The effects of UVB on ORAI1 channel and the role of ORAI1 channel on the UVB-induced epidermal hyperplasia and upregulation of TSLP and COX-2

Seung Joon Oh

Department of Medicine

The Graduate School, Yonsei University

The effects of UVB on ORAI1 channel and the role of ORAI1 channel on the UVB-induced epidermal hyperplasia and upregulation of TSLP and COX-2

Directed by Professor Seung Hun Lee

The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Master of Medical Science

Seung Joon Oh

December 2015

This certifies that the Master's Thesis
of
Seung Joon Oh is approved.

Thesis Supervisor : Seung Hun Lee

Thesis Committee Member #1 : Kyung Hee Chun

Thesis Committee Member #2: Sang Eun Lee

The Graduate School
Yonsei University

December 2015

ACKNOWLEDGEMENTS

The final outcome of this study required much guidance and inspiration from Professor Seung Hun Lee. I would like to express my great appreciation to her for her support and encouragement along the completion of this thesis.

I also owe my profound gratitude to Professor Kyung Hee Chun and Professor Sang Eun Lee who took keen interest on this study and offered invaluable professional advices and guidance.

I specially thank to Song-Ee Kim for her kind advices and encouragement.

Last but not least, I thank my family for their unchanging presence in my life.

Seung Joon Oh

TABLE OF CONTENTS

LIST OF FIGURES

Abstract

The effects of UVB on ORAI1 channel and the role of ORAI1 channel on the UVB-induced epidermal hyperplasia and upregulation of TSLP and COX-2

Seung Joon Oh

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Seung Hun Lee)

Epidermal keratinocytes, components of the outermost skin layer, are always exposed to the ultraviolet (UV) light from the sun. UV radiation induces a number of adverse biological effects, including inflammation and cancer. UV irradiation activates numerous signaling pathways that alter transcription including keratinocyte proliferation via activation of epidermal growth factor receptor (EGFR) augments, inflammation, and carcinogenesis.

Calcium (Ca^{2+}) signaling is a major second messenger essential to a variety of intracellular signaling pathways. ORAI1 and its sensor STIM1 are

activated by endoplasmic reticulum (ER) stress and induce Ca^{2+} influx from the extracellular medium, which is named store-operated calcium entry (SOCE).

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine belonging to the Th2 cytokine family which has a key role in the initiation and maintenance of allergic inflammatory diseases. Cyclooxygenase-2 (COX-2) increases production of PGE2 known to promote skin carcinogenesis.

At present, nothing is known about the functional role of ORAI1 channel for UV induced biological effects in the skin. In this paper we have shown that UVB induces the expression of ORAI1. In addition, using topical application of ORAI1 inhibitor, we have confirmed functional roles of ORAI1 in UVB-induced change such as epithelial proliferation, TSLP induction and cyclooxygenase 2 (COX-2) induction. Thus, this study revealed that ORAI1 is a central modulator of the UV response of the skin that regulates cell proliferation, inflammation and even carcinogenesis. ORAI1 may represent a new therapeutic target for negative effects of UVB.

Key words : ultraviolet (UV) light, calcium, ORAI1, STIM1, cyclooxygenase 2 (COX-2), thymic stromal lymphopoietin (TSLP)

The effects of UVB on ORAI1 channel and the role of ORAI1 channel on the UVB-induced epidermal hyperplasia and upregulation of TSLP and COX-2

Seung Joon Oh

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Seung Hun Lee)

I. INTRODUCTION

The skin is the organ that acts as a barrier between the outer and inner environments of the body. Skin is exposed not only to a variety of physical, chemical, and thermal threats from the outside but also to inside endogenous stimuli. The integrity of differentiation process of keratinocytes (KCs) is critical for skin barrier function. Calcium (Ca^{2+}) signals are known to control keratinocyte proliferation and differentiation.[1,2] The marked Ca^{2+} gradient presents in the epidermis, higher in the stratum granulosum than in the basal layer.[3] This Ca^{2+} gradient is very important for keratinocyte proliferation,

differentiation and barrier homeostatsis. Ca^{2+} pumps and channels play an important role in matinining this Ca^{2+} gradient and barrier homeostasis.[4] Defects of Ca^{2+} pumps can cause skin diseases such as Darier disease and Hailey-Hailey disease involving abnormal keratinocyte differentiation and barrier dysfunction.[5,6]

ORAI1 channel is one of the well-known Ca^{2+} selective ion channel.[7] Reduction in endoplasmic reticulum (ER) Ca^{2+} is sensed by calcium sensor STIM1, that then transactivates plasma membrane Ca^{2+} channel ORAI1 channel and induces Ca^{2+} influx from the extracellular medium, which is named store-operated calcium entry (SOCE).[8,9] SOCE is a major Ca^{2+} influx pathway in most non-excitable cells and involved in cell proliferation and differentiation processes.[10,11] Epidermal growth factor (EGF) signaling in keratinocytes also involves SOCE mediated by ORAI1 channels.[7] Recently, the topical application of ORAI1 activator was reported to reverse the epidermal atrophy induced by corticosteroids in mice.[12] In addition, ORAI1 channel, signaling via the NFAT pathway, regulates thymic stromal lymphopoietin (TSLP) which is a cytokine released from keratinocytes and plays an important role in the pathogenesis of atopic dermatitis.[13,14] TSLP evoked sensory neurons triggering pruritus and stimulates skin-resident group 2 innate lymphoid cells to induce interleukin (IL) -5 and IL-13 expression leading to eosinophil recruitment and inflammation.[15] ORAI1-mediated NFκB activation is also reported to play a role in the regulation of cyclooxygenase-2

(COX-2).[16] Prostaglandin, which is an important pro-inflammatory molecule involved in the pathogenesis of a variety of diseases, is metabolized from arachidonic acid by the COX-2.[16]

Exposure of skin to ultraviolet (UV) radiation from the sun induces a number of adverse biological effects, including inflammation and carcinogenesis. UV irradiation activates numerous signaling pathways that alter transcription. UV-induced activation of EGF receptor augments keratinocyte proliferation and suppresses apoptosis, leading to epidermal hyperplasia, associated with increased G1 cyclin expression and suppression of cyclin dependent kinase (CDK).[17] In contrast, UVB also induces an immediate reactive oxygen species (ROS) generation that resulted in emptying of ER Ca^{2+} stores inducing ER stress and activation of RNA dependent protein kinase like ER kinase (PERK)-phosphorylated eukaryotic initiation factor 2α (peIF2α)-C/EBP-homologous protein (CHOP) pathway.[18] CHOP plays a convergent role in unfolded protein response (UPR) and has been identified as one of the most important mediators of ER stress induced apoptosis.[19] UV exposure also causes inflammatory responses in the skin. UV radiation activates the transcription factor NFκB, which stimulates transcription of pro-inflammatory cytokine genes, such as IL-1β, TNF-α, IL-6, and IL-8.[20] UVB also induces TSLP expression through a HIF-1α-dependent mechanism.[21] Another inflammatory molecule, COX-2 is induced by UV exposure through NFκB signaling.[22]

Those previous reports imply the distinct possibility that ORAI1-mediated Ca^{2+} influx could also be involved in the regulation of cellular functions in particular under stress conditions such as UV-irradiation. At present, nothing is known about the functional role of ORAI1 channels for UV induced biological effects in the skin. The aim of this study was to determine the effects of UVB on the expression of ORAI1 and its functional role in UVB-induced epidermal hyperplasia, and TSLP and COX-2 upregulation.

