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A novel PP2A enhancer induces caspase-independent apoptosis of MKN28 gastric cancer cells with high MEK activity

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ABSTRACT

The newly synthesized phosphatidylinositol (PI) derivative 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidyl-D-1-inositol (diDCP-LA-PI) significantly enhanced protein phosphatase 2A (PP2A) activity in the cell-free assay. This prompted to assess the antitumor effect of diDCP-LA-PI. diDCP-LA-PI attenuated phosphorylation of mitogen-activated protein kinase (MAPK) kinase (MEK) in Lu65 human lung cancer and MKN28 human gastric cancer cells with high MEK activity. diDCP-LA-PI reduced cell viability in Lu65 and MKN28 cells, but otherwise such effect was not found in 786-O human renal cancer and HUH-7 human hepatoma cells with relatively low MEK activity. For Lu65 and MKN28 cells diDCP-LA-PI increased terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive cells, but no significant activation of caspase-3, -8, or -9 was obtained. For MKN28 cells diDCP-LA-PI-induced reduction of MEK phosphorylation and cell viability was prevented by knocking-down PP2Ac. Taken together, these results indicate that diDCP-LA-PI induces caspase-independent apoptosis of Lu65 and MKN28 human cancer cells, for the latter cells by suppressing MEK activity through PP2A-catalyzed dephosphorylation.

1. Introduction

Phosphatidylinositol (PI) regulates a variety of cell processes, which include cell proliferation, differentiation, migration, chemotaxis, phagocytosis, and survival [1]. PI is also implicated in the regulation of vesicular trafficking, membrane dynamics, actin cytoskeleton organization, activation of ion channels, and transporters [2,3]. Moreover, PI metabolites serve as critical messengers in the diverse signaling pathways. Phosphatidylinositol 4,5-biphosphate (PIP_2) is hydrolyzed into diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) by phospholipase C [4,5]. IP_3 releases Ca^{2+} through IP_3-gated calcium channel on the endoplasmic reticulum, an intracellular calcium store, and classical PKCs PKCα, -βI, - βII, and -γ are activated by intracellularly released Ca^{2+} and diacylglycerol [4,5]. PIP_2, alternatively, is phosphorylated by phosphatidylinositol 3-kinase (PI3K) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which binds to and activates 3-phosphoinositide-dependent protein kinase 1 (PDK1), followed by activation of Akt and Rac1/Cdc42.

So far we have been doing an intensive and extensive research about lipid signaling. During its process we have synthesized a variety of *cis*-unsaturated free fatty acid derivatives, which exhibit stable bioactivities in the *in vitro* and *in vivo* systems, and of them the greatest hit was the linoleic acid derivative 8-[2-(2-pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid (DCP-LA), with cyclopropane rings instead of *cis*-double bonds [6–8]. To gain further insight into phospholipid signaling, we have newly synthesized phospholipid derivatives (DCP-LA phospholipids) such as 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidylethanolamine (diDCP-LA-PE), 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidyl-L-serine (diDCP-LA-PS), 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidylcholine (diDCP-LA-PC), and 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidyl-D-1-inositol (diDCP-LA-PI) (Fig. 1), with DCP-LA on the α and β position. All the DCP-LA phospholipids serve as an activator of protein kinase C (PKC) [9]. In addition, diDCP-LA-PE and diDCP-LA-PI serve as an inhibitor for protein

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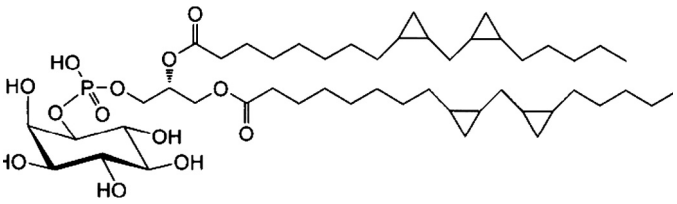

Fig. 1. Chemical structure of diDCP-LA-PI.

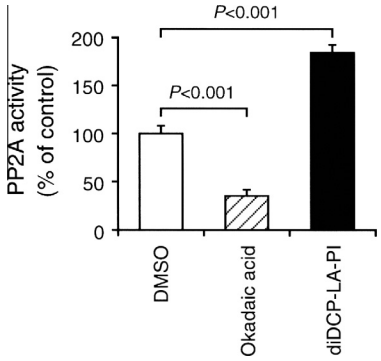

Fig. 2. Effect of diDCP-LA-PI on PP2A activity. In a cell-free system, PP2A was reacted with pNPP in the presence and absence of okadaic acid (2 nM) or diDCP-LA-PI (100 μM), and dephosphorylated pNPP was quantified. Dimethyl sulfoxide (DMSO) was used as a vehicle control. In the graph, each value represents the mean (±SEM) percentage of basal phosphatase activity (control) ($n = 4$ independent experiments). *P* values, Dunnett's test.

