



Kahweol from Coffee Induces Apoptosis by Upregulating Activating Transcription Factor 3 in Human Colorectal Cancer Cells

Gwang Hun Park, Hun Min Song and Jin Boo Jeong*

Department of Bioresource Sciences, Andong National University, Andong 36729, Republic of Korea

Abstract

Kahweol as a coffee-specific diterpene has been reported to induce apoptosis in human cancer cells. Although some molecular targets for kahweol-mediated apoptosis have been elucidated, the further mechanism for apoptotic effect of kahweol is not known. Activating transcription factor 3 (ATF3) has been reported to be associated with apoptosis in colorectal cancer. The present study was performed to investigate the molecular mechanism by which kahweol stimulates ATF3 expression and apoptosis in human colorectal cancer cells. Kahweol increased apoptosis in human colorectal cancer cells. It also increased ATF3 expression through the transcriptional activity. The responsible cis-element for ATF3 transcriptional activation by kahweol was CREB located between -147 to -85 of ATF3 promoter. ATF3 overexpression increased kahweol-mediated cleaved PARP, while ATF3 knockdown attenuated the cleavage of PARP by kahweol. Inhibition of ERK1/2 and GSK3 β blocked kahweol-mediated ATF3 expression. The results suggest that kahweol induces apoptosis through ATF3-mediated pathway in human colorectal cancer cells.

Key Words: Activating transcription factor 3, Apoptosis, Coffee, Human colorectal cancer, Kahweol

INTRODUCTION

Epidemiological studies have shown that the coffee consumption can reduce the incidence of human colorectal cancer (Giovannucci, 1998). A meta-analysis that combined the results of 12 case-control studies and five prospective cohort studies have shown that daily coffee consumption can lower a risk of human colorectal cancer by 24% compared to abstinence from it (Giovannucci, 1998).

Although many components of coffee beans have been reported to exert cancer chemoprotective effects (Cavin *et al.*, 2002), kahweol as a diterpene molecule from coffee beans shows anti-cancer activity in human colorectal cancer (Choi *et al.*, 2015). Apart from human colorectal cancer, kahweol has anti-cancer properties against oral squamous cancer (Chae *et al.*, 2014), breast cancer (Cardenas *et al.*, 2014), pleural mesothelioma (Lee *et al.*, 2012), renal carcinoma (Um *et al.*, 2010) and lung cancer (Kim *et al.*, 2009). In mechanistic studies for apoptotic effect of kahweol, it stimulated the cleavage of caspase-3 and poly ADP-ribose polymerase (PARP) by inhibiting the phosphorylation of a signal transducer and activator of transcription 3 (STAT3) in lung adenocarcinoma cells (Kim *et al.*, 2009). In addition, kahweol has been reported to

sensitize TRAIL-induced apoptosis through down-regulation of Bcl-2 and c-FLIP in renal carcinoma cells (Um *et al.*, 2010). In human colorectal cancer cells, kahweol mediated apoptosis by suppressing heat shock protein 70 expression (Choi *et al.*, 2015). Although some molecular targets for kahweol-mediated apoptosis have been elucidated, the further mechanism for apoptotic effect of kahweol is not known.

Activating transcription factor 3 (ATF3) as a member of the ATF/CREB subfamily of the basic-region leucine zipper (bZIP) family (Hai and Hartman, 2001) exhibits tumor suppressor function in the development of human colorectal cancer. ATF3 can enhance the activation of p53 (Yan *et al.*, 2005), suppress Ras-mediated tumorigenesis, and down-regulate cyclin D1 (Lu *et al.*, 2006) and MMP-2 expression (Chen and Wang, 2004). A number of phytochemicals showing anti-cancer activity such as indole-3-carbinol (Lee *et al.*, 2005), conjugated linoleic acid (Lee *et al.*, 2006), epicatechin gallate (Baek *et al.*, 2004), and resveratrol (Whitlock *et al.*, 2011) have been reported to induce apoptosis by activating ATF3 expression. Furthermore, ATF3 is a target of some anticancer drugs such as cisplatin (St Germain *et al.*, 2010) and bortezomib (Bruning *et al.*, 2009).

In this report, we investigated the molecular mechanism of

Open Access <https://doi.org/10.4062/biomolther.2016.114>

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received May 25, 2016 Revised Jul 27, 2016 Accepted Aug 23, 2016
Published Online Nov 25, 2016