II. MATERIALS AND METHODS

1. Cell culture and treatments

The immortalized human keratinocytes cell line, HaCaT (Cell Lines Service GmbH, Germany) was grown in DMEM medium, supplemented with 10% fetal bovine serum, 200 U/mL penicillin and 270 mg/mL streptomycin (Sigma–Aldrich, St. Louis, MO, USA). The cells were maintained in a humidified chamber at 37.8 °C and 5% CO_2.

Before UVB exposure, HaCaT cells were seeded with 4×10^5 cells/well on a 35-mm dish and were incubated for 2 hours with 10 μM of GSK-7975A (AOBIOUS) or 1 μM of BTP2 (Sigma) which were reported to block the ORAI1 channel.

For UVB exposure, HaCaT cells (about 80% confluence) were washed with phosphate buffered saline (PBS) twice and exposed to UVB irradiation (Bio-Sun; Vilber lourmet). The total energy dose of UVB irradiation was 30 mJ/cm^2. After UVB exposure, cells were harvested up to 24 hours, and quantitative real-time polymerase chain reaction (qRT-PCR) was done.

2. Mice and treatments

Female hairless mice (SKH1/Hr), 6-7 weeks of age, were purchased from Orient Bio (Seoul, Korea). All animal experiments described were conducted in accord with accepted with standards of humane animal care and approved by the local animal research committee. The mice were killed and their skin samples were taken at 30 min, 4 hours, 24 hours and 72 hours after UVB irradiation. Each group has 2 mice.

A UVB lamp (PL-S 9W/01/2P; Philips, Warszawa, Poland) with a 280- to 320-nm emission was used. The UVB fluence rate was simultaneously measured and integrated using a radiometer (XD-9521 from Daavlin) placed at the same distance from the UVB source. A dose of 100 mJ/cm^2 or 200 mJ/cm^2 was used in the study, because this dose yielded the optimal effect without significantly affecting cell viability. One minimal erythema dose (MED) equals approximately 80 mJ/cm^2 in this species.[23]

Mice were treated by a topical application (100 μl) of ORAI1 inhibitor, BTP2 (Sigma) 1 μM dissolved in ethanol solvent. In the morning and the evening (8 hours apart) daily ethanol (control) was applied on the right side of back and BTP2 was applied on the left side of back immediately after UVB irradiation.

3. Quantification of epidermal thickness and hyperplasia

Thickness of the epidermal nucleated cell layers from the epidermal–dermal junction to the distal edge of the stratum granulosum was measured in at least five randomly selected regions from each hematoxylin and eosin stained slide using ocular and stage micrometers. For quantification of epidermal hyperplasia following UV exposure, the number of nucleated epidermal cell layers was counted in the same regions. Measurements were performed with the investigator blinded as to the identity of the samples.

4. Immunohistochemical staining

Skin sections from mouse were either cryoconserved by including in OCT (Sakura Finetek Europe, Alphen aan den Rijn, The Neherlands) or dehydrated and embedded in paraffin. ORAI1 immunostainings were performed on paraffin-embedded sections. Sections were rehydrated, antigens were unmasked by boiling sections in 10mM citrate buffer or 1 nM EDTA, and then stained with anti-ORAI1 antibody (Sigma, 08264, Carlsbad, CA). An anti-rabbit biotinylated antibody was used as secondary antibody to detect ORAI1. Sections were then incubated with a ready-to-use streptavidin-HRP solution (DAKO) and revealed with 3,39-diaminobenzidine (Sigma). Images were acquired with a Zeiss microscope (Jena, Germany). To avoid irrelevant

variations of the staining intensity in the immunohistochemistry, all conditions were stained with the same solutions and timing.

5. qRT-PCR for mRNA expression

Mouse: The epidermis was separated from the dermis by scraping the skin on ice with a size 21 scalpel blade and then homogenized with a polytron in buffer (50 mM Tris, pH 7.4, 1% Tween-20) containing 100 mg/mL phenylmethylsulfonyl fuoride and a protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN). Total RNA was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA), following the manufacturer's protocol. Reverse-transcription reactions were performed using 1 mg total RNA. The cDNA samples were analyzed for TSLP (Mm01157588_m1) and COX-2 (Mm00478374_m1).

HaCaT: For the relative mRNA expression analysis of selected genes, total RNA was isolated TRIzol (Invitrogen), according to the manufacturer's instructions. The RNA was qua ntified using a NanoDrop spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). RNA (4 μg) was reverse transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen), and aliquots were kept at −20°C. qRT−PCR was performed using an ABI 7500 Fast Real-Time PCR System with

commercially available TaqMan site-specific primers and probes (Applied Biosystems, Foster City, CA, USA). The cDNA samples were analyzed for ORAI1 (Hs00375481_m1), TSLP (Hs00263639_m1), and COX-2 (Hs00153133_m1). The results were normalized to the glyceraldehyde 3-phosphate dehydrogenase levels. The data were analyzed using 7500 Fast System SDS Software version 1.3.1 (Applied Biosystems). All of the data were obtained from more than two independent experiments carried out in triplicate.

6. Western blotting

Total protein was extracted from epidermal extracts with lysis buffer (50 mM Tris, 5 mM EDTA, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 1mM sodium orthovanadate, 100 mg/ml phenylmethanesulfonylfluoride, and protease inhibitors). Protein concentrations were determined with the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL) using BSA as the standard. Protein lysates (30 mg) were resolved by 10–12% SDS–PAGE and transferred to polyvinylidene difluoride membranes (Millipore). After blocking, the polyvinylidene difluoride membranes were incubated overnight with anti-ORAI1 antibody (Sigma, 08264, Carlsbad, CA) on a shaker at 4°C. After extensive washing, the membranes were incubated with secondary peroxidase-linked IgG (1:5,000, Abcam) for 1 hour. After washing three times

for 10 minutes with Tris-buffered saline with Tween 20 at room temperature, the immunoreactivity was detected by enhanced chemiluminescence (ECL kit, Amersham Pharmacia Biotech, Piscataway, NJ).

