Chimyl Alcohol Suppresses PGE₂ Synthesis by Human Epidermal Keratinocytes through the Activation of PPAR- γ

Mariko Yokota^{1,2*}, Shoichi Yahagi¹, Yoshihiro Tokudome² and Hitoshi Masaki³

¹ NIKKOL GROUP Cosmos Technical Center Co., Ltd. 3-24-3 Hasune, Itabashiku, TOKYO 174-0046, JAPAN

² Laboratory of Dermatological Physiology, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University. 1-1 Keyakidai, Sakado-shi, SAITAMA 350-0295, JAPAN

³ School of Bioscience and Biotechnology, Tokyo University of Technology. 1404-1 Katakuracho, Hachioji, TOKYO 192-0982, JAPAN

Abstract: Alkyl glyceryl ethers (AKGs) are widely used as emulsion stabilizers, and their anti-inflammatory effects are well known. Daily exposure to environmental stresses, such as chemicals, low humidity and ultraviolet light (UV), can initiate and promote the development of various skin problems. Among those stresses, it has been established that UV induces skin pigmentation and accelerates premature skin aging due to the inflammation that results. Here, we investigated whether chimyl alcohol (CA), which is an AKG, suppresses the inflammatory process. The suppression of cell damage and the reduction of intracellular levels of reactive oxygen species (ROS) in normal human epidermal keratinocytes (NHEKs) after UVB exposure was evaluated using the Neutral red (NR) and the 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assays, respectively. Moreover, the expression levels of mRNAs and proteins related to inflammation were evaluated by Real-time RT-PCR and ELISA assays, respectively. CA suppressed prostaglandin E₂ (PGE₂) production in UVB-exposed NHEKs according to the down-regulated expression level of cyclooxygenase-2 (COX-2) mRNA. Furthermore, CA up-regulated the mRNA expression levels of peroxisome proliferator-activated receptor (PPAR)- γ , nuclear factor E2-related factor 2 (Nrf2) and γ -glutamyl cysteine synthase (γ -GCS) in NHEKs. Finally, we examined the effects of CA on siPPAR- γ transfected NHEKs. siPPAR- γ transfection of NHEKs abolished the mRNA expression levels of Nrf2 and UVB-stimulated PGE₂ secretion that were regulated by CA. Hence, CA suppresses the UVB-induced COX-2 mRNA expression and PGE₂ production through PPAR- γ as an agonist. We conclude that CA provides useful protection and/or alleviation against UV damage.

Key words: UVB, PPAR- γ , Nrf2, γ -GCS, chimyl alcohol

1 INTRODUCTION

Excess exposure of the skin to ultraviolet light (UV) commonly causes inflammation due to the generation of reactive oxygen species (ROS)¹⁾. It is well known that ROS can trigger the progress of skin pigmentation, premature skin aging and skin cancer²⁾. Irradiation of the epidermis with UVB increases the synthesis of prostaglandin E₂ (PGE₂) due to the up-regulation of cyclooxygenase-2 (COX-2) mRNA expression^{3, 4)}. PGE₂ contributes to UVB-induced erythema by collaborating with nitric oxide⁵⁾. It has been reported that PGE₂ also stimulates melanin synthesis and regulates the proliferation and the dendrite elongation of melanocytes⁶⁻⁹⁾. In the dermis, PGE₂ suppresses collagen synthe-

sis in fibroblasts by partially interfering with transforming growth factor- β signaling¹⁰⁾. In this process, lymphocytes invading the dermis secrete matrix metalloproteases (MMPs), which decompose dermal matrix components such as collagens and elastins¹¹⁾. In fact, PGE₂ levels in fibroblasts are increased in elderly skin, and result in degradation of the dermal extracellular matrix¹²⁾. Therefore, the regulation of PGE₂ production accompanied by the up-regulation of COX-2 induced by UV-irradiation is important to protect the skin against photo-aging.

