Biochemical and Biophysical Research Communications 459 (2015) 591–596

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Chikusetsusaponin IVa methyl ester induces cell cycle arrest by the inhibition of nuclear translocation of β-catenin in HCT116 cells

Kyung-Mi Lee [a], Ji Ho Yun [b], Dong Hwa Lee [c], Young Gyun Park [b], Kun Ho Son [c], Chu Won Nho [b,*], Yeong Shik Kim [a,**]

[a] Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Republic of Korea
[b] Natural Products Research Center, Korea Institute of Science and Technology, Gangneung, 210-340, Republic of Korea
[c] Department of Food Science and Nutrition, Andong National University, Andong 760-749, Republic of Korea

ARTICLE INFO

Article history:
Received 23 February 2015
Available online 5 March 2015

Keywords:
Chikusetsusaponin IVa methyl ester
β-catenin
Cell cycle arrest
Apoptosis
Wnt

ABSTRACT

We demonstrate that chikusetsusaponin IVa methyl ester (CME), a triterpenoid saponin from the root of *Achyranthes japonica*, has an anticancer activity. We investigate its molecular mechanism in depth in HCT116 cells. CME reduces the amount of β-catenin in nucleus and inhibits the binding of β-catenin to specific DNA sequences (TCF binding elements, TBE) in target gene promoters. Thus, CME appears to decrease the expression of cell cycle regulatory proteins such as Cyclin D1, as a representative target for β-catenin, as well as CDK2 and CDK4. As a result of the decrease of the cell cycle regulatory proteins, CME inhibits cell proliferation by arresting the cell cycle at the G0/G1 phase. Therefore, we suggest that CME as a novel Wnt/β-catenin inhibitor can be a putative agent for the treatment of colorectal cancers.

1. Introduction

Colorectal cancer is caused by multiple transition steps, including the accumulation of genetic errors [1,2]. Previous studies discovered several critical genes and pathways that regulate the progression of colorectal cancers [3]. Among those mutated genes, genetic mutations in the Wnt/β-catenin signaling pathway increase the progression of colorectal cancer through the up-regulation of β-catenin transcriptional activity [4–6]. The Wnt/β-catenin signaling pathway affects cellular developmental processes and human carcinogenesis through the activation of genes associated with cell proliferation, such as c-jun, c-myc, fibronectin, cyclinD1 and fra-1 [7–11]. Thus, inhibition of the Wnt/β-catenin signaling pathway can be an important candidate for drug targeting for the treatment of colorectal cancers.

Inhibition of the Wnt/β-catenin signaling pathway may be a useful treatment for colorectal cancer through the induction of cell cycle arrest. Some compounds from natural products inhibit the Wnt/β-catenin signaling pathway. For example, quercetin, fisetin, curcumin, resveratrol and genistein are well-known inhibitors of the pathway. Moreover, lignans, which are cancer chemopreventive agents, have also been reported to inhibit the Wnt/β-catenin signaling pathway [12–16]. These previous studies imply that natural compounds, including dietary compounds, are potential inhibitors of the Wnt/β-catenin signaling pathway and may inhibit colon cancer proliferation. Among natural compounds, saponins are well known to have an anticancer activity [17–19]. However, it is not well known that saponins may serve as an inhibitor for Wnt/β-catenin signaling.

In this study, we investigated whether chikusetsusaponin IVa methyl ester (CME), a triterpenoid saponin from the root of *Achyranthes japonica*, disrupts Wnt/β-catenin signaling to inhibit cell proliferation in colon cancer cells. We elucidated the underlying mechanism of the regulation by treating cells with CME. We used a molecular assay to observe the cell cycle distribution and apoptosis and measured the ability of β-catenin binding, its transcriptional activity and changes in protein expression in HCT116 cells treated with CME.

2. Materials and methods

2.1. Plant material, extraction and isolation

The root of *A. japonica* was purchased and identified by Professor Jehyun Lee (College of Oriental Medicine, Dongguk

* Corresponding author. Fax: +82 33 650 3679.
** Corresponding author. Fax: +82 2 765 4768.
E-mail addresses: cwnho@kist.re.kr (C.W. Nho), kims@snu.ac.kr (Y.S. Kim).

http://dx.doi.org/10.1016/j.bbrc.2015.02.152
0006-291X/

University, Gyeongju, Korea). The dried root of *A. japonica* (12.15 kg) was extracted with methanol (60 ℓ) under reflux 3 times, and the filtrate was concentrated under reduced pressure to give the methanol extract (962.16 g). The methanol extract was suspended in H_2O and partitioned with hexane (20 ℓ), ethylacetate (20 ℓ) and butanol (20 ℓ) to yield hexane (45.04 g), ethylacetate (28.81 g), butanol (79.90 g) and H_2O (770.62 g) extracts, respectively. A portion of the butanol extract (76.37 g) was separated by RP-18 column chromatography with a CH_3CN:H_2O (gradient) to give 11 fractions. Fraction 4 was chromatographed again by silica gel column chromatography with $CHCl_3$: methanol (gradient) and an RP-18 column eluted with a gradient mixture of methanol and H_2O to give chickusetsusaponin IVa methyl ester. It's structure was characterized and identified based on the previous report [20].

