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SHORT COMMUNICATION

Delphinidin-3-glucoside suppresses lipid accumulation in HepG2 cells

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Abstract Delphinidin is an anthocyanidin commonly found in various fruits and vegetables. Delphinidin has been known to possess many functions, such as an antioxidant, and anti-inflammatory, anti-cancer and anti-muscular atrophy agent. In this study, we attempted to evaluate the effects of delphinidin on lipid accumulation in hepatocytes. The results showed that palmitic acid (PA)-induced cellular senescence in HepG2 cells and reduced the expression of SMARCD1, which is known to regulate senescence-associated lipid accumulation in hepatocytes. However, delphinidin-3-glucoside (D3 g) suppressed PA-induced senescence and reversed the expression of SMARCD1 to the level of untreated HepG2 cells. Consequently, D3 g inhibited PA-induced lipid accumulation through the restoration of the expression of SMARCD1 and fatty acid oxidation genes. Taken together, our results suggest that D3 g suppresses the lipid accumulation induced by hepatocyte senescence.

Keywords Delphinidin · Senescence · Lipid · HepG2 · SMARCD1

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Introduction

Delphinidin is an anthocyanidin commonly found in pigmented fruits and vegetables and is present in the pericarp, testa, and bark of various plants (Wang et al. 2017). Delphinidin possesses many biological functions, such as antioxidant, and has anti-inflammatory, anti-cancer, and anti-muscular atrophy effects (Murata et al. 2017; Wang et al. 2017; Kang et al. 2018; Chen et al. 2018). Recently, delphinidin has been shown to possess beneficial effects on obesity. Rahman et al. (2016) showed the effects of delphinidin on adipogenesis and the related underlying molecular mechanisms. In this study, we evaluated the effects of delphinidin on the lipid accumulation in hepatocytes and the underlying molecular mechanisms.

SMARCD1 is a member of the SWI/SNF chromatin remodeling complex family, which regulates the transcription of target genes (Zhang et al. 2016). Previously, we identified SMARCD1 as a senescence-associated gene (Udono et al. 2015), and demonstrated that PGC-1α-mediated SMARCD1 has roles in the fatty acid oxidation and palmitic acid (PA)-induced hepatic senescence (Inoue et al. 2017; Kang et al. 2018). In the present study, we investigated whether SMARCD1 is a target molecule of delphinidin to suppress lipid accumulation in hepatocytes.

Materials and methods

Cell culture and reagents

HepG2 cells (a human cell line derived from hepatocyte carcinoma, RIKEN, BRC, Tsukuba, Japan) were cultured in DMEM medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Gaithersburg, MD, USA) at 37 °C in a 95% air/5% CO_2 atmosphere. Palmitic acid (PA) was purchased from Chem Service (West Chester, PA, USA). PA complexed with bovine serum albumin (BSA) was prepared as described previously (Zhang et al. 2016). Cells were treated with 0.1 mM PA. Delphinidin-3-glucoside (D3 g) and resveratrol were purchased from Tokiwa Phytochemical (Chiba, Japan) Wako (Osaka, Japan), respectively. D3 g and resveratrol were dissolved in dimethyl sulfoxide (DMSO) and added to the cells at a concentration of 10 μM.

Quantitative reverse transcriptase PCR (qRT-PCR)

RNA was extracted using the High Pure RNA Isolation kit (Roche Diagnostics GmbH, Mannheim, Germany), and cDNA was prepared using ReverTra Ace (Toyobo, Osaka, Japan). The qPCR was performed using the KAPA SYBR Fast qPCR kit (KAPA Biosystems, Woburn, MA, USA) and a Thermal Cycler Dice Real Time System TP-800 (TaKaRa, Shiga, Japan) as described previously (Inoue et al. 2017). The samples were analyzed in triplicates and target gene levels were normalized to the expression levels of β-actin (reference gene). PCR primers used in the study are described in the Supplementary Table 1.

