Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dermal fibroblasts

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Abstract **Ultraviolet (UV) irradiation regulates UV-responsive genes, including matrix metalloproteinases (MMPs). Moreover, UV-induced MMPs cause connective tissue damage and the skin to become wrinkled and aged. Here, we investigated the effect of eicosapentaenoic acid (EPA), a dietary ω-3 fatty acid, on UV-induced MMP-1 expression in human dermal fibroblasts (HDFs). We found that UV radiation increases MMP-1 expression and that this is mediated by p44 and p42 MAP kinase (ERK) and Jun-N-terminal kinase (JNK) activation but not by p38 activation. Pretreatment of HDFs with EPA inhibited UV-induced MMP-1 expression in a dose-dependent manner and also inhibited the UV-induced activation of ERK and JNK by inhibiting ERK kinase (MEK1) and SAPK/ERK kinase 1 (SEK1) activation, respectively. Moreover, inhibition of ERK and JNK by EPA resulted in the decrease of c-Fos expression and c-Jun phosphorylation/expression induced by UV, respectively, which led to the inhibition of UV-induced activator protein-1 DNA binding activity. This inhibitory effect of EPA on MMP-1 was not mediated by an antioxidant effect. We also found that EPA inhibited 12-*O*-tetradecanoylphorbol-13-acetate- or tumor necrosis factor-α-induced MMP-1 expression in HDFs and UV-induced MMP-1 expression in HaCaT cells. In conclusion, our results demonstrate that EPA can inhibit UV-induced MMP-1 expression by inhibiting the MEK1/ERK/c-Fos and SEK1/JNK/c-Jun pathways. Therefore, EPA is a potential agent for the prevention and treatment of skin aging.**—Kim, H. H., C. M. Shin, C-H. Park, K. H. Kim, K. H. Cho, H. C. Eun, and J. H. Chung. **Eicosapentaenoic acid inhibits UV-induced MMP-1 expression in human dermal fibroblasts. *J. Lipid Res.* 2005.** 46: **1712–1720.**

Supplementary key words ultraviolet radiation • matrix metalloproteinase-1 • polyunsaturated fatty acid

The skin aging process is commonly associated with increased wrinkling, sagging, and laxity and is considered to consist of two components, intrinsic aging and extrinsic aging, the latter of which is referred to as photoaging (1). Histological and ultrastructural studies have revealed that the major alterations in photoaged skin are localized in dermal connective tissue. The matrix metalloproteinases (MMPs) are a family of structurally related matrix-degrading enzymes that play important roles in various destructive processes, including inflammation (2), tumor invasion (3, 4), and skin aging (5–7). Moreover, the expression of various ultraviolet (UV)-induced MMPs in dermal fibroblasts leads to the breakdown of collagen and other extracellular matrix proteins and is thus related to photoaging in human skin (5, 8, 9).

Researchers have investigated the molecular mechanisms underlying the induction of MMPs after exposure to UV irradiation. Fisher and Voorhees (8) suggested that UV radiation activates growth factor receptors, which induce the activation of protein kinase cascades, such as the mitogen-activated protein kinase (MAPK) cascade. This activation is then succeeded by an increase in the expression of c-Jun and c-Fos, which form the activator protein-1 (AP-1) complex. Transcription of several MMPs, including MMP-1, MMP-3, and MMP-9, is regulated by AP-1. For AP-1 complex formation, Jun proteins (c-Jun, Jun B, and Jun D) form homodimers or heterodimers with Fos proteins (c-Fos, Fos B, Fra-1, and Fra-2) (10). The transcriptional activity of AP-1 is also dependent on the degree of phosphorylation of c-Jun and expression of c-Fos as well as their abundance. Moreover, increased AP-1 activity is re-

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Abbreviations: AA, arachidonic acid; AP-1, activator protein-1; COX-2, cyclooxygenase-2; DCF-DA, 2′-7′-dichlorofluorescein diacetate; DHA, docosahexaenoic acid; EMSA, electrophoretic mobility shift assay; EPA, eicosapentaenoic acid; ERK, p44 and p42 MAP kinase; JNK, Jun-N-terminal kinase; HDF, human dermal fibroblast; LA, linolenic acid; MAPK, mitogen-activated protein kinase; MEK1, MAP or ERK kinase; MMP-1, matrix metalloproteinase-1; NAC, N-acetyl cysteine; OA, oleic acid; PGE, prostaglandin E; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species; SEK, SAPK/ERK kinase; TNF-α, tumor necrosis factor-α; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

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sponsible for the degradation of extracellular matrix proteins, such as collagen, by inducing MMPs.