III. Results

1. Upregulation of ORAI1 expression by UVB irradiation in murine epidermis

To determine whether UVB induces ORAI1 expression, mice were irradiated with different UVB doses (0, 100, and 200 mJ/cm^2) and incubated up to 72 hours. ORAI1 expression was increased 1 day after 100 or 200 mJ/cm^2 of UVB irradiation (Figure 1,2). It showed time-dependent increases in ORAI1 expression. In addition, 200 mJ/cm^2 of UVB showed more prominent staining results compared with 100 mJ/cm^2 of UVB. To confirm the levels of ORAI1 protein expression induced by UVB in epidermis, we used a western blotting. ORAI1 protein expression was increased in epidermis irradiated with 100 mJ/cm^2 and 200 mJ/cm^2 of UVB compared with the control (Figure 3). It also showed time-dependent increases in ORAI1 expression. And similary to immunohistochemical staining result, 200 mJ/cm^2 of UVB showed more increased protein levels than 100 mJ/cm^2 of UVB

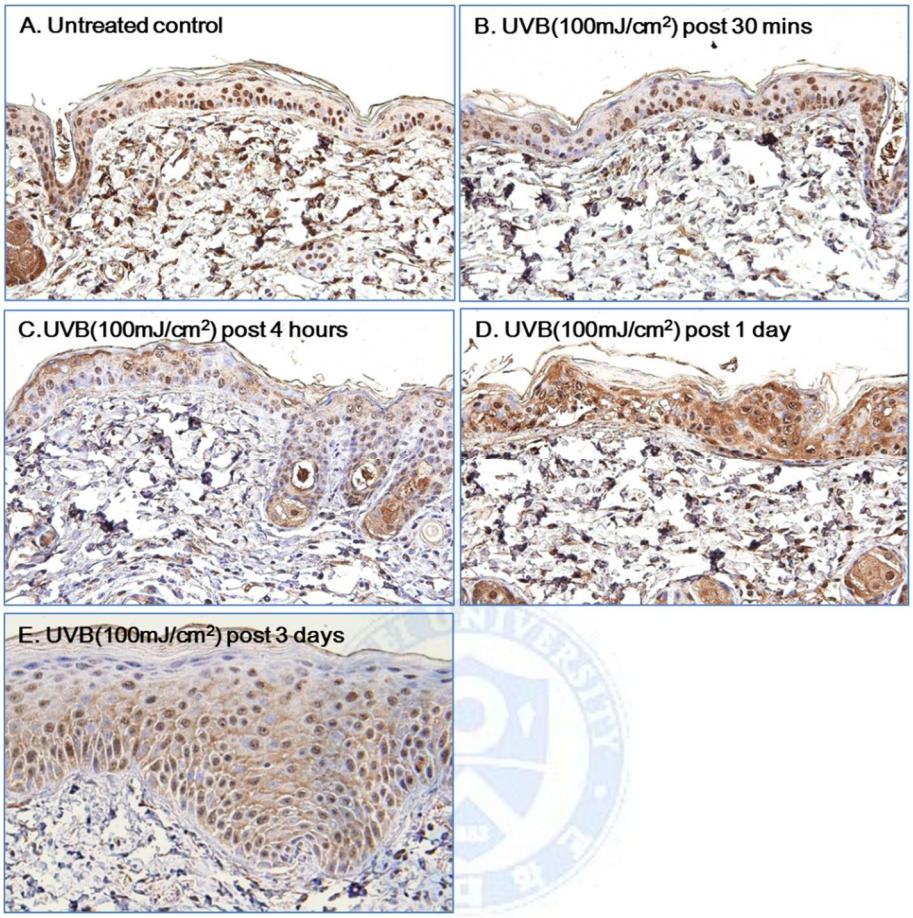

Figure 1. ORAI1 immunohistochemical staining after 100 mJ/cm^2 of UVB irradiation. **(A)** ORAI1 expression in the untreated mouse skin. ORAI1 was mostly expressed in basal layer as previous reports. **(B-E)** ORAI1 level is increased at 1 day and 3 days after UVB irradiation compared with untreated control. Original magnification: X200.

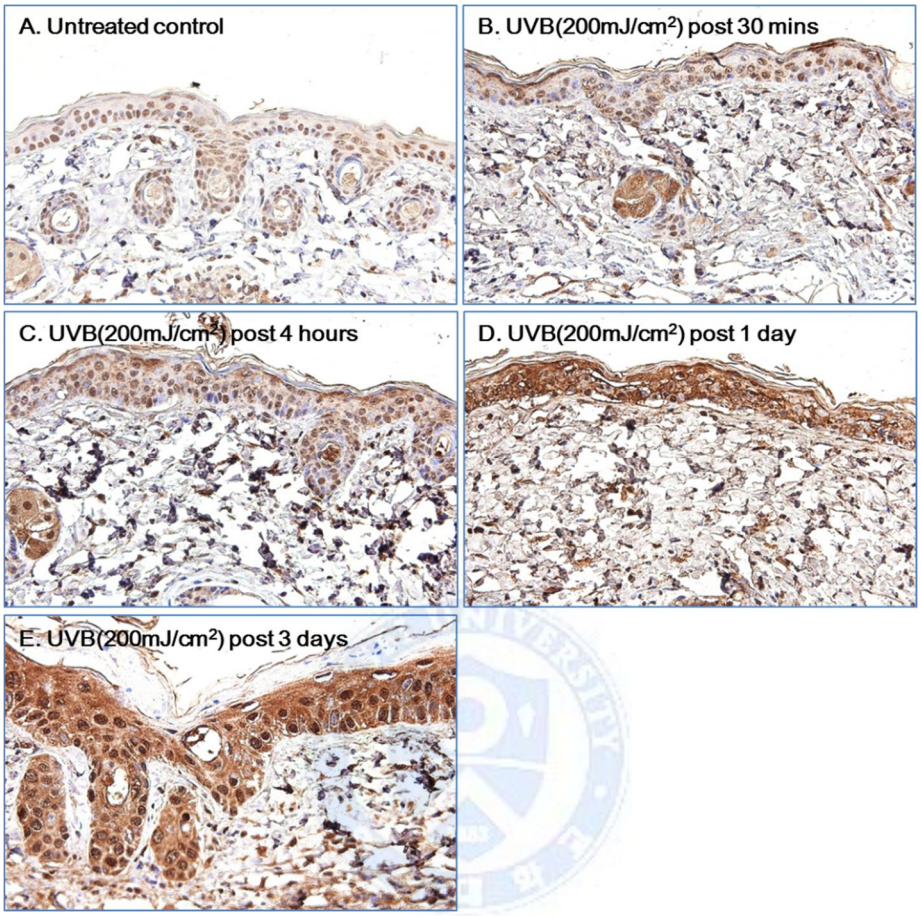

Figure 2. ORAI1 immunohistochemical staining after 200 mJ/cm^2 of UVB irradiation. **(A)** ORAI1 expression in the untreated mouse skin. **(B-E)** ORAI1 level is increased from 4 hours after UVB irradiation compared with untreated control. Original magnification: X200.