*Corresponding Author

E-mail: jjb0403@anu.ac.kr

Tel: +82-54-820-7757, Fax: +82-54-820-6252

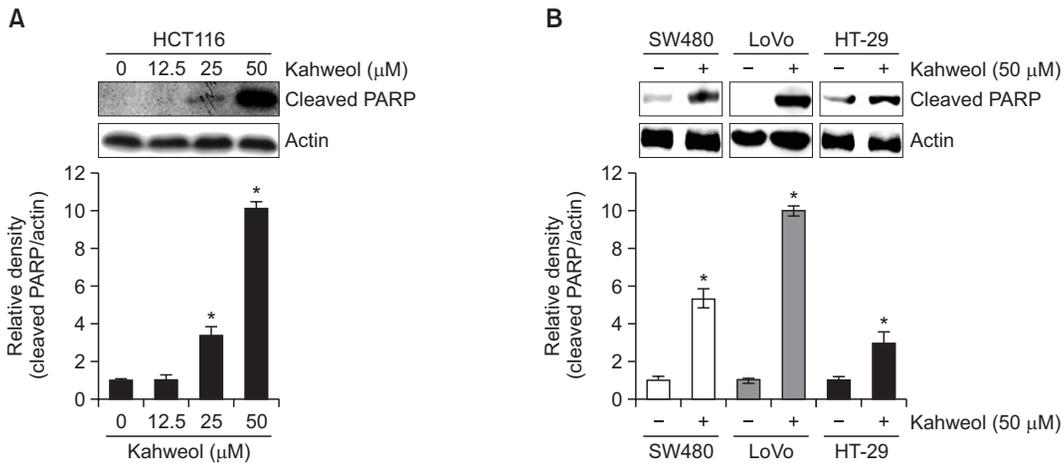


Fig. 1. The effect of kahweol on apoptosis in human colorectal cancer cells. (A) HCT116 cells were treated with 0, 12.5, 25 and 50 μM of kahweol for 24 h. (B) SW480, LoVo or HT-29 cells were treated with 50 μM of kahweol for 24 h. Cell lysates were subjected to SDS-PAGE and Western blot was performed using antibodies against cleaved PARP and actin. **p*<0.05 compared to cells without kahweol. All experiments were repeated three times.

kahweol-mediated ATF3 expression and apoptosis in human colorectal cancer cells.

MATERIALS AND METHODS

Chemical reagents

Kahweol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle medium (DMEM)/F-12 1:1 Modified medium (DMEM/F-12) was purchased from Lonza (Walkersville, MD, USA). Antibodies against ATF3, Poly ADP ribose polymerase (PARP) and β-actin were purchased from Cell Signaling (Beverly, MA, USA). PD98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor) and LiCl (GSK3β inhibitor) were purchased from Calbiochem (San Diego, CA, USA). ATF3 siRNA was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ATF3 promoter constructs used in this study were kindly provided by Dr. Seong Ho Lee (University of Maryland, College Park, MD, USA). All chemicals were purchased from Fisher Scientific (Hampton, NH, USA), unless otherwise specified.

Cell culture and treatment

Human colorectal cancer cell lines (HCT116, SW480, LoVo and HT-29) were purchased from Korean Cell Line Bank (Seoul, Korea) and grown in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were maintained at 37°C under a humidified atmosphere of 5% CO₂. Kahweol was dissolved in dimethyl sulfoxide (DMSO) and treated to cells. DMSO was used as a vehicle and the final DMSO concentration did not exceed 0.1% (v/v).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

After kahweol treatment, total RNA was prepared using a RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and 1 μg of total RNA was reverse-transcribed using a Verso cDNA Kit (Thermo Scientific, Pittsburgh, PA, USA) according to the manufacturer's protocol for cDNA synthesis. PCR was performed

using PCR Master Mix Kit (Promega, Madison, WI, USA) with human primers for ATF3 and GAPDH as followed: human ATF3: 5'-GTTTGAGGATTTTGCTAACCTGAC-3', and reverse 5'-AGCTGCAATCTTATTTCTTTCTCGT-3'; human GAPDH: forward 5'-ACCCAGAAGACTGTGGATGG-3' and reverse 5'-TTCTAGACGGCAGGTCAGGT-3'.

Transient transfections

Transient transfections were performed using the PolyJet DNA transfection reagent (SigmaGen Laboratories, Ijamsville, MD, USA) according to the manufacturers' instruction. Cells were plated in 12-well plates at a concentration of 2×10⁵ cells/well. After growth overnight, plasmid mixtures containing 1 μg of ATF3 promoter linked to luciferase and 0.1 μg of pRL-null vector were transfected for 24 h. The transfected cells were cultured in the absence or presence of kahweol. The cells were then harvested in 1×luciferase lysis buffer, and luciferase activity was normalized to the pRL-null luciferase activity using a dual-luciferase assay kit (Promega).

Transfection of small interference RNA (siRNA)

HCT116 cells were plated in 6-well plates and incubated overnight. HCT116 cells were transfected with control siRNA and ATF3 siRNA for 48 h at a concentration of 100 nM using TransIT-TKO transfection reagent (Mirus, Madison, WI, USA) according to the manufacturer's instruction. Then the cells were treated with 50 μM of kahweol for 24 h.

Expression vector

ATF3 expression vector was provided from Addgene (Cambridge, MA, USA). Transient transfection of the vector was performed using the PolyJet DNA transfection reagent (SigmaGen Laboratories) according to the manufacturers' instruction.