Fluorescence senescence-associated β-galactosidase (SA-β-Gal) assay

Fluorescence SA-β-Gal assay was performed as described previously (Udono et al. 2012). The images were acquired using the IN Cell Analyzer 1000 (GE Healthcare, Little Chalfont, UK), and analyzed using the IN Cell Developer Toolbox 1.9 (GE Healthcare). The imaging data were reported as SA-β-Gal intensity (mean fluorescence intensity per cell) and the number of SA-β-Gal positive/negative cells. The threshold of SA-β-Gal intensity was set to a value so that approximately 75% of the control cells were negative.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 15 min at room temperature, and blocked with a blocking solution (5% goat serum, 0.3% Triton-X 100) for 1 h. Next, cells were incubated overnight at 4 °C with anti-phospho-p38 (#4511, Cell Signaling, Beverly, MA, USA); anti-p16 (ab81278; Abcam, Cambridge, MA, USA); anti-phospho-histone H2A.X (#9718, Cell Signaling) antibodies. Cells were further incubated with the secondary antibody (Alexa Fluor 555 F(ab') fragment of goat anti-rabbit IgG; Life Technologies) at room temperature for 1 h. Finally, cells were incubated with 1 μg/mL of Hoechst 33342 solution (Dojindo, Kumamoto, Japan) at room temperature for 10 min. The images from the immunofluorescence were analyzed using the IN Cell Developer Toolbox 1.9, and the results were depicted using SpotFire DecisionSite Client 8.2 software (PerkinElmer, Waltham, MA, USA).

Quantification of intracellular lipid

HepG2 cells were fixed with 10% formaldehyde for 10 min at room temperature, and stained with 0.06 μg/mL BODIPY 493/503 (Life Technologies) and 1 μg/mL Hoechst 33342 solution. Fluorescence images were obtained using the IN Cell Analyzer 1000, fluorescence intensity and area of lipid droplets were quantitatively determined using the IN Cell Developer Toolbox 1.9 (Inoue et al. 2017).

Statistical analysis

All experiments were performed at least 3 times. The results are presented as mean ± standard deviation. Statistical significance was determined using a two-sided Student's *t* test. Multiple comparisons between groups were made by one-way ANOVA with Turkey's post hoc test. Statistical significance was defined as $P < 0.05$.

Results and discussion

Delphinidin-3-glucoside (D3 g) suppressed the palmitic acid (PA)-induced cellular senescence in HepG2 cells.

