의학박사 학위논문

Roles of activin A receptor type 1C in UV-induced decrease of lipogenesis and calcium-induced differentiation in human epidermal keratinocytes

사람 표피 각질형성세포에서 자외선에 의한 지방생성감소와 칼슘에 의한 표피분화에서의 ACVR1C 역할

2021 년 8 월

서울대학교 대학원
의과학과 의과학 전공

Tian Yu-Dan

사람 표피 각질형성세포에서 자외선에 의한 지방생성감소와 칼슘에 의한 표피분화에서의 activin A receptor type 1C 역할

지도교수 정진호

이 논문을 의학박사 학위논문으로 제출함
2021 년 7 월

서울대학교 대학원
의과학과 의과학 전공
Tian Yu-Dan

Tian Yu-Dan 의 의학박사 학위논문을 인준함
2021 년 7 월

위원장 _____
부위원장 _____
위원 _____
위원 _____
위원 _____

Abstract

Roles of activin A receptor type 1C in UV-induced decrease of lipogenesis and calcium-induced differentiation in human epidermal keratinocytes

Tian Yu-Dan

Department of Biomedical Sciences

The Graduate School Seoul National University

Activin A receptor type 1C (ACVR1C), a type I transforming growth factor-β (TGF-β) receptor, is involved in the regulation of metabolic homeostasis as well as in the proliferation and differentiation of human epidermal keratinocytes. Thus, alteration of ACVR1C expression can cause skin tissue dysfunction including lipid metabolism and calcium signaling. Although ACVR1C is known to be reduced in sensitive skin and psoriasis patients, the exact role of ACVR1C in the skin has not been fully investigated.

Ultraviolet (UV) radiation, in particular, causes various skin disorders including premature skin aging, inflammation and skin cancer, and disruption of epidermal lipid metabolism and epidermal differentiation. Expectedly, UV irradiation decreased the expression of ACVR1C in human skin *in vivo* and in normal human epidermal keratinocytes (NHEK) *in vitro*. Epidermal triglyceride (TG)

synthesis was also decreased in UV-irradiated NHEK, which was consistent with its tendency in UV-irradiated human skin. Among the major lipogenic genes, the mRNA expression of sterol regulatory element binding protein-1 (SREBP1) and acetyl-CoA carboxylase (ACC) were most significantly decreased in UV-irradiated NHEK, suggesting the involvement of ACVR1C in the regulation of epidermal TG synthesis. Consistently, the knockdown of ACVR1C with short hairpin RNA (shRNA) led to a greater decrease in SREBP1 and ACC in response to UV irradiation and the overexpression of ACVR1C attenuated the UV-induced decreases in both genes. In addition, the UV-irradiated reduced phosphorylation of SMAD2, which is phosphorylated by activated type I TGF-β receptors, and the SMAD2 phosphorylation was further suppressed when the ACVR1C expression was knockdown, along with decreased expression of SREBP1 and ACC in NHEK, suggesting that ACVR1C plays important roles in homeostasis of lipid metabolism in the skin.

Alteration of ACVR1C expression was also observed in differentiation of HaCaT keratinocyte. During calcium-induced differentiation, ACVR1C expression in HaCaT keratinocytes was increased. The knockdown of ACVR1C showed higher expression levels of various differentiation markers of keratinocytes, such as keratin 1, involucrin and loricrin. Conversely, overexpression of ACVR1C attenuated levels of keratin 1 and loricrin, suggesting that

ACVR1C may negatively regulate the differentiation of keratinocytes.

Taken together, these results suggest that reduced expression of ACVR1C may be one of the causes of UV-induced disruptions in lipid metabolism and an abnormality in human epidermal differentiation, and suggest ACVR1C as a potential therapeutic target for preventing or treating skin disorders.

Keywords : activin A receptor type 1C; photoaging; epidermal lipogenesis; epidermal differentiation;

Student Number : 2015-22410

Contents

List of figures

Introduction

Activin A receptor type 1C (ACVR1C)

Activin A receptor type 1C (ACVR1C) is a type I receptor of the transforming growth factor (TGF)−β superfamily that consists of (TGF)−βs, activins, bone morphogenetic proteins (BMPs) and nodal. ACVR1C is also known as activin receptor−like kinase 7 (ALK7) (Graham and Peng, 2006). ACVR1C is activated by various ligands, such as nodal, growth/differentiation factor 3 (GDF3), activin B, and activin AB (Bu et al., 2018, Namwanje and Brown, 2016, Xu et al., 2004). ACVR1C mainly activates the SMAD−dependent pathway including SMAD2 and 3, and combines with SMAD4 for translocation into the nucleus (Tsuchida et al., 2009), and also activates the SMAD−independent pathways such as MAPK, Ras−ERK, AKT/PI3K pathways, thereby regulating various biological processes (Derynck and Zhang, 2003).

ACVR1C is expressed in various tissues, such as the brain, pancreas, heart, and adipose tissues, and activates downstream signaling to regulate physiological processes in different types of cells. Activation of ACVR1C has been shown to suppress cell growth and proliferation, and induce apoptosis (Hu et al., 2017, Shi et al., 2020, Zhong et al., 2009). ACVR1C also play a critical role in the metabolic regulation in pancreas (Bertolino et al., 2008), adipose tissue (Andersson et al., 2008) and brain (Sandoval−

Guzman et al., 2012). Furthermore, defects in ACVR1C are related to various diseases, including metabolic disorders (Carlsson et al., 2009), cardiac hypertrophy (Huang et al., 2015), and tumor progression and metastasis (Michael et al., 2019). ACVR1C, as a marker of adipocyte differentiation, is abundantly expressed in adipose tissue and is reduced in obesity (Carlsson et al., 2009). ACVR1C knockout mice exhibits reduced insulin sensitivity, liver steatosis, impaired glucose tolerance (Bertolino et al., 2008), and also contributes to fat accumulation by suppressing lipolysis (Yogosawa et al., 2013). Notably, ACVR1C is also expressed in human skin, and its expression is decreased in sensitive skin and psoriasis (Kim et al., 2016, Swindell et al., 2014).

Ultraviolet radiation

According to the wavelength, ultraviolet (UV) radiation is classified into UVA (315–400 nm), UVB (290–320 nm) and UVC (100–280 nm). Among them, both UVA and UVB are capable of penetrating the skin. UVA deeply penetrates into the dermis, and UVB is mostly absorbed by the epidermis (D'Orazio et al., 2013). UV can induce various detrimental processes, including inflammation, carcinogenesis, and premature skin aging (Bald et al., 2014, Laikova et al., 2019, Trautinger, 2001). In addition, UV exposure contributes to skin lipid deregulation (Olivier et al., 2017), and also leads to the vitamin D synthesis, which is involved in the

regulation of immune response, calcium homeostasis (Mostafa and Hegazy, 2015).

Triglycerides synthesis in skin epidermis

The human skin epidermis is rich in lipids, including neutral lipids, phospholipids, and sphingolipids, and is the main site of lipid synthesis in the skin. These lipids contribute to structural components and are involved in cell growth, differentiation and signal transduction (Jia et al., 2018). Triglycerides (TG) belong to the group of neutral lipids, they are a major forms of energy reserve and are essential for membrane lipid synthesis (Athenstaedt and Daum, 2006). The disruption of TG metabolism is related to metabolic diseases in several organs and adverse skin conditions, such as skin barrier abnormalities and skin photoaging (Alves-Bezerra and Cohen, 2017, Kim et al., 2010, Radner and Fischer, 2014, Smith et al., 2000). TG metabolism is a dynamic process, with lipogenic and lipolytic enzymes participate in its synthesis and degradation (Coleman and Mashek, 2011). TG synthesis is regulated by sterol regulatory element-binding proteins (SREBPs), those are involved in lipid biosynthesis. SREBPs consist of three isoforms (SREBP1a, 1c, and SREBP2), and SREBP1c preferentially induces the expression of genes involved in the production of TG, including acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), and

glycerol-3-phosphate acyltransferase (GPAT) (Horton et al., 2002).

Skin epidermal differentiation

The skin protects human body from external insults such as UV radiation. The epidermis is the outermost layer of skin, and is composed of stratum corneum, stratum granulosa, stratum spinosum, and stratum basale. The epidermis is a dynamic structure mainly constituted by keratinocytes that undergo a complex program of differentiation, a process in which keratinocytes moving from the basal to the suprabasal layers, and maintains epidermal homeostasis (Fuchs and Raghavan, 2002). There is calcium gradient in the epidermis, with lowest levels in the basal layer and the highest levels in the granular layer. Calcium is the well-known pro-differentiating element for keratinocytes and regulates signaling pathways associated with differentiation (Bikle et al., 2012). Differentiation-related proteins, such as keratins, involucrin, loricrin, and filaggrin can be induced by calcium, which have been known to be expressed in the four distinct layers of the epidermis. Keratin 5 and keratin 14 are expressed in the basal layer, and keratin1, keratin 10, transglutaminase, and involucrin are mainly synthesized in spinous layer. Furthermore, loricrin and filaggrin appear at the granular layer (Elsholz et al., 2014). Dysregulation of keratinocyte differentiation can be observed in several skin diseases, such as ichthyosis, atopic dermatitis and psoriasis

(Hoffjan and Stemmler, 2007), these skin diseases are accompanied by the disappearance of the calcium gradient (Elias et al., 2002). In addition, the abnormalities of epidermal calcium distribution as well as keratinocyte differentiation can be induced under harmful stimuli such as UV exposure (Jiang et al., 2007). Furthermore, aberrant keratinocyte differentiation is involved in the pathology of several skin diseases, such as psoriasis, atopic dermatitis and skin cancer (Bikle et al., 2016, Elsholz et al., 2014).

In skin epidermis, UV decreases TG synthesis as well as the expression of related lipogenic genes, suggesting that altered TG synthesis plays an important role in photoaged skin (Kim et al., 2010). ACVR1C is also involved in lipid metabolism by regulating lipolysis process (Yogosawa et al., 2013). However, it is not clear whether ACVR1C affects the process of lipogenesis, particularly in the epidermis.

In addition, defect of ACVR1C contributes to enhance calcium influx enhancement, and causes abnormal skin hypersensitivity (Kim et al., 2016). ACVR1C is reduced in skin disorders such as sensitive skin and psoriasis. However, the effect of ACVR1C in the abnormality of the skin has not been revealed.