phosphatase 1 (PP1) and protein tyrosine phosphatase 1B (PTP1B), diDCP-LA-PS as a PTP1B inhibitor, diDCP-LA-PI as a protein phosphatase 2A (PP2A) enhancer, and diDCP-LA-PC as a PTP1B enhancer [9]. PP2A is recognized to dephosphorylate and inactivate mitogen-activated protein kinase (MAPK) and MAPK kinase (MEK). Then, we postulated that diDCP-LA-PI might exert its antitumor action by inactivating MAPK and MEK in association with enhanced PP2A activity. The present study aimed at obtaining evidence for this hypothesis.

2. Materials and methods

2.1. Synthesis of diDCP-LA-PI

diDCP-LA-PI was originally synthesized in our laboratory. *N,N*-Diisopropylmethylphosphonamidic chloride (0.028 ml, 0.14 mmol) was added to a solution of 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycerol (0.080 g, 0.12 mmol) and triethylamine (0.033 ml, 0.24 mmol) in CH_2Cl_2 (2 ml) under ice-cooling. After stirring for 10 min at room temperature, (-)-2,3,4,5,6-penta-*O*-benzyl-D-1-inositol (0.11 g, 0.18 mmol) and 1*H*-tetrazole (0.033 g, 0.48 mmol) were added, and 70% (v/v) aqueous solution of *tert*-butyl peroxide (0.16 ml, 1.2 mmol) was added to the reaction mixture and stirred for 20 min at the same temperature. After adding 10% (w/v) aqueous solution of $Na_2S_2O_3$, the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by a silica gel column chromatography (hexane: diethyl ether = 1:1) to give *O*-(1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-3-glyceryl) *O*-methyl *O*-(2′,3′,4′,5′,6′-penta-*O*-benzyl-D-1′-inositol) phosphate (30 mg, 17%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ -0.33- -0.21 (m, 4H), 0.52–0.85 (m, 12H), 0.87–0.95 (m, 6H), 0.96–1.70 (m, 44H), 2.24 (t, $J = 7.3$ Hz, 2H), 2.26 (t, $J = 7.3$ Hz, 2H), 3.46–3.54 (m, 2H), 3.67 (d, $J = 11.4$ Hz, 3H), 3.88 (dd, $J = 11.9$ and 6.0 Hz, 1H), 3.94 (ddd, $J = 6.8$, 6.4 and 5.0 Hz, 1H), 4.00–4.15 (m, 4H), 4.24 (ddd, $J = 7.7$, 7.4 and 2.1 Hz, 1H), 4.34 (t, $J = 2.1$ Hz, 1H), 4.67 (d, $J = 11.4$ Hz, 1H), 4.73 (d, $J = 11.4$ Hz, 1H), 4.75–4.85 (m, 4H), 4.85–4.95 (m, 3H), 4.95 (d, $J = 11.4$ Hz, 1H), 5.00–5.07 (m, 1H).

NaI (0.017 g, 0.11 mmol) was added to a solution of *O*-(1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-3-glyceryl) *O*-methyl *O*-(2′,3′,4′,5′,6′-penta-*O*-benzyl-D-1′-inositol) phosphate (30 mg, 0.020 mmol) in 2-butanone (2 ml). After stirring for 2 h at 80 °C, 2 N HCl was added to the reaction mixture, and the aqueous layer was extracted with chloroform. The organic layer was washed with H_2O and brine, and the combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give *O*-(1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-3′-glyceryl) *O*-(2′,3′,4′,5′,6′-penta-*O*-benzyl-D-1′-inositol) phosphate.

To a solution of *O*-(1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-3′-glyceryl) *O*-(2′,3′,4′,5′,6′-penta-*O*-benzyl-D-1′-inositol) phosphate in ethanol (3 ml) was added 10% (w/v) palladium on activated carbon (21 mg). The resulting suspension was placed under hydrogen (1 atm) and stirred for 2 h at room temperature. The catalyst was removed through a pad of Celite, rinsed with ethyl acetate, and concentrated under reduced pressure. The resulting crude product was purified by a silica gel column chromatography (chloroform:methanol = 10:1) to give 1,2-*O*-bis-[8-{2-(2-pentyl-cyclopropylmethyl)-cyclopropyl}-octanoyl]-*sn*-glycero-3-phosphatidyl-D-1-inositol (10 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ -0.33- -0.20 (m, 4H), 0.55–0.85 (m, 12H), 0.87–0.95 (m, 6H),

