Biochemical and Biophysical Research Communications 442 (2013) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Caffeic acid phenethyl ester downregulates phospholipase D1 via direct binding and inhibition of NFκB transactivation

Mi Hee Park [a,1], Dong Woo Kang [a,1], Yunjin Jung [b], Kang-Yell Choi [c], Do Sik Min [a,*]

[a] Department of Molecular Biology, Pusan National University, Busan 609-735, Republic of Korea
[b] College of Pharmacy, Pusan National University, Busan 609-735, Republic of Korea
[c] Translational Research Center for Protein Function Control, Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 17 September 2013
Available online 5 October 2013

Keywords:
Caffeic acid phenethyl ester
Phospholipase D
Glioma cells
Invasion
Proliferation

ABSTRACT

Upregulation of phospholipase D (PLD) is functionally linked with oncogenic signals and tumorigenesis. Caffeic acid phenethyl ester (CAPE) is an active compound of propolis extract that exhibits anti-proliferative, anti-inflammatory, anti-oxidant, and antineoplastic properties. In this study, we demonstrated that CAPE suppressed the expression of PLD1 at the transcriptional level via inhibition of binding of NFκB to PLD1 promoter. Moreover, CAPE, but not its analogs, bound to a Cys837 residue of PLD1 and inhibited enzymatic activity of PLD. CAPE also decreased activation of matrix metalloproteinases-2 induced by phosphatidic acid, a product of PLD activity. Ultimately, CAPE-induced downregulation of PLD1 suppressed invasion and proliferation of glioma cells. Taken together, the results of this study indicate that CAPE might contribute to anti-neoplastic effect by targeting PLD1.

1. Introduction

Gliomas are considered the most malignant form of brain tumors, and are ranked among the most aggressive human cancers. Gliomas are composed of a complex milieu of tumor cells that interact in close proximity with the components of the tumor microenvironment, including stromal cells and extracellular matrix (ECM). Interactions between tumor cells and their microenvironment are critical to cancer cell proliferation and invasion into the brain parenchyma [1]. Accumulating evidence indicates that invasive glioma cells show resistance to apoptosis, which may contribute to their resistance to chemotherapy and radiation [2].

Degradation of ECM results in the promotion of cancer cell mobility, invasion, and metastasis, and this process is accomplished in part by tumor cell-secreted matrix metalloproteases (MMPs) [3,4]. Glioma cells express different types of MMPs to successfully cross brain structures [5].

Caffeic acid phenethyl ester (CAPE), an active component of honeybee propolis extract, has been used as a traditional medicine for many years. [6]. CAPE possesses several biological properties including antioxidant, anti-inflammatory, antiviral, immune stimulatory and anticancer activities. At the molecular level, CAPE modulates the activities of MMP-2, MMP-9, focal adhesion kinase, inducible nitric oxide synthase and cyclooxygenase-2, which all play a role in tumorigenesis [7,8]. CAPE is a well-documented inhibitor of NFκB, which may be an action mechanism for CAPE-mediated anti-inflammatory and anti-tumor effects [9]. However, molecular targets and structural analysis of CAPE via NFκB inhibition have yet to be investigated.

We recently reported that overexpression of phospholipase D (PLD) promotes invasion by enhancing MMP-2 gene transcription via NFκB-mediated signaling pathways in glioma cells [10]. PLD catalyzes the hydrolysis of phosphatidylcholine to generate free choline and phosphatidic acid (PA), a lipid mediator. Two mammalian isozymes, PLD1 and PLD2, have been identified to date [11,12].

Abnormalities in the expression and activity of PLD have been observed in many human cancers [13]; therefore, it has been suggested that the elevated activity and expression of PLD are functionally linked with invasion, a critical aspect of tumor biology. Despite gathering evidence regarding the regulation of PLD activity in cell function, little is known about regulatory mechanisms of PLD expression. In this study, we investigated the effects of CAPE on PLD1 expression and the underlying molecular mechanisms. We found that CAPE downregulates the expression and activity of PLD1 via inhibition of NFκB transactivation and direct binding to PLD1, followed by suppression of invasion and proliferation of glioma cells.

* Corresponding author. Address: Department of Molecular Biology, College of Natural Science, Pusan National University, 30 Jangjeon dong, Geumjeong gu, Busan 609-735, Republic of Korea. Fax: +82 51 513 9258.
E-mail address: minds@pusan.ac.kr (D.S. Min).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.105

2. Materials and methods

2.1. Cell lines and materials

Human U87MG glioma cells were purchased from ATCC (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum containing 1% antibiotic–antimycotic (Gibco BRL, Grand Island, NY) and 15 mmol/L HEPES (pH 7.4). Phosphatidic acid was purchased from Sigma (Sigma, St. Louis, MO). CAPE was obtained from Alexis (San Diego, CA). siRNAs for control and PLD1 were purchased from Dharmacon Research Inc. (Lafayette, CO). The siRNA sequence for human PLD1 (nucleotides 1571–1591) was as follows: 5′-AAGGUGGGACGACAAUGAGCA-3′.

2.2. Transient transfection and reporter gene assay

Cells were seeded in 24-well plates and transiently transfected with 300 ng of promoter constructs and 30 ng of pRL-TK (internal control). The activities of *firefly* and *Renilla* luciferase in the cellular extracts were subsequently measured using a Dual-Luciferase Assay kit (Promega, WI) according to the manufacturer's instructions. Wild type PLD1 promoter (pGL4-PLD1-Luc) and NFκB binding site mutant of PLD1 promoter have been described elsewhere [14]. pGL2–3X NFκB containing three tandem repeats of the NFκB-binding motif was used for the NFκB transactivation assay.

