Stemness and differentiation potential-recovery effects of sinapic acid against ultraviolet-A-induced damage through the regulation of p38 MAPK and NF-κB

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INTRODUCTION

▶ Ultraviolet (UV) A (320–380 nm) irradiation is believed to be deleterious or beneficial to cells and tissues. Various types of cells such as fibroblasts, keratinocytes, melanocytes, cardiomyocytes, vascular endothelial cells, smooth-muscle cells, and osteoblasts respond to UVA irradiation. Several reports suggest that UVA may affect the hypodermis where adipose tissue-derived mesenchymal stem cells, preadipocytes and adipocytes exist. In addition, the direct effects of UVA irradiation on adipose tissue-derived mesenchymal stem cells (hAMSCs), especially the stemness of hAMSCs, have recently been elucidated by our group.

▶ In this study, we investigated effects of sinapic acid on UVA irradiation-induced damages to stemness and differentiation potential of human adipose tissue-derived mesenchymal stem cells (hAMSCs). We also examined the UVA-antagonizing mechanisms of sinapic acid.

▶ These findings suggest that sinapic acid may ameliorate UVA-irradiation-induced reduced stemness and differentiation potential of hAMSCs. Therefore, sinapic acid might have potential as an antagonist agent to attenuate damages caused by UVA.

MATERIALS & METHODS

▶ Cell proliferation: Cell proliferation was measured using ELISA (BrdU Cell Proliferation Assay Kit, Cell Signaling Technology, Danvers, MA, USA) .

▶ Enzyme-linked immunosorbent assay (ELISA)

▶ Real-time RT-PCR (TaqMan™) analysis

▶ Differentiation assays: Oil Red O staining

▶ Immunoblotting analysis

▶ Luciferase reporter assay

RESULTS

▶ ***Effects of UVA irradiation on proliferative potential and self-renewal of hAMSCs.*** Effects of sinapic acid (SA) on the UVA-induced reduction of stemness in hAMSCs were investigated, using cell proliferation assay and real time PCR analysis. As shown in Fig. 1B, SA recovered UVA-induced reduction of proliferation in a dose dependent manner. SA also did not show any apoptotic effects at the treated concentration (Fig. 1C). In addition, as shown in Fig. 1D, reduced expression levels of OCT4, NANOG, and SOX2 by UVA irradiation were all increased by SA, suggesting that SA attenuates the effects of UVA irradiation on hAMSCs.

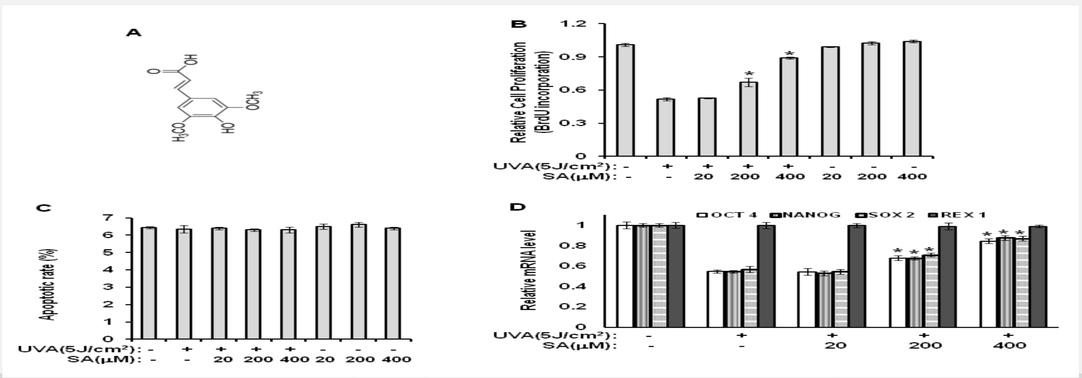

Fig. 1 Sinapic acid(SA) attenuates the effects of UVA irradiation on proliferative potential and self-renewal of hAMSCs. hAMSCs were irradiated with 5J/cm² UVA and then incubated for three days in the presence of the indicated concentrations of SA under serum-free conditions. (A) Chemical structure of SA. (B) After three days, cell proliferation was evaluated using the BrdU incorporation assay. (C) The apoptotic effects of UVA irradiation were determined by Hoechst 33332 staining. (D) After three days of incubation under serum-free conditions, total RNA was isolated and the mRNA levels of the indicated genes were measured by real-time quantitative RT-PCR.