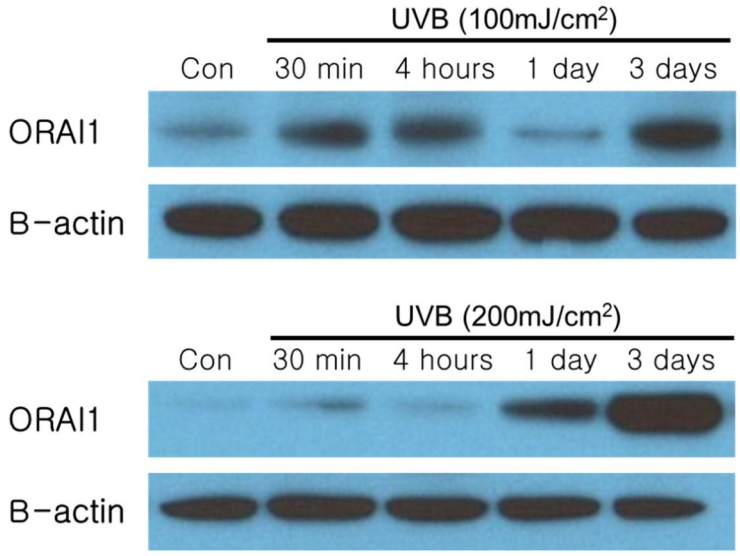

Figure 3. Western blotting of ORAI1 reveled time-dependent and dose-dependent increases in ORAI1 protein expression.

2. Mediation of UVB-induced epidermal hyperplasia by ORAI1 in murine epidermis

As 100 mJ/cm^2 UVB irradiation showed more epidermal hyperplasia, this amount was selected for this experiment. UVB is reported to induce epidermal hyperplasia through EGF receptor activation. On the basis of previous reports, the thickness of epidermis was increased at 3 days after UVB irradiation (Figure 4A-D). To confirm the role of ORAI1, we applied BTP2 on a half of back twice a day daily immediately after UVB irradiation. BTP2 attenuates UVB-induced epidermal hyperplasia in mice skin (Figure 4E). These findings suggest that UVB-induced ORAI1 activation may increase the proliferation of keratinocyte in murine epidermis.

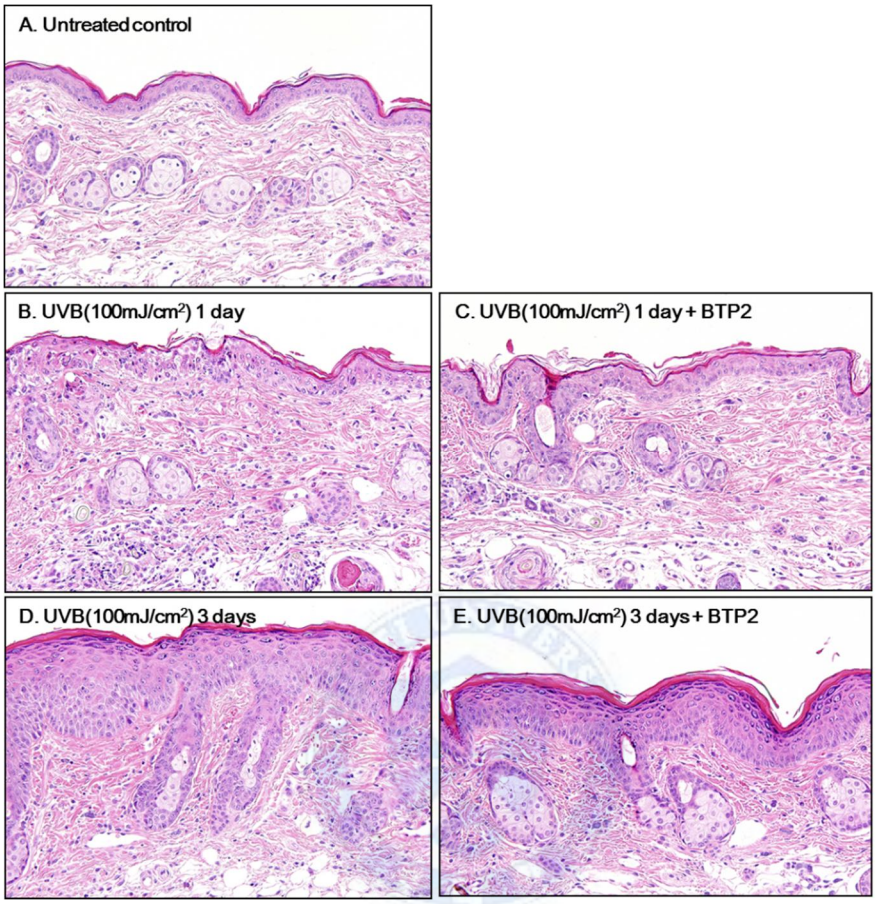

Figure 4. Epidermal thickness of UVB-irradiated mice treated with or without ORAI1 inhibitor, BTP2. **(A)** Epidermis of untreated mouse skin. **(B,C)** There is no difference of epidermal thickness between mice treated or not treated with BTP2 at a day after UVB irradiation. **(D,E)** UVB induced epidermal hyperplasia **(D)** and BTP2 suppressed UVB-induced epidermal hyperplasia **(E)** at 3 days. Original magnification: X200.

3. Mediation of UVB-induced TSLP and COX-2 upregulation by ORAI1 in murine epidermis

As 200 mJ/cm^2 UVB irradiation showed more prominent results, this amount was selected for subsequent experiments. To definitively demonstrate the role of ORAI1 in TSLP induction, we analyzed the effect of ORAI1 on TSLP expression by applying BTP2 on a half of back. At 3 days after UVB irradiation, TSLP upregulation by UVB was attenuated on the BTP2-treated flank compared to the contralateral, non-treated flank (Figure 5). Thus, the effect of UVB on TSLP upregulation is mediated by ORAI1 activation.