2.2. Chemicals and reagents

The CDK4, CDK2, p21, β-actin and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against cyclin D1 and β-catenin were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against Lamin B1 were purchased from Abcam (UK).

2.3. Cell culture

HCT116 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). RPMI1640 was used for cell cultivation. The media was supplemented with 10% FBS (Hyclone, Logan, UT), penicillin (100 units/mL) and streptomycin (100 μg/mL). The cells were maintained at sub-confluence in 95% air and 5% CO_2 in a humidified atmosphere at 37 °C.

2.4. Flow cytometry analysis

To measure the cell cycle, we prepared samples as described previously [18]. The samples were analysed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA).

2.5. Transient transfection and luciferase assay

To measure β-catenin/TCF transcriptional activity, we performed a luciferase reporter assay (reporter plasmids; TOP Flash, FOP Flash, and pRL-CMV) as described previously [12].

2.6. Oligo pull down assay

To measure β-catenin binding activity in the nucleus, we performed an oligo pull-down assay as described previously [16].

2.7. Reverse transcription polymerase chain reaction

Total RNA (1 μg) was converted to cDNA by PCR (Genius FGEN05TD, Teche, England) using the iScript™ cDNA Synthesis Kit (BIO-RAD, Hercules, CA) according to the manufacturer's instructions. Reverse-transcription polymerase chain reaction (RT-PCR) analysis for cyclin D1, c-myc and β-actin was performed using an Applied Biosystems 7300 Real-Time PCR system and software (Applied Biosystems, Carlsbad, CA). The quantitative PCR was conducted in 0.2-mL PCR tubes with forward and reverse primers and a SYBR Green working solution (iTaq™ Universal SYBR Green Supermix, BIO-RAD, Hercules, CA) using a custom PCR master mix and the following conditions: followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. The sequences of the PCR primers were as follows: β-actin, 5′-CCCACTC CTAAGAGGAGGATG-3′ and 5′-AGGG AGACCAAAGCCTTCAT-3′; cyclin D1, 5′-AGCTCCTGTGCTGCGAAG TGGAAAC-3′ and 5′-AGTGTTCAATGAAATCGTG CGGGGT-3′; and c-myc, 5′- CTTCTCTCCGTCCTCGGATTCT-3′ and 5′- GAAGGTGATCC AGACTCTGACCTT-3′.

2.8. Western blot analysis

The cells were treated for different time periods with the indicated compounds or with vehicle only as a control. The cells were collected and lysed in lysis buffer (20 mM HEPES, 350 mM NaCl, 20% glycerol, 1% Nonidet P-40, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, protease inhibitor cocktail (Sigma Chemical, Saint Louis, MO) and a phosphatase inhibitor cocktail (Sigma Chemical, Saint Louis, MO). Total cell lysates were obtained following centrifugation at 12,000 g for 30 min at 4 °C. The protein concentration was determined using a protein assay kit (BIO-RAD, Hercules, CA). Nuclear proteins and cytoplasmic fractions were extracted using a nuclear extraction kit (Sigma Chemical, Saint Louis, MO) according to the manufacturer's instructions. Total cell lysates and nuclear fractions were separated by electrophoresis on 10% SDS-PAGE gels, and western blotting was carried out using standard protocols. The signal was detected with WEST-SAVE Up luminal-based ECL reagent (AB Frontier, Korea). Western blots were quantified by densitometry using a Las-4000 image reader and Multi Gauge 3.1 software (Fuji Photo Film Co., Japan).

2.9. Immunofluorescence assay

The cellular distribution of β-catenin was evaluated by an immunofluorescence assay using confocal microscopy (Leica, Wetzlar, Germany) as previously described [21]. In brief, HCT116 (7×10^3 cells/well) cells were seeded onto glass cover slips in 24-well plates and were incubated for 24 h prior to treatment. The primary antibody for β-catenin (1:150, Cell Signaling Technology, Beverly, MA) was incubated overnight at 4 °C. The secondary AlexaFluor488-conjugated anti-rabbit antibody (Invitrogen, Carlsbad, CA) was used at a 1:150 dilution for 1 h at room temperature. All antibodies were diluted with PBST containing 5% normal serum and 0.3% Triton X-100. Images were obtained using a Leica TCS SP5 confocal system.

2.10. Statistical analysis

The results are presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) with Dunnett's t-test was used for the statistical analysis of multiple comparisonsand p values less than 0.05 were considered statistically significant.