Essential fatty acids are divided into two categories depending on their saturation state. ω-3 and ω-6 fatty acids have unsaturated carbons at the third and sixth carbons in the methyl terminus, respectively. The ω-3 fatty acids include α-linolenic acid (LA, 18:3), stearidonic acid (18:4), eicosatetraenoic acid (20:4), eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (22:5), and docosahexaenoic acid (DHA, 22:6) (11). Of these ω-3 PUFAs, EPA is found primarily in fish oils and used to treat inflammatory diseases and prostate and colon cancer. EPA competes with arachidonic acid (AA), an ω-6 PUFA, for incorporation into cell membrane phospholipids (12) and as a substrate of cyclooxygenase-2 (COX-2) (13). Prostaglandin Es (PGEs) are derived from membrane PUFAs and play important roles in inflammation, immune response, and wound healing (14). Whereas ω-6 PUFAs such as AA generate 2-series PGEs (PGE_2), ω-3 PUFAs such as EPA and DHA generate 3-series PGEs (PGE_3) (13). PGE_2 is more mitogenic and proinflammatory than PGE_3. It has been reported that fish oils, rich in ω-3 PUFAs, possess anti-inflammatory properties in UV-exposed skin (15). Recently, EPA and DHA were reported to reduce basal and UV-induced interleukin-8 secretion by keratinocytes and fibroblasts (16). The aims of the present study were to examine the effect of EPA on UV-induced MMP-1 expression in cultured human dermal fibroblasts (HDFs) and to reveal the signaling pathway changes through which EPA inhibits UV-induced MMP-1 expression.

MATERIALS AND METHODS

Materials

EPA, DHA, AA, LA, oleic acid (OA), and 2′-7′-dichlorofluorescein diacetate (DCF-DA) were purchased from Sigma (St. Louis, MO). All inhibitors [U0126, a ERK kinase (MEK1)-specific inhibitor; SP600126, a Jun-N-terminal kinase (JNK)-specific inhibitor; and SB203580, a p38-specific inhibitor] were purchased from Calbiochem (San Diego, CA). Anti-human MMP-1 antibody was purchased from Oncogene (Boston, MA), and anti-phospho-SAPK/ERK kinase (SEK)1, anti-phospho-JNK, anti-phospho-MEK1, anti-phospho-ERK, anti-phospho-MKK3/6, anti-phospho-p38, anti-phospho-c-Jun, anti-c-Fos, and anti-phospho-Elk-1 antibodies were purchased from Cell Signaling Technology (Beverly, MA). Anti-JNK2 and anti-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture, EPA treatment, and UV irradiation

HDFs were isolated from young foreskins and cultured in monolayers at 37°C and 5% CO_2 using DMEM containing 10% FBS. Human foreskin fibroblasts (Hs27) and immortalized human keratinocytes (HaCaT) were cultured using the same medium and conditions. For all experiments, cells were cultured to 80% confluence and then starved in DMEM containing 0.1% FBS for 36 h.

All fatty acids (EPA, DHA, AA, LA, and OA) were dissolved in DMSO (10 mM), diluted with culture media at the indicated concentrations (final DMSO concentration, 0.1%), and used to pretreat cells for 24 h before UV irradiation. After pretreatment, cells were washed with PBS and irradiated with UV in PBS.

Philips TL 20W/12 RS fluorescent sun lamps with an emission spectrum between 275 and 380 nm (peak, 310–315 nm) were used as a UV source (17), and a Kodacel filter (TA401/407; Kodak, Rochester, NY) was used to block UVC, which has wavelengths of <290 nm. UV strength was measured using a Waldmann UV meter (model 585100).

Western blot analysis

Western blotting was performed as described previously (18). Cells were lysed with lysis buffer [50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 5 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM DTT] containing 1% Triton X-100. Insoluble debris was removed by centrifugation at 12,000 rpm for 10 min, and protein content was determined using Bradford reagent (Bio-Rad, Hercules, CA). Equal amounts of protein were resolved on gradient (8–16%) SDS-PAGE gels (Invitrogen, Carlsbad, CA) and then electrophoretically transferred to polyvinylidene fluoride membranes (Immobilon P; Amersham, Buckinghamshire, UK). Membranes were subsequently blocked with 5% skim milk in TBS/T (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.05% Tween 20) and incubated with the indicated antibodies. Blotting proteins were visualized by enhanced chemiluminescence (Amersham).

AP-1 DNA binding assay

HDFs were pretreated with the indicated concentrations of EPA for 24 h, washed with PBS, and irradiated with UV (75 mJ/cm^2) in PBS. After incubation for 10 h, nuclear extracts were prepared using a modification of the method described by Liu et al. (19). Briefly, cells were lysed with lysis buffer (25 mM HEPES, pH 7.8, 50 mM KCl, 1 mM DTT, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 5 mM PMSF) containing 0.5% Nonidet P-40, cytosol fractions were removed by centrifugation, and nuclear pellets were solubilized with extraction buffer (25 mM HEPES, pH 7.8, 500 mM KCl, 1 mM DTT, 20 μg/ml aprotinin, 20 μg/ml leupeptin, and 5 mM PMSF) containing 1% Nonidet P-40. Equal amounts of nuclear protein were then subjected to gel shift assay according to the manufacturer's instructions (Gel Shift Assay System; Promega, Madison, WI). Briefly, AP-1 consensus oligonucleotides were labeled by incubating them with T4 polynucleotide kinase buffer, 10 μCi/ml [γ-^{32}P]ATP, and T4 polynucleotide kinase at 37°C for 10 min. Equal amounts of nuclear proteins were added to the reaction mixtures and incubated for 10 min at room temperature, and labeled oligonucleotide was then added. After incubation for 20 min at room temperature, samples were loaded onto 6% DNA retardation gels (Invitrogen). Gels were dried, and the labeled complex was visualized by autoradiography.