Alkyl glyceryl ethers (AKGs) are widely used as emulsion stabilizers and the basic chemical structure of AKGs is shown in Fig. 1. AKGs contain an alkyl chain bound to the

*Correspondence to: Mariko Yokota, NIKKOL GROUP Cosmos Technical Center Co., Ltd. 3-24-3 Hasune, Itabashiku, TOKYO 174-0046, JAPAN

E-mail: yokota@ns-cosmos.co.jp

Accepted November 30, 2017 (received for review July 13, 2017)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

Fig. 1 Chemical structure of AKGs; the R group of CA is shown.

hydroxyl group in glycerin with an ether bond. There are several types of AKGs, such as chimyl alcohol (CA), batyl alcohol and selachyl alcohol, depending on variations of the alkyl chain length. AKGs are abundantly contained in shark-liver oil, and in Europe, shark-liver oil has been used for the treatment of inflammation as a folk remedy. In addition, it has been reported that administration of shark-liver oil reduces lethal injury caused by radiation¹³⁾. These facts suggested to us that AKGs have potential anti-inflammatory effects. The purpose of the study is to clarify the underlying anti-inflammatory mechanism of AKGs focusing on CA, which showed the most effective anti-inflammatory effect among AKGs in a preliminary examination. We hypothesized that the anti-inflammatory effect of CA results from its signaling of peroxisome proliferator-activated receptor (PPAR)- γ , since PPAR- γ agonists have been shown to have anti-inflammatory effects through the suppression of COX-2 mRNA up-regulation¹⁴⁾. Furthermore, it has been reported that PPAR- γ activation modulates UVB-induced COX-2 expression and PGE₂ synthesis¹⁵⁾. In addition, PPAR- γ directly suppresses the inflammatory process through stimulation of the defensive response against oxidative stress by the signaling of nuclear factor-E2-related factor 2 (Nrf2)¹⁶⁾.

The regulation of the intracellular antioxidant system is well known to be conducted by the Nrf2-Kelch-like ECH associated protein 1 (Keap-1) signaling pathway^{17, 18)}. Nrf2 is a key transcription factor that regulates the cellular adaptive response to oxidative stress through the cis-regulating antioxidant response element (ARE)¹⁹⁾. Although Nrf2 is inactivated by binding to Keap-1 in the cytoplasm under physiological conditions, oxidative stress releases Nrf2 from Keap1 by the oxidation of sulfhydryl groups. Nrf2 is then translocated to the nucleus and binds to the ARE^{17, 18)}. In the nucleus, Nrf2 up-regulates the transcription of antioxidant-related genes, including γ -glutamyl cysteine synthase (γ -GCS), which is the rate-limiting enzyme of glutathione (GSH) synthesis²⁰⁾.

To date, several agonists of PPAR- γ have been reported. Among them, linoleic acid, which is a long chain fatty acid and is an agonist for PPAR- γ ²¹⁾, is similar to CA in chemical structure. Thus, we suspected that CA would also work as an agonist for PPAR- γ .

Here, we investigated the effects of CA on the UVB-induced inflammatory process, and further characterized the anti-inflammatory mechanism of CA focusing on its agonis-

tic effect on PPAR- γ .

2 EXPERIMENTAL

2.1 Reagents

Chimyl alcohol was obtained from Nikko Chemicals (Tokyo, Japan). Neutral red (NR) solution was purchased from Sigma-Aldrich (MO, USA). Ambion® Cells-to-CT™ TaqMan® kits, the TaqMan® Fast Universal PCR Master Mix, the Pierce™ BCA Protein Assay kit, and Lipofectamine® 3000 reagent were purchased from Thermo Fisher Scientific (Kanagawa, Japan). 2',7'-Dichlorodihydrofluorescein diacetate (DCFDA) was purchased from Calbiochem (CA, USA). The prostaglandin E₂ ELISA kit was purchased from Cayman Chemical (MI, USA).

2.2 Cell culture and UVB exposure

Normal human epidermal keratinocytes (NHEKs) were obtained from Kurabo (Osaka, Japan) and were cultured in HuMedia-KG2 (Kurabo) at 37°C in a 5% CO₂ atmosphere. NHEKs were exposed to 20 mJ/cm² UVB (TL20W/12RS lamp, Phillips, Hamburg, Germany) in Hank's buffer without Ca²⁺ and Mg²⁺.

2.3 Resistance to UVB-induced cell toxicity

NHEKs were incubated at a density of 2.0×10^4 cells per well in 96-well plates, and were pre-treated with or without CA for 24 hr and were then exposed to 20 mJ/cm² UVB. After further incubation for 24 hr post-treatment, cell viabilities were evaluated using the neutral red (NR) assay. NHEKs were incubated in 1% NR containing medium for 2 hr. The amount of NR then extracted by 100 mmol/L hydrochloric acid solution containing 30% ethanol was quantified by measuring the absorbance (550–650 nm). Cell viability is expressed as an index (%) of the non-treated control (NC).