SDS-PAGE and Western blot

After kahweol treatment, cells were washed with 1×phosphate-buffered saline (PBS), and lysed in radioimmunoprecipitation assay (RIPA) buffer (Boston Bio Products, Ashland, MA, USA) supplemented with protease inhibitor cocktail (Sigma-AI-

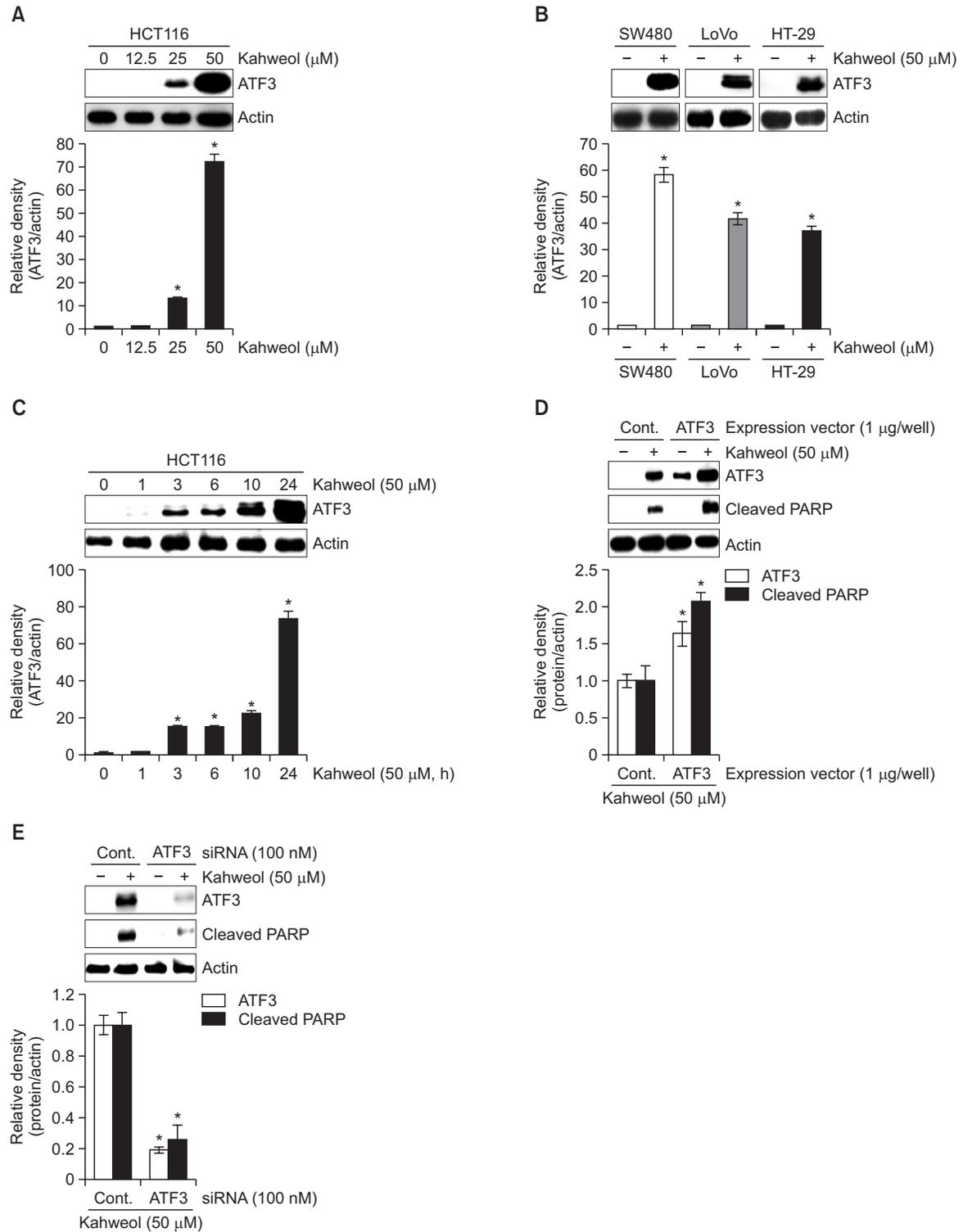


Fig. 2. The effect of kahweol on ATF3 expression and ATF3-mediated apoptosis in human colorectal cancer cells. HCT116 cells were treated with 0, 12.5, 25 and 50 μ M of kahweol for 24 h. (B) SW480, LoVo or HT-29 cells were treated with 50 μ M of kahweol for 24 h. (C) HCT116 cells were treated with 50 μ M of kahweol for the indicated times. (D) HCT116 cells were transfected with ATF3 expression vector for 24 h and then co-treated with 50 μ M of kahweol for the additional 24 h. (E) HCT116 cells were transfected with control- or ATF3 siRNA for 48 h and then co-treated with 50 μ M of kahweol for the additional 24 h. Cell lysates were subjected to SDS-PAGE and Western blot was performed using antibodies against ATF3, cleaved PARP or actin. * p <0.05 compared to cells without kahweol (A-C), control expression vector (D) or control siRNA (E). All experiments were repeated three times.