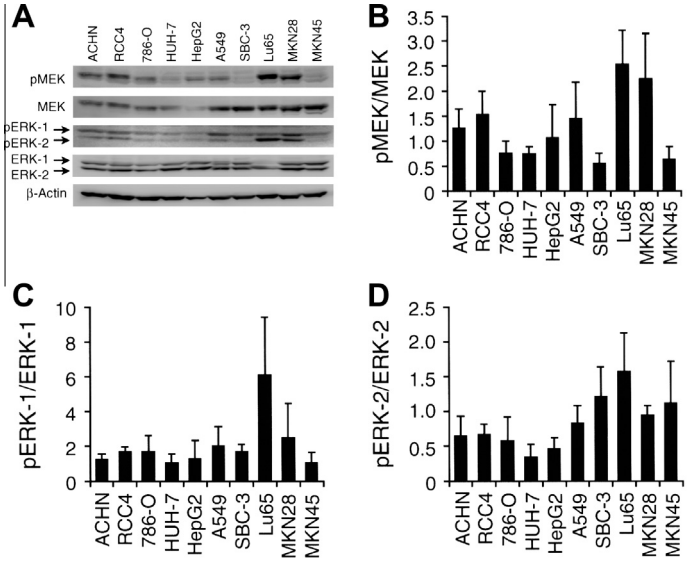

Fig. 3. Phosphorylation of MEK, ERK1, and ERK2 in a variety of human cancer cell lines. Western blotting was carried out in ACHN, RCC4-VHL, 786-O, HUH-7, HepG2 A549, SBC-3, Lu65, MKN28, and MKN45 cells (A). In the graphs, each column represents the mean (±SEM) ratio of signal intensity of phosphorylated MEK (B), ERK1 (C), and ERK2 (D) relative to that of total MEK, ERK1, and ERK2, respectively ($n = 4$ independent experiments).

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0.96–1.70 (m, 44H), 2.20–2.43 (m, 4H), 3.80–4.51 (m, 5H), 5.18–5.32 (m, 1H); ESI-HRMS (negative ion, sodium formate) calculated for $C_{49}H_{86}O_{13}P$ ([M-H]$^-$) 913.5811; found 913.5806.

2.2. Cell-free assay of PP2A activity

Activity of PP2A under cell-free conditions was assayed by the method as previously described [10]. Human recombinant PP2A was purchased from Wako Pure Chemical Industries (Osaka, Japan). PP2A activity was assayed by reacting with *p*-nitrophenyl phosphate (pNPP) (Sigma, St. Louis, MO, USA) as a substrate. Enzyme was preincubated at 37 °C for 30 min in a reaction medium [50 mM Tris–HCl, 0.1 mM EGTA, 0.1% (v/v) 2-mercaptoethanol, pH 7.0] in the presence and absence diDCP-LA-PI (100 μM) or okadaic acid. Then, pNPP at a concentration of 500 μM was added to the reaction medium followed by 60-min incubation, and the reaction was terminated by adding 0.1 N NaOH. Dephosphorylated pNPP was quantified at an absorbance of 405 nm with a SpectraMax PLUS384 (Molecular Devices, Sunnyvale, CA, USA).

2.3. Cell culture

Human renal cancer cell lines ACHN, RCC4-VHL, and 786-O cells and human hepatoma cell lines HUH-7 and HepG2 cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS). Hunan lung cancer cell lines A549 and SBC-3 cells were cultured in minimum essential medium (MEM) with 0.1 mM non-essential amino acids, and another human lung cancer cell line Lu65 cells and human gastric cancer cell lines MKN28 and MKN45 cells were grown in RPMI-1640 medium supplemented with 10% (v/v) FBS. Penicillin (final concentration, 100 U/ml) and streptomycin (final concentration, 0.1 mg/ml) were added to the all the cultured medium, and cells were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

2.4. Western blotting

Cells were untreated and treated with diDCP-LA-PI, and then lysed in a lysate solution [150 mM NaCl, 20 mM Tris, 0.1% (v/v) Tween-20 and 0.1% (w/v) sodium dodecyl sulfate (SDS), pH 7.5] containing 1% (v/v) protease inhibitor cocktail and 1% (v/v) phosphatase inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). The lysates were centrifuged at 3000 rpm for 5 min at 4 °C. Proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. Blotting membranes were blocked with TBS-T [150 mM NaCl, 0.1% (v/v) Tween-20 and 20 mM Tris, pH 7.5] containing 5% (w/v) bovine serum albumin and subsequently reacted with antibodies against phospho-MEK at Ser217/Ser221 (pMEK) (Cell Signaling Technology, Danvers, MA, USA), MEK (Cell Signaling Technology), phospho-ERK1/2 at (Thr202/Tyr204)/(Thr185/Tyr187) (pERK1/2)(Santa Cruz Biotechnology, Santa Cruz, CA, USA), ERK1/2 (Santa Cruz Biotechnology), PP2Ac (Cell Signaling Technology), and β-actin (Sigma). After washing, membranes were reacted with a horseradish peroxidase-conjugated goat anti-mouse IgG or goat anti-rabbit IgG antibody. Immunoreactivity was detected with an ECL kit (Invitrogen, Carlsbad, CA, USA) and visualized using a chemiluminescence LAS-4000mini detection system (GE Healthcare, Piscataway, NJ, USA). Protein concentrations for each sample were determined with a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