Chapter 1

UV-induced reduction of ACVR1C decreases SREBP1 and ACC expression by the suppression of SMAD2 phosphorylation in normal human epidermal keratinocytes

Materials and Methods

Human skin samples and UV irradiation

Healthy human volunteers provided sun-protected and UV-irradiated skin samples. An F75/85W/UV21 fluorescent lamp (emission range 285-350 nm, peak at 310-315 nm) was used to irradiate sun-protected buttock skin. UV-C (< 290 nm) was filtered. In this study, UV irradiation included both UV-A and UV-B light. The MED (minimal erythema dose) means the amount of UV radiation required to produce a minimal erythema, and the MED value depends on the individual's skin sensitivity. The MED was determined 24 h after UV irradiation. Sun-protected buttock skin was irradiated with UV (2MED), and non-irradiated and UV-irradiated samples were obtained by punch biopsy at the indicated times. The epidermis was separated from whole skin specimens following incubation at 55° C in phosphate-buffered saline (PBS) for 2 min. This study was approved by the Institutional Review Board at Seoul National University Hospital (IRB No. 1610-097-801), and all subjects provided written informed consent. The study was conducted according to the Principles of the Declaration of Helsinki.

Cell culture and UV irradiation

Primary NHEK were isolated from the foreskin. Cells were cultured in EpiLife™ medium containing human keratinocyte growth

supplement (Thermo Fisher Scientific, Inc., MA, USA) in a 37° C humidified, 5% CO_2 incubator. The UV source from the Philips TL 20W/12RS fluorescent sun lamps had an emission wavelength between 275 and 380 nm (peak, 310-315 nm). UV-C (< 290 nm) was filtered. The UV irradiation intensity was measured using a UV meter (Model 585100; Waldmann Co., Villingen-Schwenningen, Germany). Primary NHEK were seeded in culture dishes, and upon reaching confluence, cells were washed with phosphate-buffered saline (PBS), and irradiated with UV (75 mJ/cm^2) in PBS. After UV irradiation, cells were maintained in EpiLife™ medium, and were harvested at the indicated times after UV irradiation.

Lentivirus production and infection

A pLKO.1 transfer vector containing short hairpin RNA (shRNA) targeting sequences (#TRCN0000196633) was obtained from Sigma (St. Louis, MO, USA). To generate lentiviral particle, HEK-293TN cells were transfected with 5 μg of packaging vector (GAG), 2.5 μg of envelope vector (VSVG), and 2.5 μg shACVR1C using Lipofectamine® 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions in Opti-MEM reduced serum medium (Gibco, Thermo Fisher Scientific, Inc., MA, USA). At 6 h after the transfection, medium was replaced with Opti-MEM reduced serum medium. Viral supernatant was collected after 24 h and was further collected after 52 h. Scrambled shRNA was used as controls. Primary NHEK were infected with 1 ml of

medium containing lentiviral particles with 8 μg/ml polybrene (Santa Cruz Biotechnology, CA, USA). At 8 h after the infection, cells were washed with PBS and were maintained in culture medium. This study was approved by the Institutional Biosafety Committee at Seoul National University Hospital (SNUH-IBC-1801-001-004-2B). Cells were treated with UV (75mJ/cm^2) after reaching confluence, and were harvested at 24 h.

Plasmid transfection

For the overexpression of ACVR1C, the plasmid expressing human ACVR1C was obtained from Sino Biological Inc. (Beijing, China). Primary NHEK were transfected with 0.5 μg of pcDNA3 and 0.5 μg of pCMV-ACVR1C plasmid using Lipofectamine® 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions in Opti-MEM reduced serum medium (Gibco, Thermo Fisher Scientific, Inc., MA, USA). At 6 h after the transfection, Opti-MEM reduced serum medium was replaced with culture medium. Cells were treated with UV (75mJ/cm^2) after reaching confluence, and were harvested at 24 h.

RNA interference

For the knockdown of SMAD2, cells were transiently transfected with SMAD2 siRNA or negative control siRNA (Bioneer, Daejeon, Republic of Korea) using Lipofectamine® 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's

instructions in Opti-MEM reduced serum media (Gibco, Thermo Fisher Scientific, Inc., MA, USA). At 6 h after the transfection, medium was replaced with culture medium. Cells were treated with UV (75mJ/cm^2) after reaching confluence, and were harvested at 24 h.

Western blot analysis and immunofluorescence staining

Primary NHEK were homogenized, and proteins were extracted using RIPA buffer (Merck Millipore, Billerica, MA, USA) containing a protease inhibitor mixture (Roche Applied Science, Penzberg, Germany) and phosphatase inhibitor mixture (Sigma-Aldrich; St. Louis, MO, USA). Equal amounts of protein lysates were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted onto PVDF membranes. After blocking in 5% non-fat milk diluted with Tris-buffered saline containing 0.1% Tween 20 (TBST), the membrane was incubated overnight at 4° C with primary antibodies against ACVR1C (Atlas Antibodies, Stockholm, Sweden), SREBP1 (Santa Cruz Biotechnology, CA, USA), ACC (Cell Signaling, MA, USA), p-SMAD2, and t-SMAD2 (Cell Signaling, MA, USA), β-actin (Thermo Fisher Scientific, Inc., MA, USA). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Bands were detected using an enhanced chemiluminescence detection system (Biomax Co. Ltd., Seoul, South Korea). Band density was quantified using ImageJ (NIH,

Bethesda, MD, USA). Skin specimen sections (4 mm) were stained with a primary ACVR1C (MyBioSource, Inc. CA, USA) rabbit polyclonal antibody in a humidified chamber at 4° C for 18 h. After washing in PBS, the sections were incubated with a secondary Alexa 488-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) antibody for 1 h at room temperature. Nuclei were counterstained with DAPI.

Real-time quantitative PCR (RT-PCR)

Total RNA was prepared from the separated epidermis or NHEK using RNAiso Plus (Takara Bio Inc., Shiga, Japan), and 1 μg of total RNA was converted to complementary DNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. To quantitatively estimate the mRNA expression level of each gene, PCR was performed on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. The sequences of primers are as following: ACVR1C (forward, 5' - AGT CGG AGG AAT TGT TGA GGA -3' ; reverse, 5' - CTC GGA GTG CTT CAC AAC TTT -3'), SREBP1c (forward, 5' - GCC ATG GAT TGC ACT TT -3' ; reverse, 5' - CAA GAG AGG AGC TCA ATG - 3'), ACC (forward, 5' - CCA CTT GGC TGA GCG ATT -3' ; reverse, 5' - CCA GGT CCT CCA GCA GAA - 3'), FAS (forward, 5' - CCG AGG AAC TCC CCT CAT -3' ;

reverse, 5’ – GCC AGC GTC TTC CAC ACT –3’), SCD (forward, 5’ – TGG AGC CAC CGC TCT TAC –3’; reverse, 5’ – GCC ACG TCG GGA ATT ATG –3’), 36B4 (forward, 5’ – TCG ACA ATG GCA GCA TCT AC –3’; reverse, 5’ – TGA TGC AAC AGT TGG GTA GC –3’).

Triglyceride contents

NHEK were homogenized with chloroform/methanol/water (1:2:0.8, v/v/v) by Bligh and Dyer method, the lipids of the lower phase were collected after phase separation, and triglyceride contents were determined by a fluorescent enzymatic method (Asan Pharmaceutical Co. Ltd., Seoul, South Korea) according to the manufacturer’s instructions, and normalized to the protein content by the Bradford method (Bio–Rad, Hercules, CA, USA).

Statistical analysis

Data were presented as means ± SEM. Significance was analyzed using the paired *t*–test or Student’s *t*–test. Differences were considered significant when $p < 0.05$.

Results

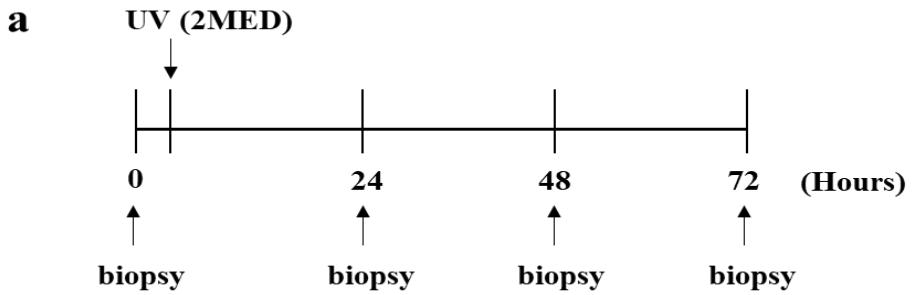
UV irradiation decreases the expression of ACVR1C in the epidermis

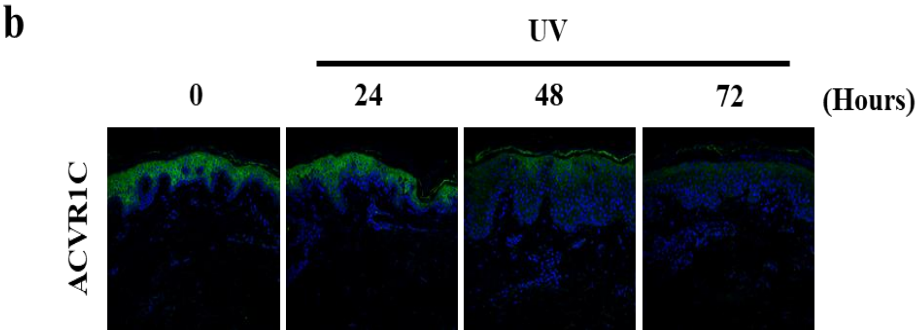
Previous studies have shown found that the expression of ACVR1C is decreased in skin disorders, such as sensitive skin and psoriasis (Kim et al., 2016, Swindell et al., 2014).

I evaluated the effects of UV irradiation on ACVR1C expression at the protein and mRNA levels in the human epidermis.

Sun-protected buttock skin was irradiated with 2 minimal erythema dose (MED) of UV and evaluated at 24, 48, and 72 h after irradiation. The epidermis was separated from the dermis of the whole skin sample. ACVR1C protein expression was decreased markedly after UV irradiation, as determined by immunofluorescence staining (Figure 1b). In addition, the mRNA expression of ACVR1C was significantly reduced by UV irradiation, as analyzed by quantitative RT-PCR (Figure 1c).