Detection of reactive oxygen species production

HDFs were pretreated with the indicated concentrations of EPA for 24 h, washed with PBS, and stained with 30 μM DCF-DA for 30 min. The cells were then irradiated with UV (75 mJ/cm^2) while in PBS and harvested by trypsinization. Cells were then washed twice with PBS and analyzed using a FACScan flow cytometer (FACStar; Becton-Dickinson, San Jose, CA). All experiments were performed in triplicate, and reactive oxygen species (ROS) levels are described as fold increase versus the vehicle-treated control.

Statistical analysis

Statistical analyses were performed using the Wilcox rank sum test. $P < 0.05$ was considered statistically significant. All analyses were performed using Statistical Analysis Software (SAS, Inc., Cary, NC).

RESULTS

UV-induced MMP-1 expression was mediated by ERK and JNK activation but not by p38 activation

To examine UV-induced MMP-1 expression in cultured HDFs, cells were irradiated with UV (0–75 mJ/cm^2), which did not influence cell viability (data not shown). Seventy-two hours after UV radiation, MMP-1 protein levels in culture media were determined by Western blotting. As expected, UV radiation induced MMP-1 expression in a dose-dependent manner (**Fig. 1A**).

Three MAPKs (ERK, JNK, and p38) were activated by UV within 15 min after UV irradiation and then declined to the basal level in HDFs (Fig. 1B). To investigate the roles of MAPKs in UV-induced MMP-1 expression, HDFs were pretreated with U0126 (10 μM; a MEK1-specific inhibitor), SP600125 (25 μM; a JNK-specific inhibitor), or SB203580 (10 μM; a p38-specific inhibitor) for 30 min and then irradiated with UV (75 mJ/cm^2). UV-induced MMP-1 expression was inhibited by pretreating with U0126 and SP600125 by 82 ± 6% and 98 ± 3%, respectively, but not by SB203580 (Fig. 1C). These results suggest that the activation of the ERK and JNK pathways mediates UV-induced MMP-1 expression in cultured HDFs.

EPA inhibits UV-induced MMP-1 expression by inhibiting ERK and JNK activation

To determine whether EPA inhibits UV-induced MMP-1 expression, HDFs were pretreated with various concentrations of EPA for 24 h and then irradiated with UV (75 mJ/cm^2). Pretreatment with EPA inhibited UV-induced MMP-1 expression in a dose-dependent manner (**Fig. 2A**) by 33 ± 9% at 5 μM and 79 ± 1% at 10 μM, versus the UV-only-treated group (Fig. 2A). Moreover, pretreatment with EPA also inhibited basal MMP-1 expression.

As ERK and JNK activation are required for UV-induced MMP-1 expression in dermal fibroblasts (Fig. 1C), we investigated the effects of EPA on UV-induced ERK and JNK activation. Cells were pretreated with EPA for 24 h and then irradiated with UV (75 mJ/cm^2). Levels of phospho- and total MEK1/2, ERK, SEK1, and JNK proteins were determined by Western blot analysis at 30 min after UV treatment. Pretreatment with EPA inhibited both ERK and JNK activation in a dose-dependent manner without altering total ERK or JNK levels (Fig. 2B, C). We also found that pretreatment with EPA inhibited the UV-induced phosphorylation of MEK1/2 and SEK1, which are the upstream kinases of ERK and JNK, respectively (Fig. 2B, C). Pretreatment with EPA also inhibited both basal MEK1/ERK and SEK1/JNK activation, which resulted in the inhibition of basal MMP-1 expression. These results suggest that inhibition of the MEK1/ERK and SEK1/JNK pathways by EPA might attenuate UV-induced MMP expression.

EPA inhibited UV-induced AP-1 activation, c-Jun phosphorylation, and c-Fos expression

AP-1 activation induced by UV was found to increase MMP-1 expression because MMP-1 promoters carry the AP-1 binding site (20). To investigate the effects of EPA on UV-induced AP-1 activity, HDFs were pretreated with EPA for 24 h and then irradiated with UV (75 mJ/cm^2). Pretreatment with EPA inhibited UV-induced AP-1 DNA binding activity in a dose-dependent manner at 10 h after UV