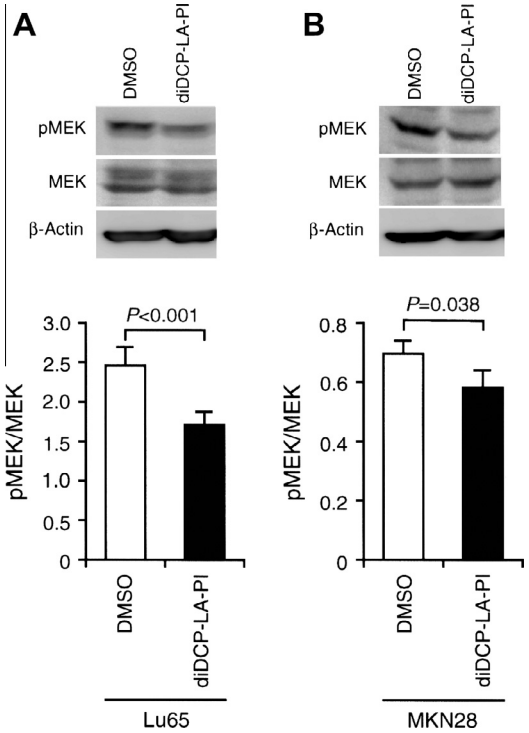

Fig. 4. Effect of diDCP-LA-PI on MEK phosphorylation. Lu65 (A) and MKN28 (B) cells were treated with DMSO or diDCP-LA-PI (100 μM) for 24 h, followed by Western blotting. In the graphs, each column represents the mean (±SEM) ratio of signal intensity of phosphorylated MEK relative to that of total MEK ($n = 4$ independent experiments). *P* values, unpaired *t*-test.

2.5. Cell viability

Cell viability was evaluated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) by the method previously described [11].

2.6. Construction and transfection of small interfering RNA (siRNA)

The siRNA to silence PP2A catalytic subunit α gene (PP2Ac siRNA) was obtained from Santa Cruz Biotechnology and the negative control siRNA (NC siRNA) was obtained from Ambion (Carlsbad, CA, USA). The NC siRNA had the scrambled sequence, the same GC content, and nucleic acid composition. The PP2Ac siRNA or the NC siRNA were transfected into cells using a Lipofectamine reagent (Invitrogen). Cells were used for experiments 48 h after transfection.

2.7. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining

TUNEL staining was performed to detect in situ DNA fragmentation as a marker of apoptosis using an In Situ Apoptosis Detection Kit (Takara Bio, Otsu, Japan). Briefly, fixed and permeabilized cells were reacted with terminal deoxynucleotidyl transferase and fluorescein isothiocyanate (FITC)-deoxyuridine triphosphate for 90 min at 37 °C. FITC signals were visualized with a confocal scanning laser microscope (LSM 510, Carl Zeiss Co., Ltd., Oberkochen, Germany).

2.8. Enzymatic assay of caspase activity

Caspase activity was measured using a caspase fluorometric assay kit: Ac-Asp-Glu-Val-Asp-MCA for a caspase-3 substrate peptide, Ac-Ile-Glu-Thr-Asp-MCA for a caspase-8 substrate peptide, and Ac-Leu-Glu-His-Asp-MCA for a caspase-9 substrate peptide. Briefly, cells were harvested before and after treatment with diDCP-LA-PI, and then centrifuged at 3000 rpm for 5 min at 4 °C. The pellet was incubated on ice in cell lysis buffer for 10 min, and then centrifuged at 10,000 g for 1 min at 4 °C. The supernatant was reacted with the fluorescently labeled tetra-