These data showed that acute UV irradiation reduces the protein and mRNA levels of ACVR1C in human epidermis *in vivo*.


a
UV (2MED)
0
24
48
72
(Hours)
biopsy
biopsy
biopsy
biopsy


b
UV
0
24
48
72
(Hours)
ACVR1C

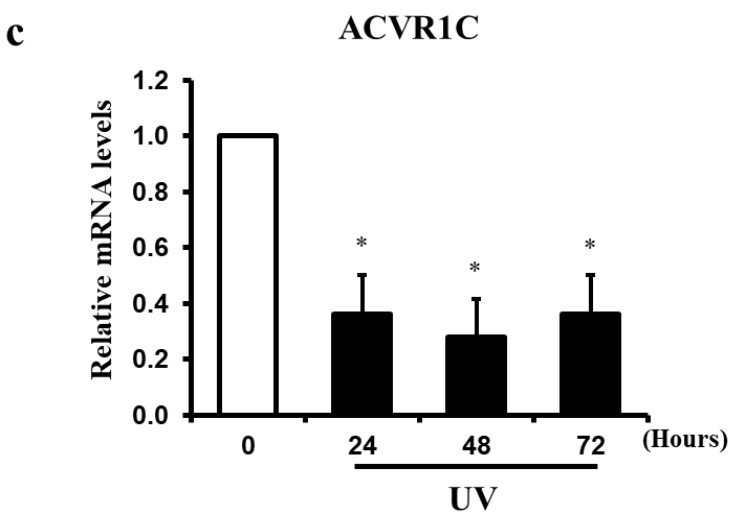

c
ACVR1C
Relative mRNA levels
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
0
24
48
72
(Hours)
UV

Figure 1. UV irradiation decreased ACVR1C expression in human skin epidermis *in vivo*. (a) Sun-protected buttock skin was irradiated with UV (2 MED) and biopsied at the indicated times after UV irradiation. (b) ACVR1C protein levels were visualized by immunofluorescence staining (representative images, n = 3); (c) ACVR1C mRNA levels were measured by quantitative RT-PCR (n = 4). Data are presented as means ± SEM of the expression of ACVR1C gene relative to 36B4. $*p < 0.05$ versus the non- irradiated control.

UV irradiation decreases the expression of ACVR1C as well as lipogenic genes in primary NHEK

Previous study showed that UV irradiation decreases the expression of genes involved in TG synthesis in the human epidermis *in vivo* (Kim et al., 2010). Consistent with the previous findings, I demonstrated that the protein and mRNA expression levels of ACVR1C were significantly decreased by UV irradiation (Figure 2a and b). Furthermore, the lipogenic genes ACC and its transcriptional activator SREBP1 were also significantly reduced by UV irradiation (Figure 2a and b). In addition, the mRNA expression levels of genes encoding other lipogenic enzymes regulated by SREBP1, such as fatty acid synthase (FAS) and stearoyl CoA desaturase (SCD), were significantly reduced by UV irradiation (Figure 2b). Moreover, the TG content, the final product of lipogenesis, was decreased at 24 h in UV-irradiated primary NHEK (Figure 2c).

These data indicated that UV irradiation reduced the levels of ACVR1C and lipogenic genes in primary NHEK, consistent with expression patterns in the epidermis *in vivo.*

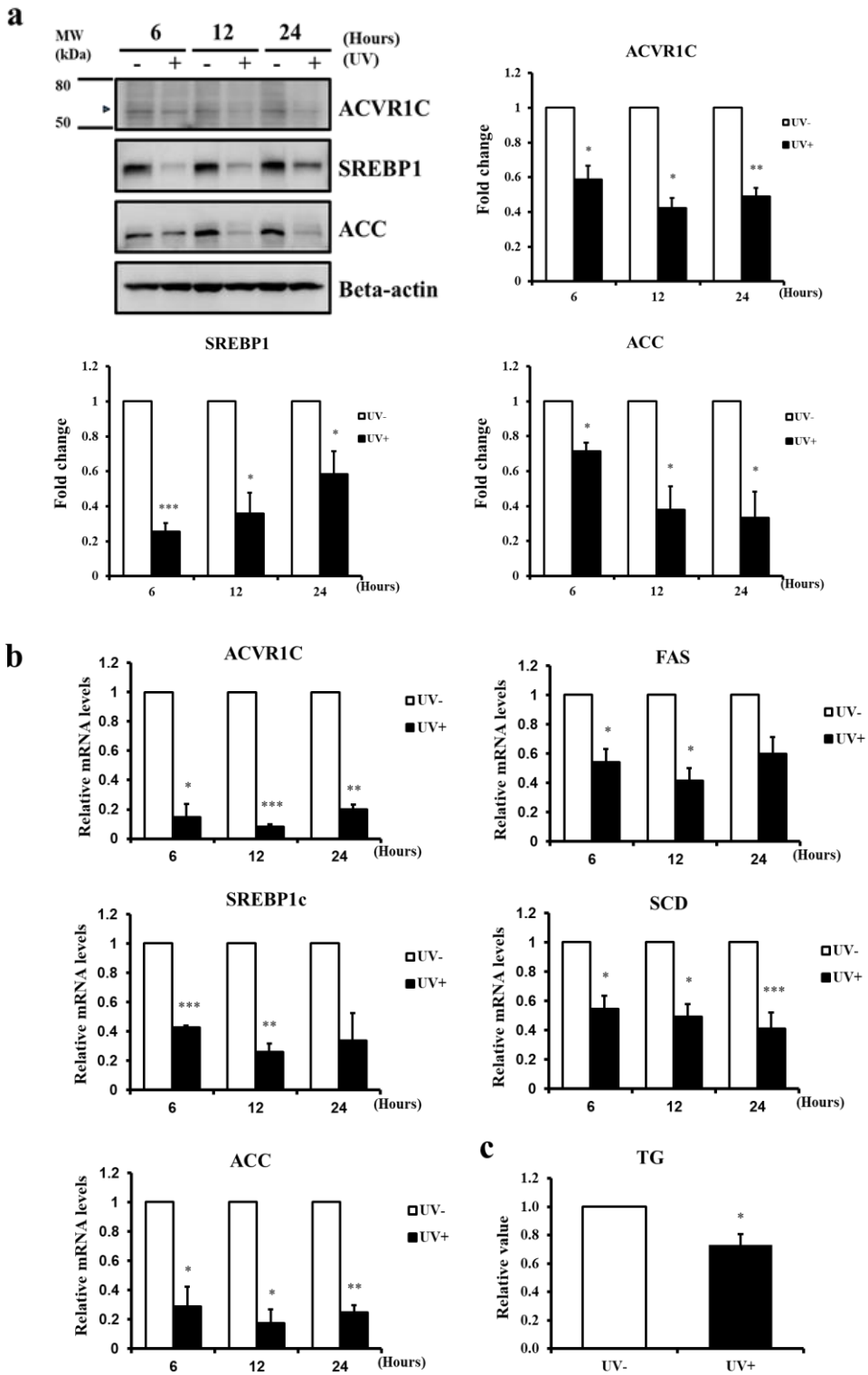

a
MW (kDa)
6
12
24
(Hours)
(UV)
- + - + - +
80
50
ACVR1C
SREBP1
ACC
Beta-actin
ACVR1C
Fold change
UV-
UV+
(Hours)
SREBP1
ACC
b
ACVR1C
FAS
SREBP1c
SCD
ACC
Relative mRNA levels
c
TG
Relative value
UV-
UV+

Figure 2. UV irradiation decreased the expression of ACVR1C as well as lipogenic genes in primary NHEK. (**a**) Primary NHEK were irradiated with UV (75 mJ/cm^2) and the protein levels of ACVR1C, SREBP1, and ACC were measured by western blotting. β −actin was used as a loading control (n = 3); (**b**) mRNA levels of *ACVR1C* and lipogenic genes, such as *SREBP1c*, *ACC, FAS,* and *SCD,* were measured by quantitative RT−PCR after UV irradiation. Data are presented as means ± SEM of the level of each gene relative to 36B4 (n = 3); (**c**) Total lipids were extracted from primary NHEK with chloroform/methanol/water (1:2:0.8, v/v/v). TG contents were measured by a fluorescent enzymatic method and normalized against the protein contents (n = 4). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. the non−irradiated control.

Knockdown of ACVR1C decreases the expression of SREBP1 and ACC in both non-irradiated and UV-irradiated NHEK.

To examine whether decreased ACVR1C is associated with reduced SREBP1 and ACC, primary NHEK cells were transfected with ACVR1C short hairpin RNA (shRNA) or a scrambled control, followed by UV irradiation. ACVR1C protein expression levels were significantly reduced by ACVR1C shRNA in both non-irradiated and UV-irradiated cells (Figure 3a and b). The protein expression levels of SREBP1 and ACC after transfection with ACVR1C shRNA were significantly lower than those in cells treated with control shRNA in both non-irradiated and UV-irradiated cells (Figure 3a, c, and d). The UV-induced decreases in SREBP1 and ACC were exacerbated in ACVR1C shRNA-treated cells (Figure 3a, b, and d).

These results suggest that a reduction of ACVR1C may decrease the expression of SREBP1 and ACC in both UV-irradiated and non-irradiated NHEK.

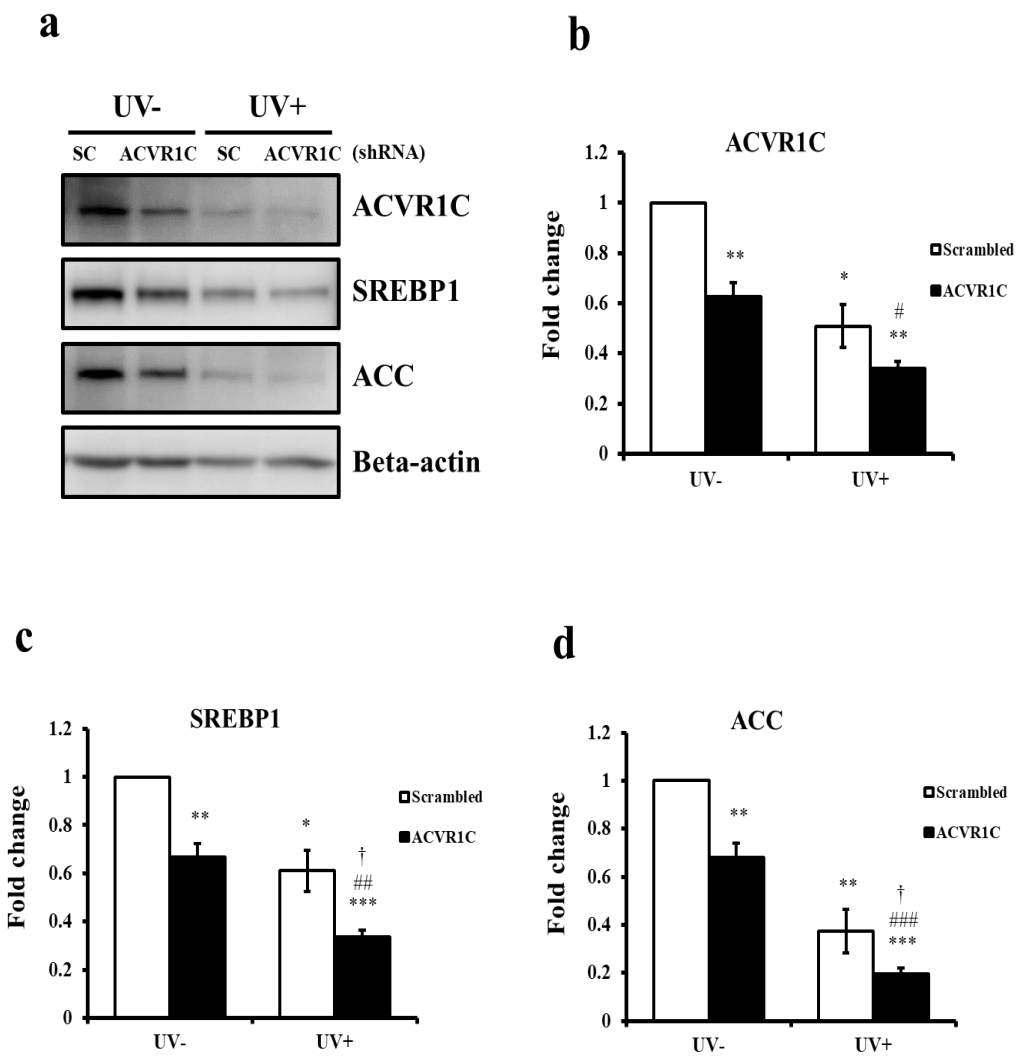

a
UV-
UV+
SC
ACVR1C
SC
ACVR1C
(shRNA)
ACVR1C
SREBP1
ACC
Beta-actin
b
ACVR1C
Fold change
1.2
1
0.8
0.6
0.4
0.2
0
**
*
#
**
Scrambled
ACVR1C
UV-
UV+
c
SREBP1
Fold change
1.2
1
0.8
0.6
0.4
0.2
0
**
*
†
##
***
Scrambled
ACVR1C
UV-
UV+
d
ACC
Fold change
1.2
1
0.8
0.6
0.4
0.2
0
**
**
†
###
***
Scrambled
ACVR1C
UV-
UV+

