

Association of a Methanol Extract of *Rheum undulatum* L. Mediated Cell Death in AGS Cells with an Intrinsic Apoptotic Pathway

Noo Ri Hong[#], Hyun Soo Park[#], Tae Seok Ahn, Myeong Ho Jung, Byung Joo Kim*

Division of Longevity and Biofunctional Medicine, Healthy Aging Korean Medical Research Center, School of Korean Medicine, Pusan National University, Busan, Korea

Key Words

AGS human gastric adenocarcinoma cells, anti-proliferative activity, apoptosis, cell death, intrinsic apoptotic pathway, *Rheum undulatum* L.

Abstract

Objectives: *Rheum undulatum* L. has traditionally been used for the treatment of many diseases in Asia. However, its anti-proliferative activity in cancer has still not been studied. In the present study, we investigated the anti-cancer effects of methanol extract of *Rheum undulatum* L. (MERL) on human adenocarcinoma gastric cell lines (AGS).

Methods: To investigate the anti-cancer effect of MERL on AGS cells, we treated the AGS cells with varying concentrations of MERL and performed 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays. Cell cycle analyses, measurements of the mitochondrial membrane potential (MMP), caspase activity assays and Western blots were conducted to determine whether AGS cell death occurred by apoptosis.

Results: Treatment with MERL significantly inhibited growth of AGS cells in a concentration dependent manner. MERL treatment in AGS cells led to increased accumulation of apoptotic sub G1 phase cells in a concentration dependent manner. In control cultures, 5.38% of the cells were in the sub G1 phase. In MERL

treated cells, however, this percentage was significantly increased (9.95% at 70 µg/mL, 15.94% at 140 µg/mL, 26.56% at 210 µg/mL and 38.08% at 280 µg/mL). MERL treatment induced the decreased expression of pro-caspase-8 and -9 in a concentration dependent manner, whereas the expression of the active form of caspase-3 was increased. A subsequent Western blot analysis revealed increased cleaved levels of poly (ADP-ribose) polymerase (PARP) protein. Also, treatment with MERL increased the activities of caspase-3 and -9 compared with the control. MERL treatment increased the levels of the pro-apoptotic truncated Bid (tBid) and Bcl2 Antagonist X (Bax) proteins and decreased the levels of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein, whose is the stabilization of mitochondria. However, inhibitions of p38, extracellular signal regulated kinases (ERKs) and C-Jun N-terminal kinases (JNK) by MERL treatment did not affect cell death.

Conclusion: These results suggest that MERL mediated cell death is associated with an intrinsic apoptotic pathway in AGS cells.

1. Introduction

Recently, many researchers have been looking for better anti-cancer agents from traditional medicinal herb [1]. Among cancers, gastric cancer in men is the fourth most frequently diagnosed cancer and the third most frequent cause of cancer death worldwide [2, 3]. Although cancer treatment including surgery, radi-

Received: Feb 09, 2015 Reviewed: Mar 03, 2015 Accepted: Mar 04, 2015

© This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

© This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 and ANSI/NISO Z39.48-1992 (Permanence of Paper).

*Corresponding Author

Byung Joo Kim, Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Beomeori, Mulgeum-eup, Yangsan, Gyeongsangnamdo, 626-870, Korea.

Tel: +82-51-510-8469 Fax: +82-51-510-8420

E-mail: vision@pusan.ac.kr

[#]Contributed equally to this work and should be considered joint first authors.

© 2015 Korean Pharmacopuncture Institute

<http://www.journal.ac>

ation therapy and chemotherapy has advanced in recent years, these therapies have many limitations because of low response and poor survival [4]. Therefore, the development of more effective strategies to improve the survival rate for gastric cancer patients is important.

Rheum undulatum L. (MERL), a well-known traditional Chinese medicine, has been widely used for thousands of years for the treatment of many diseases [5]. In particular, the root of the species is widely used as a purgative and anti-inflammatory agent in East Asia [6-8]. In addition, according to some reports, *Rheum undulatum* L. is used traditionally in Korea for the treatment of dental disease [9, 10].