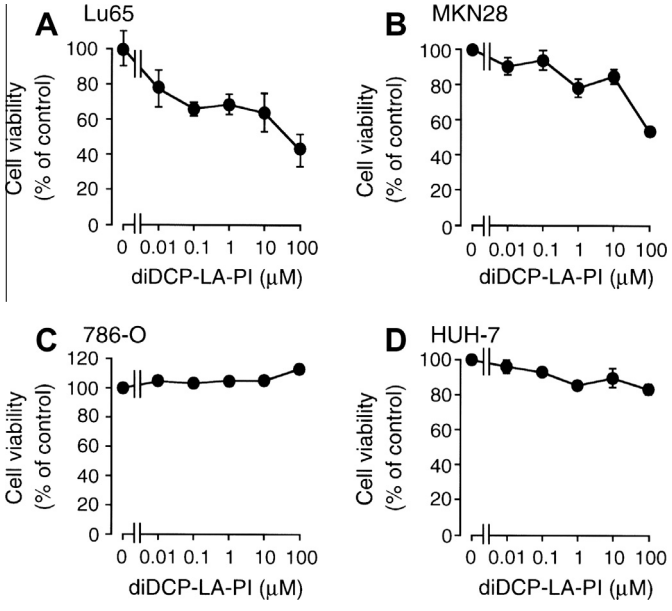

Fig. 5. Effect of diDCP-LA-PI on Lu65 and MKN28 cell viability. Lu65 (A), MKN28 (B), 786-O (C), and HUH-7 (D) cells were treated with DMSO or diDCP-LA-PI at the concentrations as indicated for 48 h, followed by MTT assay. In the graphs, each point represents the mean (±SEM) percentage of control cell viability (MTT intensities of cells untreated with diDCP-LA-PI) ($n = 4$ independent experiments).

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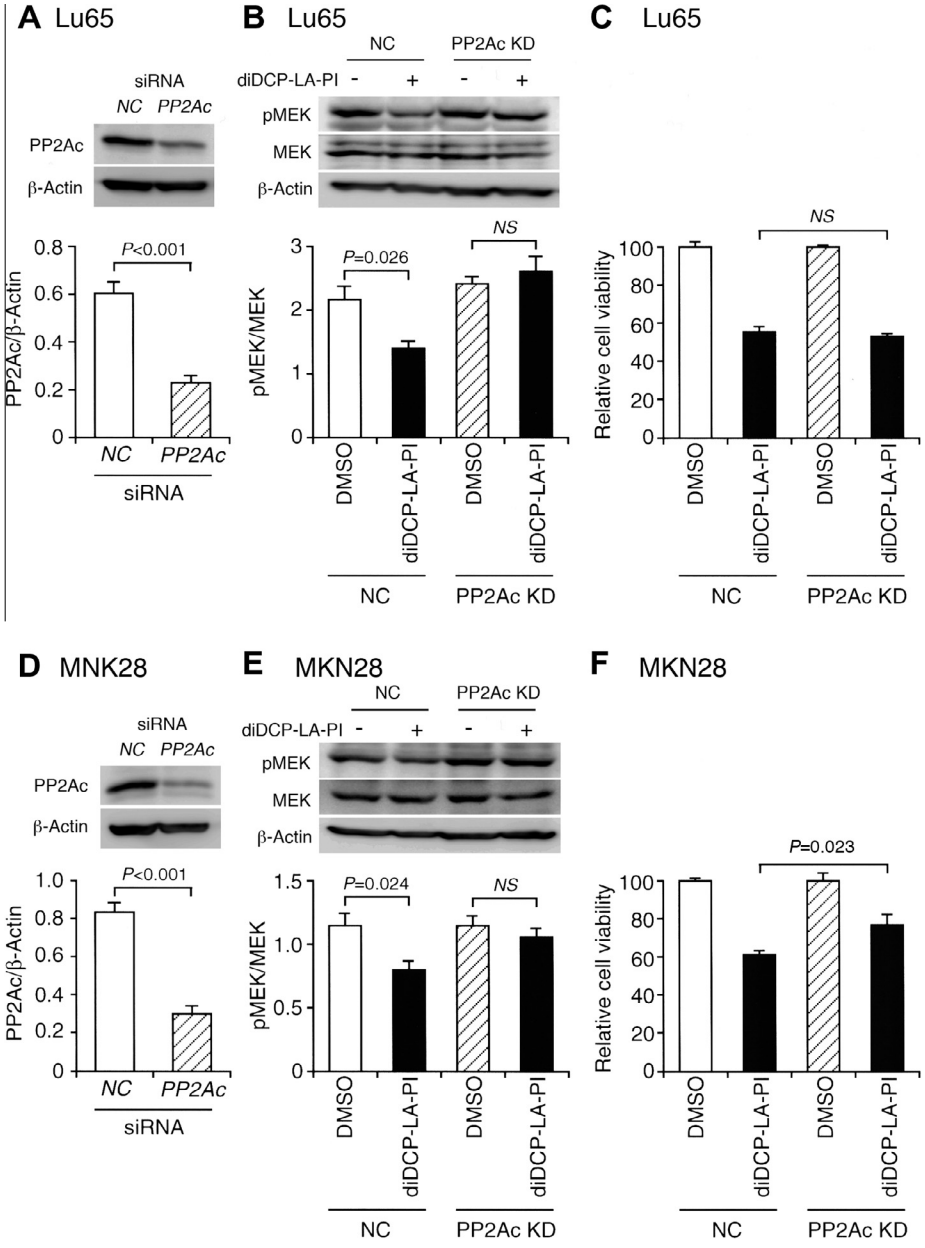