2.4 Detection of intracellular ROS levels after UVB exposure

NHEKs were incubated at a density of 2.0×10^4 cells per well in 96-well plates, and were pre-treated with or without CA for 24 hr. Cells preloaded with DCFDA for 45 min in Hank's buffer were exposed to 20 mJ/cm² UVB. Intracellular ROS levels were quantified by measuring the fluorescence intensities of lysates of NHEKs extracted using Triton X-100 (Ex. 485 nm/Em. 530 nm). Protein concentrations of NHEK extracts were determined using a BCA protein assay kit. Intracellular ROS levels are expressed as fluorescence intensity per protein.

2.5 Quantification of PGE₂ secreted from NHEKs after UVB exposure

NHEKs were incubated at a density of 2.0×10^4 cells per

well in 96-well plates, and were pre-treated with or without CA for 24 hr and were then exposed to 20 mJ/cm² UVB. After further incubation for 24 hr post-treatment, amounts of PGE₂ in the medium were determined using an ELISA kit.

2.6 Real-time RT-PCR analysis

NHEKs were incubated at a density of 1.5×10^4 cells per well in 96-well plates, were cultured with or without CA for the times noted in the text. Total RNAs were extracted from NHEKs and cDNAs were synthesized using an Ambion® Cells-to-CT™ TaqMan® Kits. Real-time RT-PCR was performed with TaqMan® Fast Universal PCR Master Mix using the Step One Plus™ Real-time PCR system. Primer sets and Assay IDs for Gene expression assays are as follows; PPAR-γ (Hs00234592_m1), Nrf2 (Hs00975961_g1), γ-GCS (Hs00155249_m1), COX-2 (Hs00153133_m1) and Cyclophilin (PPIA control mix).

2.7 RNA interference

NHEKs were inoculated at a density of 1.0×10^5 cells per well in 24-well plates, and were transfected with PPAR-γ small interference RNA (siPPAR-γ) or a random sequence siRNA as a control (siNC) using Lipofectamine® 3000 reagent. Cells were further incubated with CA for 24 hr in fresh medium. mRNA expression levels of PPAR-γ and Nrf2 were evaluated using Real-time RT-PCR analysis.

2.8 Statistical analysis

Statistical analyses were performed using a two-tailed paired t-test. Differences are considered significant if $p < 0.050$ (indicated by * $p < 0.050$, ** $p < 0.010$).

3 RESULTS

3.1 CA protects against cell damage induced by UVB exposure

To evaluate the effects of CA on cell viability, NHEKs were exposed to a dose of UVB at 20 mJ/cm², which caused 60% survival and was used throughout this study. Pre-treatment of NHEKs with CA showed a significant dose-dependent protection against UVB-induced cell damage compared to untreated controls (Fig. 2).

3.2 Reduction of intracellular ROS levels increased in NHEKs following UVB exposure

It was reported that UVB exposure induces the elevation of intracellular ROS levels¹⁾. To analyze the effects of CA on intracellular ROS levels, we examined the effects of CA on intracellular ROS levels of UVB-irradiated NHEKs. NHEKs were pre-treated with or without CA for 24 hr and were then preloaded with DCFDA for 45 min in Hank's buffer and were then exposed to 20 mJ/cm² UVB. CA sig-

Fig. 2 Resistance to UVB-induced cell toxicity in NHEKs treated with CA.

NHEKs were pre-treated with or without CA for 24 hr, then were exposed to 20 mJ/cm² UVB and further incubated for 24 hr as a post-treatment. Cell viabilities were evaluated using the NR assay and are expressed as fold index (%) compared with the NC without UVB. Results are expressed as means $\pm$ S.D. (n = 4). Significance (Student t-test); * $p < 0.050$, ** $p < 0.010$.

nificantly suppressed the elevation of intracellular ROS levels elicited by UVB exposure (Fig. 3).