▶ ***UVA irradiation-induced downregulation of HIF-1a is restored by SA*** In a luciferase reporter assay, SA increased reduced HRE-luciferase reporter activity induced by UVA irradiation (Fig. 2A). In addition, treatment with SA attenuated the reduction in the expression of HIF-1α that occurred in response to treatment with UVA irradiation (Fig. 2B). However, SA did not affect the expression of HIF-2α (Fig. 2B). Consistent with these findings, the reduction in the protein level of HIF-1α that was induced by UVA irradiation was also significantly recovered by SA (Fig. 2C). Taken together, these findings suggest that SA recovered the reduced stemness of hAMSCs due to UVA irradiation through the upregulation of HIF-1α.

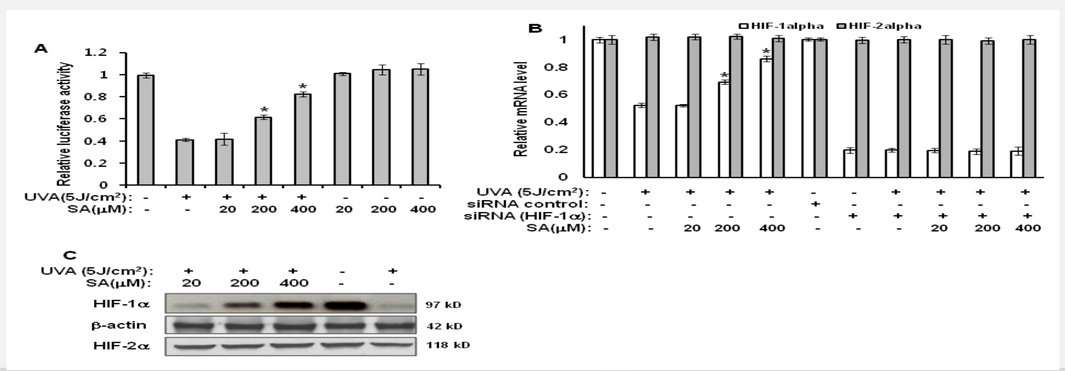

Fig. 2 SA increases downregulated expression of HIF-1α by UVA irradiation. A: Luciferase reporter assay(HRE-Luc reporter), B: Real-time quantitative RT-PCR for HIF-1α and HIF-2α genes, C: Western blot assay using the HIF-1α and HIF-2α antibodies. SA: sinapic acid.

▶ **SA reduces *UVA-induced suppression of adipogenic differentiation through downregulating MIF-AMPK-KLF2 signaling*** In Oil Red O staining and triglyceride accumulation assays, SA reduced the anti-adipogenic effects of UVA, thus having differentiating potential-protection effect (Fig. 7A & 7B). In addition, SA reduced both mRNA and protein levels of MIF which were induced by UVA (Fig. 7C & 7D). As shown in Fig. 8A, UVA-induced phosphorylation of AMPK, a downstream molecule of MIF, was also suppressed by SA. In addition, while SA reduced the mRNA levels of KLF2, the mRNA levels of PPARγ was increased by SA. Furthermore, the activity of NF-κB, but not JNK or p42/44 MAPK, was reduced by sinapic acid (Fig. 8C).

