL-60 cells following Rh2-treatment under physiological conditions. The microarray analysis indicated that the classical MAP kinase pathway was inhibited in Rh2-treated HL-60 cells. The mRNA levels of platelet-derived growth factor (*PDGF*), *Ras*, and *Raf* were down-regulated, which confirmed that Rh2 inhibited the activation of extracellular signal-regulated kinase [31]. PI3K/Akt signaling pathway was also inhibited by Rh2 [30]. These mechanisms may explain the ginsenoside Rh2-mediated effects on inhibition of survival, invasion, migration, and angiogenesis of cancer cells.

Supplementary Data

Supplementary data is available at *ABBS* online.

Funding

The work was supported by the grants from the National Natural Science Foundation of China (Nos. 81071821, 30572208, and 30470189), the Key Hunan Provincial Natural Science Foundation of China (Nos. 13JJ2016 and 07JJ5096), the Science and Technology Program of Hunan Province of China (No. 2014SK4050), and Changsha National High Tech Zone Innovation Leading Talent Program (No. 20143701).

References

1. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. *Biochem Pharmacol* 1999, 58: 1685–1693.
2. Chang YS, Seo EK, Gyllenhaal C, Block KI. Panax ginseng: a role in cancer therapy? *Integr Cancer Ther* 2003, 2: 13–33.
3. Lee CH, Kim J. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. *J Ginseng Res* 2014, 38: 161–166.
4. Li C, Qin G, Shi R, Zhang M, Lv J. Ginsenoside Rg1 reduces toxicity of PM2.5 on human umbilical vein endothelial cells by upregulating intracellular antioxidative state. *Environ Toxicol Phar* 2013, 35: 21–29.
5. Lee I, Hyam SR, Jang S, Han MJ, Kim D. Ginsenoside Re ameliorates inflammation by inhibiting the binding of lipopolysaccharide to TLR4 on macrophages . *J Agr Food Chem* 2012, 60: 9595–9602.
6. Wu Y, Yu Y, Szabo A, Han M, Huang XF. Central inflammation and leptin resistance are attenuated by ginsenoside Rb1 treatment in obese mice fed a high-fat diet. *PLos One* 2014, 9: e92618.
7. Li J, Zhong W, Wang W, Hu S, Yuan J, Zhang B, Hu T, *et al*. Ginsenoside metabolite compound K promotes recovery of dextran sulfate sodium-induced colitis and inhibits inflammatory responses by suppressing NF-kappaB activation. *PLos One* 2014, 9: e87810.
8. Hwang JT, Kim SH, Lee MS, Kim SH, Yang HJ, Kim MJ, Kim HS, *et al*. Anti-obesity effects of ginsenoside Rh2 are associated with the activation of AMPK signaling pathway in 3T3-L1 adipocyte. *Biochem Biophys Res Commun* 2007, 364: 1002–1008.
9. Cho Y, Hur S, Kim J, Kim J, Lee D, Choe J, Won M, *et al*. Specific activation of insulin-like growth factor-1 receptor by ginsenoside Rg5 promotes angiogenesis and vasorelaxation. *J Biol Chem* 2015, 290: 467–477.
10. Shin K, Seo C, Cho H, Oh S, Hong S, Yoo H, Hong J, *et al*. Ginsenoside compound K inhibits angiogenesis via regulation of sphingosine kinase-1 in human umbilical vein endothelial cells. *Arch Pharm Res* 2014, 37: 1183–1192.
11. Chen W, Qiu Y. Ginsenoside Rh2 targets EGFR by up-regulation of miR-491 to enhance anti-tumor activity in hepatitis B virus-related hepatocellular carcinoma. *Cell Biochem Biophys* 2015, 72: 325–331.
12. Zhang Y, Zhang R, Xu H, Yu X, Qu S, Sui D. 20(S)-Protopanaxadiol triggers mitochondrial-mediated apoptosis in human lung adenocarcinoma A549 cells via inhibiting the PI3K/Akt signaling pathway. *Am J Chin Med* 2013, 41: 1137–1152.