3.3 Suppression of the up-regulation of COX-2 mRNA expression in NHEKs induced by UVB exposure

It was reported that UVB-irradiated keratinocytes express COX-2 mRNA and protein³⁾. We examined whether CA could suppress the up-regulation of UVB-induced COX-2 mRNA expression. NHEKs were pre-treated with or without CA for 24 hr, then were exposed to 20 mJ/cm² UVB and were further incubated for 6 hr before analysis by Real-time RT-PCR. CA exhibited a significant suppression of the UVB-induced up-regulation of COX-2 mRNA expression, and even suppressed COX-2 mRNA expression in sham-irradiated NHEKs (Fig. 4).

3.4 CA suppresses PGE₂ production by NHEKs after UVB exposure

It is well known that COX-2 is a rate-limiting enzyme in the arachidonic acid cascade. It was reported that COX-2 expression enhances PGE₂ production by UVB-irradiated keratinocytes¹⁵⁾. To characterize the anti-inflammatory effects of CA, we examined the secretion of PGE₂ from NHEKs exposed to UVB. NHEKs were pre-treated with or

Fig. 3 Influence of CA on intracellular ROS levels in NHEKs after UVB exposure.

NHEKs were pre-treated with or without CA for 24 hr, then were preloaded with DCFDA for 45 min in Hank's buffer and exposed to 20 mJ/cm² UVB, after which intracellular ROS levels were quantified. ROS levels are expressed as fold index (%) compared with the NC without UVB. Results are expressed as means ± S.D. (n = 4). Significance (Student t-test); ***p* < 0.010.

without CA for 24 hr, then were exposed to 20 mJ/cm² UVB and were further incubated for 24 hr as a post-treatment. PGE₂ production was increased by UVB exposure and CA significantly suppressed the UVB-induced PGE₂ production in a dose-dependent manner (Fig. 5).

3.5 Stimulation of the intracellular defense system against oxidative stress

It has been reported that PPAR-γ is involved in adipose cell differentiation, inflammation and keratinocyte differentiation²¹. We initially focused on PPAR-γ activation because a previous study showed that the UVB-induced the expression of COX-2 and the production of PGE₂ are regulated by PPAR-γ activation¹⁴. Thus, we examined the involvement of Nrf2 in the stimulation of GSH synthesis, and found that CA up-regulated the expression levels of PPAR-γ and Nrf2 mRNAs (Fig. 6a-b). Also, GSH is an intracellular antioxidant²³, which was stimulated following Nrf2 activation¹⁶. GSH is synthesized by γ-GCS, which is the rate-limiting enzyme of de novo GSH synthesis¹⁸. In order to address the effects of CA, we examined γ-GCS mRNA expression in NHEKs treated with CA. CA up-regulated γ-GCS mRNA expression in a dose-dependent manner (Fig. 6c).

Fig. 4 Inhibition of UVB-induced COX-2 up-regulation in NHEKs treated with CA.

NHEKs were pre-treated with or without CA for 24 hr, then were exposed to 20 mJ/cm² UVB and further incubated for 6 hr as a post-treatment prior to Real-time RT-PCR analysis. COX-2 mRNA expression was normalized against cyclophilin mRNA expression and is expressed as fold induction compared with the NC. Results are expressed as means ± S.D. (n = 4). Significance (Student t-test); **p* < 0.050, ***p* < 0.010.

3.6 Activation of PPAR-γ-Nrf2 signaling

In order to clarify the involvement of PPARγ-Nrf2 signaling in the suppressive effects of CA on the UVB-stimulated secretion of PGE₂, we examined the effect of CA on NHEKs where PPAR-γ was knocked down using transfection with siPPAR-γ. PPAR-γ mRNA expression levels in siPPAR-γ transfected NHEKs were significantly decreased to approximately 20% of the siNC control level (i.e. NHEKs transfected with a random sequence) (Fig. 7a). siPPAR-γ transfected NHEKs abolished the response to CA on the up-regulation of Nrf2 mRNA (Fig. 7b). Furthermore, although CA still suppressed PGE₂ secretion in siNC transfected NHEKs following UVB exposure (Fig. 7c), PPAR-γ knock-down NHEKs failed to show any suppressive effects of CA on the PGE₂ secretion increased by UVB (Fig. 7d).