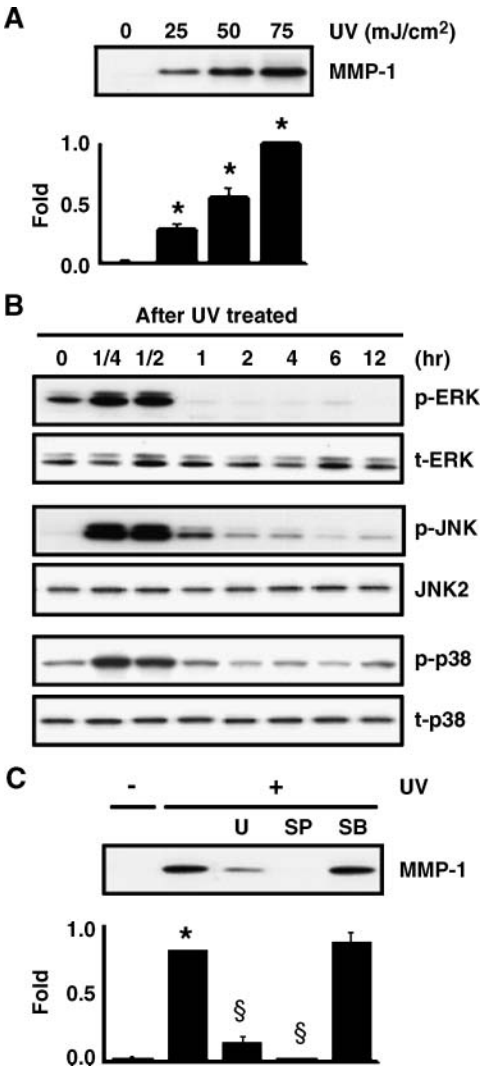

Fig. 1. Ultraviolet (UV)-induced matrix metalloproteinase-1 (MMP-1) expression is mediated by ERK and Jun-N-terminal kinase (JNK) activation but not by p38 activation. Human dermal fibroblasts (HDFs) were cultured in DMEM until 80% confluent and then starved with DMEM containing 0.1% FBS for 36 h. A: Cells were washed with PBS and irradiated with various intensities of UV (0–75 mJ/cm^2). The UV-exposed cells were cultured for 72 h, and MMP-1 expression was determined in culture media by Western blotting. B: HDFs were irradiated with UV (75 mJ/cm^2) and harvested at the indicated times. Total cell lysates were prepared as described in Materials and Methods and subjected to Western blotting using phospho-specific (p-) ERK, JNK, and p38 antibodies. Levels of total (t-) ERK, JNK2, and p38 were used as loading controls. C: HDFs were pretreated with U0126 [(U) a ERK kinase (MEK1) inhibitor; 10 μM], SP600125 [(SP) a JNK inhibitor; 25 μM], and SB203580 [(SB) a p38 inhibitor; 10 μM] for 30 min. Cells were washed with PBS and irradiated with UV (75 mJ/cm^2) in PBS. Irradiated cells were cultured for 72 h, and MMP-1 expression was determined in culture media by Western blotting. All experiments were performed in triplicate. Values shown are means ± SEM (n = 3). * $P < 0.05$ versus the control, § $P < 0.05$ versus UV-irradiated cells.

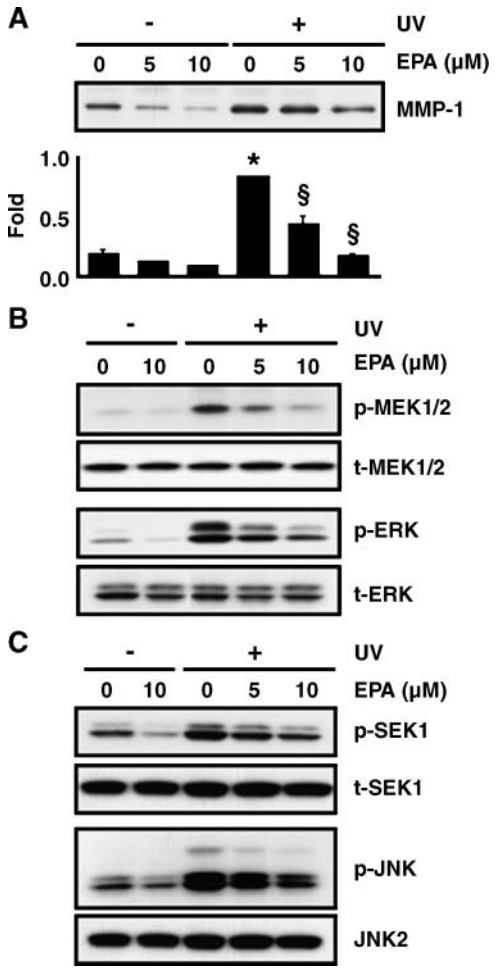

Fig. 2. Eicosapentaenoic acid (EPA) inhibits UV-induced MMP-1 expression by inhibiting ERK and JNK activation. HDFs were cultured in DMEM until 80% confluent and starved with DMEM containing 0.1% FBS for 36 h. A: Cells were then pretreated with the indicated concentrations of EPA for 24 h, washed with PBS, and irradiated with UV (75 mJ/cm^2) in PBS. UV-exposed cells were cultured with EPA for 72 h, and MMP-1 expression was determined in culture media by Western blotting. B, C: HDFs were pretreated with the indicated concentrations of EPA for 24 h and then irradiated with UV (75 mJ/cm^2). After incubation for 30 min, total cell lysates were prepared as described in Materials and Methods. Western blotting was performed using phospho-specific (p-) MEK1/ERK (B) and SAPK/ERK kinase (SEK)1/JNK (C) antibodies. Levels of total (t-) ERK and JNK2 were used as loading controls. All experiments were performed in triplicate. Values shown are means ± SEM (n = 3). * $P < 0.05$ versus the control, § $P < 0.05$ versus UV-irradiated cells.

treatment (**Fig. 3A**). When pretreated with 10 μM EPA, AP-1 DNA binding activity was reduced to 23 ± 3% of that in the UV-irradiated group.