Fig. 6. Effects of diDCP-LA-PI on MEK phosphorylation and cell viability in Lu65 and MKN28 cells with PP2Ac knock-down. Western blotting for Lu65 (A) and MKN28 (D) cells transfected with the NC siRNA or the PP2Ac siRNA 48 h after transfection using an anti-PP2Ac antibody. Signal intensities for PP2Ac protein were normalized by those for β-actin. In the graphs, each column represents the mean (±SEM) PP2Ac protein intensity ($n = 4$ independent experiments). *P* values, unpaired *t*-test. Lu65 (B) and MKN28 (E) cells transfected with the NC siRNA or the PP2Ac siRNA were treated with DMSO or diDCP-LA-PI (100 μM) for 24 h, followed by Western blotting using antibodies against pMEK, MEK, and β-actin. In the graphs, each column represents the mean (±SEM) ratio of signal intensity of phosphorylated MEK relative to that of total MEK ($n = 4$ independent experiments). *P* values, ANOVA followed by a Bonferonni correction. *NS*, not significant. MTT assay was carried out in Lu65 (C) and MKN28 (F) cells transfected with the NC siRNA or the PP2Ac siRNA untreated (DMSO) and treated with diDCP-LA-PI (100 μM) for 48 h. In the graphs, each column represents the mean (±SEM) cell viability relative to that for untreated control cells transfected with each siRNA) ($n = 4$ independent experiments). *P* value, Dunnett's test. *NS*, not significant.

peptide at 37 °C for 2 h. Fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nm for caspase-3, -8, and -9 with a fluorescence microplate reader (TECAN Infinite, Männedorf, Switzerland).

2.9. Statistical analysis

Statistical analysis was carried out using Dunnett's test, unpaired *t*-test, and analysis of variance (ANOVA) followed by a Bonferonni correction.

3. Results

3.1. diDCP-LA-PI enhances PP2A activity

We initially examined the effect of diDCP-LA-PI on PP2A activity in a cell-free system. Okadaic acid (2 nM), an inhibitor of PP2A, clearly reduced PP2A activity (Fig. 2), confirming a reliable PP2A assay. As previously found [9], diDCP-LA-PI (100 μM) significantly enhanced PP2A activity, reaching nearly 1.8 fold of the control levels (Fig. 2).

3.2. diDCP-LA-PI suppresses phosphorylation of MEK in Lu65 and MKN28 cells

Extracellular signal-regulated kinase (ERK), a MAPK, is activated through a pathway along the receptor tyrosine kinase (RTK)/Ras/Raf/MEK/ERK axis, and promotes cell growth and proliferation of cancer cells. We next examined activity of MEK and ERK1/2 in a variety of human cancer cell lines such as renal cancer cell lines

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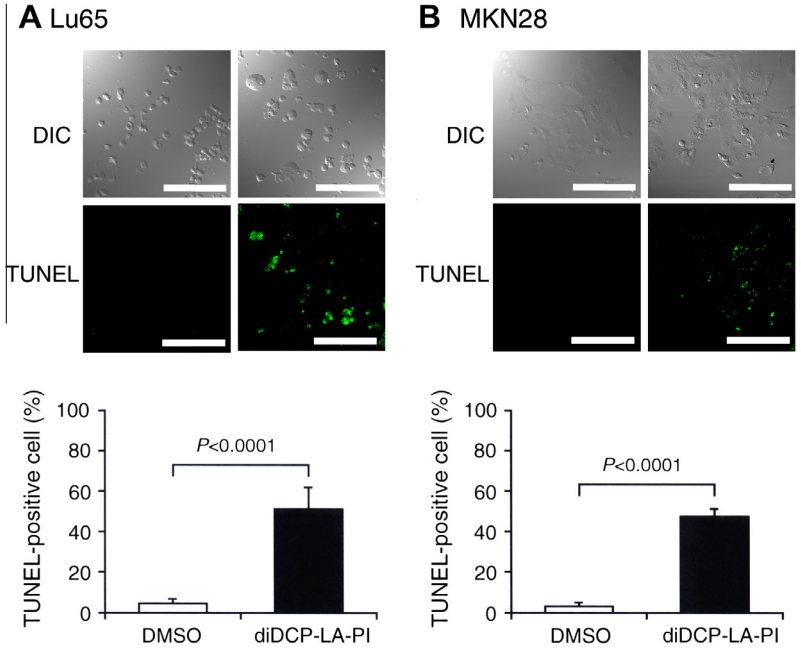