4 DISCUSSION

UVB-induced erythema is caused by the excess synthesis of PGE₂ through the up-regulation of COX-2 mRNA expression. The UVB-induced up-regulation of COX-2 mRNA has been demonstrated to result from p38 MAPK activation

Fig. 5 PGE₂ secretion from NHEKs pre-treated with CA before UVB exposure. NHEKs were pre-treated with or without CA for 24 hr, then were exposed to 20 mJ/cm² UVB and further incubated for 24 hr as a post-treatment. PGE₂ secretion into the medium was quantified using an ELISA kit. Results are expressed as means ± S.D. (n = 4). Significance (Student t-test); ***p* < 0.010.

triggered by intracellular elevations of ROS²³⁾. These facts confirm the importance of reducing intracellular ROS levels to reduce UVB-induced erythema¹⁴⁾. In this study, we investigated the anti-inflammatory effects of CA on UVB-in-

duced erythema focusing on COX-2 expression and PGE₂ synthesis through the reduction of intracellular levels of ROS. Firstly, we found that CA protects NHEKs against the damage caused by UVB (Fig. 2). Although UVB-irradiation of NHEKs gave significant increases of intracellular ROS levels, CA reduced those ROS levels in a dose-dependent manner (Fig. 3). In addition, CA significantly decreased the secretion of PGE₂ based on the down-regulation of COX-2, which is up-regulated by ROS¹⁾ (Figs. 4-5). Furthermore, CA even showed a significant down-regulation of COX-2 mRNA expression levels in sham-irradiated NHEKs (Fig. 4). The sum of those results shows that CA suppresses inflammation in UVB-exposed skin. In fact, we previously conducted a human volunteer test on the anti-inflammatory effects of CA on UVB-induced erythema using a minimal erythema dose (MED) of UVB as a parameter to measure effects. A cream containing CA significantly increased the MED in each subject compared with the placebo cream (data not shown).

To elucidate the detailed mechanism of the anti-inflammatory effects of CA, we conducted further examinations focusing on the involvement of PPAR-γ. It has been reported that a PPAR-γ agonist has an anti-inflammatory effect¹⁵⁾, and further, that UVB-induced COX-2 expression and PGE₂ synthesis are regulated by PPAR-γ signaling¹⁴⁾. In general, it is known that UVB irradiation up-regulates COX-2 mRNA expression via NF-κB signaling²⁵⁻²⁷⁾. Since PPAR-γ activation by agonists suppresses the up-regulation of COX-2 expression induced by lipopolysaccharide (LPS) or interleukin-1β (IL-1β), and directly stimulates Nrf2 signaling¹⁶⁾, we investigated the interaction of CA with PPAR-γ and Nrf2. In general, intracellular defenses against ROS are achieved by antioxidants and enzymes such as GSH and catalase. Since

Fig. 6 Induction of PPAR-γ (a), Nrf2 (b) and γ-GCS (c) mRNA expression in NHEKs treated with CA. NHEKs were treated with or without CA for 24 hr prior to Real-time RT-PCR analysis. PPAR-γ, Nrf2 and γ-GCS mRNA levels normalized against cyclophilin mRNA expression are expressed as fold induction compared with the NC. Results are expressed as means ± S.D. (n = 4). Significance (Student t-test); **p* < 0.050, ***p* < 0.010.

Fig. 7 Effects of CA on Nrf2 mRNA expression and UVB-stimulated PGE₂ secretion in PPAR- γ knockdown NHEKs. NHEKs were transfected with siPPAR- γ or with an siRNA to a random sequence (siNC) and were further incubated with CA for 24 hr. a) mRNA expression levels of PPAR- γ in siNC or siPPAR- γ transfected NHEKs, b) mRNA expression levels of Nrf2 in siNC or siPPAR- γ transfected NHEKs treated with CA for 24 hr, PPAR- γ and Nrf2 mRNA levels were normalized against cyclophilin mRNA levels and are expressed as fold induction compared with the NC. c-d) PGE₂ secretion from siNC or siPPAR- γ transfected NHEKs exposed to 20 mJ/cm² UVB. CA at various concentrations was pre-treated for 24 h before UVB irradiation. Results are expressed as means $\pm$ S.D. (n = 4). Significance (Student t-test); N.S., Not Significance, * p < 0.050, ** p < 0.010

those genes are coded downstream of Nrf2, we examined the role of CA on mRNA expression levels of PPAR- γ and Nrf2 and its downstream gene, γ -GCS. CA up-regulated the expression levels of mRNAs encoding PPAR- γ , Nrf2 and γ -GCS (Fig. 6). These results are consistent with the fact that CA decreases intracellular ROS levels elevated by UVB exposure (Fig. 3). To demonstrate the interaction of CA and PPAR- γ , we conducted further examinations using NHEKs with knocked-down expression of PPAR- γ mRNA using siPPAR- γ transfection. In PPAR- γ down-regulated NHEKs, the effects of CA on the dose-dependent regula-

tion of mRNA expression levels of Nrf2 were abolished (Fig. 7). These results indicate that the crucial mechanism for the anti-inflammatory effect of CA originates from its activation of PPAR- γ -Nrf2 signaling (as shown schematically in Fig. 8).