In general, apoptosis has two major pathways: the death receptor pathway (extrinsic) and the mitochondria dependent pathway (intrinsic). The interaction between apoptosis inducing ligands and death receptors initiates an extrinsic pathway at the cell's membrane and subsequently activates caspase-8 by the formation of a death induced signaling complex (DISC). The activated caspase-8 directly activates effector caspases such as caspase-3, or cleaves BH3 interacting domain death agonist (Bid) to truncated Bid (tBid), leading to intrinsic pathway activation via mitochondrial dysfunction. As a result, cytochrome *c* is released from mitochondria, causing activation of caspase-9 and effector caspases, eventually leading to apoptotic cell death [11-13]. However, until now, no informations about the effects of *Rheum undulatum* L. on human adenocarcinoma gastric cells (AGS) has been available. Therefore, we investigated the anti-cancer effects of a methanol extract of *Rheum undulatum* L. on AGS human gastric adenocarcinoma cells. Our results demonstrated that MERL triggers apoptosis of AGS cells through activation of caspases and mitochondrial dysfunction.

2. Materials and Methods

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), propidium iodide (PI) and 5,5', 6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1) used in this study were purchased from Sigma (St. Louis, MO). Fetal bovine serum (FBS) and caspase activity assay kits were obtained from GIBCO-BRL (Gaithersburg, MD) and R&D Systems (Minneapolis, MN), respectively. The extracellular signal-regulated kinase (ERK)-specific inhibitor, PD98059, the c-Jun N-terminal Kinase (JNK)-specific inhibitor, SP600125, and the p38 mitogen activated protein kinase (MAPK) specific inhibitor, SB203580 were purchased from Calbiochem (San Diego, CA). Enhanced chemiluminescence (ECL) kits were purchased from Amersham (Arlington Heights, IL). All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

For the preparation of MERL, the powder of a methanol extract of *Rheum undulatum* L. (Catalog number: CA04-008) was obtained from the plant extract bank at the Korea Research Institute of Bioscience and Biotechnology (KRIBB) (Daejeon, Korea). The powder was then immersed in methanol, sonicated for 15 minutes, and extracted for 72 hours. The extract was filtered through non fluorescent cotton and evaporated under reduced pressure by using a

rotary evaporator (N-1000SWD, Eyela, Japan) in 45°C. The condensed extract was then lyophilized by using a Modul Spin 40 dryer (Biotron Corporation, Calgary, Canada) for 24 hours. Finally, the yield of lyophilized powder (MERL) was 12.3%. The MERL was dissolved in dimethyl sulfoxide (DMSO) as a stock solution at a 100 mg/mL concentration and stored at 4°C. The stock solution was then diluted with a medium to the desired concentration prior to use.

The AGS human gastric adenocarcinoma cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained at 37°C in air at 95% humidity with 5% CO₂ in RPMI1640 supplemented with 10% heat inactivated FBS, 2 mM glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin.

To investigate cell viability, we seeded the cells in 6 well plates at a density of 2×10^5 cells per well and stabilized them for 24 hours. The cells were then treated with various dose of MERL for the desired times. The MTT working solution (0.5 mg/mL) was then added to the culture plates, and the cells were incubated continuously at 37°C for 2 hours. The culture supernatant was completely removed from the wells, and DMSO was added to completely dissolve the formazan crystals. The absorbance of each well was measured at a wave length of 540 nm by using a microplate reader (Molecular Devices, Palo Alto, CA). The effect of MERL on the inhibition of cell growth was assessed on the basis of the percentage of cell viability, where the vehicle treated cells were considered 100% viable.

Following treatment with MERL, cells were trypsinized, washed with phosphate buffered saline (PBS), fixed in 75% ethanol, and stored at 4°C overnight. Prior to analysis, cells were again washed with PBS, suspended in a cold PI (Sigma) solution, and incubated at room temperature in the dark for 30 minutes. A fluorescence activated cell sorting (FACS) can flow cytometry system (Becton-Dickinson, San Jose, CA) was used for the performance of flow cytometry analyses.