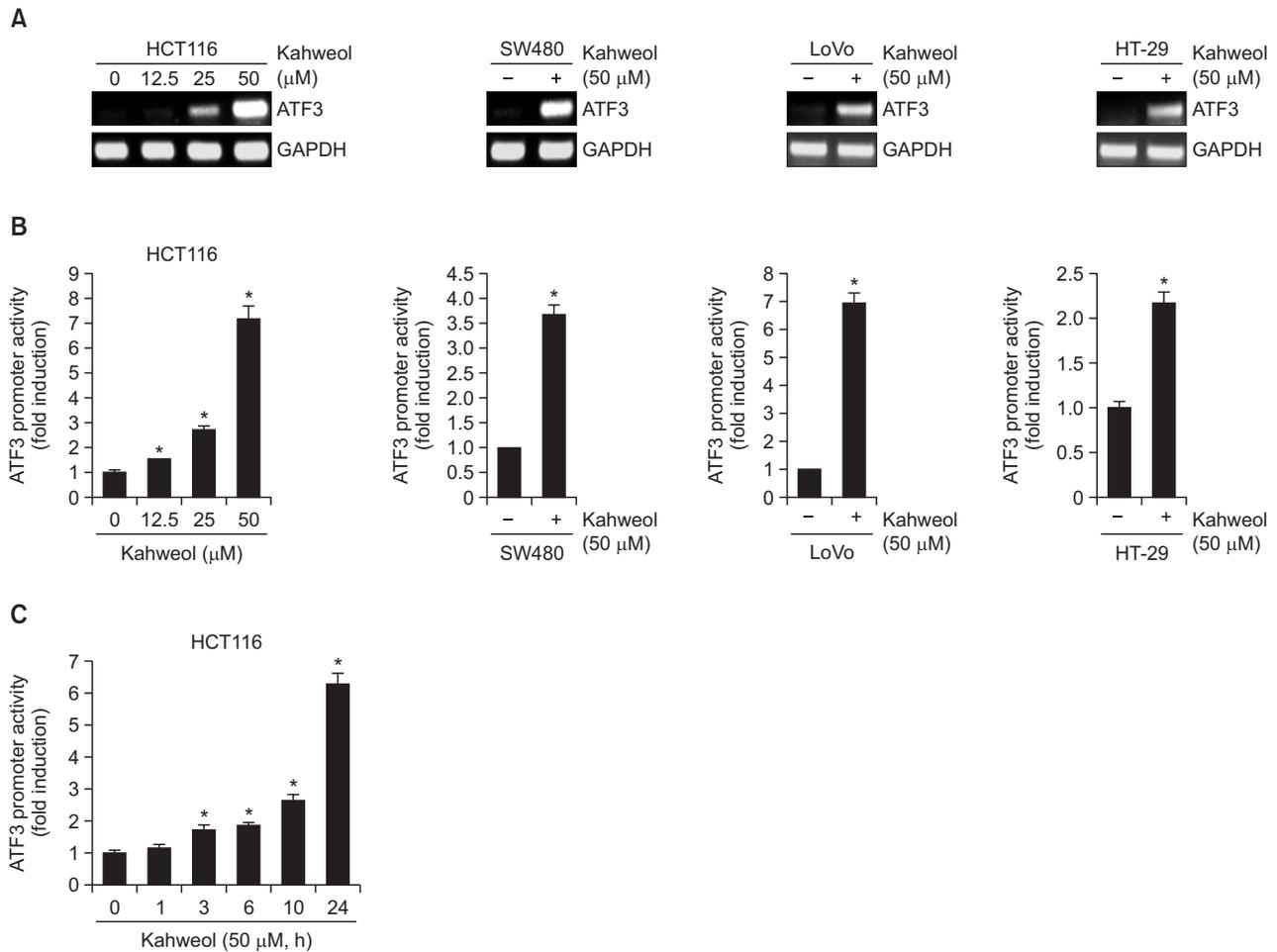


Fig. 3. The effect of kahweol on ATF3 transcriptional activation. (A) HCT116, SW480, LoVo or HT-29 cells treated with kahweol for 24 h and then total RNA was isolated and RT-PCR was performed. (B) The pATF3-1420/+34 construct (1 μg) was co-transfected with pRL-null vector (0.1 μg) into HCT116, SW480, LoVo or HT-29 cells. The cells were treated with kahweol for 24 h and then luciferase activity was measured. (C) The pATF3-1420/+34 construct (1 μg) was co-transfected with pRL-null vector (0.1 μg) into HCT116 cells. The cells were treated with 50 μM of kahweol for the indicated times and then luciferase activity was measured. **p*<0.05 compared to cells without kahweol. All experiments were repeated three times.

drich) and phosphatase inhibitor cocktail (Sigma-Aldrich), and centrifuged at 15,000×g for 10 min at 4°C. After determining protein concentration by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA), the proteins were separated on SDS-PAGE and transferred to PVDF membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked for non-specific binding with 5% non-fat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) for 1 h at room temperature and then incubated with specific primary antibodies in 5% non-fat dry milk at 4°C overnight. After three washes with TBS-T, the blots were incubated with horse radish peroxidase (HRP)-conjugated immunoglobulin G (IgG) for 1 h at room temperature and chemiluminescence was detected with ECL Western blotting substrate (Amersham Biosciences, Piscataway, NJ, USA) and visualized in Polaroid film.

Statistical analysis

All the data are shown as mean ± SEM (standard error of mean). Statistical analysis was performed with one-way ANOVA followed by Dunnett’s test. Differences with **p*<0.05 were

considered statistically significant.

RESULTS

Kahweol mediates apoptosis in human colorectal cancer cells

To determine pro-apoptotic effect of kahweol in human colorectal cancer cells, HCT116 cell were treated with 0, 12.5, 25 and 50 μM of kahweol for 24 h. After kahweol treatment, Western blot was performed against the cleaved PARP. As shown in Fig. 1A, cleaved PARP was slightly increased in HCT116 cells treated with 25 μM of kahweol. However, treatment of kahweol at 50 μM significantly increased the cleaved PARP. In addition, 50 μM of kahweol induced the cleaved PARP in SW480, LoVo and HT-29 cells (Fig. 1B). These findings indicate that kahweol may induce apoptosis in human colorectal cancer cells.