In addition, at 1 day after UVB irradiation, COX-2 upregulation by UVB was delayed on the BTP2-treated flank compared to the contralateral, non-treated flank (Figure 6). Taken together, these results demonstrate that the role of ORAI1 in UVB-induced harmful effects on epidermis.

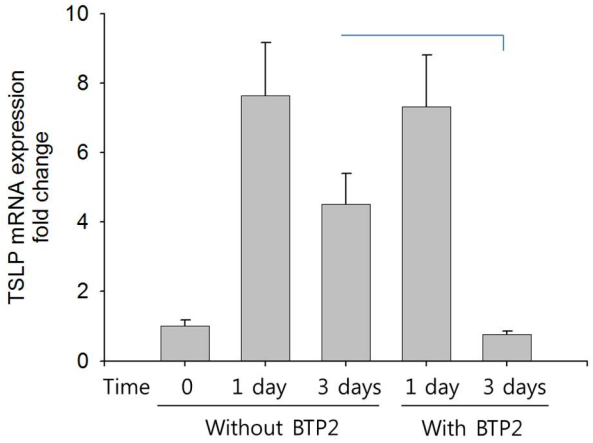

Figure 5. TSLP mRNA was upregulated by UVB irradiation. BTP2 attenuated TSLP upregulation 3 days after UVB irradiation.

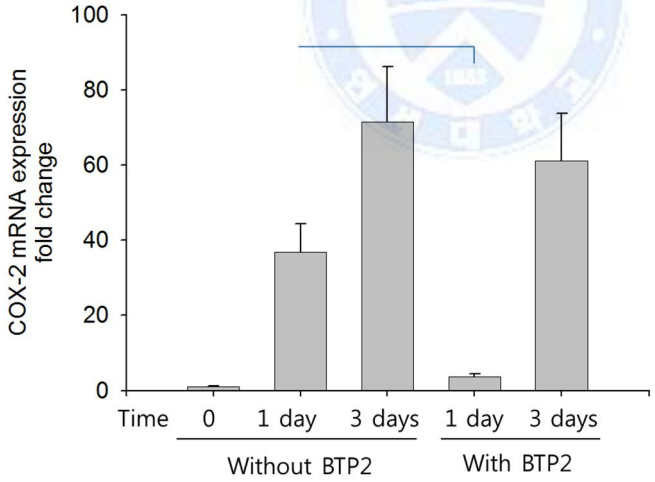

Figure 6. COX-2 mRNA was upregulated by UVB. BTP2 attenuated COX-2 upregulation 1 day after UVB irradiation.

4. Mediation of UVB-induced ORAI1 and COX-2 upregulation by ORAI1 in HaCaT

To confirm the upregulation of ORAI1 by UVB-irradiation and the role of ORAI1, we conducted in vitro experiments with HaCaT. To clarify the specific role of ORAI1, we used two ORAI1 inhibitors, whether GSK-7975A or BTP2. At 1 hour after UVB irradiation, ORAI1 mRNA expression was increased about 2-folds (Figure 7). And we found both inhibitors reversed upregulation of ORAI1 mRNA expression. In the same manner, COX-2 mRNA expression was increased about 4-folds and both inhibitors reversed upregulation of COX-2 mRNA expression (Figure 8). However, TSLP failed to show upregulation (Data are not shown). Although we used a 30 mJ/cm^2 of UVB, HaCaT lost their viability at 24 hours after UVB irradiation. Thus, these results confirm the role of ORAI1 in UVB-induced effects on epidermis.

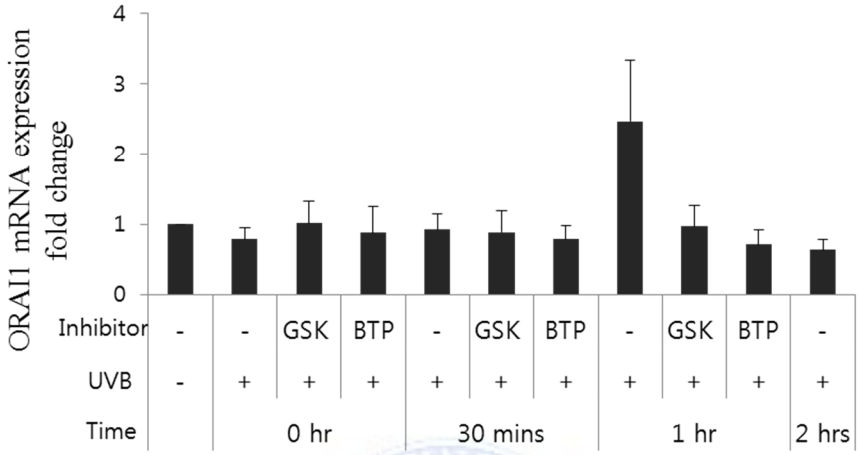

Figure 7. Quantitative real-time PCR for ORAI1 in UVB-irradiated HaCaT treated with or without ORAI1 inhibitor (GSK: GSK-7975A, BTP: BTP2). 1 hour after UVB exposure, HaCaT showed upregulated ORAI1 mRNA expression. UVB-induced ORAI1 upregulation is reversed by either GSK or BTP.

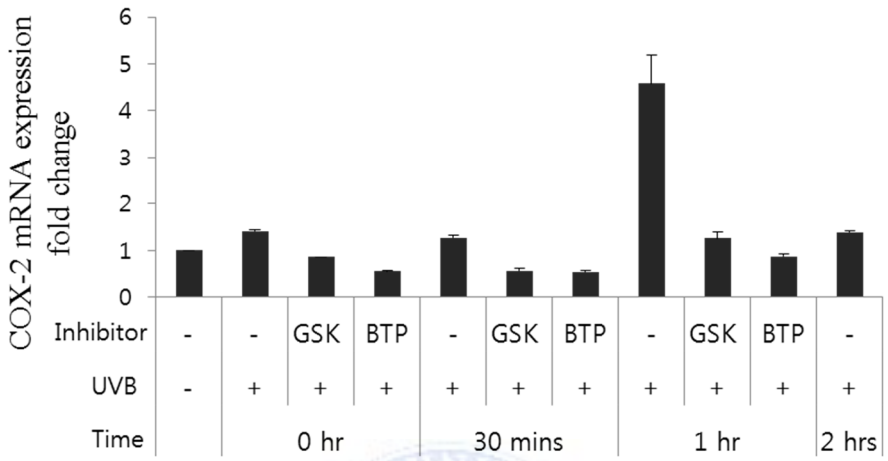

Figure 8. Quantitative real-time PCR for COX-2 in UVB-irradiated HaCaT treated with or without ORAI1 inhibitor (GSK: GSK-7975A, BTP: BTP2). 1 hour after UVB exposure, HaCaT showed upregulated COX-2 mRNA expression. UVB-induced COX-2 upregulation is reversed by either GSK or BTP.