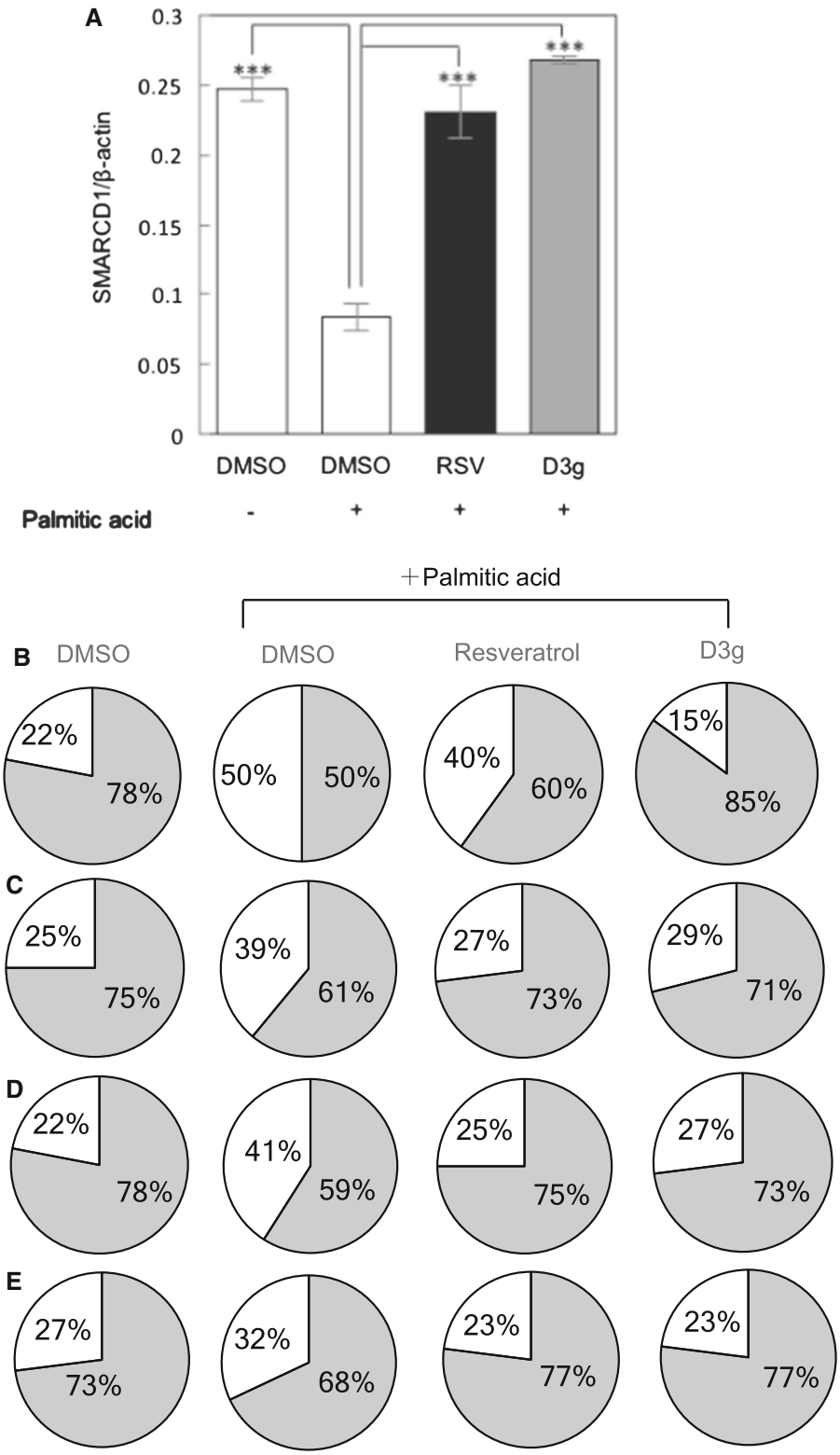
As reported previously (Inoue et al. 2017), PA induced cellular senescence in HepG2 cells, as evidenced by the augmented SA-β-Gal activity, increased expression of phospho-p38, p16 and γH2AX (Fig. 1b–e). Concurrently, PA also reduced the expression of SMARCD1 possibly through the induction of cellular senescence, which is known to be a member of the SWI/SNF chromatin remodeling complex family and regulates the senescence-associated lipid accumulation in hepatocytes (Inoue et al. 2017) (Fig. 1a). Resveratrol is known to suppress cellular senescence, then we used it as a positive control (Ido et al. 2015). Actually, resveratrol and D3 g, an anthocyanidin present in many fruits including pomegranates, suppressed the PA-induced cellular senescence in HepG2 cells. In the present study, we used D3 g, because Wang et al. reported that the glycoside form of anthocyanin showed a high inhibitory activity on triglyceride accumulation in HepG2 cells (Wang et al. 2016). We should confirm whether D3 g directly affects hepatic cells without metabolizing to its aglycon form in a future study. On the other hand, activity and expression of cellular senescence markers were reversed to the state similar to non-treated cells upon treatment with D3 g as well as resveratrol (Fig. 1b–e). Furthermore, D3 g as well as resveratrol also reversed the expression of SMARCD1 to the level of non-treated cells possibly through the suppression of cellular senescence. All these results suggest that D3 g as well as resveratrol suppressed the PA-induced cellular senescence in HepG2 cells.