Figure 3. Knockdown of ACVR1C decreased the expression of SREBP1 and ACC in both non-irradiated and UV-irradiated primary NHEK. Primary NHEK were transfected with ACVR1C or scrambled shRNA and then irradiated with UV (75 mJ/cm^2). (a) ACVR1C, SREBP1, and ACC protein levels were measured by western blotting at 24 h after UV irradiation; (b – d) Protein levels were quantified using ImageJ. β-actin was used as a loading control (n = 4). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ vs. non-irradiated scrambled shRNA, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. non-irradiated ACVR1C shRNA, $^{\dagger}p < 0.05$ vs. UV-irradiated scrambled shRNA.

Overexpression of ACVR1C ameliorates UV-induced decreases in SREBP1 and ACC protein expression in primary NHEK.

To further validate the role of ACVR1C in the UV-induced reductions in SREBP1 and ACC, primary NHEK cells were transfected with an ACVR1C expression vector and then irradiated with UV. An increase in ACVR1C protein expression was confirmed at 24 h after UV irradiation by transfection with the *ACVR1C* gene (Figure 4a and b). The protein expression levels of SREBP1 and ACC were also measured at 24 h after UV irradiation. Although the overexpression of ACVR1C did not lead to a significant change of the SREBP1 and ACC protein expression in non-irradiated cells, UV-induced reductions in SREBP1 and ACC protein expression were ameliorated in cells overexpressing ACVR1C than in control vector-transfected groups (Figure 4a, c, and d).

These results suggest that the upregulation of ACVR1C might prevent UV-induced reductions in SREBP1 and ACC.

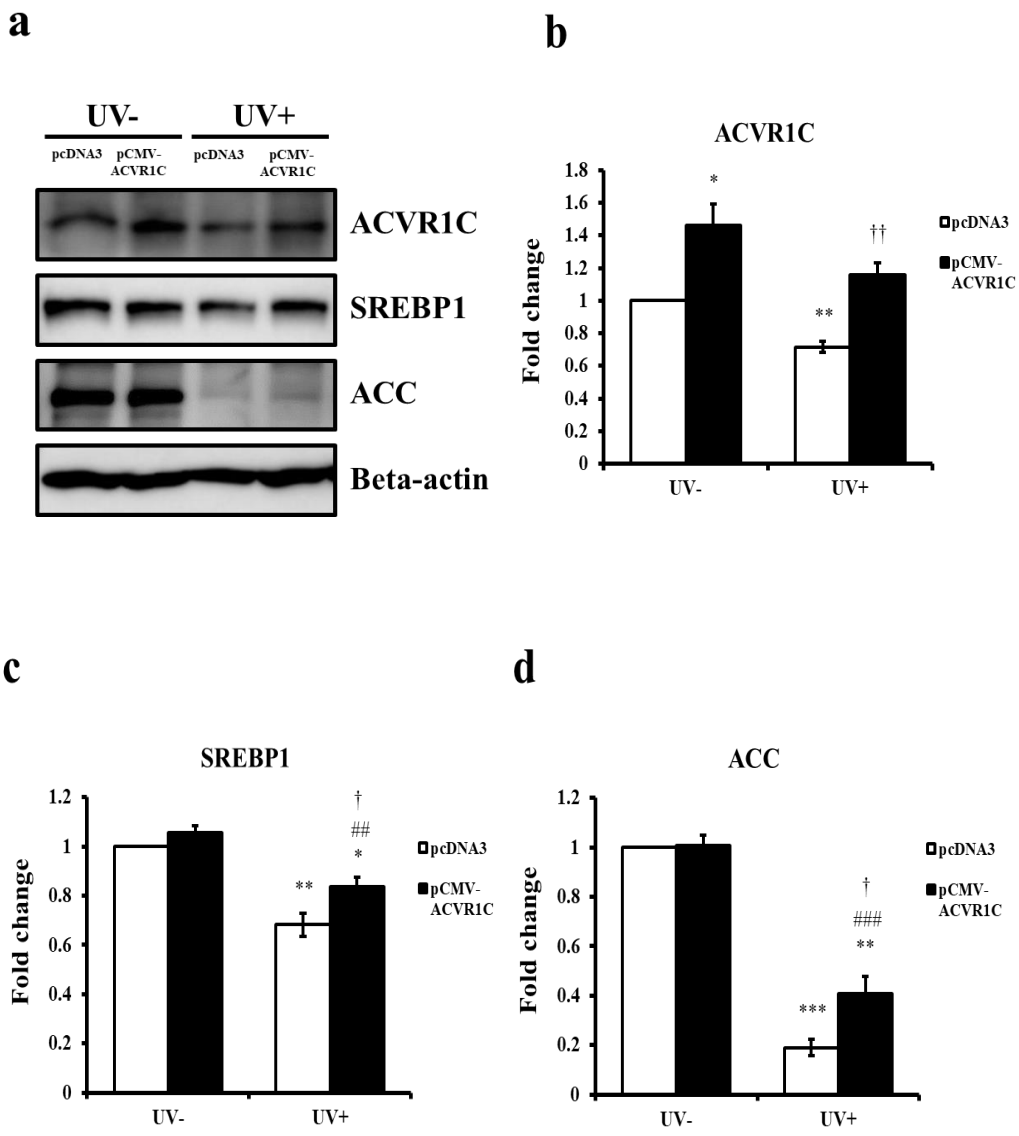
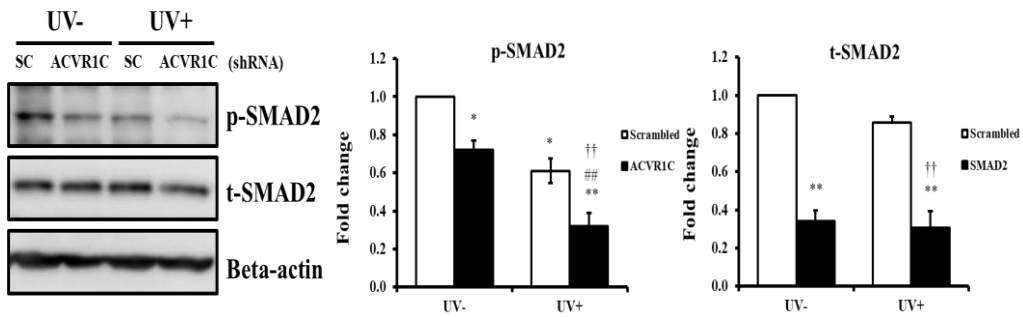

a
UV-
UV+
pcDNA3
pCMV-ACVR1C
pcDNA3
pCMV-ACVR1C
ACVR1C
SREBP1
ACC
Beta-actin
b
ACVR1C
Fold change
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
**
††
UV-
UV+
pcDNA3
pCMV-ACVR1C
c
SREBP1
Fold change
1.2
1
0.8
0.6
0.4
0.2
0
**
†
##
*
UV-
UV+
pcDNA3
pCMV-ACVR1C
d
ACC
Fold change
1.2
1
0.8
0.6
0.4
0.2
0
***
†
###
**
UV-
UV+
pcDNA3
pCMV-ACVR1C

Figure 4. ACVR1C overexpression ameliorated UV-induced decreases in SREBP1 and ACC protein expression in primary NHEK. Primary NHEK were transfected with an expression vector containing the *ACVR1C* gene and then irradiated with UV (75 mJ/cm^2). (**a**) ACVR1C, SREBP1, and ACC protein levels were measured by western blotting at 24 h after UV irradiation; (**b-d**) The protein levels were quantified using ImageJ. β-actin was used as a loading control (n = 4). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. non-irradiated pcDNA3, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. non-UV-irradiated pCMV-ACVR1C, $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs. UV-irradiated pcDNA3.

ACVR1C regulates the expression of SREBP1 and ACC protein via the suppression of SMAD2 phosphorylation.

ACVR1C controls the transcription of target genes by phosphorylating receptor-regulated SMAD2 and SMAD3 (Tsuchida et al., 2009), and SMAD2 deficiency is related to the disruption of lipid metabolism (Yang et al., 2014). To investigate the mechanism by which ACVR1C modulates the UV-induced reduction of SREBP1 and ACC protein expression, we examined SMAD2 phosphorylation in UV-irradiated NHEK after transfection with ACVR1C shRNA. We observed that the phosphorylation of SMAD2 was decreased after UV irradiation (Figure 5). In addition, SMAD2 phosphorylation was suppressed in both non-irradiated and UV-irradiated cells by transfection with ACVR1C shRNA (Figure 5a). We also demonstrated that the levels of SREBP1 and ACC were decreased in both non-irradiated and UV-irradiated groups after transfection with SMAD2 siRNA (Figure 5b). Taken together, the UV-induced reduction of ACVR1C might regulate the expression of SREBP1 and ACC protein by suppressing SMAD2 phosphorylation.