3. Results

3.1. CME inhibits cell proliferation in HCT116 cells

To investigate the anticancer activity of CME (the chemical structure shown in Fig. 1A) in colorectal cancer cells, we used HCT116 cells, which are colon cancer cells with mutations in the β-catenin gene [6]. First, we performed the cell viability assay that measures cellular mitochondrial dehydrogenase activity. We treated the cells with different concentrations of CME, as shown in Fig. 1B. CME decreased the cell proliferation of HCT116 cells in the range of 25 μM and 50 μM and caused severe cell death at concentrations above 50 μM (Fig. 1B) in HCT116 cells.

We next evaluated whether CME induces cell cycle arrest to inhibit cell proliferation by analysing the DNA content by flow cytometry. As shown in Fig. 1C, the proportion of cells in the G0/G1 phase changed from 40.9 % to 78.5 % at 20 μM CME and 63.0% at 30 μM CME in HCT116 cells. Also, CME increased the sub G1

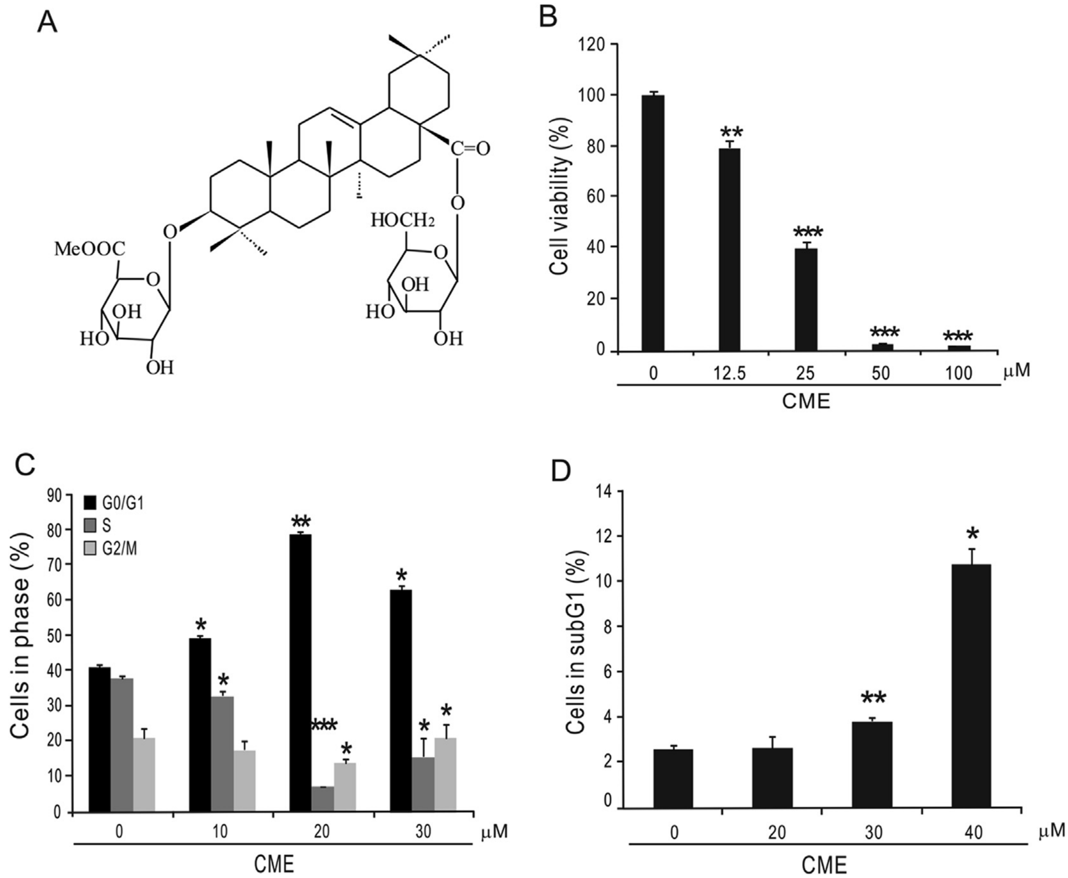

Fig. 1. Inhibition of cell proliferation by chikusetsusaponin IVa methyl ester (CME) in HCT116 cells. (A) The chemical structure of chikusetsusaponin IVa methyl ester. (B) Cell survival was determined using a cell viability assay. HCT116 cells were treated with CME at the indicated concentrations for 24 h. (C) Cell cycle distribution was measured by flow cytometry DNA content analysis (FACS) in HCT116 cells. (D) The sub G1 population was analysed by FACS in HCT116 cells. Bars represent the mean ± SD of three replicates; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, significant versus vehicle-treated control.

population of cells from 2.57% at 0 μM to 10.73% at 40 μM in HCT116 cells (Fig. 1D). These results suggested that CME inhibits cell proliferation by inducing cell cycle arrest at G0/G1 phase.