Cells were harvested and washed twice in PBS at 4°C. Total cells lysates were lysed in lysis buffer. The supernatants were collected and the protein concentrations were then measured by using protein assay reagents (Bio-Rad Laboratories, Hercules, CA). For Western blotting, equal amounts of protein extracts were denatured by boiling at 95°C for 3 minutes in sample buffer (0.5 M Tris-HCl, pH 6.8, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol) in a ratio of 1 : 1, subjected to 10% — 13% SDS-polyacrylamide gels, and transferred to polyvinylidene difluoride membranes (Schleicher & Schuell, Keene, NH) by electroblotting. The membranes were blocked with 5% non fat dry milk in PBS with Tween 20 buffer (PBS-T) (20 mM Tris, 100 mM NaCl, pH 7.5, and 0.1% Tween 20) for 50 minutes at room temperature. Membranes were then incubated overnight at 4°C with the primary antibodies, probed with enzyme linked secondary antibodies, and visualized using an ECL kit according to the manufacturer's instructions.

The activities of the caspases were determined by using colorimetric assay kits, which utilize synthetic tetrapeptides (Asp-Glu-Val-Asp DEVD for caspase-3, and Leu-Glu-His-Asp (LEHD) for caspase-9) labeled with p-nitroaniline (p-NA). Briefly, MERL treated and untreated cells were

lysed in the supplied lysis buffer. The supernatants were collected and incubated with the supplied reaction buffer containing dithiothreitol (DTT) and DEVD-pNA or LEHD-pNA as substrates at 37°C. The reactions were measured by using a microplate reader to record the changes in the absorbance at a wavelength of 405 nm.

Mitochondrial membrane potential (MMP) was determined by using the dual emission potential sensitive probe JC-1. The cells were collected and incubated with 10 μ M JC-1 for 20 minutes at 37°C in the dark. The cells were then washed once with PBS and analyzed by using a flow cytometer [14].

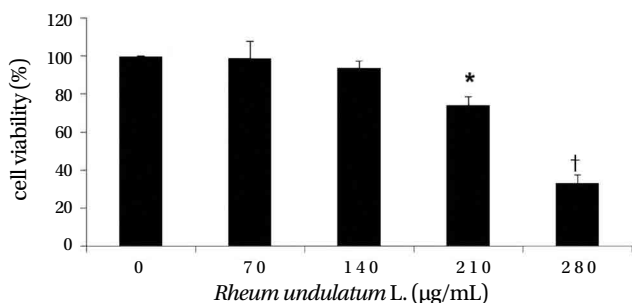


Figure 1 Induction of apoptosis by MERL treatment in AGS cells. The cells were treated with the indicated concentrations of MERL for 24 hours. The cell viability was measured by using a MTT assay. Bars represent the mean \pm S.D. * $P < 0.05$. † $P < 0.01$.

MERL, methanol extract of *Rheum undulatum* L.; AGS, adenocarcinoma gastric cells; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; S.D., standard deviation.

Unless otherwise indicated, each result is expressed as the mean \pm standard deviation (S.D.) of data obtained from triplicate experiments. A statistical analysis was performed by using a paired Student's *t*-test. Differences at $P < 0.05$ were considered statistically significant.

3. Results

To determine the effect of MERL on cell growth in AGS cells, we treated the cells various concentrations of MERL for 24 hours; then, we used an MTT assay to measure the cell's viability. As shown in Fig. 1, the cell's viability was significantly decreased as a result of the MERL treatment in a concentration dependent manner. These results suggested that exposure of the AGS cells to MERL decreased cellular viability in a concentration dependent manner (Fig. 1).

We next performed a flow cytometric analysis to detect apoptotic dead cells to determine whether MERL induced cell death resulted from apoptosis. As indicated in Fig. 2, MERL treatment of AGS cells led to increased accumulation of apoptotic sub G1 phase cells in a concentration dependent manner. In the control cultures, 5.38% of cells were in the sub G1 phase. In MERL treated cells, however, this percentage was significantly increased (9.95% at 70 μ g/mL, 15.94% at 140 μ g/mL, 26.56% at 210 μ g/mL and 38.08% at 280 μ g/mL). These results suggest that MERL induced growth inhibition was related to the induction of apoptosis in AGS cells.