Because the AP-1 complex is composed of Jun and Fos family members (21, 22), we investigated the effects of EPA on UV-induced c-Jun phosphorylation/expression and c-Fos expression. UV irradiation induced c-Jun phosphorylation/expression and c-Fos expression in HDFs (Fig. 3B), and pretreatment with EPA inhibited UV-induced c-Jun phosphorylation by 37 ± 4% at 5 μM and 82 ± 8% at 10 μM and c-Jun expression by 19 ± 4% at 5 μM and 46 ± 8% at 10 μM. Pretreatment with EPA also inhibited c-Fos expression by 27 ± 6% at 5 μM and 74 ± 9% at 10 μM (Fig. 3B). To determine how EPA inhibited UV-induced c-Fos expression, we investigated whether EPA inhibited UV-induced Elk-1 activation, which is closely related to c-Fos expression (23–25). UV radiation rapidly induced the phosphorylation of Elk-1, and pretreatment with EPA dramatically inhibited UV-induced Elk-1 phosphorylation in a dose-dependent manner (Fig. 3C).

The present study demonstrates that the inhibition of ERK activity by U0126 (a MEK1 inhibitor) prevented UV-induced c-Fos expression in cultured HDFs without inhibiting UV-induced c-Jun phosphorylation (Fig. 3D). On the other hand, the inhibition of JNK activity by SP600125 prevented UV-induced c-Jun phosphorylation without inhibiting UV-induced c-Fos expression (Fig. 3D). These results suggested that MEK1/ERK pathways are involved in UV-induced c-Fos expression and that SEK1/JNK pathways are involved in UV-induced c-Jun phosphorylation. Therefore, our results indicate that EPA suppresses UV-induced AP-1 activation by inhibiting the MEK1/ERK/c-Fos and SEK1/JNK/c-Jun pathways.

EPA does not have an antioxidant effect

To investigate whether EPA inhibits UV-induced MMP-1 expression via an antioxidant effect, ROS levels were measured after EPA treatment. UV radiation significantly increased ROS generation by 380 ± 43% versus the control (**Fig. 4**). However, EPA did not affect this UV-induced ROS generation or basal ROS levels (Fig. 4). This result suggests that the inhibition of UV-induced MMP-1 expression by EPA is not associated with the inhibition of ROS production or any antioxidant effect of EPA.

Inhibition of MMP-1 expression by EPA is not cell type- or stimuli-specific

As various cytokines and chemicals can induce MMP-1 expression, we investigated whether EPA can also inhibit MMP-1 induction by tumor necrosis factor-α (TNF-α) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA). We found that TNF-α or TPA induced MMP-1 expression in HDFs and that pretreatment with EPA inhibited TNF-α- or TPA-induced MMP-1 expression in a dose-dependent manner (**Fig. 5A**). To investigate whether the inhibition of MMP-1 expression by EPA is cell type-specific event, the effects of EPA on UV-induced MMP-1 expression were also examined in Hs27 (human foreskin fibroblasts) and HaCaT (immortalized human immortalized keratinocytes) cells. Hs27 and HaCaT cells were pretreated with the indicated concentrations of EPA for 24 h and then irradiated with UV (75 and 30 mJ/cm^2 for Hs27 and HaCaT, respectively). In both cell lines, pretreatment with EPA inhibited UV-induced MMP-1 expression in a dose-dependent manner (Fig. 5B). These results suggest that the inhibition of UV-induced MMP-1 expression by EPA is not cell type- or stimulus-specific.

n-3 and n-9 PUFAs, but not n-6 PUFAs, inhibited UV-induced MMP-1 expression

To investigate the effects of other fatty acids, such as n-3 DHA, n-6 AA, n-6 LA, and n-9 OA, on UV-induced MMP-1