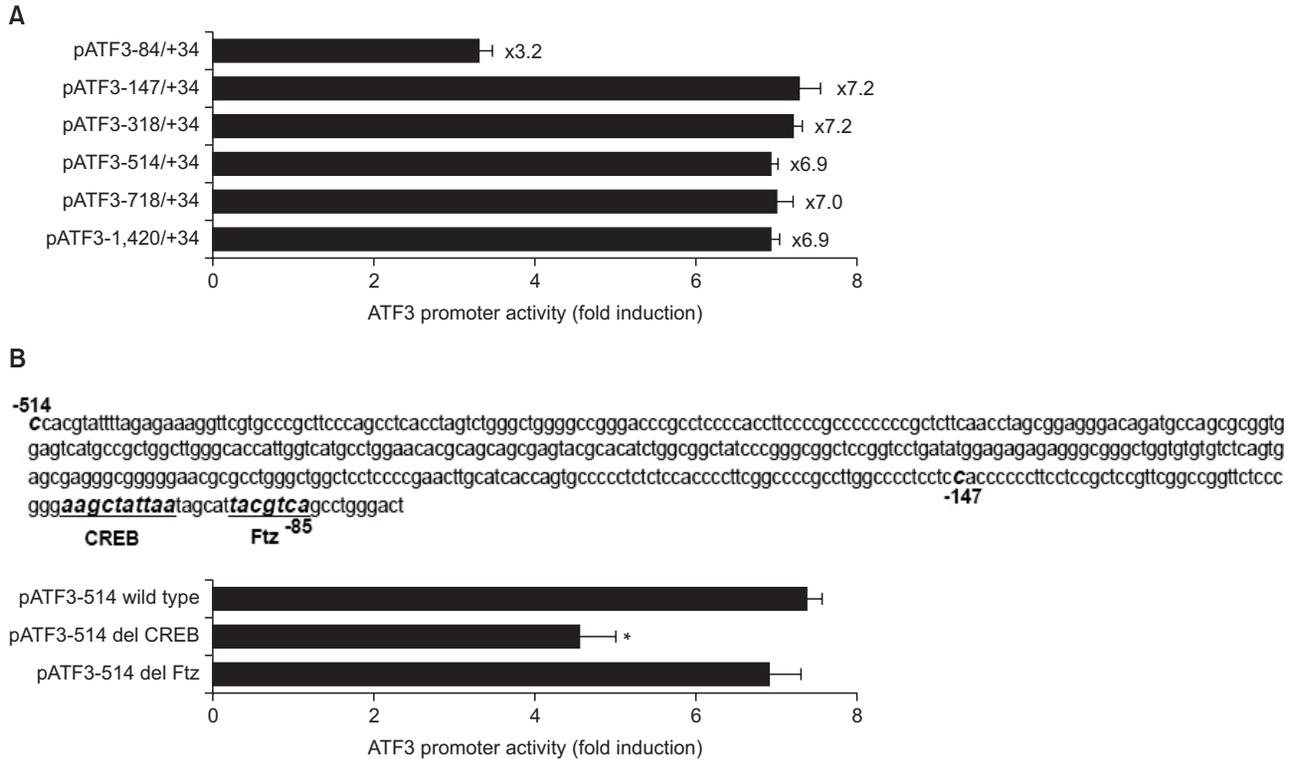


Fig. 4. Identification of ATF3 promoter sites responsible for kahweol-induced ATF3 transcriptional activation. (A, B) Each indicated construct of the ATF3 promoter (1 μ g) was co-transfected with 0.1 μ g of pRL-null vector into HCT116, and then cells were treated with 50 μ M of kahweol. Luciferase activity was measured. * $p < 0.05$ compared to cells transfected with luciferase construct containing -1420 to +34 of human ATF3 promoter region or pATF3-514 wild type. All experiments were repeated three times.

ATF3 expression contributes to kahweol-mediated apoptosis

To investigate whether kahweol affects ATF3 expression, the cells were treated with kahweol for 24 h and then Western blot was performed using ATF3 antibody. Kahweol dose-dependently increased ATF3 protein level in HCT116 cells (Fig. 2A). In addition, increased ATF3 protein level was observed in SW480, LoVo and HT-29 cells treated with kahweol (Fig. 2B). In time-course experiment, ATF3 protein level started increasing at 3 h after kahweol treatment (Fig. 2C). Next, we investigated the effect of ATF3 expression by kahweol on apoptosis using ATF3 expression construct and ATF3 siRNA. As shown in Fig. 2D, ATF3 overexpression increased PARP cleavage by kahweol. Furthermore, ATF3 knockdown by ATF3 siRNA attenuated kahweol-mediated cleavage of PARP (Fig. 2E). These findings indicate that kahweol-mediated ATF3 expression may contribute to apoptosis in human colorectal cancer cells.