Caspases are known to be important mediators of apop-

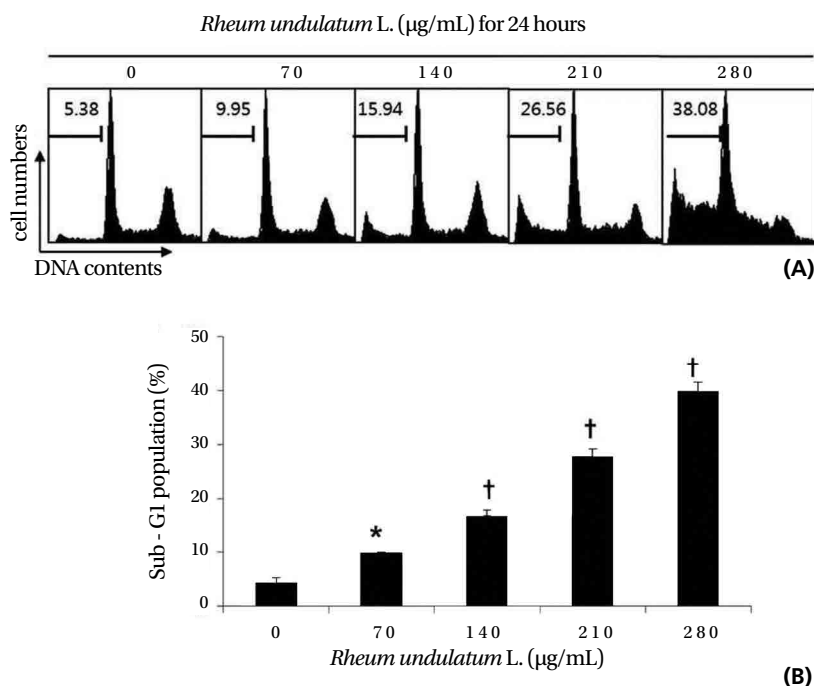


Figure 2 MERL increases the activity of sub G1 phase cells in AGS cells. To quantify the degree of apoptosis induced by MERL, we evaluated the cells by using flow cytometry to determine the sub G1 DNA content, which represents the cells undergoing apoptotic DNA degradation. Bars represent the mean \pm SD. * $P < 0.05$. † $P < 0.01$.

MERL, methanol extract of *Rheum undulatum* L.; AGS, adenocarcinoma gastric cells; S.D., standard deviation.

tosis in both the intrinsic and the extrinsic pathways. This executionary phase induces activation of cytoplasmic endonuclease, which degrades nuclear material, activation of proteases, which degrades cytoskeletal proteins and nuclei, and cleavage of various substrates including polymerase (PARP), which serves as an apoptosis marker [15]. As shown in Fig. 3(a), MERL treatment induced a decrease in the expression of pro-caspase-8 and -9 in a concentration dependent manner, whereas the active form of caspase-3 was increased. Subsequent Western blot analyses revealed increased cleaved levels of the PARP protein. We next attempted to quantify the proteolytic activation of caspases

due to the MERL treatment. As shown in Fig. 3(b), treatment with MERL increased the activities of caspase-3 and -9 compared with the levels for the controls. These results indicate that MERL treatment induces apoptosis via activation of caspases in AGS cells.

Mitochondria, which are specialized organelles, play a critical role in apoptosis. Intermembrane spaces of mitochondria contain many pro-apoptotic proteins including cytochrome *c*. Disruption of the outer mitochondrial membrane, which can be caused by several events, results in the release of cytochrome *c*, leading to the activations of caspase-9 and effector caspases, which eventually caus-