2.3. Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed as previously described [10], with minor modifications. The PLD1 promoter region that contains putative NFκB binding sites was analyzed by PCR using the following primers: for NFκB-A, 5′-CCT TAT CTA TAA AGA GGG GAT GGC-3′ (forward) and 5′-GTC GGG AGA AAG AGA CTG TGT TTG G-3′ (reverse); for NFκB-B, 5′-CAC ACA GAG CAG GCT GAA TTG-3′ (forward) and 5′-GCT CAG ATC ATC CGT CTT TAC C-3′ (reverse).

2.4. Western blotting

Immunoblot analysis was performed as previously described [15], and enhanced chemiluminescence was used for signal detection. Anti-α-tubulin and anti-MMP-2 antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). A polyclonal anti-PLD antibody that recognizes both PLD1 and PLD2 was generated as previously described [16].

2.5. PLD activity assay

PLD activity was assessed by measuring the formation of [^{3}H] phosphatidylbutanol, the product of PLD-mediated transphosphatidylation, in the presence of 1-butanol. Briefly, U87MG cells were seeded and serum-starved in the presence of 3 μCi of [^{3}H] myristic acid/ml for 12 h, after which they were washed and pretreated with CAPE for 20 min and 0.3% 1-butanol was added. After 10 min, the cells were treated with the drugs for 30 min. Extraction and characterization of the lipids by thin-layer chromatography were then performed as previously described [15].

2.6. In vitro PLD1 capture assay

Biotinylated-CAPE or CAPE analogues were incubated with cell lysates or GST-PLD1 fragments. After 2 h, streptavidin beads

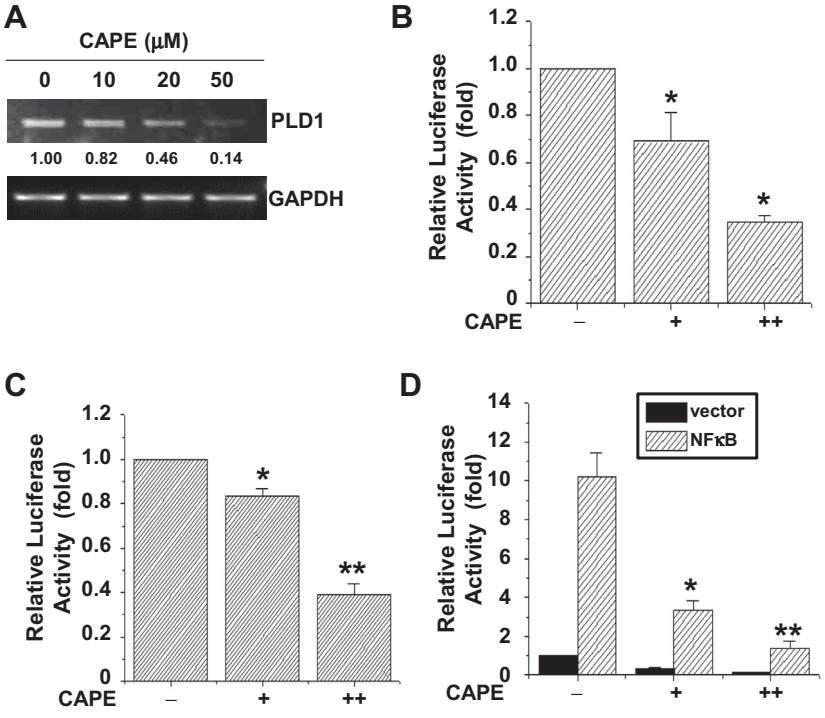

Fig. 1. CAPE inhibits PLD1 expression by suppression of NFkB transactivation. (A) U87 cells were treated with the indicated concentrations of CAPE for 24 h, after which total RNA was isolated and subjected to RT-PCR and the mRNA level of PLD1 was determined by densitometer analysis. (B) U87 cells were transfected with pGL4-PLD1 for 24 h and treated with CAPE (10 μM, 20 μM) for 24 h, after which the luciferase activity was measured.*$P < 0.001$ *versus* non-treatment. (C) U87 cells were transfected with pGL2–3X NFκB for 24 h and then treated with CAPE for 24 h, after which the luciferase activity was measured. Data are expressed as the means ± SD of four independent experiments.*$P < 0.01$ or **$P < 0.005$ *versus* control. (D) The U87 cells were cotransfected with pGL4-PLD1 and NFκB (p65) and the luciferase activity was measured. Data are expressed as the means ± SD of six independent experiments. *$P < 0.001$ or **$P < 0.05$ *versus* NFκB-transfected cells. (E) The U87 cells were transfected with wild type or NFκB-binding site mutant of pGL4-PLD1 and then treated with 20 μM of CAPE, after which the luciferase activity was measured. Data are expressed as the means ± SD of six independent experiments. NS non-significant *$P < 0.001$ or **$P < 0.005$ *versus* vehicle-treated cells. (F) ChIP assay was performed using preimmune IgG or anti-NFκB antibody in glioma cells treated with vehicle or CAPE. Data are representative of three independent experiments.