3.2. CME reduces cyclin D1, a representative target of Wnt/β-catenin signaling, to induce G0/G1 cell cycle arrest

As shown in Fig. 1C, we observed that CME induced cell cycle arrest in HCT116 cells through FACS analysis. To determine whether CME regulates cell cycle regulatory proteins to induce cell cycle arrest, we measured the expression of cell cycle regulatory proteins by western blot. We observed the expression of cyclin D1, which is a target of the Wnt/β-catenin signaling pathway and is known to regulate cell cycle arrest at G0/G1 phase [14,22]. HCT116 cells treated with CME showed a significant decrease in cyclin D1 expression in a time- and dose-dependent manner (Fig. 2A, B).

3.3. CME regulates cell cycle regulatory proteins including CDK2, CDK4, and p21

Next, we investigated whether CME also affects the expression of cell cycle regulatory proteins that are involved in the regulation of G1 progression and G1/S transition [22,23]. Therefore, we measured the expression level of other cell cycle regulatory proteins, including CDK2, CDK4, and p21. The expression of CDK4 and CDK2 was decreased, whereas the expression of p21 appeared to be increased in HCT116 cells treated with CME (Fig. 2A, B). These results showed that CME plays a crucial role in the co-regulation of cyclin D1 as well as cell cycle regulatory proteins, such as CDK 2, CDK 4 and p21.

3.4. CME inhibits TCF/β-catenin-dependent transcriptional activity by decreasing β-catenin binding to TCF binding element (TBE)

HCT116 cells have a mutated β-catenin that is not degraded by the proteasome system, resulting in abnormal cell proliferation [6]. We observed that CME significantly reduces the cyclin D1 protein, one of the main targets of the Wnt/β-catenin signaling pathway (Fig. 2A, B). In the present work, we investigated whether cell cycle arrest by CME involves the regulation of the Wnt/β-catenin signaling pathway. First, we transiently co-transfected cells with reporter plasmids, including TOP flash and FOP flash as a negative control and pRL-CMV for normalization. β-catenin transcriptional activity was decreased by CME in dose-dependent manner (Fig. 3A). To confirm the decrease of β-catenin transcriptional activity, we measured the binding of β-catenin to specific DNA sequences, called TCF-binding elements (TBE), in CME treated HCT116 cells (Fig. 3B). CME decreased the binding activity of β-catenin in a dose-dependent manner.

3.5. CME suppresses translocation of β-catenin to nucleus to reduce the expression of cyclin D1

β-catenin usually translocates from the cytoplasm to the nucleus to increase the transcriptional activity of target genes, such as cyclin D1 and c-myc [9,11]. To examine the accumulation of β-