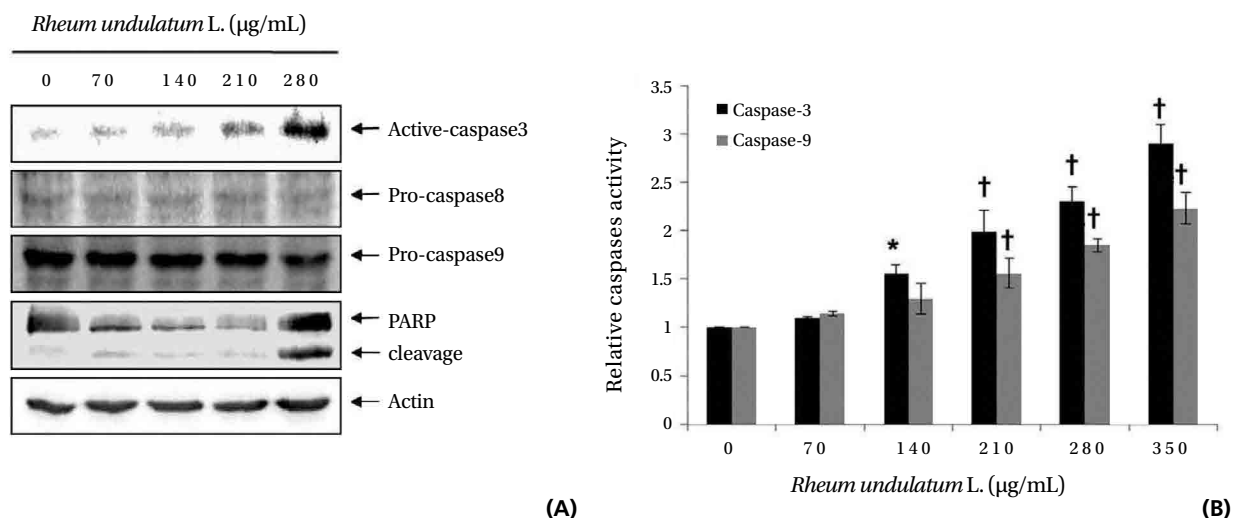


Figure 3 Activation of caspases and degradation of the PARP protein due to MERL treatment of AGS cells. (A) AGS cells were treated with the indicated concentrations of MERL for 24 hours. Actin was used as an internal control. (B) After a 24 hours incubation with the indicated concentrations of MERL, the cells were lysed and aliquots were assayed for the *in-vitro* caspase-3 and -9 activities with DEVD-pNA and LEHD-pNA as substrates, respectively. Bars represent the mean \pm SD. * $P < 0.05$. † $P < 0.01$.

PARP, polymerase; MERL, methanol extract of *Rheum undulatum* L.; AGS, adenocarcinoma gastric cell lines; DEVD, Asp-Glu-Val-Asp; p-NA, p-nitroaniline; LEHD, Leu-Glu-His-Asp; S.D., standard deviation.

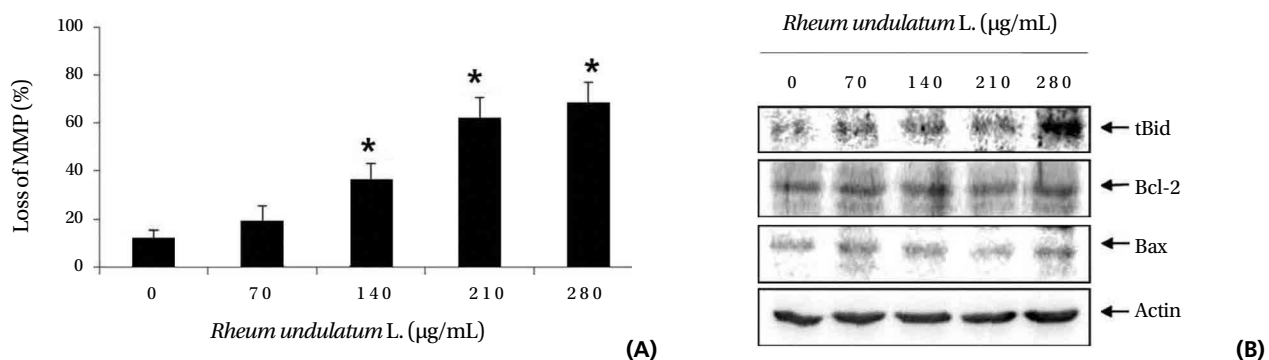


Figure 4 Effects of MERL on the MMP values and the levels of tBid, Bcl-2 and Bax proteins in AGS cells. (A) Cells were treated with the indicated concentration of MERL for 24 hours. Cells were collected and incubated with JC-1 (10 μ M) for 20 minutes at 37°C in the dark. The cells were washed once with PBS and analyzed by a DNA flow cytometer. (B) The cell lysates obtained from cells grown under the same conditions as (A) were separated by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Actin was used as an internal control. Bars represent the mean \pm S.D. * $P < 0.01$.