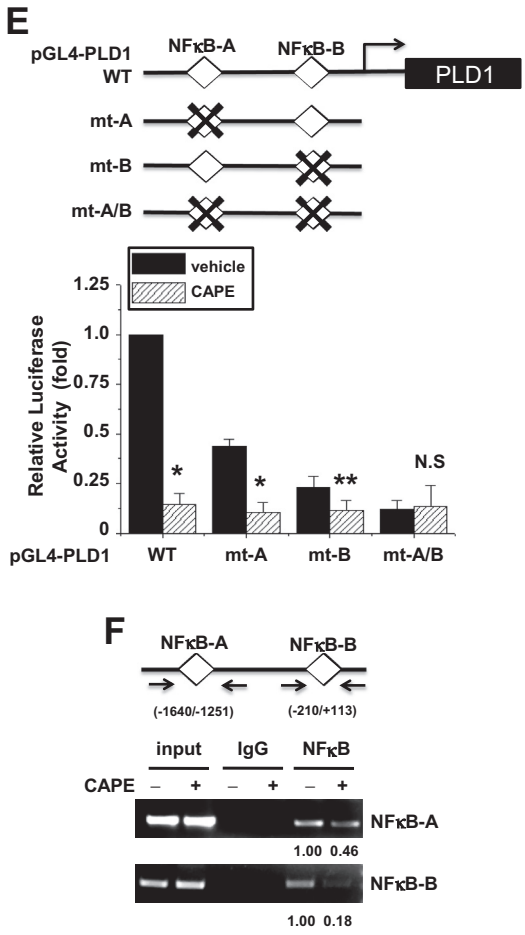

Fig. 1 (*continued*)

(Pierce, Rockford, IL) were added and the samples were incubated overnight at 4 °C. The beads were then washed and SDS Laemmli buffer was added, followed by immunoblot with antibody to PLD.

2.7. Gelatin zymography

U87MG cells were seeded and refreshed with serum-free DMEM, after which the activity of electrophoretically separated gelatinolytic enzymes in the conditioned media was analyzed. Briefly, equal amounts of conditioned media were mixed with 2× zymogram buffer and then electrophoresed on SDS–PAGE gels containing 1 mg/ml gelatin. Following electrophoresis, the gels were washed with 2.5% Triton X-100 and incubated with developing buffer, then stained with 2% Coomassie blue solution. Zones of gelatinolytic activity were detected as clear bands on a dark background.

2.8. Invasion assay

An invasion assay was conducted using Boyden chambers with a polycarbonate nucleopore membrane (Corning, Corning, NY). Cells were seeded onto the upper portion of a 24-well Matrigel chamber in serum-free media, while the lower compartment contained DMEM with 10% FBS. Cells that migrated to the lower surface of the filter were fixed and stained with crystal violet, then counted in five random fields per well, and the extent of invasion was expressed as the average number of cells per microscopic field.

3. Results

3.1. CAPE downregulates PLD1 expression via inhibition of NFκB transactivation in glioma cells

Since CAPE is known to inhibit NFκB activation [18] and PLD1 expression is upregulated via NFκB (11), we examined whether CAPE regulates PLD1 expression in U87MG glioma cells. CAPE decreased the mRNA level of PLD1 in a dose-dependent manner (Fig. 1A). CAPE also inhibited the promoter activity of PLD1 (Fig. 1B). To investigate whether NFκB is involved in CAPE-induced PLD1 suppression, we examined its effects on NFκB transactivation using a luciferase reporter gene containing a triple tandem repeat of an NFκB-binding motif. CAPE decreased NFκB transactivation (Fig. 1C). We further examined whether CAPE-induced inhibition of NFκB transactivation is responsible for downregulation of PLD1 and found that CAPE attenuated NFκB (p65)-dependent transcriptional activation of PLD1 (Fig. 1D). We recently identified two NFκB binding sites on the PLD1 promoter, NFκB-A; −1640/−1251 and NFκB-B; −210/+113 [11]. Mutagenesis of the NFκB binding sites significantly inhibited PLD1 promoter activity (Fig. 1E). When either NFκB binding site was mutated, CAPE still exerted an inhibitory effect on PLD1 promoter activity. However, when both NFκB binding sites were mutated, CAPE showed no inhibitory effect on PLD1 promoter activity (Fig. 1E). We further examined whether CAPE suppresses binding of NFκB to the PLD1 promoter. As shown in Fig. 1F, CHIP assay revealed that CAPE abolished the binding of NFκB to the PLD1 promoter. Taken together, these results suggest that CAPE downregulates PLD1 expression via suppression of NFκB transactivation.

3.2. Both the catechol moiety and MRA are required for inhibitory potency of PLD1 expression

CAPE decreased the protein level of PLD1 in a dose and time-dependent manner in U87MG glioma cells (Fig. 2A). Downregulation of PLD1 protein was detected after 12 h of CAPE treatment. In glioma cells, PLD1 is a major isoform of PLD, and PLD2 could not be detected by antibody to PLD, which recognizes both PLD1 and PLD2. We also found that CAPE significantly inhibited the expression of PLD1 in various cancer cell lines including breast, colon, and lung cancer cells (data not shown). To analyze the structure of CAPE needed to inhibit PLD1 expression, CAPE analogues showing variation in the aromatic substituent or side chain, namely dihydroxydihydrocinnamic acid phenethylester (DHHC) and dimethoxycinnamic acid phenethyl ester (DMC), were prepared (Fig. 2C). While CAPE has electrophiles, i.e., Michael reaction acceptor (MRA) and catechol moiety, DMC (with MRA) and DHHC (with catechol) each have only one electrophilic functional group. The ability of the analogues to inhibit PLD1 expression was examined by transfecting glioma cells with PLD1-luciferase reporter constructs and then treating them with the analogues. As shown in Fig. 2D, the inhibitory potency of CAPE was greater than that of DHHC and DMC. We further confirmed the effect of CAPE and its analogues on the expression of PLD1 protein (Fig. 2E). When glioma cells were treated for 24 h, CAPE showed the greatest inhibitory effect on PLD protein level, while the potency of DHH and DMC was lower than that of CAPE. Taken together, these results suggest that both the catechol moiety and MRA are involved in inhibition of PLD1 expression.