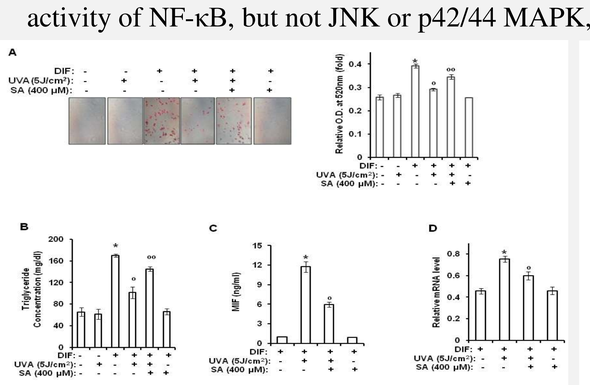

Fig. 7 SA attenuates UVA-induced suppression of adipogenic differentiation through downregulation of MIF gene expression. A: Oil Red O staining, B: Triglyceride content assay C: ELISA for MIF, D: Real-time quantitative RT-PCR for MIF

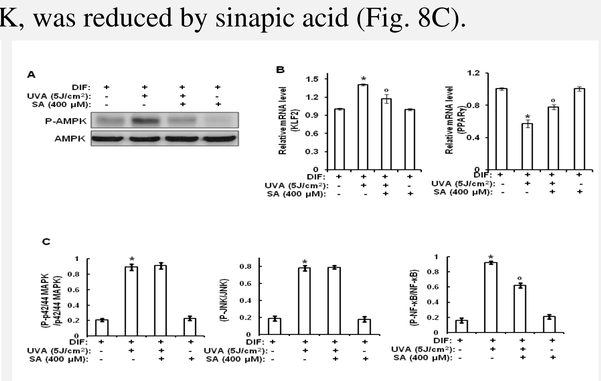

Fig. 8 Sinapic acid attenuates UVA-induced suppression of adipogenic differentiation through downregulation of MIF-AMPK-KLF2 signaling by inhibiting NF-κB. A: Western blot analysis for AMPK, B: Real-time quantitative RT-PCR for KLF2 and PPARγ genes, C: ELISA for MAPKs

▶ ***Sinapic acid (SA) reduces UVA-induced production of PGE_2 and cAMP through inhibition of JNK and p38 MAPK*** In this study, treatment of hAMSCs with SA was found to lead to a significant decrease in the production of PGE_2 and cAMP when compared to the UVA-irradiated controls (Fig. 3A and 3B). To investigate the action mechanisms of SA, luciferase reporter assays for AP-1, NF-κB, or CRE were conducted. As shown in Fig. 4A, 4B, and 4C, SA reduced the UVA-induced activation of the NF-κB, AP-1, and CRE promoters. These results suggest that SA attenuated UVA-induced production of PGE_2 through inhibition of AP-1, CRE, and NF-κB activities. In addition, among the three types of MAPKs evaluated, the activity of p38 MAPK, but not p42/44 MAPK, and JNK, were found to be reduced by SA when compared to the UVA-irradiated controls (Fig. 4D).

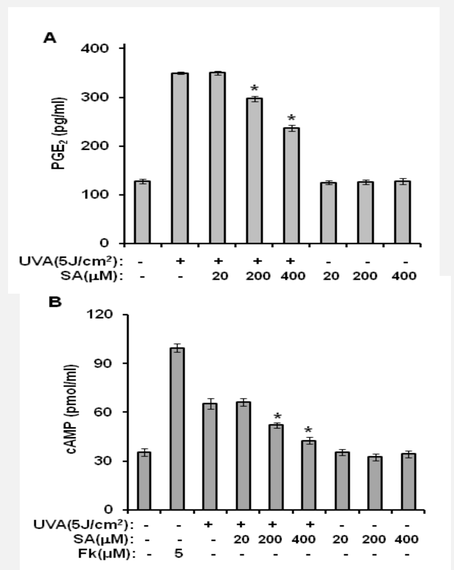

Fig. 3. SA reduces UVA-induced production of PGE_2 and cAMP. hAMSCs were irradiated with 5J/cm² UVA and then incubated for three days in the presence of the indicated concentrations of SA under serum-free conditions. (A and B) After three days of incubation, the supernatants were harvested for PGE_2 (A) and cAMP (B) measurement. SA: sinapic acid.