MERL, methanol extract of *Rheum undulatum* L.; MMP, mitochondrial membrane potential; tBid, truncated Bid; Bcl-2, B-cell lymphoma 2; Bax, Bcl2 Antagonist X; AGS, adenocarcinoma gastric cell lines; JC-1, 5,5', 6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; S.D., standard deviation.

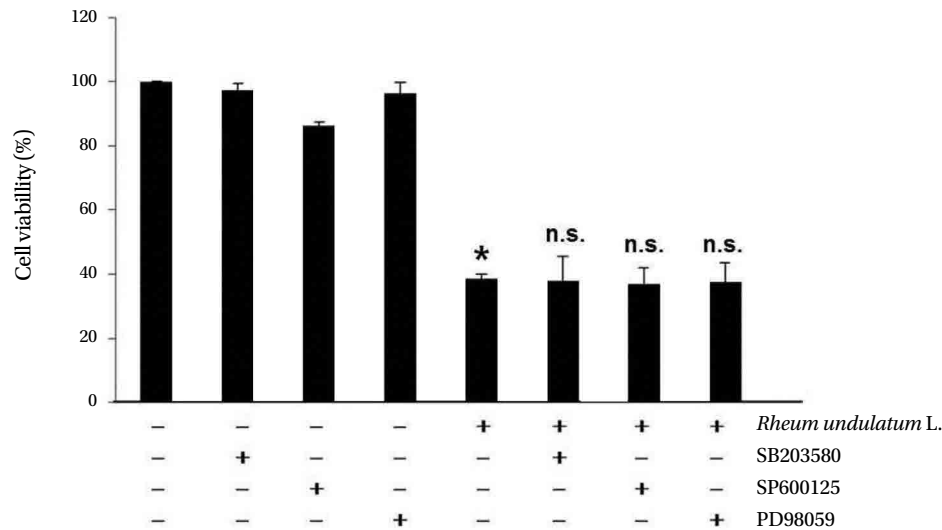


Figure 5 Effects of a specific inhibitor of MAPK on MERL induced cell death in AGS cells. (A) Cells were pretreated with the indicated MAPK inhibitors (SB203580 (20 μ M), SP600125 (20 μ M) and PD98059 (50 μ M)) for 1 hour and then treated with MERL (350 μ g/mL) for 24 hours. The cell's viability was measured by using the MTT assay. Bars represent the mean \pm SD. * $P < 0.01$.

MAPK, mitogen activated protein kinase; MERL, methanol extract of *Rheum undulatum* L.; AGS, adenocarcinoma gastric cell lines; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; S.D., standard deviation; n.s., not significant *vs.* MERL treated cells.

es apoptotic cell death [16-19]. For this study, the effects of MERL on the levels of MMP were measured by using a flow cytometer with a JC-1, mitochondrial specific probe. As shown in Fig. 4(a), MERL treatment increased the loss of MMP in a concentration dependent manner, indicating that treatment with MERL causes depolarization of the mitochondrial membrane. We next determined the changes in the levels of the anti- or the pro-apoptotic proteins. As indicated in Fig. 4(b), MERL treatment increased the levels of the pro-apoptotic tBid and Bcl2 Antagonist X (Bax) proteins and decreased the levels of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein. These results suggest that MERL treatment induces mitochondrial dysfunction via decreased levels of Bcl-2 and increased levels of tBid and Bax.

The MAPK signaling pathway functions as a critical regulator of cell survival and proliferation [20]. The MAPKs, including p38 MAPK, ERKs and JNKs also play fundamental roles in survival, proliferation, and apoptosis [21]. To determine whether these signaling pathways played a role in the MERL induced apoptotic response, we pretreated the cells with a specific inhibitor of MAPK and then measured the cell's viability by using the MTT assay. As shown in Fig. 5, pretreatment with SB203580 (a specific inhibitor of p38 MAPK), SP600125 (a potent inhibitor of JNK), or PD98059 (a potent inhibitor of ERK) did not have an effect on the response to MERL treatment. Taken together, these results suggest that MERL induced cell death is associated with the intrinsic apoptotic pathway in AGS cells.