3.3. CAPE covalently binds to PLD1 and inhibits PLD1 activity

CAPE was recently reported to covalently bind to cysteine residues of Keap1 protein and release Nrf2 from Keap1 repression,

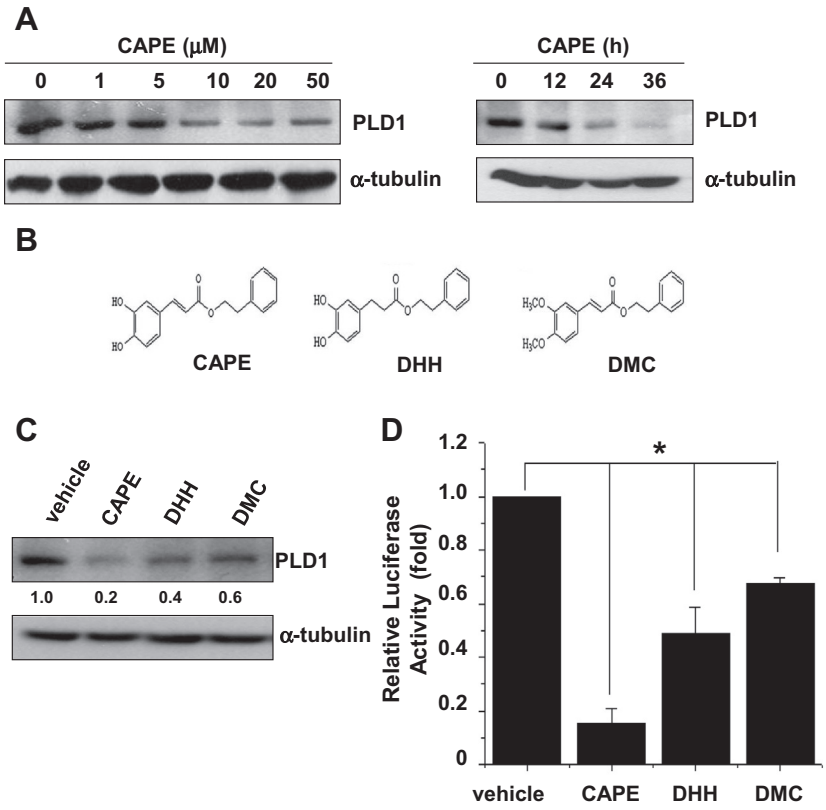

Fig. 2. Catechol moiety and MRA are involved in inhibitory potency of PLD1 expression. (A) U87 cells were treated with the indicated concentrations of CAPE for 24 h or 20 μM CAPE for the indicated times and the cell lysates were subjected to Western blot using anti-PLD antibody. Data are representative of three independent experiments. (B) The chemical structure of CAPE analogues. (C) U87 cells were treated with 20 μM of CAPE for 24 h and the cell lysates were subjected to Western blot. (D) U87 cells were transfected with pGL4-PLD1 and then treated with the drugs for 24 h, after which the luciferase activity was measured. $^{*}P < 0.05$ *versus* non-treatment.

which was followed by activation of the Nrf2 pathway [17]. Thus, we examined whether CAPE was able to bind to PLD1. To accomplish this, caffeic acid was conjugated with biotin to generate biotin-tagged caffeic acid (BTCA). The lysates from U87MG glioma cells were then treated with BTCA, after which they were precipitated by BTCA-bound proteins using streptavidin beads. Next, immobilized proteins on the beads were subjected to Western blot analysis. As shown in Fig. 3A, PLD1 was detected in the precipitated proteins, suggesting that CAPE covalently binds to PLD1. To confirm CAPE binding to PLD1, the same experiment was performed following pretreatment with excess CAPE. As shown in Fig. 3A, pretreatment with CAPE dramatically suppressed precipitation of PLD1, indicating that CAPE prevented binding of BTCA to PLD1. We next attempted to identify the region of PLD1 interacting with CAPE. To accomplish this, various glutathione-S-transferase (GST)-fused PLD1 fragments were incubated with BTCA and then precipitated with streptavidin beads. BTCA was found to bind to F6 fragment of PLD1 (amino acids 826–927) (Fig. 3B). Since electrophiles bind to thiol(s) and CAPE has electrophilic groups (MRA), we investigated which cysteine residue(s) of PLD1 are involved in binding with CAPE. We found two putative cysteine residues (837 and 921 amino acids) present in the F6 fragment of PLD1 and conducted site-directed mutagenesis of the residues. As shown Fig. 3C, wild-type GST-PLD1-F6 and mutant GST-PLD1-F6 (C921A) interacted with BTCA, whereas GST-PLD1-F6 (C837A) containing a mutation of cysteine 837 into alanine did not, suggesting that Cys837 of PLD1 is a critical residue for binding to CAPE.