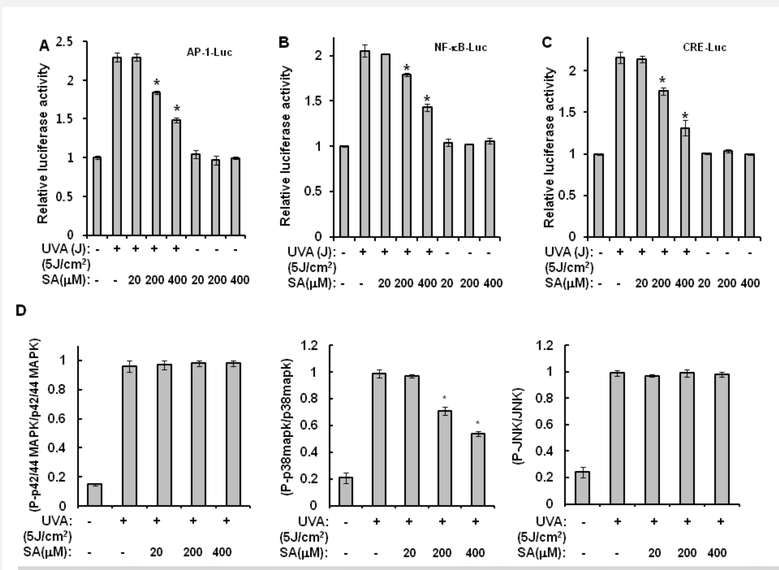

Fig. 4. SA reduces UVA-induced production of PGE_2 and cAMP through the inhibition of p38 MAPK, CRE, and NF-κB. (A, B, and C) hAMSCs were transfected with AP-Luc, NF-κB-Luc, or CRE-Luc reporters. After incubation for 24 h, the cells were irradiated with 5J UVA and then further incubated in the presence of the indicated concentrations of SA under serum-free conditions for 14 h. These cells were assayed. (D) hAMSCs were irradiated with 5J/cm² UVA and then treated with the indicated concentrations of SA for 1 h under serum-free conditions. After 1 h of incubation, the cell lysates were analyzed. SA: sinapic acid.

▶ ***Antagonizing effects of SA against UVA-induced downregulation of stemness genes are mediated by downregulating PGE_2-cAMP-HIF-1α signaling through inhibition of AP-1, NF-κB, and CRE.*** Until now, SA was found to enhance expression of stemness genes by downregulating PGE_2-cAMP-HIF-1α signaling through inhibition of p38 MAPK, CRE, and NF-κB. These results were further confirmed by experiments using siRNA for HIF-1α, PGE_2, and cAMP. As shown in Fig. 5, SA treatment reduced the effects of UVA on the expression of stemness-related genes. Specifically, the reduced expression of both HIF-1α gene (Fig. 5A) and OCT4, NANOG, and SOX2 genes (Fig. 5B) caused by UVA irradiation was increased by SA treatment. However, the effects of SA were attenuated by treatment of PGE_2 and cAMP as well as knock-down of the HIF-1α gene. These results indicate that SA operates upstream of PGE_2, cAMP and HIF-1α molecules, suggesting that SA effects are mediated by downregulating PGE_2-cAMP-HIF-1α signaling through inhibition of AP-1, CRE, and NF-κB. The action mechanisms of SA were shown in detail in Fig. 6.