D3 g suppressed the PA-induced accumulation of lipid droplets in HepG2 cells.

We previously reported that cellular senescence induction triggers lipid accumulation in hepatocytes (Inoue et al. 2017), which suggests that D3 g and resveratrol inhibit PA-induced lipid accumulation in HepG2 cells through suppressing the induction of cellular senescence. As shown in Fig. 2a–d, PA suppressed the expression of fatty acid oxidation (FAO) genes, including acyl-CoA oxidase 1 (ACOX1), acetyl-CoA acyltransferase 1 and 2 (ACAA1 and ACAA2), and carnitine palmitoyltransferase 1A (CPT1A) (Fig. 2a–d). SMARCD1 is involved in the physiological regulation of hepatic fat oxidation (Li et al. 2008) and plasma cholesterol levels (Meng et al. 2015). Furthermore, we demonstrated a PGC-1α-mediated SMARCD1 plays a role in the fatty acid oxidation and PA-induced hepatic senescence. In our previous study, inhibition of SMARCD1 expression induced the accumulation of lipid droplets through the inactivation of PGC-1α-dependent transcription and suppressing the FAO gene expression (Inoue et al. 2017). In the present study, PA suppressed the expression of SMARCD1 in HepG2 cells, which result in the reduction of the FOA gene expression (Fig. 2a–d). Concurrently, D3 g as well as resveratrol reversed the expression of SMARCD1, resulting in a reversal of FAO gene expression in the PA-treated HepG2 cells.

Finally, we tested for the effects of D3 g and resveratrol on the PA-induced accumulation of lipid droplets. As shown in Fig. 2e, PA induced the accumulation of lipid droplets in HepG2 cells, while D3 g and resveratrol suppressed the PA-induced accumulation of lipid droplets. All these results suggest that D3 g as well as resveratrol suppresses the lipid accumulation induced by hepatocyte senescence.

To date, delphinidin has been reported to reduce intracellular lipid accumulation and promote lipolysis through inhibiting the pre-adipocyte differentiation (Rahman et al. 2016). Rahman et al. reported that delphinidin suppressed the expression of early adipogenic transcription factors and effectively inhibited adipogenesis followed by the stabilization of β-catenin. In this study, we present a novel mechanism of delphinidin to suppress lipid accumulation. Delphinidin alleviated the PA-induced reduction of SMARCD1 expression, leading to the avoidance of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◀**Fig. 1** Delphinidin-3-glucoside (D3 g) suppresses the palmitic acid (PA)-induced cellular senescence in HepG2 cells. HepG2 cells were treated with 0.1 mM PA in the presence of 10 μM RSV or D3 g for 24 h. **a** Relative expression of SMARCD1 in HepG2 cells was analyzed by qRT-PCR. **b** Activity and expression of senescence markers (**b** SA-β-Gal, **c** phospho p38, **d** p16, **e** γH2AX) were measured in HepG2 cells. The pie charts show the ratio of senescence marker-positive (white)/ negative cells (gray)

PA-induced cellular senescence and concurrent suppression of PA-induced accumulation of lipid droplets in HepG2 cells. Thus, we demonstrated that SMARCD1 might be a promising candidate for the suppression of accumulation of lipid droplets, and therefore, the prevention of metabolic diseases, including obesity and hepatic steatosis.