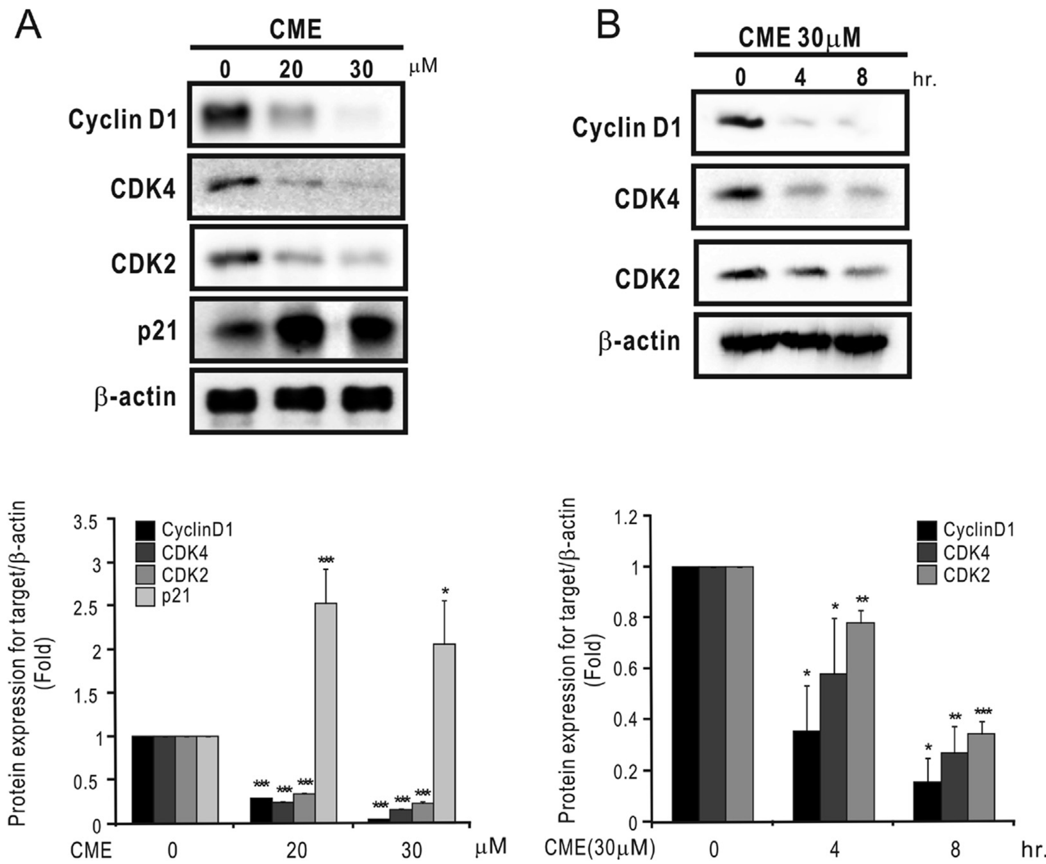

Fig. 2. Regulation of cyclin D1, a representative target of Wnt/β-catenin signaling, and cell cycle regulatory proteins by chikusetsusaponin IVa methyl ester (CME). HCT116 Cells were treated with 20 μM and 30 μM CME for 24 h (A) and 4 h, 8 h (B). Bars represent the mean ± SD of three replicates; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, significant versus each vehicle-treated control.

catenin in the nucleus, we measured the nuclear expression of β-catenin using immunofluorescence and used western blot analysis to assess the nuclear fractions (Fig. 3C and D). As shown in Fig. 3C and D, the level of β-catenin was decreased in the nucleus in both a dose- and time-dependent manner in HCT116 cells treated with CME. These results suggested that CME inhibits the Wnt/β-catenin signaling pathway by disrupting the binding of β-catenin to TBE in the promoters of target genes. After CME treatment, as the amount of β-catenin in the nucleus decreased, the mRNA expression of cyclin D1 and c-myc, which are targets of the Wnt/β-catenin signaling pathway, were decreased in HCT116 cells (Fig. 3E).

4. Discussion

Here, we report the anticancer activity of chikusetsusaponin IVa methyl ester (CME) and elucidate its molecular mechanism. CME induced cell cycle arrest by inhibiting β-catenin nuclear translocation in HCT116 cells. The Wnt/β-catenin signaling pathway is significant in human cancer because mutations of the pathway's components, such as Axin, APC and β-catenin, are common causes of cancer progression [24]. Mutations in the APC gene as a negative regulator of Wnt/β-catenin signaling are typically truncated and identified in the majority of sporadic colon cancer [24]. Additionally, mutations in the APC gene have been discovered in lung, ovarian and breast cancers [25–28]. Mutations in β-catenin, which abrogate its regulation by APC, represent an alternative method of Wnt activation and occur in sporadic cancers, including colon cancers and melanoma [6,29,30]. Mutations in the regulatory region of β-catenin or loss of APC function have been identified in human colon cancers [6].

Inhibitors of the Wnt/β-catenin signaling pathway from natural products are thought to be potential chemotherapeutic agents for colorectal cancer [12–16]. However, although previous study showed that saponins have an anticancer activity [17–19], the effects of saponins on Wnt/β-catenin signaling have been poorly understood. One recent report has described the regulation of the Wnt/β-catenin signaling pathway. It showed that the inhibitory effect of ginsenoside Rg3, a red ginseng saponin, is, at least in part, caused by diminishing nuclear translocation of the β-catenin protein and hence decreasing transcriptional activity of it [31]. In the present study, we found that CME strongly regulates the Wnt/β-catenin signaling pathway in HCT116 cells.

CME inhibited the transcriptional activity of β-catenin in HCT116 cells by disrupting the binding of β-catenin to the TCF binding element (TBE) (Fig. 3). Thus, CME caused G0/G1 arrest by inhibiting the nuclear translocation of β-catenin to the nucleus, inducing target genes, such as cyclin D1 or c-myc, which are involved in cell cycle regulation, to induce proliferation [7–9]. Like CME, curcumin inhibited the transcriptional activity of β-catenin to induce cell cycle arrest and apoptosis in HCT116 colon cancer cells [32]. However, the mechanism by which they disrupt nuclear translocation of β-catenin is still unclear. Investigating the detailed mechanism of the inhibition of β-catenin binding or β-catenin nuclear translocation by natural products may be an important approach for searching potential anticancer agents, such as CME, in colon cancer.