IV. Discussion

UVB radiation is well known to induce ER stress and activate UPR[24]. UVB damages the human skin, causing premature aging and severe skin pathologies, including cancer.[25] Previous study reported ORAI1 as a main player in UV-induced melanogenesis.[26] STIM2-regulated ORAI1 was identified as one of the major SOCE pathways in primary melanocytes, being activated by endothelin-1 which can be released by UVB irradiation.[26] However, our study is the first study which shows direct effects of UVB on the expression and function of ORAI1 in keratinocytes. In mouse skin, ORAI1 level is increased after UVB irradiation of 100 or 200 mJ/cm^2. ORAI1 expression showed time-dependent and dose-dependent increase. As reported previously, the UVB irradiated epidermis of skin was slightly thickened compared with intact mice skin.[17] The proliferative effects of UV[17] and of ORAI1[12] in the skin have been independently documented. We showed ORAI1 inhibitor attenuated UVB-induced epidermal hyperplasia. Thus, present study showed UVB-induced epidermal hyperplasia is dependent on ORAI1. ORAI1 might suppress UVB-induced apoptosis although the anti-apoptotic role of ORAI1 and STIM1 has been reported controversially.[27,28]

TSLP is an IL-7-like cytokine belonging to the Th2 cytokine family.[29] TSLP acts as a master switch for Th2 immune responses and may have a key role in

the initiation and maintenance of allergic inflammatory diseases, including AD.[30] Recent studies have shown that UVB radiation exposure increases The present study shows that UVB irradiation can induce TSLP expression via ORAI1 signaling, too. ORAI1 mediates NFAT-dependent cytokine expression during immune cell activation and the loss of function mutations in ORAI1 and STIM1 leads to severe combined immunodeficiencies in patients.[9,31] ORAI1 may play more general role in the pathogenesis of inflammatory disease. Although a role for the ORAI1 in atopic dermatitis had not been studied, SNPs in the ORAI1 gene have been linked to susceptibility to atopic disease in humans.[32] Thus, ORAI1 may represent a new therapeutic target for inflammatory skin disease such as sun exposure-related dermatitis or atopic dermatitis.

COX-2 is a key inducible enzyme known to be involved in producing prostaglandins and other eicosanoids in the inflammatory reaction. COX-2 increases production of PGE2 known to modulate cell proliferation, cell death, and tumor invasion in many types of cancer. PGE2 production by UV induced COX-2 is thought to promote skin carcinogenesis, as well as contribute to even the earliest stages of UV-induced skin damage.[33] Topical and systemic treatment with celecoxib, a COX-2-selective inhibitor has also been shown to inhibit UV-induced skin tumorigenesis.[34-36] Thus, the present findings suggest that ORAI1 may be involved in the pathogenesis of UVB-mediated skin cancer through COX-2 regulation. This assumption is supported by reports

showing upregulation of ORAI and STIM proteins in cancer growth, migration, and metastasis.[37-40] Findings from our present study show that topical treatment of BTP2 immediately after UV exposure inhibited UV-induced epidermal hyperplasia and upregulation of TSLP and COX-2. Therefore, our results suggest that suppression of UV induced changes by BTP2 may prevent UV-induced skin damage and inflammation.

ROS such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) play a role in physiological and pathophysiological processes through oxidizing proteins, lipids and polynucleotides.[41] Increasing evidence indicates that ROS and Ca^{2+} interacts together to finely tune the homeostasis. Ca^{2+} is released from ER mediated by ryanodine recptors (RyR) and 1,4,5-inositol-triphosphate receptors (IP_3R). It has been reported that ROS enhances RyR and IP_3-induced Ca^{2+} release from endo/sarcoplasmic reticulum (ER/SR).[42] Not only IP_3R, but also RyR are expressed in epidermal keratinocytes.[43] In addition, ROS inhibits Ca^{2+} transport by ER/SR Ca^{2+}-ATPases (SERCA) which replenish ER/SR Ca^{2+} stores in smooth muscle cells.[41] These reports suggest that UV-induced ROS may activate ORAI1 through ER calcium depletion.

As we could not find any references with topical treatment of BTP2 to the skin, we decided the concentration of BTP2 considering absorption of the skin. In our experiments, applying twice a day with 100 μl of BTP2 1 μM dissolved

in ethanol solvent was enough to inhibit ORAI1 activation. UVB-induced skin damage may let BTP2 to be absorbed more easily through epidermis. Further study will be needed to clarify the proper dose and efficient form of BTP2.

Human keratinocytes express not only ORAI1, but also TRP which are non-selective ion channels with only a very modest Ca^{2+} selectivity. TRP has been reported to interact with STIM/ORAI. ORAI and TRP interfere with each other in skin barrier formation, atopic dermatitis pathogenesis, and even melanogensis.[11,26,32,44,45] TRPM1 and TRPA1 has been reported to be associated with UV radiation, but they are mainly expressed in melanocytes, not keratinocytes.[46,47] Among TRP expressed in keratinocytes, TRPV1 was reported to be associated with UV-induced cutaneous changes. Topical application of TRPV1 blocker reversed UV-induced epidermal hyperplasia, and upregulation of metalloproteinases, pro-inflammatory cytokines, and COX-2.[20] In addition, TRPV1 expression is increased in photoaged human skin.[48] Taken together, STIM1/ORAI1 may interact with other TRP channels such as TRPV1 in UV-induced cutaneous response.

Hair loss, thinner epidermis and alopecia have been reported in mice lacking ORAI1.[49] In addition, ectodermal dysplasia with anhydrosis was observed in very few human patients with mutations in ORAI1 and STIM1 genes (severe combined immunodeficiency patients).[50] These previous results clearly suggest ORAI1 is an important calcium channel regulates skin homeostasis.

However, it was unknown whether UV-induced keratinocyte associated changes are regulated by ORAI1 until this study. Although it has not been reported, patients with mutation in ORAI1 and STIM1 genes may have different sensitivity to UVB. In future, a comparison of UV-induced change in humans with defective ORAI and/or STIM function with healthy controls could provide further evidence for the role of ORAI1 and Ca^{2+} signaling in UV-induced changes.

V. Conclusion

This research demonstrates that ORAI1 channel is a major regulator of the UVB response in skin. Keratinocyte proliferation and epidermal hyperplasia following UVB exposure were dependent on ORAI1 activation. In addition, the present findings suggest ORAI1 plays more general role in UVB-induced inflammation or carcinogenesis through TSLP or COX-2.