4. Discussion

Rheum undulatum L. contains many anthraquinones and their glycosides, including aloe-emodin, rhein, emo-

din, and chrysophanol, which are thought to be the major active components for inhibiting tumor growth in various cancer cells [10, 22-25]. However, no study has been performed to investigate the effect of *Rheum undulatum* L. on the growth of human cancer cells. Therefore, this present study aimed to demonstrate the anti-cancer effect of *Rheum undulatum* L. and its the related mechanism.

In this study, we used a methanol extract of *Rheum undulatum* L., and investigated whether treatment with MERL could induce apoptosis in the human gastric adenocarcinoma AGS cell line. Our results suggested that MERL treatment reduced cell viability in a concentration dependent manner, due to apoptosis. MERL induced apoptosis was confirmed by the increases in the sub G1 population and in the cleaved PARP protein, which is an apoptotic marker (Figs. 1, 2). Also, MERL treatment induced mitochondrial dysfunction via decreased levels of Bcl-2 and increased levels of tBid and Bax (Fig. 4).

Apoptosis can be initiated by two pathways, the extrinsic and the intrinsic pathways. The extrinsic apoptotic pathway can be triggered by interactions between transmembrane death receptors and their cognate ligands. These interactions result in recruitment of the associated death domain (FADD) and caspase-8 to the DISC, leading to activation of caspase-8 [15, 26]. The intrinsic apoptotic pathway involve non receptor mediated stimuli that produce mitochondrial mediated signals, resulting in an opening of mitochondrial permeability transition (MPT) pore, loss of the mitochondrial membrane potential and release of pro-apoptotic proteins such as cytochrome *c*. The Bcl-2 family of proteins, which includes anti-apoptotic proteins such as Bcl-2 and Bcl-xL and pro-apoptotic proteins such as Bax, Bid and Bcl-2-associated death promoter (Bad) regulates the release of cytochrome *c* from mitochondria via control of the mitochondrial membrane's permeabil-

ity. Release of cytochrome *c* contributes to the activation of caspase-9 followed by sequential activation of caspase-3 and cleavage of PARP [11, 12, 27]. Our results showed that MERL induced apoptosis of AGS cells was associated with the caspase cascade reaction, the down regulation of the Bcl-2 level and the up regulations of the tBid and the Bax levels, as well as the loss of MMP (Figs. 2, 3, 4), indicating that MERL induced mitochondrial dysfunction through regulation of the Bcl-2 family, leads to apoptosis.

5. Conclusions

Our study showed that the MERL reduces cell proliferation and induced apoptosis, which was confirmed by an increased accumulation of the sub G1 phase. Furthermore, MERL induced apoptosis was shown to be associated with activation of caspases, and mitochondrial dysfunction based on the decreased Bcl-2 and the increased tBid and Bax proteins levels. In addition, specific inhibitors of MAPK had no effects on MERL induced apoptosis in AGS cells. These findings suggest that MERL should be considered to be a useful potential therapeutic agent for use in gastric cancer treatment.

Acknowledgements

This research was supported by the Basic Science Research Program of the Korean National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (Grant no. 2010-0021347).

Conflict of interest

The authors declare that there are no conflicts of interest.

ORCID

Byung Joo Kim. <http://orcid.org/0000-0001-8835-9103>.