We measured the expression level of other crucial G1 phase cell cycle regulatory proteins in cells treated with CME. CME decreased the expression of not only cyclin D1 but also CDK4 and CDK2 which play a crucial role as cyclin-dependent kinases that control the G1/S

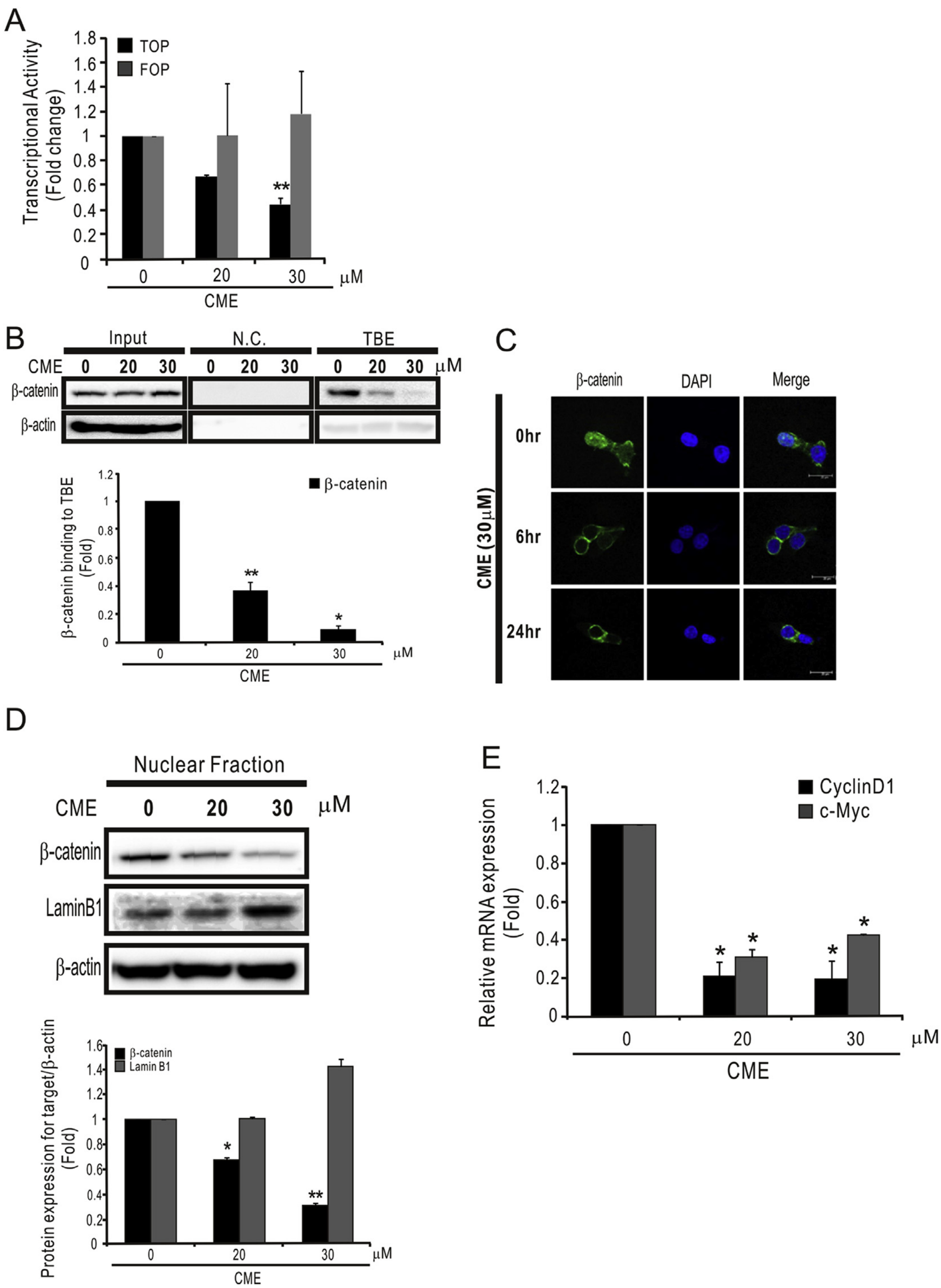

Fig. 3. Inhibition of TCF/β-catenin-dependent transcriptional activity by decreasing β-catenin binding to TCF binding element (TBE) and translocation to the nucleus. (A) Luciferase reporter assay. HCT116 cells were treated with luciferase reporter plasmids and then treated with 20 μM or 30 μM CME for 24 h. (B) Oligo pull-down assay in HCT116 cells with TBE elements. After cells were treated with 20 μM and 30 μM CME for 24 h, cell lysates were used for the oligo pull-down assay. Oligo with mutations in TBE elements is used as a negative control (N.C.). (C) Immunofluorescence staining of β-catenin nuclear translocation under 30 μM CME for 0 h, 6 h and 24 h in HCT116 cells (Bar = 20 μm). (D) Western blot analysis of nuclear fraction in HCT116 cells with 20 μM and 30 μM CME for 24 h. (E) Real-time PCR analysis of cyclin D1 and c-myc expression in HCT116 cells. The cells were treated with 20 μM and 30 μM CME for 24 h. Bars represent the mean ± SD of three replicates; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, significant versus vehicle-treated control.

transition and G1 progression [22,23] (Fig. 2). Interestingly, although CME attenuated the expression of p53 (data not shown), CME increased the expression of p21 (Fig. 2A), which is involved in cell cycle arrest regulation and is a target for p53 [22,23]. We suggested that the expression of p21 increased by CME in a p53-independent manner. Our results indicated that CME regulates multiple genes associated with the cell cycle and inhibits the Wnt/β-catenin signaling pathway.