a


UV-
UV+
SC
ACVR1C
SC
ACVR1C
(shRNA)
p-SMAD2
t-SMAD2
Beta-actin
p-SMAD2
Fold change
Scrambled
ACVR1C
UV-
UV+
t-SMAD2
Fold change
Scrambled
SMAD2
UV-
UV+

b

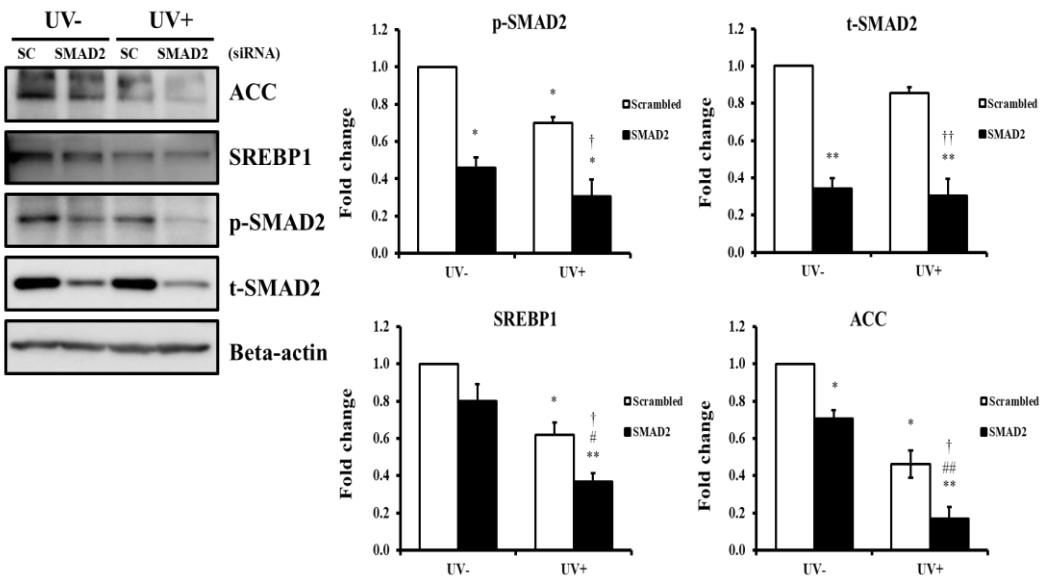

UV-
UV+
SC
SMAD2
SC
SMAD2
(siRNA)
ACC
SREBP1
p-SMAD2
t-SMAD2
Beta-actin
p-SMAD2
Fold change
Scrambled
SMAD2
UV-
UV+
t-SMAD2
Fold change
Scrambled
SMAD2
UV-
UV+
SREBP1
Fold change
Scrambled
SMAD2
UV-
UV+
ACC
Fold change
Scrambled
SMAD2
UV-
UV+

Figure 5. ACVR1C regulated the expression of SREBP1 and ACC protein via the suppression of the SMAD2 phosphorylation. (**a**) Primary NHEK were transfected with ACVR1C shRNA or scrambled control and then irradiated with UV (75 mJ/cm^2). SMAD2 phosphorylation was examined at 24 h after UV irradiation (n = 3). (**b**) Primary NHEK were transfected with SMAD2 siRNA or scrambled control and then irradiated with UV (75 mJ/cm^2). Protein levels were measured by western blotting and quantified using ImageJ. β−actin is an equal volume of cell lysates was used as a loading control (n = 3). $*p < 0.05$ and $**p < 0.01$ vs. non−irradiated scrambled siRNA, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. non−UV−irradiated ACVR1C shRNA or SMAD2 siRNA, $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs. UV−irradiated scrambled shRNA or scrambled siRNA.

Chapter 2

The negative role of ACVR1C in calcium-induced differentiation of human HaCaT keratinocytes

Materials and Methods

Cell culture and differentiation

An immortalized human HaCaT keratinocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) purchased from Welgene (Gyeongsan, South Korea) supplemented with penicillin (400 U/mL), streptomycin (50 mg/mL) purchased from Life Technologies (Rockville, MD, USA), and 10% FBS purchased from Welgene (Gyeongsan, South Korea) in a humidified 5% CO2 atmosphere at 37 ^{0}C. Cells were seeded in culture dishes, and upon reaching confluence, the medium was added with 1.8mM calcium to induce differentiation, and was replaced every two days. Cells were harvested at the indicated times.

Lentivirus production and infection

A pLKO.1 transfer vector containing short hairpin RNA (shRNA) targeting sequences (#TRCN0000196633) was obtained from Sigma (St. Louis, MO, USA). To generate lentiviral particle, HEK-293TN cells were transfected with 5 μg of packaging vector (GAG), 2.5 μg of envelope vector (VSVG), and 2.5 μg shACVR1C using Lipofectamine® 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions in Opti-MEM reduced serum medium (Gibco, Thermo Fisher Scientific, Inc., MA, USA). 6 h after the transfection, medium was replaced with Opti-MEM reduced serum medium. Viral supernatant was collected after

24 h and then was collected after 52 h. Scrambled shRNAs were used as controls. HaCaT keratinocytes were infected with 1 ml of medium containing lentiviral particles with 8 μg/ml polybrene (Santa Cruz Biotechnology, CA, USA). 8 h after the infection, cells were washed with PBS and were maintained in culture medium. Infected HaCaT keratinocytes were selected by 10 μg /ml of puromycin (Sigma-Aldrich; St. Louis, MO, USA) for 48 h. This study was approved by the Institutional Biosafety Committee at Seoul National University Hospital (SNUH-IBC-1801-001-004-2B). The infected cells were seeded in culture dishes, and upon reaching confluence, cells were harvested at day 0, the medium was added with 1.8mM calcium to induce differentiation, and was replaced at day 2. Cells were harvested at day 3.

Plasmid transfection

For the overexpression of ACVR1C, the plasmid expressing human ACVR1C was obtained from Sino Biological Inc. (Beijing, China). HaCaT keratinocytes were transfected with 0.5 μg of pcDNA3 and 0.5 μg of pCMV-ACVR1C plasmid using Lipofectamine® 3000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions in Opti-MEM reduced serum media (Gibco, Thermo Fisher Scientific, Inc., MA, USA). 6 h after the transfection, the medium was replaced with culture medium. And upon reaching confluence, cells were harvested at day 0, the

medium was added with 1.8mM calcium to induce differentiation, and was replaced at day 2. Cells were harvested at day 3.

Western blot analysis and immunofluorescence staining

HaCaT keratinocytes were homogenized, and proteins were extracted using RIPA buffer (Merck Millipore, Billerica, MA, USA) containing a protease inhibitor mixture (Roche Applied Science, Penzberg, Germany) and phosphatase inhibitor mixture (Sigma-Aldrich; St. Louis, MO, USA). Equal amounts of protein lysates were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted onto PVDF membranes. After blocking in 5% non-fat milk diluted with Tris-buffered saline containing 0.1% Tween 20 (TBST), the membrane was incubated overnight at 4° C with primary antibodies against ACVR1C (Atlas Antibodies, Stockholm, Sweden), involucrin (Biomedical Technologies, Stoughton, MA, USA), loricrin (BioLegend Way. San Diego, CA, USA), α-tubulin (Santa Cruz Biotechnology, CA, USA). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Bands were detected using an enhanced chemiluminescence detection system (Biomax Co. Ltd., Seoul, South Korea).

Real-time quantitative PCR (RT-PCR)

Total RNA was prepared from the separated epidermis or NHEK using RNAiso Plus (Takara Bio Inc., Shiga, Japan), and 1 μg of total RNA was converted to complementary DNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. To quantitatively estimate the mRNA expression level of each gene, PCR was performed on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. The sequences of primers are as following: ACVR1C (forward, 5' - AGT CGG AGG AAT TGT TGA GGA -3' ; reverse, 5' - CTC GGA GTG CTT CAC AAC TTT -3'), keratin 1 (forward, 5' - CCA GGA GCT GAT GAA CAC CAA -3' ; reverse, 5' - GAG GGT CCT GTA GGT GGC AAT -3'), involucrin (forward, 5' - GTG GGG GAG AGA GGG AAT TA -3' ; reverse, 5' - CTC ACC TGA GGT TGG GAT TG -3'), loricrin (forward, 5' - CAT GAT GCT ACC CGA GGT TT -3' ; reverse, 5' - ACT GGG GTT GGG AGG TAG TT -3'), 36B4 (forward, 5' - TCG ACA ATG GCA GCA TCT AC -3' ; reverse, 5' - TGA TGC AAC AGT TGG GTA GC -3').

Statistical analysis

Data were presented as means ± SEM. Significance was analyzed using the paired *t*-test or Student's *t*-test. Differences were considered significant when $p < 0.05$.

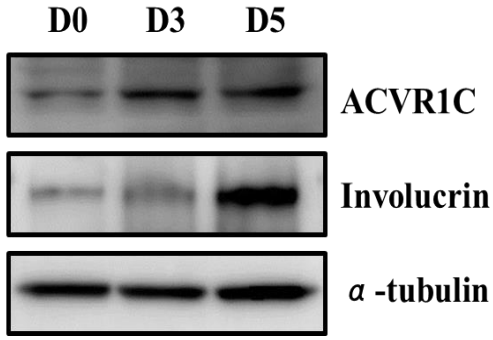
Results

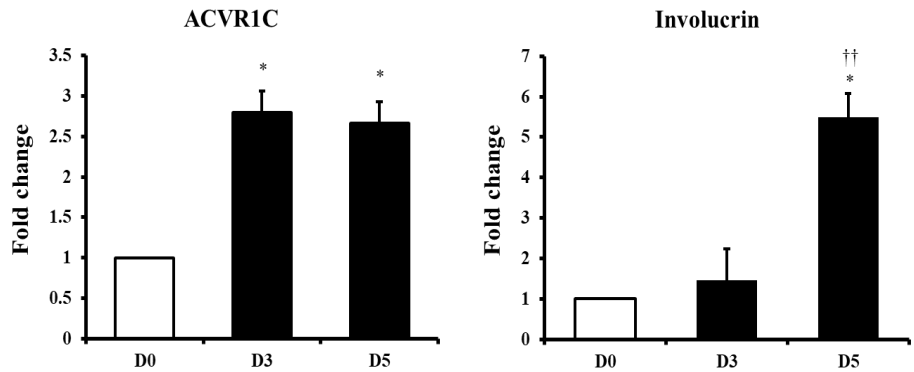
ACVR1C expression is increased during differentiation of human HaCaT keratinocytes.

ACVR1C is fully expressed in human skin epidermis. In chapter 1, I found that UV-induced reductions of ACVR1C was involved in UV-irradiated decreased lipogenesis in skin epidermal keratinoyctes (Tian et al., 2021). To further examine the function of ACVR1C in skin epidermis, I have checked the ACVR1C expression at the protein and mRNA levels during calcium-induced differentiation of HaCaT keratinocytes. The protein expression of ACVR1C was increased at the indicated time after calcium induction, and involucrin, a marker of HaCat keratinocytes differentiation, was also increased, as determined by western blot (Figure 6a). ACVR1C mRNA expression was also significantly increased at indicated time after calcium induction, as analyzed by quantitative RT-PCR (Figure 6b).