We further examined whether binding of CAPE to PLD1 affects PLD activity. We found that BTCA, but not biotin-tagged DHHC and DMC, bound to PLD1 (Fig. 3D). Moreover, treatment with CAPE for 1 h inhibited phorbol myristate acetate (PMA)-induced PLD activation, whereas other analogues did not affect the enzymatic activity of PLD (Fig. 3E). Taken together, these results suggest that CAPE covalently binds to a cysteine residue of PLD1 and inhibits PLD activity rapidly.

3.4. CAPE-induced PLD1 downregulation suppresses invasion and proliferation of glioma cells

PLD is known to upregulate MMP-2 and induce invasion of glioma cells [10]; therefore, we examined whether CAPE inhibits MMP-2 activation. To accomplish this, U87MG cells were treated with CAPE and conditioned medium was analyzed by zymography to measure the activity of secreted MMP-2. CAPE inhibited MMP-2 activity in a dose-dependent manner (Fig. 4A). Moreover, MMP-2 activation by PA, a product of PLD activity, was suppressed by CAPE in a dose-dependent manner (Fig. 4B). We also found that CAPE inhibited the expression of MMP-2 in glioma cells (Fig. 4C). Additionally, an MMP-2 promoter assay revealed that CAPE significantly suppressed both basal and PLD1-induced MMP2 expression (Fig. 4D). Furthermore, CAPE and PLD1 depletion inhibited invasion of the glioma cells (Fig. 4E and F). Treatment of PLD1-depleted cells with CAPE did not significantly affect invasion when compared with treatment of PLD1-depleted cells. Taken together, these findings suggest that CAPE-induced suppression of MMP-2 and invasion were mediated by downregulation of PLD1 by CAPE in glioma cells. We also found that PLD1 depletion decreases the viability of glioma cells, but that treatment of PLD1-depleted cells with CAPE had no effect on the viability of glioma cells when compared with that of PLD1-depleted cells. These results suggest that

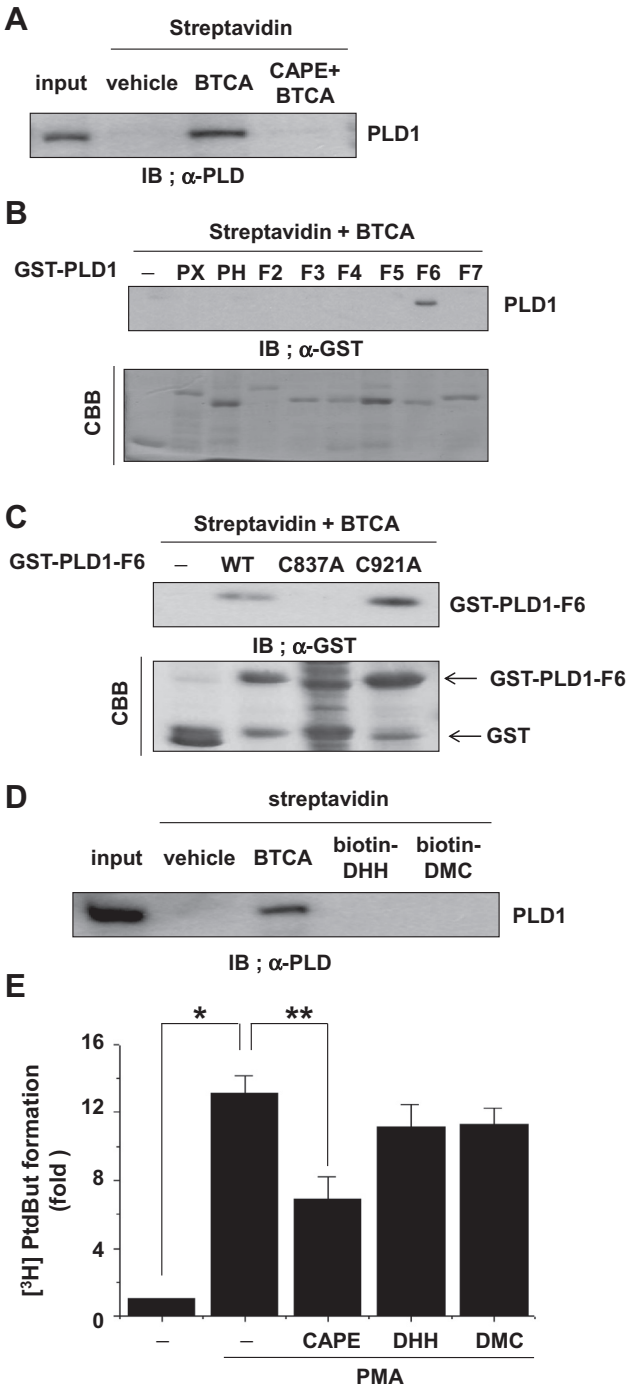

Fig. 3. CAPE inhibits PLD1 activity via interaction with PLD1. (A) BTCA was incubated with U87 cell lysates in the presence or absence of CAPE for 2 h, after which streptavidin beads were added and incubated overnight. The bound complex was then boiled and analyzed using anti-PLD1 antibody. (B) GST-PLD1 fragments were incubated with BTCA for 2 h and streptavidin beads were then added, after which the bound complex was detected by Western blot using antibody to PLD1. (C) Wild type or mutant GST-PLD1-F6 fragments were incubated with BTCA for 2 h and streptavidin beads were added. The bound complex was analyzed by Western blot. (D) U87 cell lysates were incubated with biotinylated-CAPE or CAPE analogues for 2 h and streptavidin beads were added, after which the bound complex was analyzed by Western blot. (E) U87 cells were labeled with [^{3}H] myristate for 12 h, pretreated with CAPE or CAPE analogues and then treated with PMA (100 nM) for 1 h, after which PLD activity was measured. $^*P < 0.001$ *versus* non-treatment; $^{**}P < 0.05$ *versus* PMA-treatment.