5 CONCLUSION

Our findings indicate that CA reduces UVB-induced cellular damage via its suppression of COX-2 mRNA expres-

Fig. 8 Scheme showing the anti-inflammatory effects of CA : involvement of PPAR-γ activation.

ROS; Reactive oxygen species, PLA₂; Phospholipase A₂, PGH₂; Prostaglandin H₂, PGE₂; Prostaglandin E₂, COX-2; Cyclooxygenase-2, CA; Chimyl alcohol, PPAR-γ; Peroxisome proliferator-activated receptor-γ, Nrf2; nuclear factor E2-related factor 2, γ-GCS; γ-Glutamyl cysteine synthase.

sion and PGE₂ synthesis. Moreover, CA effectively reduces intracellular ROS levels in UVB-exposed NHEKs due to stimulation of the intracellular defense system against ROS through Nrf2 signaling activated via a PPAR-γ-dependent mechanism. We propose CA as an effective chemical that prevents skin damage induced by UVB exposure.

References

- Masaki, H. Role of antioxidants in the skin: anti-aging effects. *J. Dermatol. Sci.* **58**, 85-90 (2010).
- Bickers, D.R.; Athar, M. Oxidative stress in the pathogenesis of skin disease. *J. Invest. Dermatol.* **126**, 2565-2575 (2006).
- Tripp, C.S.; Blomme, E.A.; Chinn, K.S.; Hardy, M.M.; LaCelle, P.; Pentland, A.P. Epidermal COX-2 induction following ultraviolet irradiation: suggested mechanism for the role of COX-2 inhibition in photoprotection. *J. Invest. Dermatol.* **121**, 853-861 (2003).
- Isoherranen, K.; Punnonen, K.; Jansen, C.; Uotila, P. Ultraviolet irradiation induces cyclooxygenase-2 expression in keratinocytes. *Br. J. Dermatol.* **140**, 1017-1022 (1999).
- Rhodes, L.E.; Belgi, G.; Parslew, R.; McLoughlin, L.; Clough, G.F.; Friedmann, P.S. Ultraviolet-B-induced erythema is mediated by nitric oxide and prostaglandin E₂ in combination. *J. Invest. Dermatol.* **117**, 880-885 (2001).
- Sauk, J.J.; White, J.G.; Witkop, C.J. Influence of prostaglandins E₁, E₂, and arachidonate on melanosomes in melanocytes and keratinocytes of anagen hair bulbs *in vitro*. *J. Invest. Dermatol.* **64**, 332-337 (1975).
- Tomita, Y.; Iwamoto, M.; Masuda, T.; Tagami, H. Stimulatory effect of prostaglandin E₂ on the configuration of normal human melanocytes *in vitro*. *J. Invest. Dermatol.* **89**, 299-301 (1987).
- Tomita, Y.; Maeda, K.; Tagami, H. Melanocyte-stimulating properties of arachidonic acid metabolites: possible role in postinflammatory pigmentation. *Pigment Cell Res.* **5**, 357-361 (1992).
- Scott, G.; Jacobs, S.; Leopardi, S.; Anthony, F.A.; Learn, D.; Malaviya, R.; Pentland, A. Effects of PGF₂α on human melanocytes and regulation of the FP receptor by ultraviolet radiation. *Exp. Cell Res.* **304**, 407-416 (2005).
- Sandulache, V.C.; Parekh, A.; Li-Korotky, H.; Dohar, J.E.; Hebda, P.A. Prostaglandin E₂ inhibition of keloid fibroblast migration, contraction, and transforming growth factor (TGF)-β₁-induced collagen synthesis. *Wound Repair Regen.* **15**, 122-133 (2007).
- Yano, K.; Kadoya, K.; Kajiya, K.; Hong, Y.K.; Detmar, M. Ultraviolet B irradiation of human skin induces an angiogenic switch that is mediated by upregulation of vascular endothelial growth factor and by downregulation of thrombospondin-1. *Br. J. Dermatol.* **152**, 115-121 (2005).
- Li, Y.; Lei, D.; Swindell W.R.; Xia, W.; Weng, S.; Fu, J.; Worthen, C.A.; Okubo, T.; Johnston, A.; Gudjonsson, J.E.; Voorhees, J.J.; Fisher, G.J. Age-associated increase in skin fibroblast-derived prostaglandin E₂ contributes to reduced collagen levels in elderly human skin. *J. Invest. Dermatol.* **135**, 2181-2188 (2015).
- Brohult, A.; Holmberg, J. Alkylglycerols in the treatment of leucopenia caused by irradiation. *Nature* **174**, 1102-1103 (1954).
- Du, H.; Chen, X.; Zhang, J.; Chen C. Inhibition of COX-2 expression by endocannabinoid 2-arachidonoylglycerol is mediated via PPAR-γ. *Br. J. Pharmacol.* **163**, 1533-1549 (2011).
- Yoshizaki, N.; Fujii, T.; Masaki, H.; Okubo, T.; Shimada, K.; Hashizume, R. Orange peel extract, containing high