These data showed ACVR1C expression is increased during differentiation of HaCaT keratinocytes.

a


D0
D3
D5
ACVR1C
Involucrin
α -tubulin


ACVR1C
Fold change
3.5
3
2.5
2
1.5
1
0.5
0
*
*
D0
D3
D5
Involucrin
Fold change
7
6
5
4
3
2
1
0
††
*
D0
D3
D5

b

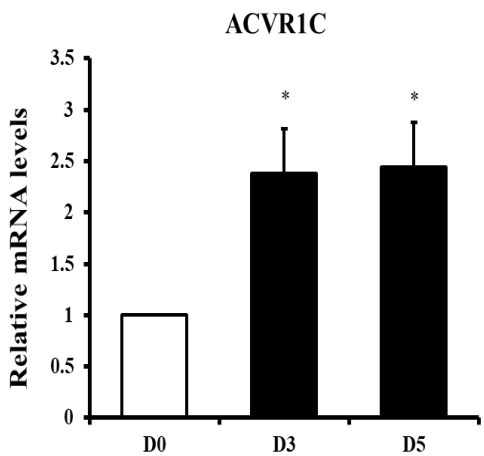

ACVR1C
Relative mRNA levels
3.5
3
2.5
2
1.5
1
0.5
0
*
*
D0
D3
D5

Figure 6. ACVR1C and involucrin expression are increased during differentiation of human HaCaT keratinocytes. Human HaCaT keratinocytes were differentiated by adding 1.8mM calcium. (a) ACVR1C and involucrin protein levels were visualized by western blot, on day 3 and day 5 after calcium induction, Protein levels were quantified using ImageJ. α-tubulin was used as a loading control (n=3); (b) ACVR1C mRNA levels were examined by quantitative RT-PCR, on day 3 and day 5 after calcium induction (n=3). Data are presented as means ± SEM of the expression of ACVR1C gene relative to 36B4. $*p < 0.05$ versus control, $^{\dagger}p < 0.05$ vs. day 3 after calcium induction.

Knockdown of ACVR1C increases differentiation markers in human HaCaT keratinocytes.

To examine whether ACVR1C is associated with differentiation of HaCaT keratinocytes, HaCaT cells were transfected with ACVR1C short hairpin RNA (shRNA) or with scrambled control, and were differentiated by adding 1.8mM calcium. ACVR1C protein and mRNA expression levels were decreased by ACVR1C shRNA (Figure 2). The protein expression levels of differentiation markers including involucrin, and loricrin were increased after transfection with ACVR1C shRNA than those in cells treated with control shRNA at day3 after calcium treatment (Figure 7a). The mRNA expression levels of differentiation markers including keratin 1, involucrin, and loricrin were increased after transfection with ACVR1C shRNA than those in cells treated with control shRNA at day3 after calcium treatment (Figure 7b).

These data suggested that decreased expression of ACVR1C accelerated differentiation of HaCaT keratinocytes.

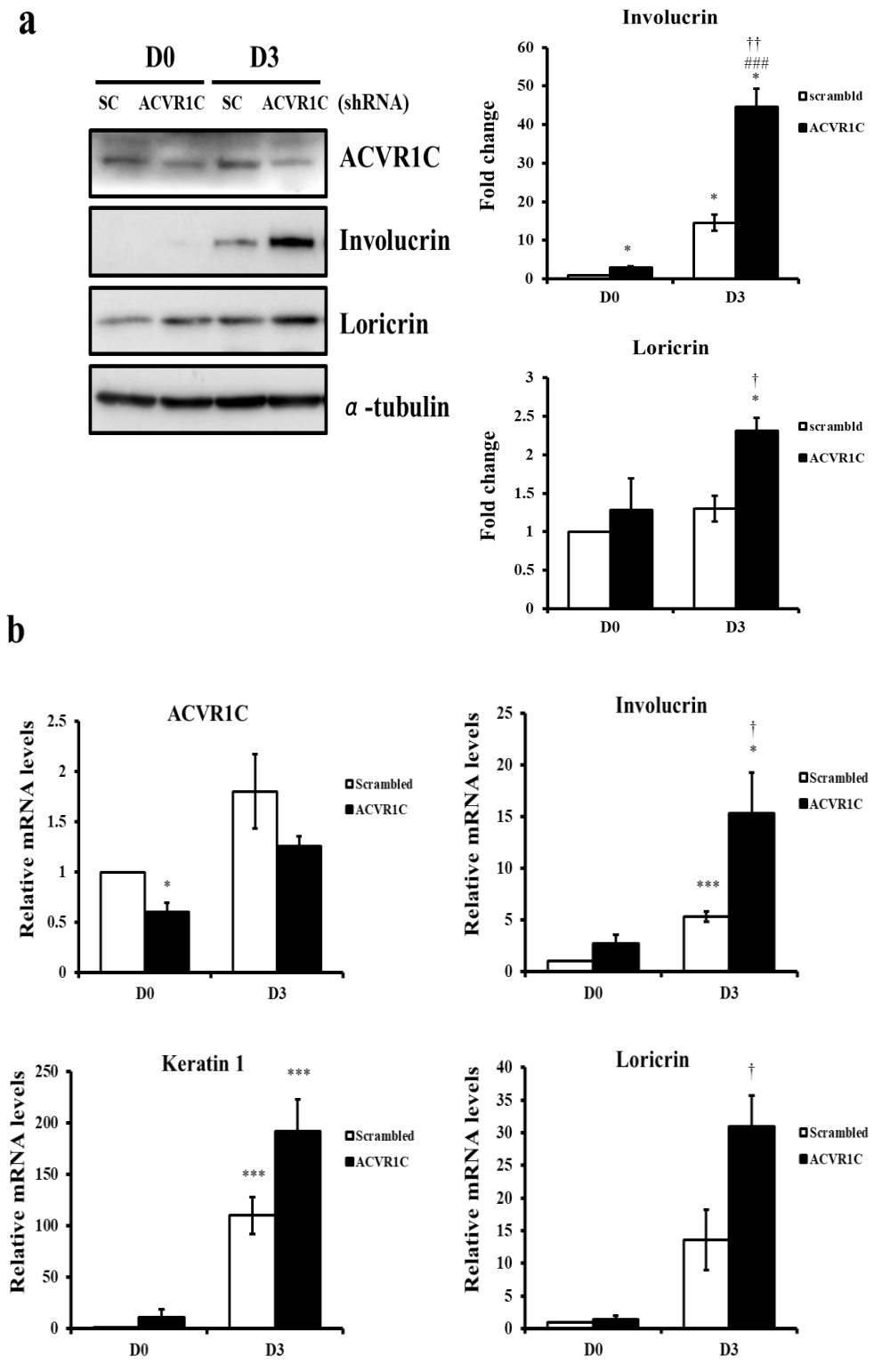

a
D0
D3
SC
ACVR1C
SC
ACVR1C
(shRNA)
ACVR1C
Involucrin
Loricrin
α -tubulin
Involucrin
Fold change
scrambld
ACVR1C
D0
D3
Loricrin
Fold change
scrambld
ACVR1C
D0
D3
b
ACVR1C
Relative mRNA levels
Scrambled
ACVR1C
D0
D3
Involucrin
Relative mRNA levels
Scrambled
ACVR1C
D0
D3
Keratin 1
Relative mRNA levels
Scrambled
ACVR1C
D0
D3
Loricrin
Relative mRNA levels
Scrambled
ACVR1C
D0
D3

Figure 7. Knockdown of ACVR1C increased differentiation markers in human HaCaT keratinocytes. HaCaT keratinocytes were transfected with ACVR1C or scrambled shRNA, and were differentiated by adding 1.8mM calcium. (a) Involucrin and loricrin protein levels were measured by western blotting at the indicated time after calcium induction. Protein levels were quantified using Image J. α-tubulin was used as a loading control (n=3); (b) Kelatin 1, involucrin and loricrin mRNA levels were measured by quantitative RT-PCR. 36b4 was used as a loading control (n =4). $^{*}p < 0.05$ and $^{***}p < 0.001$ vs. non calcium-induced control, $^{\#\#\#}p < 0.001$ vs. non calcium-induced ACVR1C shRNA, $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs. calcium-induced scrambled shRNA.

Overexpression of ACVR1C repressed the differentiated markers in human HaCaT keratinocytes

To further evaluate the role of ACVR1C in the HaCaT keratinocytes differentiation, cells were transfected with an ACVR1C expression vector and were differentiated by adding 1.8mM calcium. ACVR1C mRNA expression levels were increased at 24 h after transfection with the ACVR1C expression vector (Figure 8b). The expression levels of differentiation markers including keratin 1, involucrin, and loricrin were measured at day3 after calcium treatment. The protein level of loricrin was repressed in cells overexpressing ACVR1C than in control vector-transfected groups at day3 after calcium treatment (Figure 8a). The mRNA levels of keratin 1, and loricrin were repressed in cells overexpressing ACVR1C than in control vector-transfected groups at day3 after calcium treatment (Figure 8b). These results suggest that high level of ACVR1C suppress the differentiation markers during calcium-induced differentiation in HaCaT keratinocytes.