Fig. 7. TUNEL staining. TUNEL staining was carried out on Lu65 (A) and MKN28 (B) cells treated with DMSO or diDCP-LA-PI (100 μM) for 48 h. DIC, Differential interference contrast. Bars = 100 μm. TUNEL-positive cells were counted in an area (0.4 mm × 0.4 mm) selected at random. In the graphs, each column represents the mean (±SEM) percentage of TUNEL-positive cells relative to total number of cells ($n = 4$ independent experiments). *P* values, unpaired *t*-test.

ACHN, RCC4-VHL, and 786-O cells, hepatoma cell lines HUH-7 and HepG2 cells, lung cancer cell lines A549, SBC-3, and Lu65 cells, and gastric cancer cell lines MKN28 and MKN45 cells. Of them Lu65 cells exhibited the highest phosphorylation of MEK, ERK1, and ERK2 and MKN28 cells revealed relatively high phosphorylation of those proteins (Fig. 3A–D). This implies that MEK and ERK1/2 in Lu65 and MKN28 cells are highly activated.

We postulated that diDCP-LA-PI, to enhance PP2A activity, could suppress activity of MEK and the downstream effector ERK1/2 in Lu65 and MKN28 cells through their dephosphorylation. Expectedly, diDCP-LA-PI (100 μM) significantly reduced MEK phosphorylation both in Lu65 and MKN28 cells (Fig. 4A and B).

3.3. diDCP-LA-PI induces caspase-independent apoptosis in Lu65 and MKN28 cells

If diDCP-LA-PI suppresses activity of MEK, then it might induce cell death. To address this point, we subsequently monitored cell viability in the MTT assay. Indeed, diDCP-LA-PI reduced cell viability in a concentration (0.01–100 μM)-dependent manner in Lu65 and MKN28 cells (Fig. 5A and B). In contrast, such effect was not found in 786-O and HUH-7 cells with relatively low MEK phosphorylation (Fig. 5C and D). Collectively, these results suggest that diDCP-LA-PI induces cell death in Lu65 and MKN28 cells with highly activated MEK by enhancing PP2A activity and dephosphorylating MEK.

To obtain further evidence for this, the PP2Ac gene was knocked-down using the siRNA. Expression of PP2Ac protein for Lu65 and MKN28 cells transfected with the PP2Ac siRNA significantly lowered as compared with the expression for cells transfected with the NC siRNA (Fig. 6A and D), confirming PP2Ac knockdown. diDCP-LA-PI-induced reduction of MEK phosphorylation in Lu65 and MKN28 cells was cancelled by knocking-down PP2Ac (Fig. 6B and E). diDCP-LA-PI-induced reduction of MKN28 cell viability, on the other hand, was prevented by knocking-down PP2Ac (Fig. 6F), although no significant effect was found in Lu65 cells (Fig. 6C). These results support the note that diDCP-LA-PI induces MKN28 cell death in a PP2A-dependent manner. The results also

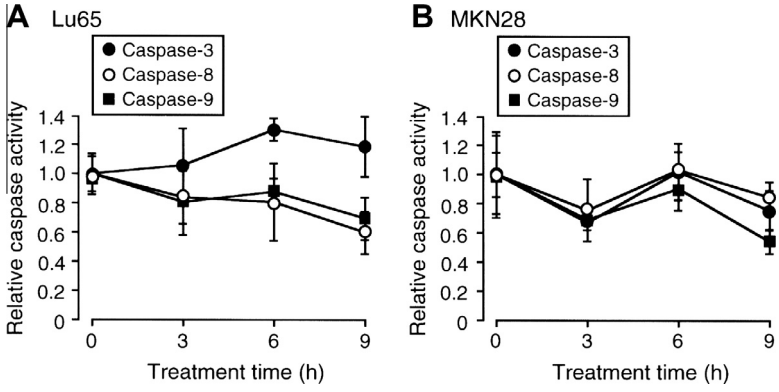

Fig. 8. Effect of diDCP-LA-PI on caspase activity. Lu65 (A) and MKN28 (B) cells were treated with DMSO or diDCP-LA-PI (100 μM) for the periods of time as indicated, and then activities of caspase-3, -8, and -9 were enzymatically assayed. In the graphs, each point represents the mean (±SEM) ratio to basal caspase activities (before treatment with diDCP-LA-PI) ($n = 4$ independent experiments).