Our data showed that CME induced apoptosis at concentrations above 30 μM CME (Fig. 1D). Although the apoptotic activity by CME

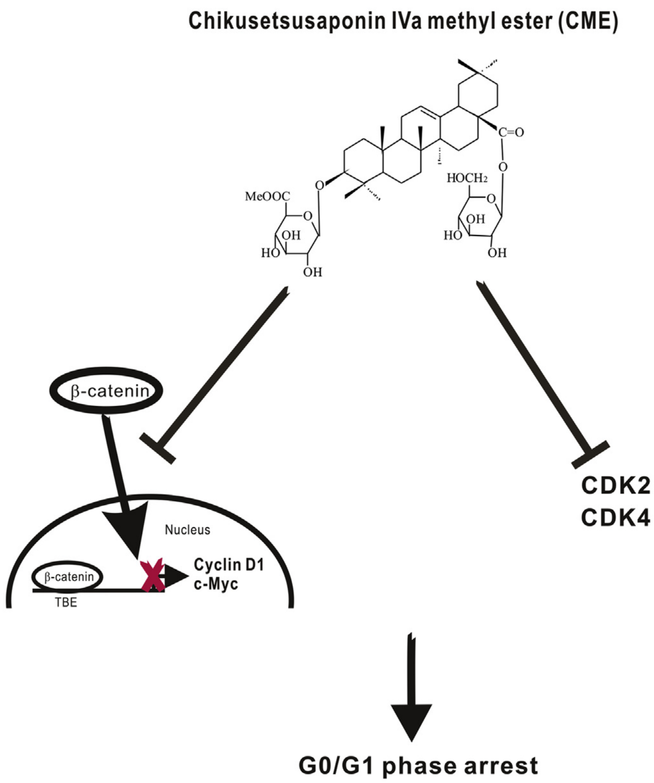

Fig. 4. Hypothetical model of chikusetsusaponin IVa methyl ester (CME)-induced cell cycle arrest. CME induces cell cycle arrest at G0/G1 phase through regulation of cell cycle regulatory proteins and inhibition of translocation of β-catenin to nucleus.

and the detailed molecular mechanisms remain unclear, we have evidence that CME inceases the transcription of SFRP1 gene, an antagonist for Wnt ligand [33] and further in-depth studies are needed.

Overall, we demonstrated that CME, isolated from the root of *A. japonica*, inhibited the Wnt/β-catenin signaling pathway to induce cell cycle arrest (Fig. 4). CME disrupted β-catenin nuclear translocation and the transcriptional activity of β-catenin also repressed in HCT116 cells. Thus, cyclin D1, a target gene for β-catenin, also decreased and was accompanied by a decrease in the expression of CDK2 and CDK4 in cell cycle arrest at the G0/G1 phase. Finally, CME strongly inhibited the cell proliferation in HCT116 cells. It is important to note that CME, a naturally occurring compound, might act as a putative anticancer drug in colon cancer even in the cases of mutations in Wnt/β-catenin signaling components.

Conflict of interest

None.

Acknowledgments

This work was supported by grants from the National Research Foundation of Korea (MRC 2009-93146) and KIST intramural research (2z04381).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.152.