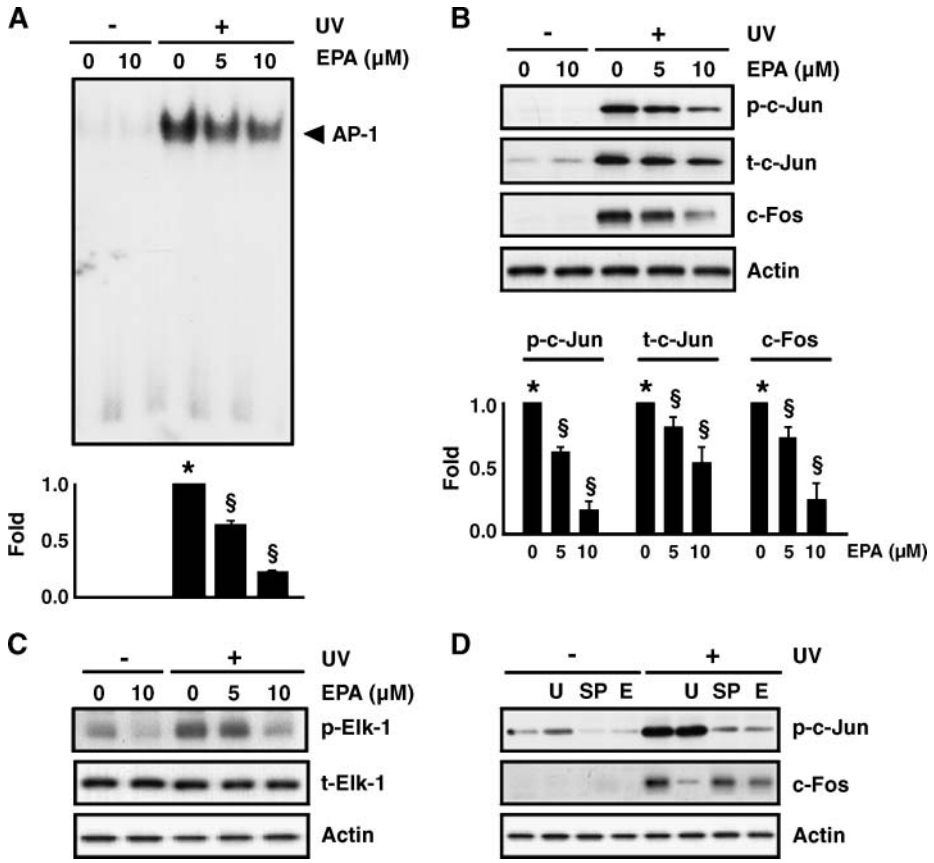

Fig. 3. EPA suppresses UV-induced activator protein-1 (AP-1) activation by inhibiting c-Jun expression/phosphorylation and c-Fos expression. A, B: HDFs were cultured in DMEM until 80% confluent and then starved with DMEM containing 0.1% FBS for 36 h. Cells were then pretreated with the indicated concentrations of EPA for 24 h. Cells were washed with PBS and irradiated with UV (75 mJ/cm^2) in PBS. After incubation for 10 h, nuclear extracts and total cell lysates were prepared for electrophoretic mobility shift assay (EMSA) (A) and Western blotting (B). Gel shift assays were performed as described in Materials and Methods, and Western blotting was performed using specific antibodies for phospho-specific (p-) c-Jun, c-Fos, and actin. Actin was used as a loading control. C: Total cell lysates were prepared 30 min after UV radiation, and Western blotting was performed using phospho-specific Elk-1 antibody. Actin was used as a loading control. D: HDFs were pretreated with U0126 [(U) 10 μM, 30 min], SP600125 [(SP) 25 μM, 30 min], or EPA [(E) 10 μM, 24 h], washed with PBS, and radiated with UV (75 mJ/cm^2) in PBS. UV-exposed cells were cultured for 10 h, and c-Jun phosphorylation and c-Fos expression levels were determined by Western blotting using actin as a loading control. All experiments were performed in triplicate. Values shown are means ± SEM (n = 3). * $P < 0.05$ versus the control, § $P < 0.05$ versus UV-irradiated cells.

expression, HDFs were pretreated with 10 μM of each fatty acid for 24 h and then irradiated with UV (75 mJ/cm^2). Pretreatment with DHA or OA significantly inhibited UV-induced MMP-1 expression (**Fig. 6**). However, pretreatment with AA or LA slightly increased or did not affect UV-induced MMP-1 expression, respectively (Fig. 6). From these results, we found that the inhibitory effect of PUFAs on UV-induced MMP-1 expression was dependent on their structure.

DISCUSSION

This study demonstrates that EPA can inhibit basal and UV-induced MMP-1 expression in HDFs and that this inhibitory effect of EPA on MMP-1 expression may be mediated by the inhibition of the ERK- and JNK-dependent pathways.

Photoaging concerns premature skin aging caused by repeated sun exposure (26–28). Recently, it was suggested that excessive matrix degradation by UV-induced MMPs secreted by various cells (e.g., keratinocytes, fibroblasts, and inflammatory cells) contributes substantially to the connective tissue damage that occurs during photoaging (28–30). UVB is known to induce the expression of MMP-1, MMP-3, and MMP-9 in the normal human epidermis in vivo (29), and UVA is known to induce the expression of MMP-1 by dermal fibroblasts and the expression of MMP-1, MMP-2, and MMP-3 in cultured HDFs (28). In the present study, we also found that UV can induce MMP-1 expression in cultured HDFs in a dose-dependent manner and that pretreatment with EPA before UV treatment inhibits UV-induced MMP-1 expression. Because collagen

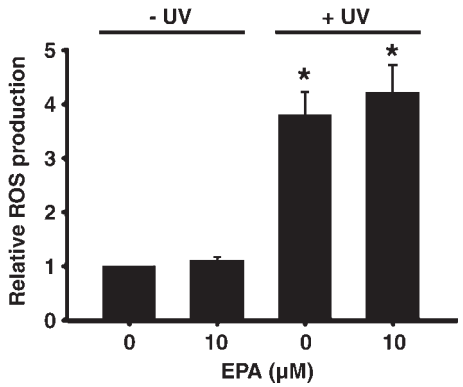

Fig. 4. EPA does not inhibit UV-induced reactive oxygen species (ROS) production. HDFs were cultured in DMEM until 80% confluent, starved with DMEM containing 0.1% FBS for 36 h, and pretreated with 10 μM EPA for 24 h. The cells were then stained with 30 μM 2′-7′-dichlorofluorescein diacetate for 30 min and irradiated with UV (75 mJ/cm^2) in PBS. Thirty minutes after UV irradiation, ROS levels were analyzed using a FACScan flow cytometer. All experiments were performed in triplicate. Values shown are means ± SEM (n = 3). * $P < 0.05$ versus the control.