13. Zhang Q, Hong B, Wu S, Niu T. Inhibition of prostatic cancer growth by ginsenoside Rh2. *Tumor Biol* 2015, 36: 2377–2381.
14. Wang J, Nao J, Zhang M, He P. 20(s)-ginsenoside Rg3 promotes apoptosis in human ovarian cancer HO-8910 cells through PI3K/Akt and XIAP pathways. *Tumor Biol* 2014, 35: 11985–11994.
15. Wen X, Zhang H, Zhao L, Yao Y, Zhao J, Tang J. Ginsenoside Rh2 differentially mediates microRNA expression to prevent chemoresistance of breast cancer. *Asian Pac J Cancer P* 2015, 16: 1105–1109.
16. Chung KS, Cho SH, Shin JS, Kim DH, Choi JH, Choi SY, Rhee YK, *et al*. Ginsenoside Rh2 induces cell cycle arrest and differentiation in human leukemia cells by upregulating TGF-expression. *Carcinogenesis* 2013, 34: 331–340.
17. Guo X, Guo Q, Li Y, Lee S, Wei X, Jin Y. Ginsenoside Rh2 induces human hepatoma cell apoptosisvia Bax/Bak triggered cytochrome C release and caspase-9/caspase-8 activation. *Int J Mol Sci* 2012, 13: 15523–15535.
18. Guan N, Huo X, Zhang Z, Zhang S, Luo J, Guo W. Ginsenoside Rh2 inhibits metastasis of glioblastoma multiforme through Akt-regulated MMP13. *Tumor Biol* 2015, 36: 6789–6795.
19. Tang X. Effects of ginsenoside Rh2 on growth and migration of pancreatic cancer cells. *World J Gastroenterol* 2013, 19: 1582.
20. An IS, An S, Kwon KJ, Kim YJ, Bae S. Ginsenoside Rh2 mediates changes in the microRNA expression profile of human non-small cell lung cancer A549 cells. *Oncol Rep* 2013, 29: 523–528.
21. Cheng C, Yang S, Huang C, Chen J, Chang W, Hsu S. Molecular mechanisms of ginsenoside Rh2-mediated G1 growth arrest and apoptosis in human lung adenocarcinoma A549 cells. *Cancer Chemoth Pharm* 2005, 55: 531–540.
22. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001, 104: 487–501.
23. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AJ. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998, 281: 1680–1683.
24. Wang L, Du F, Wang X. TNF-alpha induces two distinct caspase-8 activation pathways. *Cell* 2008, 133: 693–703.
25. He L, Lee J, Jang JH, Lee SH, Nan MH, Oh BC, Lee SG, *et al*. Ginsenoside Rh2 inhibits osteoclastogenesis through down-regulation of NF-kappaB, NFATc1 and c-Fos. *Bone* 2012, 50: 1207–1213.
26. Xia T, Wang JC, Xu W, Xu LH, Lao CH, Ye QX, Fang JP. 20S-Ginsenoside Rh2 induces apoptosis in human Leukaemia Reh cells through mitochondrial signaling pathways. *Biol Pharm Bull* 2014, 37: 248–254.
27. Chung KS, Cho SH, Shin JS, Kim DH, Choi JH, Choi SY, Rhee YK, *et al*. Ginsenoside Rh2 induces cell cycle arrest and differentiation in human leukemia cells by upregulating TGF-beta expression. *Carcinogenesis* 2013, 34: 331–340.
28. Guo XX, Guo Q, Li Y, Lee SK, Wei XN, Jin YH. Ginsenoside Rh2 induces human hepatoma cell apoptosisvia bax/bak triggered cytochrome C release and caspase-9/caspase-8 activation. *Int J Mol Sci* 2012, 13: 15523–15535.
29. Li S, Gao Y, Ma W, Guo W, Zhou G, Cheng T, Liu Y. EGFR signaling-dependent inhibition of glioblastoma growth by ginsenoside Rh2. *Tumour Biol* 2014, 35: 5593–5598.
30. Li S, Guo W, Gao Y, Liu Y. Ginsenoside Rh2 inhibits growth of glioblastoma multiforme through mTor. *Tumour Biol* 2015, 36: 2607–2612.
31. Liu ZH, Li J, Xia J, Jiang R, Zuo GW, Li XP, Chen Y, *et al*. Ginsenoside 20(s)-Rh2 as potent natural histone deacetylase inhibitors suppressing the growth of human leukemia cells. *Chem Biol Interact* 2015, 242: 227–234.