References

[1] R.J. Davies, R. Miller, N. Coleman, Colorectal cancer screening: prospects for molecular stool analysis, Nat. Rev. Cancer 5 (2005) 199–209.
[2] A.J. Watson, An overview of apoptosis and the prevention of colorectal cancer, Crit. Rev. Oncol. Hematol. 57 (2006) 107–121.
[3] E.R. Fearon, Molecular genetics of colorectal cancer, Annu. Rev. Pathol. 6 (2011) 479–507.
[4] N. Barker, H. Clevers, Mining the Wnt pathway for cancer therapeutics, Nat. Rev. Drug Discov. 5 (2006) 997–1014.
[5] A. Klaus, W. Birchmeier, Wnt signalling and its impact on development and cancer, Nat. Rev. Cancer 8 (2008) 387–398.
[6] P.J. Morin, A.B. Sparks, V. Korinek, et al., Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC, Science 275 (1997) 1787–1790.
[7] K.M. Cadigan, R. Nusse, Wnt signaling: a common theme in animal development, Genes. Dev. 11 (1997) 3286–3305.
[8] P. Polakis, Wnt signaling and cancer, Genes. Dev. 14 (2000) 1837–1851.
[9] T.C. He, A.B. Sparks, C. Rago, et al., Identification of c-MYC as a target of the APC pathway, Science 281 (1998) 1509–1512.
[10] B. Mann, M. Gelos, A. Siedow, et al., Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1603–1608.
[11] O. Tetsu, F. McCormick, Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature 398 (1999) 422–426.
[12] J.H. Yoo, H.J. Lee, K. Kang, et al., Lignans inhibit cell growth via regulation of Wnt/beta-catenin signaling, Food Chem. Toxicol. 48 (2010) 2247–2252.
[13] M.H. Teiten, F. Gaascht, M. Dicato, et al., Targeting the wingless signaling pathway with natural compounds as chemopreventive or chemotherapeutic agents, Curr. Pharm. Biotechnol. 13 (2012) 245–254.
[14] R.S. Tarapore, I.A. Siddiqui, H. Mukhtar, Modulation of Wnt/beta-catenin signaling pathway by bioactive food components, Carcinogenesis 33 (2012) 483–491.
[15] C.H. Park, J.Y. Chang, E.R. Hahm, et al., Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells, Biochem. Biophys. Res. Commun. 328 (2005) 227–234.
[16] K. Kang, K.M. Lee, J.H. Yoo, et al., Dibenzocyclooctadiene lignans, gomisins J and N inhibit the Wnt/beta-catenin signaling pathway in HCT116 cells, Biochem. Biophys. Res. Commun. 428 (2012) 285–291.
[17] J. Chun, M. Kang, Y.S. Kim, A triterpenoid saponin from Adenophora triphylla var. japonica suppresses the growth of human gastric cancer cells via regulation of apoptosis and autophagy, Tumor Biol. 35 (2014) 12021–12030.
[18] M.Y. Xu, D.H. Lee, E.J. Joo, et al., Akebia saponin PA induces autophagic and apoptotic cell death in AGS human gastric cancer cells, Food Chem. Toxicol. 59 (2013) 703–708.
[19] M.Y. Xu, S.Y. Lee, S.S. Kang, et al., Antitumor activity of jujuboside B and the underlying mechanism via induction of apoptosis and autophagy, J. Nat. Prod. 77 (2014) 370–376.
[20] S. Sakai, M. Katsumata, Y. Satoh, et al., Oleanolic acid saponins from root Bark of Aralia-Elata, Phytochemistry 35 (1994) 1319–1324.
[21] J.H. Yun, S.B. Lee, H.J. Lee, et al., Bi-functional induction of the quinone reductase and cytochrome P450 1A1 by youngiasides via Nrf2-ARE and AhR-XRE pathways, Biol. Pharm. Bull. 33 (2010) 1650–1657.
[22] J.A. Diehl, Cycling to cancer with cyclin D1, Cancer Biol. Ther. 1 (2002) 226–231.
[23] A. Eastman, Cell cycle checkpoints and their impact on anticancer therapeutic strategies, J. Cell. Biochem. 91 (2004) 223–231.
[24] P. Polakis, The many ways of Wnt in cancer, Curr. Opin. Genet. Dev. 17 (2007) 45–51.
[25] K. Furuuchi, M. Tada, H. Yamada, et al., Somatic mutations of the APC gene in primary breast cancers, Am. J. Pathol. 156 (2000) 1997–2005.
[26] H. Ohgaki, J.M. Kros, Y. Okamoto, et al., APC mutations are infrequent but present in human lung cancer, Cancer Lett. 207 (2004) 197–203.
[27] Y.L. Wallis, D.G. Morton, C.M. McKeown, et al., Molecular analysis of the APC gene in 205 families: extended genotype-phenotype correlations in FAP and evidence for the role of APC amino acid changes in colorectal cancer predisposition, J. Med. Genet. 36 (1999) 14–20.
[28] R. Wu, Y. Zhai, E.R. Fearon, et al., Diverse mechanisms of beta-catenin deregulation in ovarian endometrioid adenocarcinomas, Cancer Res. 61 (2001) 8247–8255.
[29] V. Korinek, N. Barker, P.J. Morin, et al., Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma, Science 275 (1997) 1784–1787.
[30] B. Rubinfeld, P. Robbins, M. El-Gamil, et al., Stabilization of beta-catenin by genetic defects in melanoma cell lines, Science 275 (1997) 1790–1792.
[31] B.C. He, J.L. Gao, X.J. Luo, et al., Ginsenoside Rg3 inhibits colorectal tumor growth through the down-regulation of Wnt/beta-catenin signaling, Int. J. Oncol. 38 (2011) 437–445.
[32] A.S. Jaiswal, B.P. Marlow, N. Gupta, et al., Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells, Oncogene 21 (2002) 8414–8427.
[33] H. Suzuki, D.N. Watkins, K.W. Jair, et al., Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer, Nat. Genet. 36 (2004) 417–422.