CAPE suppresses proliferation of glioma cells via downregulation of PLD1 expression.

4. Discussion

Here, we demonstrate that CAPE downregulates PLD1 expression at the transcriptional level and suppresses invasion and proliferation of glioma cells. Moreover, the covalent binding of CAPE to PLD1 is responsible for inhibition of PLD activity. CAPE is a polyphenolic natural product that activates the NFκB pathway [12]. We recently reported that mitogenic signals induce PLD1 expression by increasing the binding of NFκB to the PLD1 promoter in colon cancer and breast cancer cells [14,18]. CAPE suppressed PLD1 expression via inhibition of binding of NFκB to PLD1 promoter. Structural analysis showed that both electrophiles in CAPE, Michael reaction acceptor (MRA) and catechol moiety, are involved in the downregulation of PLD1 expression. These two functional moieties are required for NFκB transactivation, and the potency of DHHC and DMC in NFkB transactivation was lower than that of CAPE [19]. CAPE analogues with a catechol moiety (DHHC) or MRA (DMC) showed lower potency for suppression of PLD1 expression than CAPE. Although DMC and DHHC inhibits expression of PLD1, the inhibitory potency of the drugs was lower than that of CAPE, probably due to differences in NFκB transactivation. It is likely that inhibition of PLD1 activity occurs via direct binding of CAPE to PLD1, probably between the catechol moiety in CAPE and cysteine 837 in PLD1. This is supported by our findings demonstrating that (1) biotin-tagged caffeic acid, but not its analogues, precipitate with PLD1 from cell lysates, (2) the precipitation was abolished by pretreatment with CAPE, and (3) biotin-tagged dihydroxydihydrocinnamic acid (with catechol) and dimethoxycinnamic acid (with MRA) was not precipitated with PLD1. Moreover, the binding of CAPE to PLD1 for a short-term period (1 h) led to decreased PLD activity, but its analogues did not affect PLD activity. Elevated expression and activity of PLD is linked to anchorage-independent growth, tumor cell invasion, and formation of metastasis [20–23]. Despite the importance of PLD in cell proliferation and tumorigenesis, little is known about the molecules regulating expression and activity of PLD. We recently identified natural products that inhibit expression and activity of PLD, such as quercetin [24] and triptolide [25]. We also observed that rebamipide, a gastroprotective drug that is clinically used for the treatment of gastric ulcers and gastritis, suppresses the expression and activity of PLD1 in gastric cancer [26]. These compounds will be useful for investigation of PLD-mediated pathophysiology, including cancer. Invasion is a major property of various malignant tumors, which are associated with poor prognosis. Glioblastomas are characterized by aggressive invasion. Activation of MMPs in tumor invasion and metastasis has been reported to occur via degradation of the matrix surrounding the tumors, followed by tumor cells invading blood vessels and spreading to distant organs [27]. The expression and activity of MMP-2 were found to be increased in malignant tumors and involved in the development of glioblastomas. We reported that PLD is required for NFκB activation, which induces the activation and expression of MMP-2 and promotes invasion of glioma cells [10]. CAPE inhibited PA or PLD1-mediated MMP upregulation, probably via suppression of NFκB transactivation. Although CAPE decreased invasion and proliferation of glioma cells, it did not affect them in PLD1-depleted cells, suggesting that it inhibits invasion and proliferation of glioma cells through suppression of PLD1 expression. CAPE-induced PLD1 targeting might constitute a new mechanism that contributes to the anticancer properties of CAPE.