References

- Feng Y, Wang N, Zhu M, Feng Y, Li H, Tsao S. Recent progress on anticancer candidates in patents of herbal medicinal products. *Recent Pat Food Nutr Agric*. 2011;3(1):30-48.
- Li C, Tian ZN, Cai JP, Chen KX, Zhang B, Feng MY, *et al*. Panax ginseng polysaccharide induces apoptosis by targeting Twist/AKR1C2/NF-1 pathway in human gastric cancer. *Carbohydr Polym*. 2014;102:103-9.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69-90.
- Liu AN, Zhu ZH, Chang SJ, Hang XS. Twist expression associated with the epithelial-mesenchymal transition in gastric cancer. *Mol Cell Biochem*. 2012;367(1-2):195-203.
- He ZH, He MF, Ma SC, But PP. Anti-angiogenic effects of rhubarb and its anthraquinone derivatives. *J Ethnopharmacol*. 2009;121(2):313-7.
- Chang CH, Lin CC, Yang JJ, Namba T, Hattori M. Anti-inflammatory effects of emodin from *ventilago leiocarpa*. *Am J Chin Med*. 1996;24(2):139-42.
- Kuo YC, Meng HC, Tsai WJ. Regulation of cell proliferation, inflammatory cytokine production and calcium mobilization in primary human T lymphocytes by emodin from *Polygonum hypoleucum* Ohwi. *Inflamm Res*. 2001;50(2):73-82.
- Yu HM, Liu YF, Cheng YF, Hu LK, Hou M. Effects of rhubarb extract on radiation induced lung toxicity via decreasing transforming growth factor-beta-1 and interleukin-6 in lung cancer patients treated with radiotherapy. *Lung Cancer*. 2008;59(2):219-26.
- Kim JE, Kim HJ, Pandit S, Chang KW, Jeon JG. Inhibitory effect of a bioactivity-guided fraction from *Rheum undulatum* on the acid production of *Streptococcus mutans* biofilms at sub-MIC levels. *Fitoterapia*. 2011;82(3):352-6.
- Choi ES, Cho SD, Jeon JG, Cho NP. The apoptotic effect of the hexane extract of *Rheum undulatum* L. in oral cancer cells through the down-regulation of specificity protein 1 and survivin. *Lab Anim Res*. 2011;27(1):19-24.
- Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. *J Biol Chem*. 1999;274(29):20049-52.
- Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*. 2002;2(9):647-56.
- Jin Z, El-Deiry WS. Overview of cell death signaling pathways. *Cancer Biol Ther*. 2005;4(2):139-63.
- Choi JH, Choi AY, Yoon H, Choe W, Yoon KS, Ha J, *et al*. Baicalein protects HT22 murine hippocampal neuronal cells against endoplasmic reticulum stress-induced apoptosis through inhibition of reactive oxygen species production and CHOP induction. *Exp Mol Med*. 2010;42(12):811-22.
- Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516.
- Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science*. 2004;305(5684):626-9.
- Desagher S, Martinou JC. Mitochondria as the central control point of apoptosis. *Trends Cell Biol*. 2000;10(9):369-77.
- Vander Heiden MG, Thompson CB. Bcl-2 proteins: regulators of apoptosis or of mitochondrial homeostasis?. *Nat Cell Biol*. 1999;1(8):E209-16.
- Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome *c*. *Cell Death Differ*. 2000;7(12):1166-73.
- Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. *J Cell Mol Med*. 2005;9(1):59-71.
- Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev*.

- 1999;79(1):143-80.
22. Cha TL, Qiu L, Chen CT, Wen Y, Hung MC. Emodin down regulates androgen receptor and inhibits prostate cancer cell growth. *Cancer Res.* 2005;65(6):2287-95.
 23. Huang Q, Shen HM, Shui G, Wenk MR, Ong CN. Emodin inhibits tumor cell adhesion through disruption of the membrane lipid raft-associated integrin signaling pathway. *Cancer Res.* 2006;66(11):5807-15.
 24. Huang Q, Lu G, Shen HM, Chung MC, Ong CN. Anti-cancer properties of anthraquinones from rhubarb. *Med Res Rev.* 2007;27(5):609-30.
 25. Dorsey JF, Kao GD. Aloe(-emodin) for cancer? more than just a comforting salve. *Cancer Biol Ther.* 2007;6(1):89-90.
 26. Ivanov VN, Bhoumik A, Ronai Z. Death receptors and melanoma resistance to apoptosis. *Oncogene.* 2003;22(20):3152-61.
 27. Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, Earnshaw WC. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ice. *Nature.* 1994;371(6495):346-7.