In conclusion, this study confirmed that ORAI1 is a central modulator of the UVB response of the skin that regulates cell proliferation, inflammation and even carcinogenesis. Therefore, we suggest that ORAI1 inhibitors may prevent UV-induced skin damage and might be used for keeping healthy skin avoiding sun damage.

VI. REFERENCES

1 Yuspa SH, Hennings H, Tucker RW, Jaken S, Kilkenny AE, Roop DR. Signal transduction for proliferation and differentiation in keratinocytes. *Ann N Y Acad Sci* 1988;548:191-6.

2 Dotto GP. Signal transduction pathways controlling the switch between keratinocyte growth and differentiation. *Crit Rev Oral Biol Med* 1999;10:442-57.

3 Mascia F, Denning M, Kopan R, Yuspa SH. The black box illuminated: signals and signaling. *J Invest Dermatol* 2012;132:811-9.

4 Denda M, Ashida Y, Inoue K, Kumazawa N. Skin surface electric potential induced by ion-flux through epidermal cell layers. *Biochem Biophys Res Commun* 2001;284:112-7.

5 Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S *et al.* Mutations in ATP2A2, encoding a Ca2+ pump, cause Darier disease. *Nat Genet* 1999;21:271-7.

6 Hu Z, Bonifas JM, Beech J, Bench G, Shigihara T, Ogawa H *et al.* Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. *Nat Genet* 2000;24:61-5.

7 Vandenberghe M, Raphael M, Lehen'kyi V, Gordienko D, Hastie R, Oddos T *et al.* ORAI1 calcium channel orchestrates skin homeostasis. *Proc Natl Acad Sci U S A* 2013;110:E4839-48.

8 Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE, Jr. *et al.* STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* 2005;15:1235-41.

9 Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B *et al.* A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006;441:179-85.

10 Parekh AB, Putney JW, Jr. Store-operated calcium channels. *Physiol Rev* 2005;85:757-810.

11 Numaga-Tomita T, Putney JW. Role of STIM1- and Orai1-mediated Ca2+ entry in Ca2+-induced epidermal keratinocyte differentiation. *J Cell Sci* 2013;126:605-12.

12 Darbellay B, Barnes L, Boehncke WH, Saurat JH, Kaya G. Reversal of murine epidermal atrophy by topical modulation of calcium signaling. *J Invest Dermatol* 2014;134:1599-608.

13 Wilson SR, The L, Batia LM, Beattie K, Katibah GE, McClain SP *et al.* The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell* 2013;155:285-95.

14 Moniaga CS, Jeong SK, Egawa G, Nakajima S, Hara-Chikuma M, Jeon JE *et al.* Protease activity enhances production of thymic stromal lymphopoietin and basophil accumulation in flaky tail mice. *Am J Pathol* 2013;182:841-51.

15 Turner MJ, Zhou B. A new itch to scratch for TSLP. *Trends Immunol*

2014;35:49-50.

16 Huang WC, Chai CY, Chen WC, Hou MF, Wang YS, Chiu YC *et al.* Histamine regulates cyclooxygenase 2 gene activation through Orai1-mediated NFkappaB activation in lung cancer cells. *Cell Calcium* 2011;50:27-35.

17 El-Abaseri TB, Putta S, Hansen LA. Ultraviolet irradiation induces keratinocyte proliferation and epidermal hyperplasia through the activation of the epidermal growth factor receptor. *Carcinogenesis* 2006;27:225-31.

18 Farrukh MR, Nissar UA, Afnan Q, Rafiq RA, Sharma L, Amin S *et al.* Oxidative stress mediated Ca(2+) release manifests endoplasmic reticulum stress leading to unfolded protein response in UV-B irradiated human skin cells. *J Dermatol Sci* 2014;75:24-35.

19 Anand S, Chakrabarti E, Kawamura H, Taylor CR, Maytin EV. Ultraviolet light (UVB and UVA) induces the damage-responsive transcription factor CHOP/gadd153 in murine and human epidermis: evidence for a mechanism specific to intact skin. *J Invest Dermatol* 2005;125:323-33.

20 Lee YM, Kang SM, Lee SR, Kong KH, Lee JY, Kim EJ *et al.* Inhibitory effects of TRPV1 blocker on UV-induced responses in the hairless mice. *Arch Dermatol Res* 2011;303:727-36.

21 Jang Y, Jeong SH, Park YH, Bae HC, Lee H, Ryu WI *et al.* UVB

induces HIF-1alpha-dependent TSLP expression via the JNK and ERK pathways. *J Invest Dermatol* 2013;133:2601-8.

22 Jin XJ, Kim EJ, Oh IK, Kim YK, Park CH, Chung JH. Prevention of UV-induced skin damages by 11,14,17-eicosatrienoic acid in hairless mice in vivo. *J Korean Med Sci* 2010;25:930-7.

23 Hong SP, Kim MJ, Jung MY, Jeon H, Goo J, Ahn SK *et al.* Biopositive effects of low-dose UVB on epidermis: coordinate upregulation of antimicrobial peptides and permeability barrier reinforcement. *J Invest Dermatol* 2008;128:2880-7.

24 Mera K, Kawahara K, Tada K, Kawai K, Hashiguchi T, Maruyama I *et al.* ER signaling is activated to protect human HaCaT keratinocytes from ER stress induced by environmental doses of UVB. *Biochem Biophys Res Commun* 2010;397:350-4.

25 Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S *et al.* Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 2002;138:1462-70.

26 Stanisz H, Stark A, Kilch T, Schwarz EC, Muller CS, Peinelt C *et al.* ORAI1 Ca(2+) channels control endothelin-1-induced mitogenesis and melanogenesis in primary human melanocytes. *J Invest Dermatol* 2012;132:1443-51.

27 Flourakis M, Lehen'kyi V, Beck B, Raphael M, Vandenberghe M, Abeele FV *et al.* Orai1 contributes to the establishment of an

apoptosis-resistant phenotype in prostate cancer cells. *Cell Death Dis* 2010;1:e75.

28 Kondratska K, Kondratskyi A, Yassine M, Lemonnier L, Lepage G, Morabito A *et al.* Orai1 and STIM1 mediate SOCE and contribute to apoptotic resistance of pancreatic adenocarcinoma. *Biochim Biophys Acta* 2014;1843:2263-9.

29 Vu AT, Chen X, Xie Y, Kamijo S, Ushio H, Kawasaki J *et al.* Extracellular double-stranded RNA induces TSLP via an endosomal acidification- and NF-kappaB-dependent pathway in human keratinocytes. *J Invest Dermatol* 2011;131:2205-12.