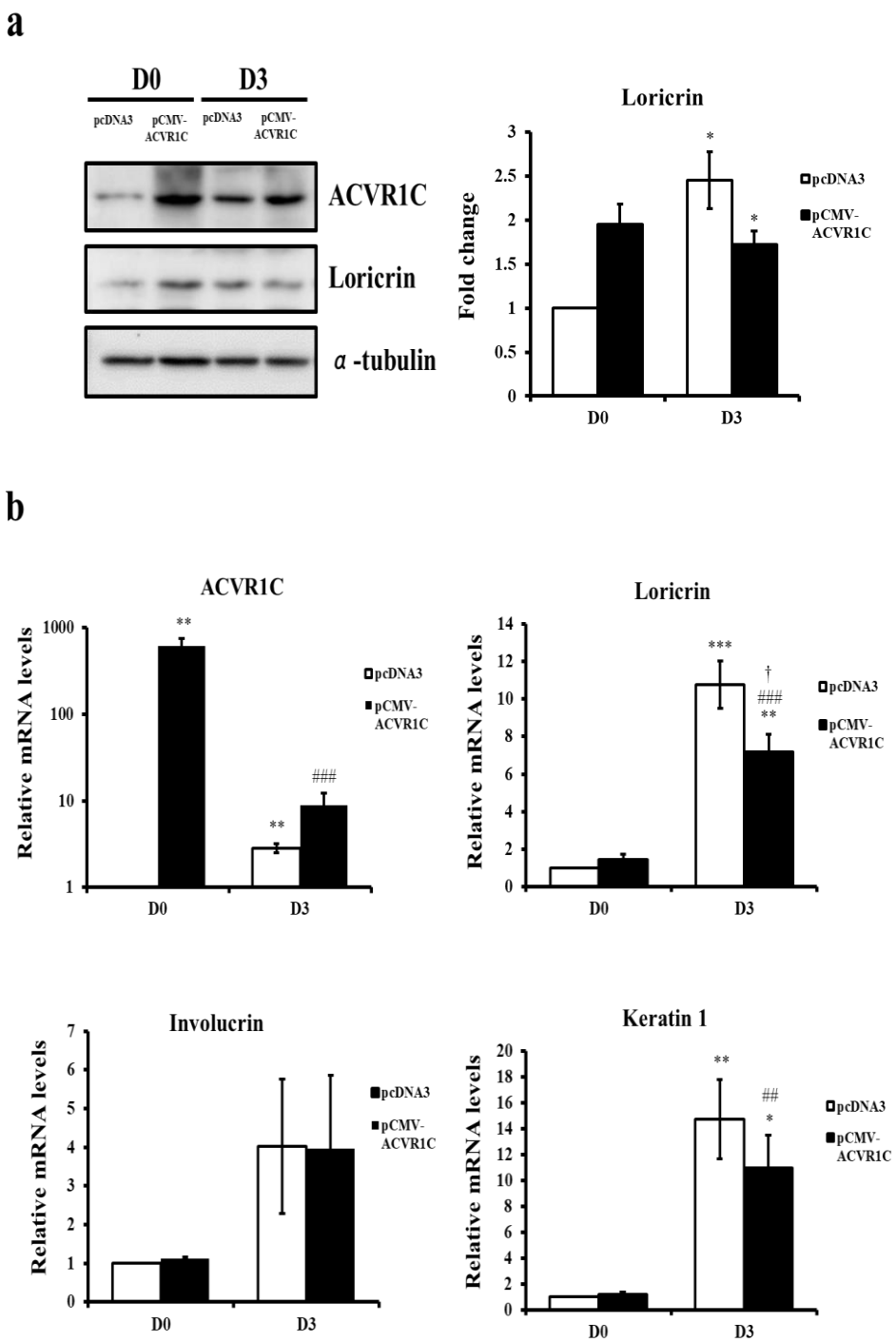

a
D0
D3
pcDNA3
pCMV-ACVR1C
pcDNA3
pCMV-ACVR1C
ACVR1C
Loricrin
α-tubulin
Loricrin
Fold change
3
2.5
2
1.5
1
0.5
0
*
*
pcDNA3
pCMV-ACVR1C
D0
D3
b
ACVR1C
Relative mRNA levels
1000
100
10
1
**
**
###
pcDNA3
pCMV-ACVR1C
D0
D3
Loricrin
Relative mRNA levels
14
12
10
8
6
4
2
0
***
†
###
**
pcDNA3
pCMV-ACVR1C
D0
D3
Involucrin
Relative mRNA levels
7
6
5
4
3
2
1
0
pcDNA3
pCMV-ACVR1C
D0
D3
Keratin 1
Relative mRNA levels
20
18
16
14
12
10
8
6
4
2
0
**
##
*
pcDNA3
pCMV-ACVR1C
D0
D3

Figure 8. Overexpression of ACVR1C repressed the differentiated markers in human HaCaT keratinocytes. HaCaT keratinocytes were transfected with an expression vector containing the ACVR1C gene and were differentiated by adding 1.8mM calcium. Loricrin protein level was measured by western blotting at the day3 after calcium treatment. Protein levels were quantified using ImageJ. α-tubulin was used as a loading control (n = 3); (b) Keratin 1, involucrin, and loricrin mRNA levels were measured by quantitative RT-PCR. 36b4 was used as a loading control (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. non calcium-induced pcDNA3. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. non calcium-induced pCMV-ACVR1C. $^{\dagger}p < 0.05$ vs. calcium-induced pcDNA3.

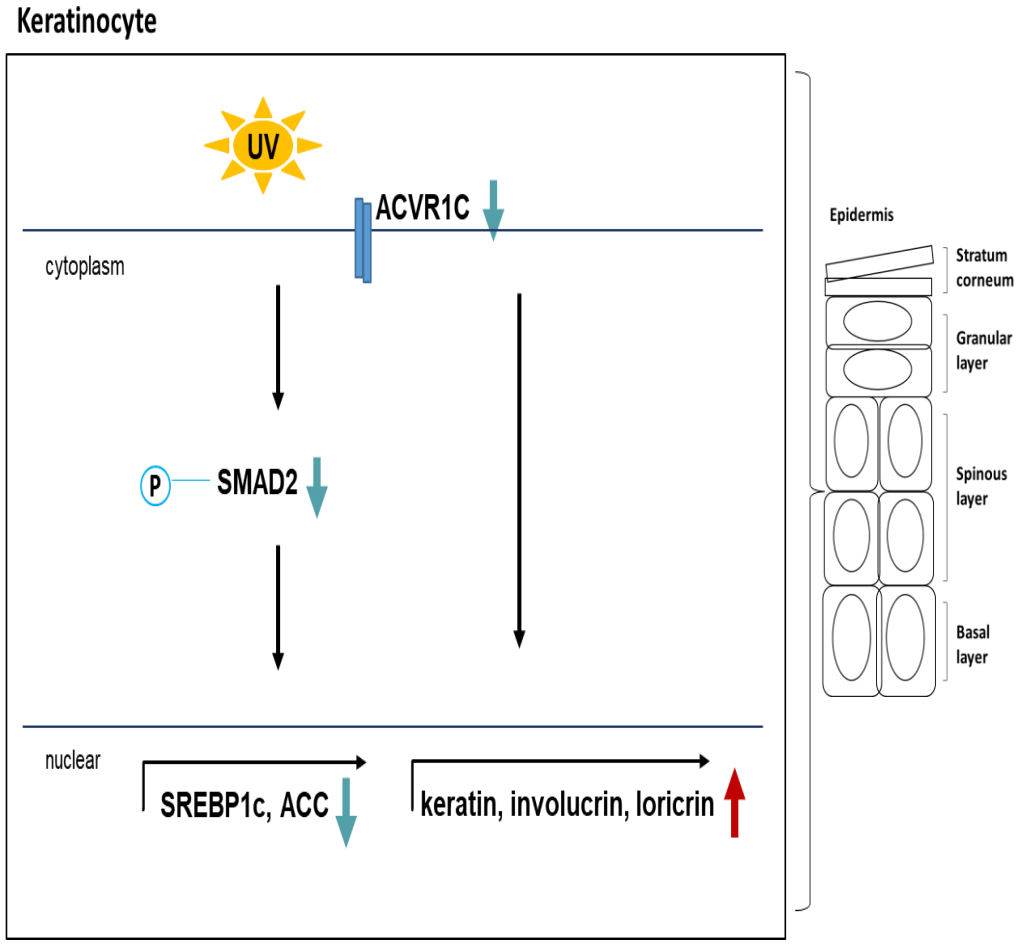

Keratinocyte
UV
ACVR1C
cytoplasm
P
SMAD2
nuclear
SREBP1c, ACC
keratin, involucrin, loricrin
Epidermis
Stratum corneum
Granular layer
Spinous layer
Basal layer

Figure 9. The diagram of the roles of ACVR1C in UV-induced decrease of lipogenesis and calcium-induced differentiation in human epidermal keratinocytes.

Discussion

Epidermal lipid metabolism plays an essential role in various skin functions. UV irradiation impairs epidermal lipid homeostasis. Previous study have demonstrated that TG content and the expression of lipogenic genes, including SREBPs, are decreased in acute UV-irradiated and photo-aged human skin (Kim et al., 2010). In the present study, I have discovered that ACVR1C, a type I TGF-β receptor, acts as a modulator of the negative effects of UV on TG synthesis. Yogosawa et al. reported that ACVR1C deficiency upregulates lipolysis and reduces the total TG content in mature adipocytes, suggesting that ACVR1C regulates fat accumulation via the lipolysis pathway (Yogosawa and Izumi, 2013, Yogosawa et al., 2013). However, little is known about the regulatory effects of ACVR1C on TG synthesis, another important pathway determining TG levels in the skin. In this study, I have showed that UV irradiation reduces the expression of ACVR1C in the human skin and identified a novel role of ACVR1C in the UV-induced reduction of TG synthesis via the downregulation of genes involved in lipogenesis, such as SREBP1 and ACC, in primary NHEK. SREBP1c, a central regulator of TG synthesis, directly regulates ACC, which is the first enzyme in the control of TG synthesis (Horton et al., 2002).

The knockdown of ACVR1C decreased SREBP1 and ACC expression as well as the phosphorylation of the downstream

mediator SMAD2. Furthermore, the knockdown of SMAD2 could downregulate SREBP1 and ACC. These data are in agreement with previous results showing that SMAD2 deficiency decreases TG levels and reduces the expression of lipogenesis-associated genes, including SREBP1c (Yang et al., 2014). UV interferes with SMAD signaling transduction in human skin, and SMAD2 phosphorylation levels are decreased in photoaged skin (Han et al., 2005). Our data suggest that the UV-induced reduction of ACVR1C triggers the downregulation of TG synthesis by the suppression of SMAD2 phosphorylation. Interestingly, I found that the overexpression of ACVR1C significantly restored the UV-induced reductions in SREBP1 and ACC levels, only when these levels were low upon UV irradiation. These findings suggest that the upregulation of ACVR1C may regulate TG metabolism more efficiently when the epidermal lipid status is compromised by external stimuli, such as UV exposure. Andersson et al. showed that ACVR1C knockout mice exhibit reduced fat accumulation only when fed with a high-fat diet but not when fed regular chow (Andersson et al., 2008), implying that ACVR1C signaling is relevant in stress conditions, such as under excess nutrients. Additional studies are warranted to elucidate the effects of known or potential ligands of ACVR1C, such as nodal, on UV-induced changes in epidermal lipid metabolism.

Epidermal differentiation is a part of process of maintaining skin epidermal homeostasis. In the present study, we found that

decreased expression of ACVR1C accelerated differentiation in human HaCaT keratinocytes via modulate the differentiation markers. Kim et al. reported that an ACVR1C deficiency can upregulate the intracellular calcium concentration, and the phenomenon can be abolished by ligand nodal (Kim et al., 2016). These findings suggest that ACVR1C may be involved in keratinocyte differentiation via modulating the intracellular calcium.

The data here has shown that the expression of ACVR1C is downregulated in UV-irradiated human skin epidermis in vivo and NHEK in vitro. I also confirmed the expression of ACVR1C is decreased in UV-irradiated HaCaT keratinocytes. Acute UV irradiation increased the keratinocyte differentiation markers both in mouse and human skin epidermis (Hong et al., 2008, Lee et al., 2002), and inducing keratinocyte differentiation can be observed during skin photoaging (Pietrzak et al., 2010, Soroka et al., 2008), Interestingly, UV irradiation also increases both the intracellular and extracellular calcium (Jiang et al., 2007). In this chapter, I identified that ACVR1C could be a negative regulator of epidermal differentiation by controlling the calcium-induced increased expression of differentiation marker. The results indicated ACVR1C may play additional roles in skin epidermis in pathological state, which is achieved in response to either internal or external stimuli.