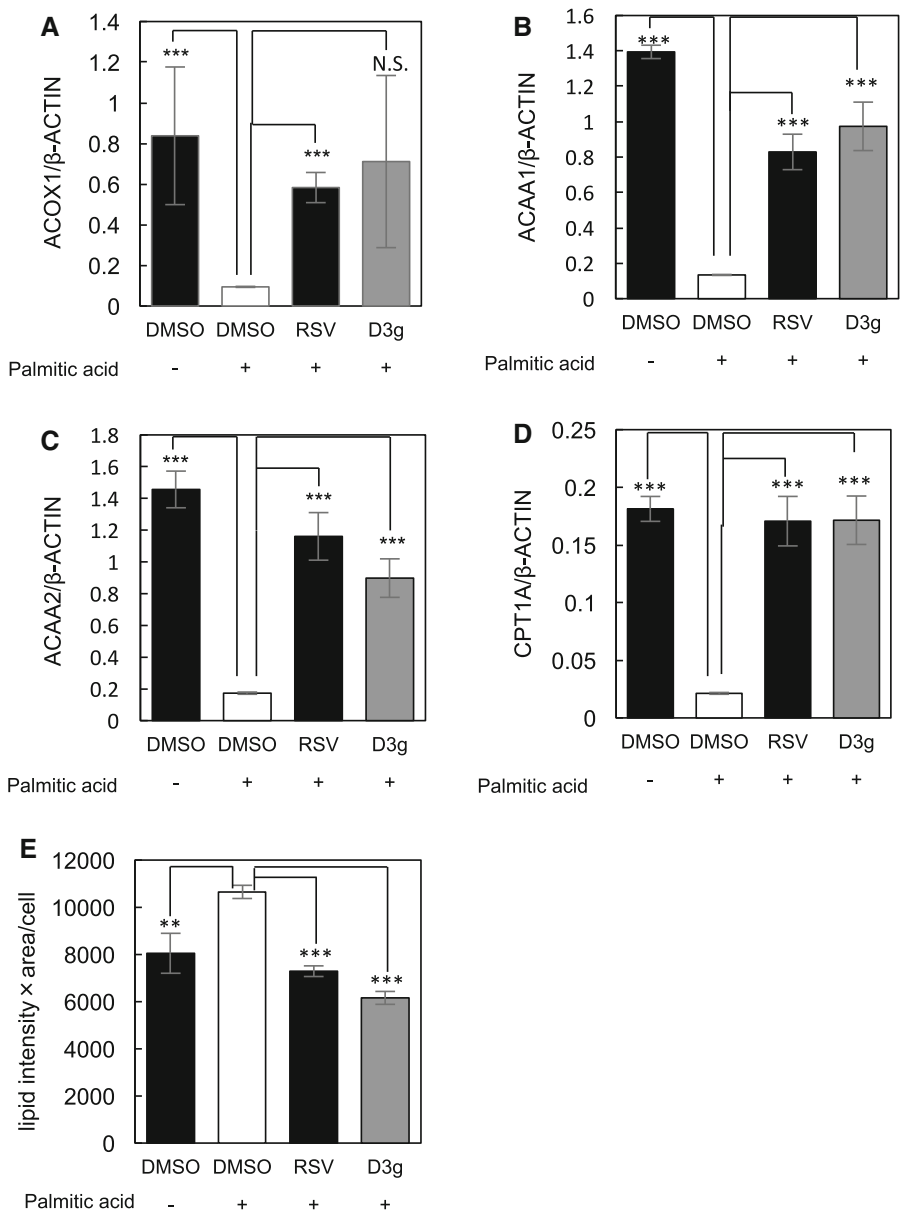

Fig. 2 D3 g suppresses the PA-induced accumulation of lipid droplets in HepG2 cells. HepG2 cells were treated with 0.1 mM PA in the presence of 10 μM RSV or D3 g for 24 h. **a**–**d**, Relative expression of FAO genes in HepG2 cells were analyzed by qRT-PCR. **e** The number of lipid droplets and lipid area in HepG2 cells were analyzed by using the IN Cell Analyzer 1000. Statistical significance was determined using a two-sided Student's *t*-test. Multiple comparisons between groups were made by one-way ANOVA with Turkey's post hoc test. Statistical significance was defined as $P < 0.05$ (**$P < 0.01$; ***$P < 0.001$). N.S. means not significant

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