30 Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673-80.

31 Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006;443:230-3.

32 Chang WC, Lee CH, Hirota T, Wang LF, Doi S, Miyatake A *et al.* ORAI1 genetic polymorphisms associated with the susceptibility of atopic dermatitis in Japanese and Taiwanese populations. *PLoS One* 2012;7:e29387.

33 Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human

malignancies. *Int J Cell Biol* 2010;2010:215158.

34 Wilgus TA, Ross MS, Parrett ML, Oberyszyn TM. Topical application of a selective cyclooxygenase inhibitor suppresses UVB mediated cutaneous inflammation. *Prostaglandins Other Lipid Mediat* 2000;62:367-84.

35 Wilgus TA, Koki AT, Zweifel BS, Kusewitt DF, Rubal PA, Oberyszyn TM. Inhibition of cutaneous ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. *Mol Carcinog* 2003;38:49-58.

36 Fischer SM, Lo HH, Gordon GB, Seibert K, Kelloff G, Lubet RA *et al.* Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcinogenesis. *Mol Carcinog* 1999;25:231-40.

37 Suyama E, Wadhwa R, Kaur K, Miyagishi M, Kaul SC, Kawasaki H *et al.* Identification of metastasis-related genes in a mouse model using a library of randomized ribozymes. *J Biol Chem* 2004;279:38083-6.

38 Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 2009;15:124-34.

39 Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, Wang Y *et al.* Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell* 2010;143:84-98.

40 Prevarskaya N, Skryma R, Shuba Y. Calcium in tumour metastasis: new roles for known actors. *Nat Rev Cancer* 2011;11:609-18.

41 Ermak G, Davies KJ. Calcium and oxidative stress: from cell signaling to cell death. *Mol Immunol* 2002;38:713-21.

42 Yan Y, Wei CL, Zhang WR, Cheng HP, Liu J. Cross-talk between calcium and reactive oxygen species signaling. *Acta Pharmacol Sin* 2006;27:821-6.

43 Denda S, Kumamoto J, Takei K, Tsutsumi M, Aoki H, Denda M. Ryanodine receptors are expressed in epidermal keratinocytes and associated with keratinocyte differentiation and epidermal permeability barrier homeostasis. *J Invest Dermatol* 2012;132:69-75.

44 Sun XD, You Y, Zhang L, Zheng S, Hong Y, Li J *et al.* The possible role of TRPC6 in atopic dermatitis. *Med Hypotheses* 2012;78:42-4.

45 Saul S, Stanisz H, Backes CS, Schwarz EC, Hoth M. How ORAI and TRP channels interfere with each other: interaction models and examples from the immune system and the skin. *Eur J Pharmacol* 2014;739:49-59.

46 Devi S, Kedlaya R, Maddodi N, Bhat KM, Weber CS, Valdivia H *et al.* Calcium homeostasis in human melanocytes: role of transient receptor potential melastatin 1 (TRPM1) and its regulation by ultraviolet light. *Am J Physiol Cell Physiol* 2009;297:C679-87.

47 Bellono NW, Kammel LG, Zimmerman AL, Oancea E. UV light

phototransduction activates transient receptor potential A1 ion channels in human melanocytes. *Proc Natl Acad Sci U S A* 2013;110:2383-8.

48 Lee YM, Kim YK, Chung JH. Increased expression of TRPV1 channel in intrinsically aged and photoaged human skin in vivo. *Exp Dermatol* 2009;18:431-6.

49 Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M *et al.* Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol Cell Biol* 2008;28:5209-22.

50 McCarl CA, Picard C, Khalil S, Kawasaki T, Rother J, Papolos A *et al.* ORAI1 deficiency and lack of store-operated Ca2+ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J Allergy Clin Immunol* 2009;124:1311-8 e7.

ABSTRACT (IN KOREAN)

자외선B가 ORAI1 채널에 미치는 영향과 자외선 B로 인한 표피증식 및 TSLP, COX-2 상향조절에서의 ORAI1 채널의 역할

<지도교수 이승헌>

연세대학교 대학원 의학과

오승준

피부는 외부 환경으로부터 보호막 역할을 하는 중요한 기관이다. 이러한 피부는 항상 태양광선으로부터 나오는 자외선B에 노출되게 된다. 자외선B 광선은 여러가지 생물학적 반응을 유발하게 되는데 특히 표피성장인자 (epidermal growth factor receptor)의 활성화를 통한 각질세포의 증식이나 염증반응, 악성종양형성 등이 대표적인 것들이다.

칼슘 신호는 대표적인 이차 신호 전달 체계로써 소포체의 스트레스 (endoplasmic reticulum stress)를 감지하는 STIM1과 이 것에 의해 활성화되는 ORAI1 칼슘채널은 세포막 외부로부터 세포 내부로 칼슘을 운송하는 역할을 한다. 이 칼슘의 흐름을 store-operated calcium entry(SOCE)라고 부른다. Thymic stromal lymphopoietin

(TSLP)는 Th2 cytokine family에 속해있는 IL-7-like cytokine으로써 알러지성 염증 질환의 발현과 유지에서 중요한 역할을 한다. Cyclooxygenase 2 (COX-2)는 프로스타글랜딘 E2의 생성을 통해 악성종양형성을 촉진시키는 것으로 알려진 물질이다.

아직까지 자외선B에 의한 ORAI1 채널의 변화와 그 역할에 대한 연구는 거의 없었다. 따라서 본 연구의 목적은 자외선B가 ORAI1 채널에 미치는 영향과 ORAI1 채널의 역할에 대하여 알아보고자 하였다. 우리는 생쥐모델을 이용하여 자외선B가 ORAI1 채널을 발현시킨다는 것을 알아냈다. 또한 ORAI1 채널의 억제제를 국소도포하는 방법을 이용하여 ORAI1 채널이 자외선 B에 의한 표피증식 및 TSLP, COX-2의 상향 조절에 관여한다는 사실을 증명하였다. 또한 HaCaT을 이용한 세포 실험으로 비슷한 결과를 얻을 수 있었다. 결론적으로 이번 연구를 통해서 ORAI1 채널이 자외선B 반응의 중요한 조절자라는 사실을 알게 되었으며 이를 통해 향후 ORAI1 채널을 표적으로 자외선B에 의한 악영향을 예방하고 감소하는 새로운 약물의 발전도 기대할 수 있을 것이다.

핵심되는 말: 자외선B, 칼슘, ORAI1, STIM1, cyclooxygenase 2 (COX-2), thymic stromal lymphopoietin (TSLP)