Kahweol-mediated ATF3 expression results from transcriptional regulation

To determine whether the upregulation of ATF3 protein by kahweol is induced by transcriptional regulation, mRNA level was tested using RT-PCR. As shown in Fig. 3A, kahweol upregulated mRNA level of ATF3 in HCT116, SW480, LoVo or HT-29 cells, which was similar to the effect of kahweol on ATF3 protein level. To confirm that kahweol activates ATF3 transcription, ATF3 promoter activity was measured and we

observed that ATF3 promoter activity was also enhanced by kahweol treatment in HCT116, SW480, LoVo or HT-29 cells (Fig. 3B). In time-course experiment, ATF3 promoter activity started being activated at 3 h after kahweol treatment (Fig. 3C). These data indicate that kahweol-mediated ATF3 promoter activation may be responsible for the upregulation of ATF3 protein level in human colorectal cancer cells.

CREB is responsible for kahweol-induced ATF3 promoter activation

To determine a specific site of ATF3 promoter associated with kahweol-induced ATF3 promoter activation, ATF3 promoter luciferase constructs (pATF3-1420/+34, pATF3-718/+34, pATF3-514/+34, pATF3-318/+34, pATF3-147/+34 and pATF3-84/+34) were transfected into HCT116 cells and treated with 50 μ M of kahweol for 24 h. As shown in Fig. 4A, the fold inductions were 7.0, 7.0, 6.9, 7.2, 7.2 and 3.2 in pATF3-1420/+34, pATF3-718/+34, pATF3-514/+34, pATF3-318/+34, pATF3-147/+34 and pATF3-84/+34, respectively. Kahweol increased ATF3 promoter activity by 7-fold using ATF3 promoter construct containing -147 to -84 region while kahweol slightly increased ATF3 promoter activity using a construct without -147 to -85 region, which suggests that kahweol-responsible elements may be between the -147 and -85 region in ATF3 promoter. The Fushi tarazu (Ftz) and CREB have been reported to be cis-acting elements in ATF3 promoter containing -147 and -85 (Cho *et al.*, 2007). To identify the role of each cis-acting element, each site-deleted ATF3 promoter constructs were

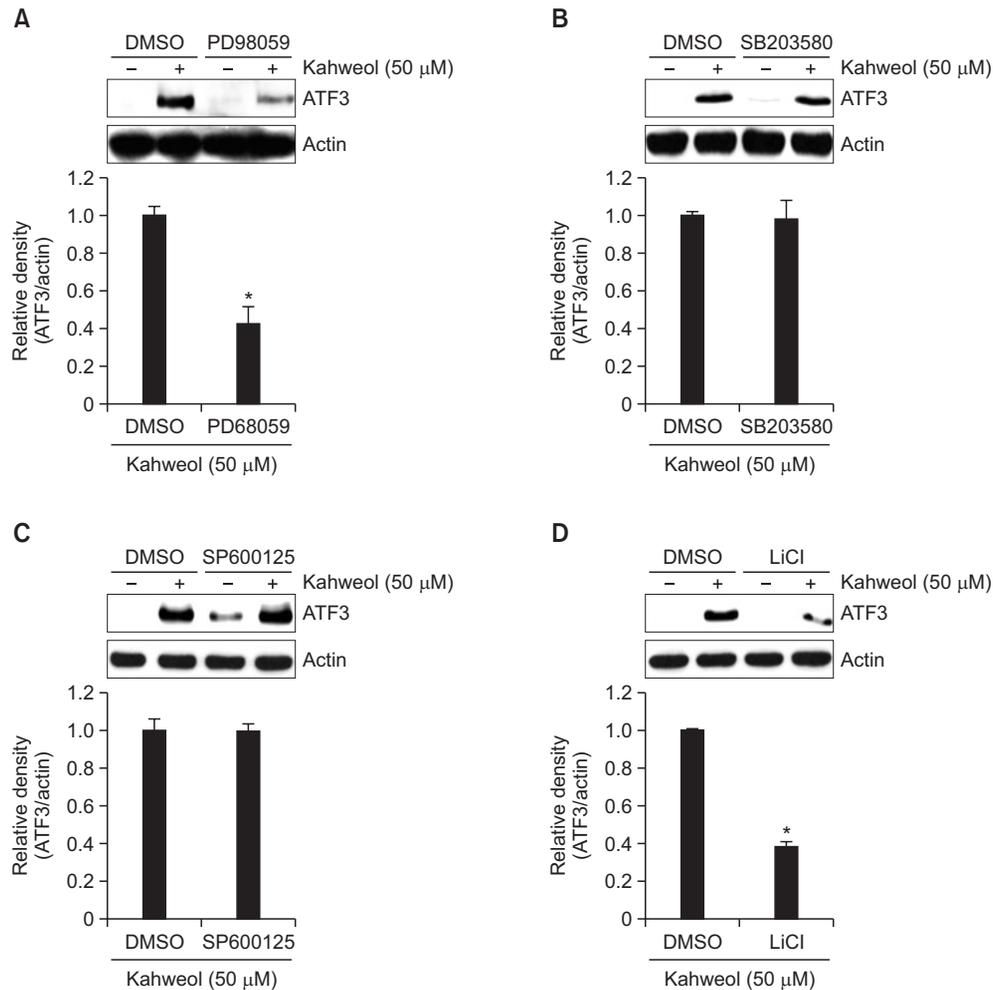


Fig. 5. Dependency of ERK1/2 and GSK3β in kahweol-mediated ATF3 expression. HCT116 cells were pretreated with PD98059 (40 μM, ERK1/2 inhibitor) (A), SB203580 (40 μM, p38 inhibitor) (B), SP600125 (40 μM, JNK inhibitor) (C) or LiCl (20 mM, GSK3β inhibitor) (D) for 2 h and then co-treated with 50 μM of kahweol for 10 h. Cell lysates were subjected to SDS-PAGE and Western blot was performed using antibodies against ATF3 or actin. **p*<0.05 compared to cells with DMSO and kahweol. All experiments were repeated three times.

transfected into HCT116 cells and treated with 50 μM of kahweol. As shown in Fig. 4B, kahweol-induced ATF3 promoter activity was significantly decreased when the CREB site was deleted. However, the deletion of Ftz sites did not affect ATF3 promoter activity by kahweol. These data indicate that CREB may be important for kahweol-induced ATF3 expression.