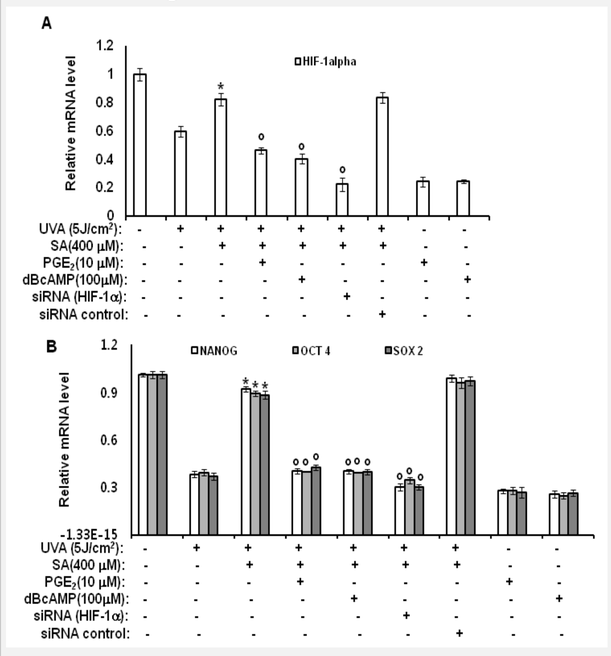

Fig. 5. SA effects against UVA-induced downregulation of stemness genes are mediated by downregulating PGE_2-cAMP-HIF-1α signaling through inhibition of AP-1, CRE, and NF-κB. hAMSCs were irradiated with 5 J/cm² UVA or transfected with the siRNA for HIF-1α and then incubated for three days with SA (400 μM) in the presence of the indicated concentration of PGE_2 or cAMP under serum-free conditions. The mRNA levels of the HIF-1α gene (A) and OCT4, NANOG, SOX2 genes (B) were measured by real-time quantitative RT-PCR. SA: sinapic acid, dBcAMP: dibutyryl cAMP.

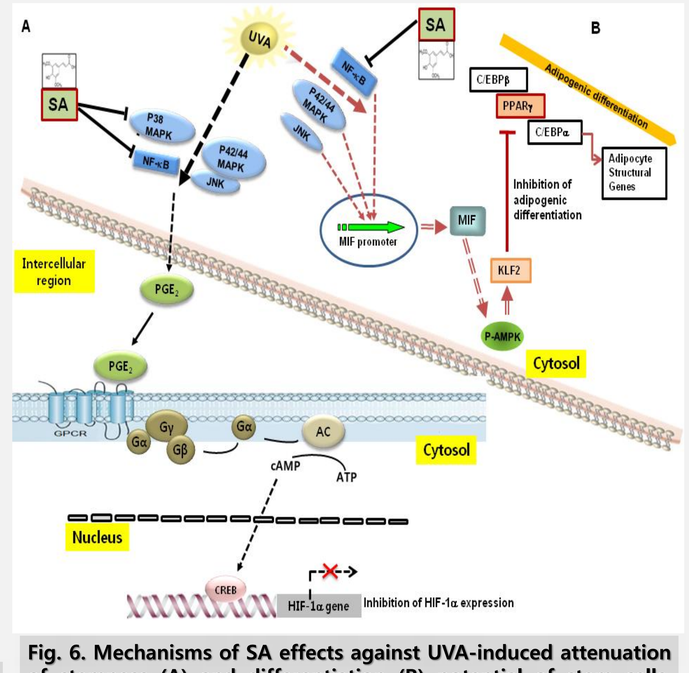

Fig. 6. Mechanisms of SA effects against UVA-induced attenuation of stemness (A) and differentiation (B) potential of stem cells. UVA irradiation induces production of PGE_2 and its downstream molecule, cAMP through activation of AP-1 and NF-κB. cAMP molecule sequentially reduces expression of HIF-1α gene through CREB activation, consequently downregulating expression of stemness genes such as NANOG, SOX2, and OCT4. In the UVA irradiation-induced signaling pathway, SA attenuates UVA-induced effects on expression of stemness genes or differentiation-related genes by inhibiting p38 MAPK and NF-κB or inhibiting just only NF-κB, respectively. The molecules are upstream of PGE_2 production. AC: adenylate cyclase.

CONCLUSIONS

▶ **Taken together, these findings suggest that SA improves reduced stemness and differentiation potential of hAMSCs induced by UVA and its effects are mediated by upregulation of HIF-1α via the inhibition of PGE_2-cAMP signaling. In addition, SA may be used as an antagonizing agent to mitigate the effects of UVA.**

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