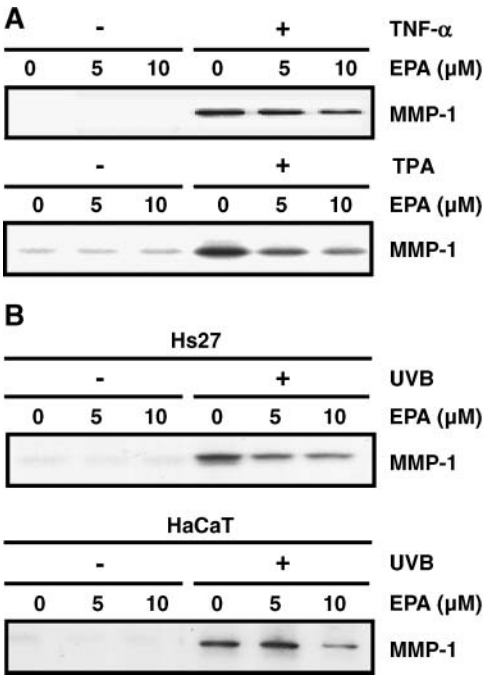

Fig. 5. The inhibition of MMP-1 expression by EPA is not cell type- or stimulus-dependent. A: HDFs were cultured in DMEM until 80% confluent and starved with DMEM containing 0.1% FBS for 36 h. Cells were then pretreated with EPA at the indicated concentrations for 24 h, treated with tumor necrosis factor-α (TNF-α; 5 ng/ml) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 10 nM), and incubated for 72 h, when MMP-1 expression was determined in culture media by Western blotting. B: Hs27 (human foreskin fibroblasts) and HaCaT (human dermal keratinocytes) cells were cultured in DMEM until 80% confluent, starved with DMEM containing 0.1% FBS for 36 h, and pretreated with EPA at the indicated concentrations for 24 h. The cells were then washed with PBS and irradiated with UV (at 75 and 30 mJ/cm^2 for Hs27 and HaCaT cells, respectively). After incubating the cells for 72 h, MMP-1 expression was determined in culture media by Western blotting.

deficiency in chronically photodamaged skin may be attributable to the increased, repetitive degradation of collagen by UV-induced MMPs, our results suggest that EPA treatment may prevent or ameliorate UV-induced connective tissue damage by preventing UV-induced MMP-1 expression.

The MAPK signal transduction pathways play an important role in regulating a variety of cellular functions (31–33), including MMP expression (34), whereas the present study shows that the ERK and JNK pathways, but not the p38 kinase pathway, are involved in UV-induced MMP-1 expression. We also found that pretreating HDFs with EPA before UV treatment inhibits the UV-induced activation of ERK and JNK in cultured fibroblasts. Therefore, our data indicate that EPA may prevent UV-induced MMP-1 expression by inhibiting the UV-induced activation of ERK and JNK. Recent studies have shown that EPA modulates the ERK1/2 signaling induced by PMA via protein kinase C-dependent and -independent pathways in human T-cells (35) and also inhibits lipopolysaccharide-induced JNK activation (36). Although p38 MAPK induces c-Jun expression by enhancing ATF2 transcriptional activity, the inhibition of p38 kinase activity by pretreating HDFs with SB203580 was not found to change UV-induced MMP-1 expression in the present study. Previously, it was reported that the activation of p38α enhances MMP-1 and MMP-3 expression by stabilizing their mRNAs (37). In contrast, it is also known that p38 MAPK negatively regulates the ERK pathway (38, 39). The reasons for these different effects of p38 kinase on MMP-1 expression remained to be determined.

The downstream effectors of the MAPKs include several transcription factors, such as Elk-1, c-Fos, and c-Jun. The transcription of several MMPs, including MMP-1, MMP-3, and MMP-9, is regulated by AP-1, which is one of several transcriptional factors activated by UV (40). The AP-1 complex is composed of Jun and Fos family members, and Jun proteins form either a homodimer or a heterodimer with Fos protein to produce the AP-1 complex. Moreover, the transcriptional activity of AP-1 is dependent not only on the abundance of c-Jun and c-Fos members but also on the degrees of phosphorylation of these components. In this study, we found that UV induced c-Jun phosphorylation/expression and c-Fos expression in HDFs and that this led to AP-1 activation by UV. In addition, EPA pretreatment inhibited UV-induced c-Jun phosphorylation/expression and c-Fos expression, and this resulted in the significant inhibition of UV-induced AP-1 activation. The inhibition of AP-1 activity by EPA has also been reported in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells (41) and TPA-stimulated mouse epidermal JB6 cells (42). Consistent with our results, EPA pretreatment was also found to attenuate increases of c-Jun and c-Fos protein levels by lipopolysaccharide and AP-1 DNA binding activity, which are all related to the inhibition of lipopolysaccharide-induced TNF-α expression by EPA (36). Moreover, phosphorylation of c-Jun by JNK is known to stimulate AP-1 transactivation activity (22, 43,

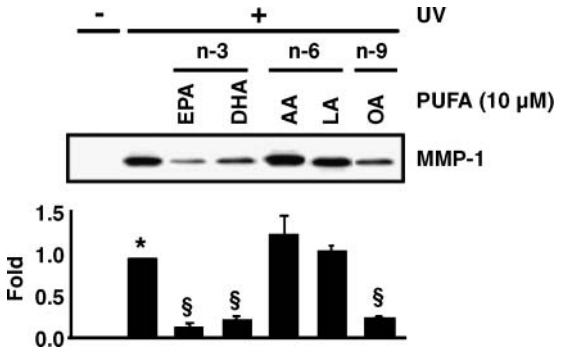

Fig. 6. n-3 and n-9 PUFAs, but not n-6 PUFAs, inhibited UV-induced MMP-1 expression. HDFs were cultured in DMEM until 80% confluent and then starved with DMEM containing 0.1% FBS for 36 h. Cells were pretreated with 10 μM EPA, docosahexaenoic acid (DHA), arachidonic acid (AA), oleic acid (OA), and linolenic acid (LA) for 24 h. Cells were washed with PBS and irradiated with UV (75 mJ/cm^2) in PBS. Irradiated cells were cultured for 72 h, and MMP-1 expression was determined in culture media by Western blotting. All experiments were performed in triplicate. Values shown are means ± SEM (n = 3). * $P < 0.05$ versus the control, § $P < 0.05$ versus UV-irradiated cells.