Kahweol-induced ATF3 expression is dependent on ERK1/2 and GSK3β

To elucidate the upstream kinases associated with ATF3 expression by kahweol, each kinase inhibitor such as PD98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor) or LiCl (GSK3β inhibitor) was pretreated in HCT116 cells and then co-treated with kahweol. As shown in Fig. 5, inhibition of ERK1/2 by PD98059 or GSK3β by LiCl attenuated kahweol-mediated ATF3 expression (Fig. 5A, 5D). However, inhibition of other kinase did not affect ATF3 expression by kahweol (Fig. 5B, 5C). These data indicate that ERK1/2 and GSK3β may be major upstream kinases for kahweol-mediated ATF3 expression.

DISCUSSION

Because many phytochemicals with anti-cancer activity act through the induction of apoptosis, induction of apoptosis has been regarded as an effective strategy in cancer chemoprevention and chemotherapy. Kahweol as a coffee-specific diterpene has been reported to induce apoptosis via downregulating STAT3 signaling pathway in human adenocarcinoma cells (Kim *et al.*, 2009). However, the other precise mechanisms of its apoptotic effect remain unclear. In the present study, we demonstrated that kahweol induces apoptosis via upregulation of ATF3 in human colorectal cancer cells. We showed that kahweol increases the cleavage level of PARP in HCT116, SW480, LoVo and HT-29 cells, indicating that human colorectal cancer cells treated with kahweol underwent apoptosis. In addition, we found that kahweol activates ATF3 expression and knockdown of ATF3 by siRNA or ATF3 overexpression by ATF3 expression vector decreases or increases kahweol-mediated cleavage of PARP, respectively. These findings suggest that ATF3 may be an important molecular target for kahweol-

mediated apoptosis. Indeed, ATF3 has been reported to play an important role for the induction of apoptosis by many anti-cancer phytochemicals such as indole-3-carbinol, conjugated linoleic acid, epicatechin gallate and resveratrol (Baek *et al.*, 2004; Lee *et al.*, 2005, 2006; Whitlock *et al.*, 2011). However, it is the first report to identify ATF3 as a molecular target in apoptotic effect of kahweol.

Furthermore, we found that kahweol-mediated increase of ATF3 protein level is through transcriptional regulation and kahweol-responsible promoter region is between the -147 and -85 region. The ATF3 promoter contains a variety of response elements (Liang *et al.*, 1996). In ATF3 promoter containing -147 to -85, Fushi tarazu (Ftz) and CREB have been reported to be cis-acting elements (Cho *et al.*, 2007). Deletion of CREB binding site attenuated ATF3 promoter activity by kahweol compared to wild type or deletion of Ftz. There is growing evidence that kahweol induces the phosphorylation of CREB (Saito *et al.*, 2015). These findings suggest that CREB is an important cis-acting element associated with kahweol-mediated ATF3 activation. However, we did not exclude other cis-acting elements containing -84 to +34 because kahweol slightly increased ATF3 promoter activity in the cells transfected with ATF3 promoter construct containing -84 to +34.

There is growing evidence that ATF3 expression is regulated by various upstream kinases including MAPK such as ERK1/2, p38 and JNK (Baek *et al.*, 2004; Lee *et al.*, 2010), and GSK3 β (Lee *et al.*, 2006). In this study, we found that inhibition of ERK1/2 by PD98059 and GSK3 β by LiCl attenuates kahweol-induced ATF3 expression, which ERK1/2 and GSK3 β may be important kinases for activating ATF3 expression by kahweol. However, further study is required to explain the more detailed information on how ATF3 interact with ERK1/2 or GSK3 β in response to kahweol.

The current data demonstrate that kahweol increases ERK1/2 or GSK3 β -dependent ATF3 expression through transcriptional regulation, which might be associated with the induction of apoptosis in human colorectal cancer cells. In addition, the current study can provide the additional information for the molecular target of kahweol's anti-cancer activity although many molecular targets for the anti-cancer activity of kahweol have been elucidated.

ACKNOWLEDGMENTS

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2014R1A1A2053448).