- levels of polymethoxyflavonoid, suppressed UVB-induced COX-2 expression and PGE₂ production in HaCaT cells through PPAR- γ activation. *Exp. Dermatol.* **23**, 18-22 (2014).
- 16) Polvani, S.; Tarocchi, M.; Galli, A. PPAR γ and oxidative stress: Con (β) catenating NRF2 and FOXO. *PPAR Res.* **2012**, 641087 (2012).
 - 17) Nguyen, T., Nioi, P.; Pickett C.B. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem.* **284**, 13291-13295 (2009).
 - 18) Zhang, D.D. Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metab. Rev.* **38**, 769-789 (2006).
 - 19) Maher, J.; Yamamoto, M. The rise of antioxidant signaling the evolution and hormetic actions of Nrf2. *Toxicol. Appl. Pharmacol.* **244**, 4-15 (2010).
 - 20) Moinova, H.R.; Mulcahy, R.T. Up-regulation of the human gamma-glutamyl cysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element. *Biochem. Biophys. Res. Commun.* **261**, 661-668 (1999).
 - 21) Xu, H.E.; Lambert, M.H.; Montana, V.G.; Parks, D.J.; Blanchard, S.G.; Brown, P.J.; Sternbach, D.D.; Lehmann, J.M.; Wisely, G.B.; Willson, T.M.; Kliewer, S.A.; Milburn, M.V. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell.* **3**, 397-403 (1999).
 - 22) Goldwasser, J.; Cohen, P.Y.; Yang, E.; Balaguer, P.; Yarmush, M.L.; Nahmias, Y. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARalpha, PPARgamma and LXRalpha. *PLoS One* **5**, e12399 (2010).
 - 23) Townsend, D.M., Tew, K.D.; Tapiero, H., The importance of glutathione in human disease, *Biomed. Pharmacother.* **57**, 145-155 (2003).
 - 24) Chen, W.; Tang, Q.; Gonzales, M.S.; Bowden, G.T. Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene* **20**, 3921-3926 (2001).
 - 25) Fisher, G.J.; Datta, S.C.; Talwar, H.S.; Wang, Z.Q.; Varani, J.; Kang, S.; Voorhees, J.J. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* **379**, 335-339 (1996).
 - 26) Breuer-McHam, J.; Simpson, E.; Dougherty, I.; Bonkora, M.; Ariizumi, K.; Lewis, D.E.; Dawson, D.B.; Duvic, M.; Cruz, P.D. Activation of HIV in human skin by ultraviolet B radiation and its inhibition by NFkappaB blocking agents. *Photochem. Photobiol.* **74**, 805-810 (2001).
 - 27) Marwaha, V.; Chen, Y.H.; Helms, E.; Arad, S.; Inoue, H.; Bord, E.; Kishore, R.; Sarkissian, R.D.; Gilchrist, B.A.; Goukassian, D.A. T-oligo treatment decreases constitutive and UVB-induced COX-2 levels through p53- and NFkappaB-dependent repression of the COX-2 promoter. *J. Biol. Chem.* **280**, 32379-32388 (2005).