44). Here, we found that JNK inhibition by the JNK-specific inhibitor SP600126 prevented UV-induced c-Jun phosphorylation but not c-Fos expression, which plays an important role in UV-induced AP-1 activity as a component of the AP-1 complex (45) and that is also induced by various other signals, including the ERK-dependent pathway (23, 24). Furthermore, it has been reported that ERK activates the ternary complex factor, Elk-1, in response to UV and increases c-Fos expression (24). In the present study, ERK inhibition by the MEK1-specific inhibitor U0126 prevented UV-induced c-Fos expression but not c-Jun phosphorylation. Our results show that EPA pretreatment inhibits UV-induced ERK activation, Elk-1 phosphorylation, and c-Fos expression and also that it suppresses UV-induced JNK activation and c-Jun phosphorylation/expression in cultured HDFs.

ROS generation plays a critical role in the MAPK-mediated signal transduction triggered by UV (46–48). In addition, ROS increases MMP-1 expression in human HDFs (49), and ROS scavengers inhibit UV-induced AP-1 activation and MMP-1 expression (50–52). Recently, it was reported that UV rapidly and significantly increases H_2O_2 levels in human skin in vivo, suggesting that an early increase in ROS may participate in the triggering of the MAPK cascade and that topical treatment with antioxidants, e.g., genistein and N-acetyl cysteine may interrupt the activation of MAPK pathways and thus inhibit UV-induced MMP expression in human skin in vivo (48). It is known that the unstable ω-3 PUFA can quench ROS and protect cellular structures from oxidative damage (16, 53, 54). However, in the present study, EPA did not reduce ROS levels induced by UV in cultured HDFs. Therefore, we suggest that these inhibitory effects of EPA on UV-induced MMP-1 expression are not mediated by its antioxidant effect.

We demonstrated that EPA also inhibited TNF-α- and TPA-induced MMP-1 expression in cultured fibroblasts and UV-induced MMP-1 expression in Hs27 and HaCaT cell lines as well as in cultured dermal fibroblasts, suggesting that the inhibitory effects of EPA on MMP-1 expression were not cell type- and stimuli-specific. Furthermore, we also demonstrated that the inhibitory effects of PUFAs on UV-induced MMP-1 expression were dependent on their structure. n-3 and n-9 PUFAs inhibited UV-induced MMP-1 expression, but n-6 PUFAs did not. Although the mechanism of these differential effects of each PUFA on UV-induced MMP-1 expression remains to be investigated, the following speculations may explain the differential effects of PUFAs. Recently, it was reported that n-3 PUFAs, but not n-6 PUFAs, inhibit TPA- and epidermal growth factor-induced AP-1 activity in JB6 cells (42). These differential inhibitory effects of PUFAs on AP-1 activity may explain the discrepancy in the regulation of MMP-1 expression by PUFAs. On the other hand, the differential effects of PUFAs on UV-induced MMP-1 expression might be related to the formation of PGE. Whereas AA is converted to 2-series PGE (PGE_2) by COX-2, EPA and DHA are converted to 3-series PGE (PGE_3) by COX-2. n-3 PUFAs such as EPA and DHA compete with AA at the level of incorporation into cell membrane phospholipids, leading to the inhibition of PGE_2 production from AA and PGE_3 synthesis from EPA and DHA. PGE_2 produced from AA induces AP-1 activation, which is closely related to MMP-1 expression (42).

In conclusion, the present study demonstrates that EPA inhibits UV-induced MMP-1 expression by suppressing UV-induced c-Jun phosphorylation/expression, c-Fos expression, and AP-1 activation, which may be mediated by the inhibition of MEK1/ERK/c-Fos and SEK1/JNK/c-Jun pathways in cultured HDFs (**Fig. 7**). Therefore, we conclude that EPA is a potential agent for the prevention and treatment of skin aging.

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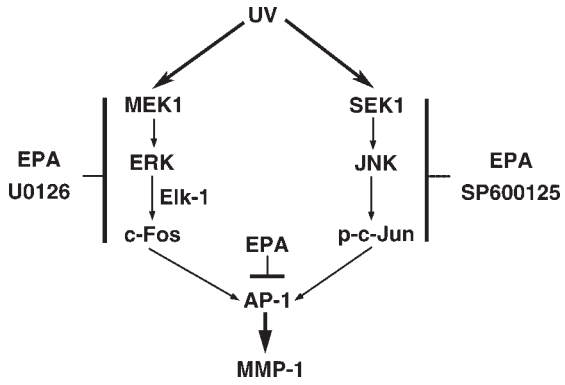

Fig. 7. Proposed mechanism for the inhibition of UV-induced MMP-1 expression by EPA.

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