REFERENCES

- Baek, S. J., Kim, J. S., Jackson, F. R., Eling, T. E., McEntee, M. F. and Lee, S. H. (2004) Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. *Carcinogenesis* **25**, 2425-2432.
- Bruning, A., Burger, P., Vogel, M., Rahmeh, M., Friese, K., Lenhard, M. and Burges, A. (2009) Bortezomib treatment of ovarian cancer cells mediates endoplasmic reticulum stress, cell cycle arrest, and apoptosis. *Invest. New Drugs* **27**, 543-551.
- Cardenas, C., Quesada, A. R. and Medina, M. A. (2014) Insights on the antitumor effects of kahweol on human breast cancer: decreased survival and increased production of reactive oxygen species and cytotoxicity. *Biochem. Biophys. Res. Commun.* **447**, 452-458.
- Cavin, C., Holzhaeuser, D., Scharf, G., Constable, A., Huber, W. W. and Schilter, B. (2002) Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. *Food Chem. Toxicol.* **40**, 1155-1163.
- Chae, J. I., Jeon, Y. J. and Shim, J. H. (2014) Anti-proliferative properties of kahweol in oral squamous cancer through the regulation specificity protein 1. *Phytother. Res.* **28**, 1879-1886.
- Chen, H. H. and Wang, D. L. (2004) Nitric oxide inhibits matrix metalloproteinase-2 expression via the induction of activating transcription factor 3 in endothelial cells. *Mol. Pharmacol.* **65**, 1130-1140.
- Cho, K. N., Sukhthankar, M., Lee, S. H., Yoon, J. H. and Baek, S. J. (2007) Green tea catechin (-)-epicatechin gallate induces tumour suppressor protein ATF3 via EGR-1 activation. *Eur. J. Cancer* **43**, 2404-2412.
- Choi, D. W., Lim, M. S., Lee, J. W., Chun, W., Lee, S. H., Nam, Y. H., Park, J. M., Choi, D. H., Kang, C. D., Lee, S. J. and Park, S. C. (2015) The cytotoxicity of kahweol in HT-29 human colorectal cancer cells is mediated by apoptosis and suppression of heat shock protein 70 expression. *Biomol. Ther. (Seoul)* **23**, 128-133.
- Giovannucci, E. (1998) Meta-analysis of coffee consumption and risk of colorectal cancer. *Am. J. Epidemiol.* **147**, 1043-1052.
- Hai, T. and Hartman, M. G. (2001) The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene* **273**, 1-11.
- Kim, H. G., Hwang, Y. P. and Jeong, H. G. (2009) Kahweol blocks STAT3 phosphorylation and induces apoptosis in human lung adenocarcinoma A549 cells. *Toxicol. Lett.* **187**, 28-34.
- Lee, K. A., Chae, J. I. and Shim, J. H. (2012) Natural diterpenes from coffee, cafestol and kahweol induce apoptosis through regulation of specificity protein 1 expression in human malignant pleural mesothelioma. *J. Biomed. Sci.* **19**, 60.
- Lee, S. H., Bahn, J. H., Whitlock, N. C. and Baek, S. J. (2010) Activating transcription factor 2 (ATF2) controls tofenamic acid-induced ATF3 expression via MAP kinase pathways. *Oncogene* **29**, 5182-5192.
- Lee, S. H., Kim, J. S., Yamaguchi, K., Eling, T. E. and Baek, S. J. (2005) Indole-3-carbinol and 3,3'-diindolylmethane induce expression of NAG-1 in a p53-independent manner. *Biochem. Biophys. Res. Commun.* **328**, 63-69.
- Lee, S. H., Yamaguchi, K., Kim, J. S., Eling, T. E., Safe, S., Park, Y. and Baek, S. J. (2006) Conjugated linoleic acid stimulates an anti-tumorigenic protein NAG-1 in an isomer specific manner. *Carcinogenesis* **27**, 972-981.
- Liang, G., Wolfgang, C. D., Chen, B. P., Chen, T. H. and Hai, T. (1996) ATF3 gene. Genomic organization, promoter, and regulation. *J. Biol. Chem.* **271**, 1695-1701.
- Lu, D., Wolfgang, C. D. and Hai, T. (2006) Activating transcription factor 3, a stress-inducible gene, suppresses Ras-stimulated tumorigenesis. *J. Biol. Chem.* **281**, 10473-10481.
- Saito, T., Abe, D. and Nogata, Y. (2015) Coffee diterpenes potentiate the cytolytic activity of KHYG-1 NK Leukemia cells. *Food Sci. Technol. Res.* **21**, 281-284.
- St Germain, C., Niknejad, N., Ma, L., Garbuio, K., Hai, T. and Dimitroulakos, J. (2010) Cisplatin induces cytotoxicity through the mitogen-activated protein kinase pathways and activating transcription factor 3. *Neoplasia* **12**, 527-538.
- Um, H. J., Oh, J. H., Kim, Y. N., Choi, Y. H., Kim, S. H., Park, J. W. and Kwon, T. K. (2010) The coffee diterpene kahweol sensitizes TRAIL-induced apoptosis in renal carcinoma Caki cells through down-regulation of Bcl-2 and c-FLIP. *Chem. Biol. Interact.* **186**, 36-42.
- Whitlock, N. C., Bahn, J. H., Lee, S. H., Eling, T. E. and Baek, S. J. (2011) Resveratrol-induced apoptosis is mediated by early growth response-1, Krüppel-like factor 4, and activating transcription factor 3. *Cancer Prev. Res. (Phila)* **4**, 116-127.
- Yan, C., Lu, D., Hai, T. and Boyd, D. D. (2005) Activating transcription factor 3, a stress sensor, activates p53 by blocking its ubiquitination. *EMBO J.* **24**, 2425-2435.