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suggest that another unknown mechanism except for enhanced PP2A activity underlies diDCP-LA-PI-induced Lu65 cell death.

To determine whether diDCP-LA-PI-induced reduction of cell viability is due to apoptosis, we performed TUNEL staining. diDCP-LA-PI (100 μM) significantly increased TUNEL-positive Lu65 and MKN28 cells (Fig. 7A and B), indicating that diDCP-LA-PI induces apoptosis of Lu65 and MKN28 cells.

We finally examined whether diDCP-LA-PI-induced apoptosis is caspase-dependent or -independent. In the enzymatic caspase assay, diDCP-LA-PI (100 μM) induced no significant activation of caspase-3, -8, and -9 in Lu65 and MKN28 cells (Fig. 8A and B). Overall, these results lead to a conclusion that diDCP-LA-PI induces caspase-independent apoptosis in Lu65 and MKN28 cells, for the latter cells by enhancing PP2A activity.

4. Discussion

PP2A, a serine/threonine phosphatase, is composed of the catalytic, scaffold and regulatory subunits. The catalytic and scaffold subunits have 2 isoforms and the regulatory subunit has 4 different families containing different isoforms. The regulatory subunit is the most diverse with temporal and spatial specificity. PP2A dephosphorylates many critical cellular molecules, which includes Akt, MEK, ERK, p53, c-Myc, and β-catenin, and regulates a variety of cellular processes such as cell proliferation, signal transduction, and apoptosis [12]. Aberrant expression, mutations, and somatic alterations of the PP2A scaffold and regulatory subunits are frequently found in human lung, breast, skin, and colon cancers [12].

Accumulating evidence has pointed to PP2A as a tumor suppressor [9]. PP2A prevents tumorgenesis by downregulating an Akt/(TSC1/TSC1)/RheB/mTOR pathway, inactivating c-Myc, or antagonizing Wnt/β-catenin [13]. PP2A, alternatively, stabilizes p53 or CDC25, to suppress tumor growth/proliferation or induce apoptosis of cancers [14]. Accordingly, recent highlight has focused upon development of anticancer drug targeting PP2A [15,16].

The present study provides direct evidence that diDCP-LA-PI has the potential to enhance PP2A activity. In the RTK signaling pathway, MEK is phosphorylated and activated through a pathway along the RTK/Ras/Raf axis, and in turn, activated MEK phosphorylates and activates the downstream effector ERK1/2 [17]. The Ras-dependent Raf/MEK/ERK1/2 signaling pathway is a major regulator of cell proliferation and survival, and aberrant activation of RTK causes hyperactivation of this pathway, responsible for progression of cancer cells [17]. This, in the light of the fact that PP2A dephosphorylates and inactivates MEK and ERK1/2, suggests that diDCP-LA-PI, to enhance PP2A activity, could inhibit cancer cell proliferation and survival.

In the present study, the degree of MEK and ERK1/2 activation varied, depending upon cancer cell types. Of them Lu65 human lung cancer and MKN28 human gastric cancer cells exhibited high activities of MEK and ERK1/2, and diDCP-LA-PI dephosphorylated and inactivated MEK. diDCP-LA-PI clearly reduced cell viability in Lu65 and MKN28 cells. In contrast, diDCP-LA-PI had no effect on cell viability in 786-O human renal cancer and HUH-7 human hepatoma cells with relative low activities of MEK and ERK1/2. diDCP-LA-PI markedly increased TUNEL-positive cells both for Lu65 and MKN28 cells, but caspase-3, -8, or -9 was not significantly activated. These results indicate that diDCP-LA-PI induces caspase-independent apoptosis of Lu65 and MKN28 cells. diDCP-LA-PI-induced reduction of MEK activity was neutralized by knocking-down PP2Ac both in Lu65 and MKN28 cells. diDCP-LA-PI-induced cell death, on the other hand, was attenuated by knocking-down PP2Ac only for MKN28 cells, but it was not affected for Lu65 cells. Overall, these results indicate that diDCP-LA-PI induces caspase-independent apoptosis of MKN28 cells by downregulating MEK/ERK signaling due to PP2A-mediated dephosphorylation of MEK and that diDCP-LA-PI-induced enhancement of PP2A activity might not be main factor for Lu65 cell apoptosis. Anyway, this represents that diDCP-LA-PI could be developed as a new type of anticancer drug.

In summary, the results of the present study demonstrate that the PI derivative diDCP-LA-PI serves as an enhancer of PP2A, to dephosphorylate and inactivate MEK, thereby inducing caspase-independent apoptosis of MKN28 human gastric cancer cells with high MEK activity.

Conflict of Interest

The present study has no financial and personal relationships with other people or organizations that could inappropriately influence their work.

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