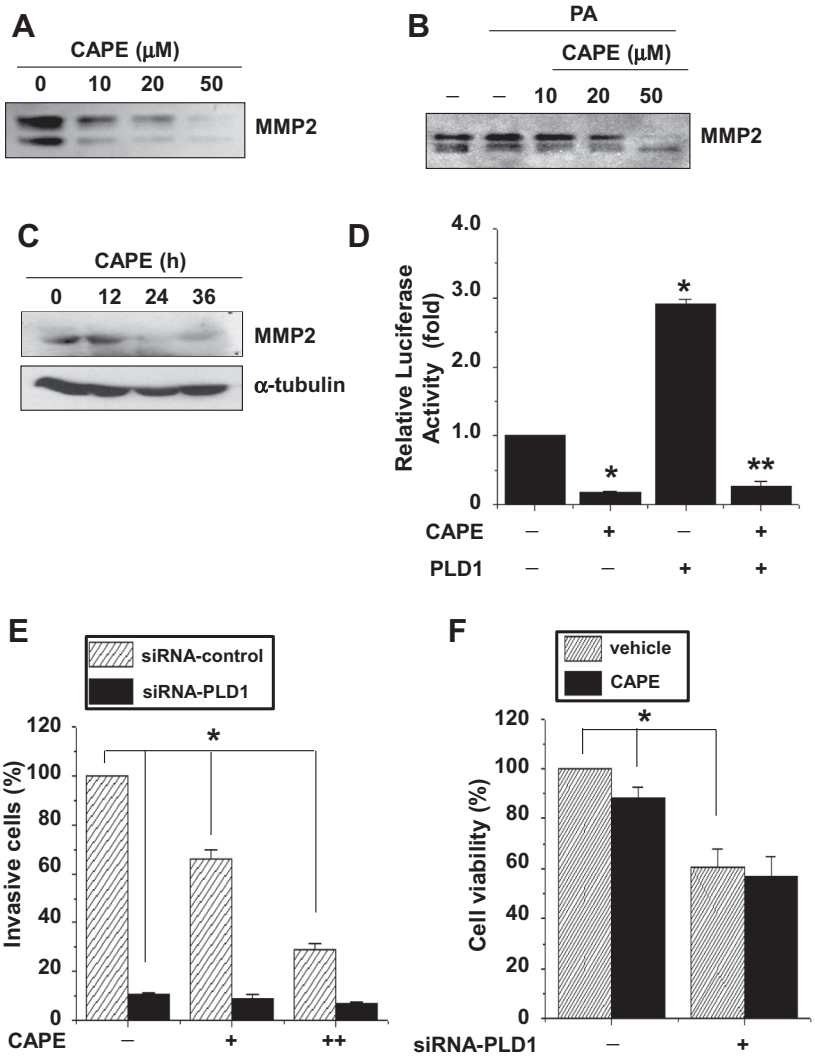

Fig. 4. CAPE-induced PLD1 downregulation suppresses invasion and proliferation of glioma cells. (A) U87 cells were incubated in serum-free media for 24 h and then treated with the indicated concentration of CAPE for 24 h, after which the conditioned media were subjected to gelatin zymography. (B) U87 cells were pretreated with the indicated concentration of CAPE for 1 h, then treated with PA (100 μM) for 24 h, after which the conditioned media were concentrated and subjected to zymography. Data are representative of three independent experiments. (C) U87 cells were treated with 20 μM of CAPE for the indicated time, after which the cell lysates were subjected to Western blot using anti-MMP-2 antibody. (D) The glioma cells were cotransfected with pGL4-MMP-2 and PLD1 and treated with CAPE (20 μM) for 24 h and the lysates were then subjected to a dual luciferase assay. $^{*}P < 0.001$ *versus* control; $^{**}P <$ *versus* PLD1-transfected cells. (E) The cells were transfected with PLD1 siRNA for 24 h and treated with CAPE (10 μM and 20 μM), after which they were subjected to Matrigel invasion assays. Data are expressed as the means ± SD of four independent experiments. $^{*}P < 0.005$ *versus* control. (F) The cells were transfected with PLD1 siRNA for 24 h and treated with 20 μM of CAPE for 24 h, after which the cell viability was measured. Each value represents the mean ± SD of four independent experiments. $^{*}P < 0.05$ *versus* control.

Acknowledgment

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (No. 2012002009) and a Translational Research Center for Protein Function Control Grant (NSF 2009-0092960) and the Financial Supporting Project of Long-term Overseas Dispatch of PNU's Tenure-track Faculty.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.105.

References

[1] F.B. Furnari, T. Fenton, R.M. Bachoo, A. Mukasa, J.M. Stommel, A. Stegh, W.C. Hahn, K.L. Ligon, D.N. Louis, C. Brennan, et al., Malignant astrocytic glioma: genetics, biology, and paths to treatment, Genes Dev. 21 (21) (2007) 2683–2710.

[2] S. Sathornsumetee, D.A. Reardon, A. Desjardins, J.A. Quinn, J.J. Vredenburgh, J.N. Rich, Molecularly targeted therapy for malignant glioma, Cancer 10 (1) (2007) 13–24.

[3] F. Lefranc, J. Brotchi, R. Kiss, Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis, J. Clin. Oncol. 23 (10) (2005) 2411–2422.

[4] T. Demuth, M.E. Berens, Molecular mechanisms of glioma cell migration and invasion, J. Neurooncol. 70 (2) (2004) 217–228.

[5] P.A. Forsyth, H. Wong, T.D. Laing, N.B. Rewcastle, D.G. Morris, H. Muzik, K.J. Leco, R.N. Johnston, P.M. Brasher, G. Sutherland, et al., Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas, Br. J. Cancer 79 (1999) 1828–1835.

[6] C.J. Weng, G.C. Yen, Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives, Cancer Treat. Rev. 38 (1) (2012) 76–87.

[7] H.J. Hwang, H.J. Park, H.J. Chung, H.Y. Min, E.J. Park, J.Y. Hong, S.K. Lee, Inhibitory effects of caffeic acid phenethyl ester on cancer cell metastasis mediated by the down-regulation of matrix metalloproteinase expression in human HT1080 fibrosarcoma cells, J. Nutr. Biochem. 17 (5) (2005) 356–362.

[8] M.J. Weyant, A.M. Carothers, M.E. Bertagnolli, M.M. Bertagnolli, Colon cancer chemopreventive drugs modulate integrin-mediated signaling pathways, Clin. Cancer Res. 6 (3) (2000) 949–956.
[9] C.E. Carrasco-Legleu, L. Márquez-Rosado, S. Fattel-Fazenda, E. Arce-Popoca, J.I. Pérez-Carreón, S. Villa-Treviño, Chemoprotective effect of caffeic acid phenethyl ester on promotion in a medium-term rat hepatocarcinogenesis assay, Int. J. Cancer 108 (4) (2004) 488–492.
[10] M.H. Park, B.H. Ahn, Y.K. Hong, D.S. Min, Overexpression of phospholipase D enhances matrix metalloproteinase-2 expression and glioma cell invasion via protein kinase C and protein kinase A/NF-kappaB/Sp1-mediated signaling pathways, Carcinogenesis 30 (2) (2009) 356–365.
[11] S.M. Hammond, Y.M. Altshuller, T.C. Sung, S.A. Rudge, K. Rose, J. Engebrecht, A.J. Morris, M.A. Frohman, Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family, J. Biol. Chem. 270 (50) (1995) 29640–29643.
[12] W.C. Lley, T.C. Sung, R. Roll, J. Jenco, S.M. Hammond, Y. Altshuller, D. Bar-Sagi, A.J. Morris, M.A. Frohman, Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization, Curr. Biol. 7 (3) (1997) 191–201.
[13] X. Peng, M.A. Frohman, Mammalian phospholipase D physiological and pathological roles, Acta Physiol. (Oxf) 204 (2) (2012) 219–226.
[14] D.W. Kang, M.H. Park, Y.J. Lee, H.S. Kim, T.K. Kwon, W.S. Park, D.S. Min, Phorbol ester up-regulates phospholipase D1 but not phospholipase D2 expression through a PKC/Ras/ERK/NF{kappa}B-dependent pathway and enhances matrix metalloproteinase-9 secretion in colon cancer cells, J. Biol. Chem. 283 (2008) 4094–4104.
[15] B.H. Ahn, G. Min, Y.S. Bae, Y.S. Bae, D.S. Min, Phospholipase D is activated and phosphorylated by casein kinase-II in human U 87 astroglioma, Exp. Mol. Med. 38 (2006) 55–62.
[16] D.S. Min, T.K. Kwon, W.S. Park, J.S. Chang, S.K. Park, B.H. Ahn, Z.Y. Ryoo, Y.H. Lee, Y.S. Lee, D.J. Rhie, et al., Neoplastic transformation and tumorigenesis associated with overexpression of phospholipase D isozymes in cultured murine fibroblasts, Carcinogenesis 22 (2001) 1641–1647.
[17] H. Kim, W. Kim, S. Yum, S. Hong, J.E. Oh, J.W. Lee, M.K. Kwak, E.J. Park, D.H. Na, Y. Jung, Caffeic acid phenethyl ester activation of Nrf2 pathway is enhanced under oxidative state: structural analysis and potential as a pathologically targeted therapeutic agent in treatment of colonic inflammation, Free Radic. Biol. Med. 65 (2013) 552–562.
[18] D.W. Kang, D.S. Min, Platelet derived growth factor increases phospholipase D1 but not phospholipase D2 expression via NFkappaB signaling pathway and enhances invasion of breast cancer cells, Cancer Lett. 294 (1) (2010) 125–133.
[19] Y. Lee, D.H. Shin, J.H. Kim, S. Hong, D. Choi, Y.J. Kim, M.K. Kwak, Y. Jung, Caffeic acid phenethyl ester-mediated Nrf2 activation and IkappaB kinase inhibition are involved in NFkappaB inhibitory effect: structural analysis for NFkappaB inhibition, Eur. J. Pharmacol. 643 (1) (2010) 21–28.
[20] P. Huang, M.A. Frohman, The potential for phospholipase D as a new therapeutic target, Expert Opin. Ther. Targets 11 (5) (2007) 707–716.
[21] D.W. Kang, K.Y. Choi, D.S. Min, Phospholipase D meets Wnt signaling: a new target for cancer therapy, Cancer Res. 71 (2) (2011) 293–297.
[22] W. Su, Q. Chen, M.A. Frohman, Targeting phospholipase D with small-molecule inhibitors as a potential therapeutic approach for cancer metastasis, Future Oncol. 5 (9) (2009) 1477–1486.
[23] S.A. Scott, P.E. Selvy, J.R. Buck, H.P. Cho, T.L. Criswell, A.L. Thomas, M.D. Armstrong, C.L. Arteaga, C.W. Lindsley, H.A. Brown, Design of isoform-selective phospholipase D inhibitors that modulate cancer cell invasiveness, Nat. Chem. Biol. 5 (2) (2009) 108–117.
[24] M.H. Park, D.S. Min, Quercetin-induced downregulation of phospholipase D1 inhibits proliferation and invasion in U87 glioma cells, Biochem. Biophys. Res. Commun. 412 (4) (2011) 710–715.
[25] D.W. Kang, J.Y. Lee, D.H. Oh, S.Y. Park, T.M. Woo, M.K. Kim, M.H. Park, Y.H. Jang, D.S. Min, Triptolide-induced suppression of phospholipase D expression inhibits proliferation of MDA-MB-231 breast cancer cells, Exp. Mol. Med. 41 (9) (2009) 678–685.
[26] D.W. Kang, G. Min, Y. Park Do, K.W. Hong, D.S. Min, Rebamipide-induced downregulation of phospholipase D inhibits inflammation and proliferation in gastric cancer cells, Exp. Mol. Med. 42 (8) (2010) 555–564.
[27] C. Gialeli, A.D. Theocharis, N.K. Karamanos, Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting, FEBS J. 278 (1) (2001) 16–27.