Bertolino et al. has reported that a ligand activin B acts as a negative regulator of calcium influx, and the influx of calcium is markdly increased by activin B only when ACVR1C is insufficeint

(Bertolino et al., 2008). Based on this finding, I have hypothesized that ACVR1C deficiency could negatively regulates keratinocyte differentiation with the participation of certain ligands. Further studies are needed to find the therapeutic potential ACVR1C ligand for treating the skin diseases.

In conclusion, in chapter 1, I have demonstrated that UV-induced reductions in ACVR1C causes a decrease in the expression of SREBP1 and ACC protein involved in TG synthesis via the suppression of SMAD2 phosphorylation. In chapter 2, I have identified that decreases in ACVR1C expression is involved in accelerated calcium-induced differentiation via modulating keratinocytes differentiation markers.

These findings suggest that ACVR1C could be a potential target for attenuating the disruption of skin lipid metabolism and epidermal differentiation by harmful stimuli.

References

Alves-Bezerra M, Cohen DE. Triglyceride Metabolism in the Liver. Comprehensive Physiology 2017;8(1):1-8.

Andersson O, Korach-Andre M, Reissmann E, Ibanez CF, Bertolino P. Growth/differentiation factor 3 signals through ALK7 and regulates accumulation of adipose tissue and diet-induced obesity. Proceedings of the National Academy of Sciences of the United States of America 2008;105(20):7252-6.

Athenstaedt K, Daum G. The life cycle of neutral lipids: synthesis, storage and degradation. Cellular and molecular life sciences : CMLS 2006;63(12):1355-69.

Bald T, Quast T, Landsberg J, Rogava M, Glodde N, Lopez-Ramos D, et al. Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma. Nature 2014;507(7490):109-13.

Bertolino P, Holmberg R, Reissmann E, Andersson O, Berggren PO, Ibanez CF. Activin B receptor ALK7 is a negative regulator of pancreatic beta-cell function. Proceedings of the National Academy of Sciences of the United States of America 2008;105(20):7246-51.

Bikle DD, Jiang Y, Nguyen T, Oda Y, Tu CL. Disruption of Vitamin D and Calcium Signaling in Keratinocytes Predisposes to Skin Cancer. Front Physiol 2016;7:296.

Bikle DD, Xie Z, Tu CL. Calcium regulation of keratinocyte differentiation. Expert Rev Endocrinol Metab 2012;7(4):461-72.

Bu Y, Okunishi K, Yogosawa S, Mizuno K, Irudayam MJ, Brown CW, et al. Insulin Regulates Lipolysis and Fat Mass by Upregulating Growth/Differentiation Factor 3 in Adipose Tissue Macrophages. Diabetes 2018;67(9):1761-72.

Carlsson LM, Jacobson P, Walley A, Froguel P, Sjostrom L, Svensson PA, et al. ALK7 expression is specific for adipose tissue, reduced in obesity and correlates to factors implicated in metabolic disease. Biochemical and biophysical research communications 2009;382(2):309-14.

Coleman RA, Mashek DG. Mammalian triacylglycerol metabolism: synthesis, lipolysis, and signaling. Chem Rev 2011;111(10):6359-86.

D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. International journal of molecular sciences 2013;14(6):12222-48.

Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003;425(6958):577-84.

Elias P, Ahn S, Brown B, Crumrine D, Feingold KR. Origin of the epidermal calcium gradient: regulation by barrier status and role of active vs passive mechanisms. The Journal of investigative dermatology 2002;119(6):1269-74.

Elsholz F, Harteneck C, Muller W, Friedland K. Calcium--a central regulator of keratinocyte differentiation in health and disease. Eur J Dermatol 2014;24(6):650-61.

Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. Nat Rev Genet 2002;3(3):199-209.
Graham H, Peng C. Activin receptor-like kinases: structure, function and clinical implications. Endocrine, metabolic & immune disorders drug targets 2006;6(1):45-58.
Han KH, Choi HR, Won CH, Chung JH, Cho KH, Eun HC, et al. Alteration of the TGF-beta/SMAD pathway in intrinsically and UV-induced skin aging. Mechanisms of ageing and development 2005;126(5):560-7.
Hoffjan S, Stemmler S. On the role of the epidermal differentiation complex in ichthyosis vulgaris, atopic dermatitis and psoriasis. The British journal of dermatology 2007;157(3):441-9.
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. The Journal of investigative dermatology 2008;128(12):2880-7.
Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of clinical investigation 2002;109(9):1125-31.
Hu T, Su F, Jiang W, Dart DA. Overexpression of Activin Receptor-like Kinase 7 in Breast Cancer Cells Is Associated with Decreased Cell Growth and Adhesion. Anticancer research 2017;37(7):3441-51.
Huang H, Tang Y, Wu G, Mei Y, Liu W, Liu X, et al. ALK7 protects against pathological cardiac hypertrophy in mice. Cardiovascular research 2015;108(1):50-61.
Jia Y, Gan Y, He C, Chen Z, Zhou C. The mechanism of skin lipids influencing skin status. Journal of dermatological science 2018;89(2):112-9.
Jiang SJ, Chu AW, Lu ZF, Pan MH, Che DF, Zhou XJ. Ultraviolet B-induced alterations of the skin barrier and epidermal calcium gradient. Experimental dermatology 2007;16(12):985-92.
Kim EJ, Jin XJ, Kim YK, Oh IK, Kim JE, Park CH, et al. UV decreases the synthesis of free fatty acids and triglycerides in the epidermis of human skin in vivo, contributing to development of skin photoaging. Journal of dermatological science 2010;57(1):19-26.
Kim EJ, Lee DH, Kim YK, Lee YM, Eun HC, Chung JH. Decreased expression of activin A receptor 1C may result in Ca(2+) -induced aberrant skin hypersensitivity. Experimental dermatology 2016;25(5):402-4.
Laikova KV, Oberemok VV, Krasnodubets AM, Gal'chinsky NV, Useinov RZ, Novikov IA, et al. Advances in the Understanding of Skin Cancer: Ultraviolet Radiation, Mutations, and Antisense Oligonucleotides as Anticancer Drugs. Molecules (Basel, Switzerland) 2019;24(8).
Lee JH, An HT, Chung JH, Kim KH, Eun HC, Cho KH. Acute effects of UVB radiation on the proliferation and differentiation of keratinocytes. Photodermatology, photoimmunology & photomedicine 2002;18(5):253-61.
Michael IP, Saghafinia S, Tichet M, Zangger N, Marinoni I, Perren A, et al. ALK7 Signaling Manifests a Homeostatic Tissue Barrier That Is

Abrogated during Tumorigenesis and Metastasis. Dev Cell 2019;49(3):409-24.e6.
Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res 2015;6(6):793-804.
Namwanje M, Brown CW. Activins and Inhibins: Roles in Development, Physiology, and Disease. Cold Spring Harb Perspect Biol 2016;8(7).
Olivier E, Dutot M, Regazzetti A, Dargere D, Auzeil N, Laprevote O, et al. Lipid deregulation in UV irradiated skin cells: Role of 25-hydroxycholesterol in keratinocyte differentiation during photoaging. The Journal of steroid biochemistry and molecular biology 2017;169:189-97.
Pietrzak A, Michalak-Stoma A, Chodorowska G, Szepietowski JC. Lipid disturbances in psoriasis: an update. Mediators Inflamm 2010;2010.
Radner FP, Fischer J. The important role of epidermal triacylglycerol metabolism for maintenance of the skin permeability barrier function. Biochimica et biophysica acta 2014;1841(3):409-15.
Sandoval-Guzman T, Gongrich C, Moliner A, Guo T, Wu H, Broberger C, et al. Neuroendocrine control of female reproductive function by the activin receptor ALK7. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2012;26(12):4966-76.
Shi Q, Dong X, Zhang M, Cheng Y, Pei C. Knockdown of ALK7 inhibits high glucose-induced oxidative stress and apoptosis in retinal pigment epithelial cells. Clinical and experimental pharmacology & physiology 2020;47(2):313-21.
Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, et al. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat Genet 2000;25(1):87-90.
Soroka Y, Ma'or Z, Leshem Y, Verochovsky L, Neuman R, Brégégère FM, et al. Aged keratinocyte phenotyping: morphology, biochemical markers and effects of Dead Sea minerals. Exp Gerontol 2008;43(10):947-57.
Swindell WR, Stuart PE, Sarkar MK, Voorhees JJ, Elder JT, Johnston A, et al. Cellular dissection of psoriasis for transcriptome analyses and the post-GWAS era. BMC medical genomics 2014;7:27-.
Tian YD, Chung MH, Quan QL, Lee DH, Kim EJ, Chung JH. UV-Induced Reduction of ACVR1C Decreases SREBP1 and ACC Expression by the Suppression of SMAD2 Phosphorylation in Normal Human Epidermal Keratinocytes. International journal of molecular sciences 2021;22(3).
Trautinger F. Mechanisms of photodamage of the skin and its functional consequences for skin ageing. Clinical and experimental dermatology 2001;26(7):573-7.
Tsuchida K, Nakatani M, Hitachi K, Uezumi A, Sunada Y, Ageta H, et al. Activin signaling as an emerging target for therapeutic interventions. Cell communication and signaling : CCS 2009;7:15.

Xu G, Zhong Y, Munir S, Yang BB, Tsang BK, Peng C. Nodal induces apoptosis and inhibits proliferation in human epithelial ovarian cancer cells via activin receptor-like kinase 7. The Journal of clinical endocrinology and metabolism 2004;89(11):5523-34.
Yang L, Roh YS, Song J, Zhang B, Liu C, Loomba R, et al. Transforming growth factor beta signaling in hepatocytes participates in steatohepatitis through regulation of cell death and lipid metabolism in mice. Hepatology 2014;59(2):483-95.
Yogosawa S, Izumi T. Roles of activin receptor-like kinase 7 signaling and its target, peroxisome proliferator-activated receptor gamma, in lean and obese adipocytes. Adipocyte 2013;2(4):246-50.
Yogosawa S, Mizutani S, Ogawa Y, Izumi T. Activin receptor-like kinase 7 suppresses lipolysis to accumulate fat in obesity through downregulation of peroxisome proliferator-activated receptor gamma and C/EBPalpha. Diabetes 2013;62(1):115-23.
Zhong Y, Xu G, Ye G, Lee D, Modica-Amore J, Peng C. Nodal and activin receptor-like kinase 7 induce apoptosis in human breast cancer cell lines: Role of caspase 3. International journal of physiology, pathophysiology and pharmacology 2009;1(1):83-96.

국문초록

ACVR1C 는 TGF-β type 1 수용체로 대사 항상성 조절과 세포 증식 및 분화에 관여한다. ACVR1C 의 결함은 지질 대사 장애와 관련이 있으며 칼슘 신호 전달 조절에도 참여한다. 최근 ACVR1C 가 민감성 피부와 건선에서 감소한다고 알려져 있다. 그러나 피부에서 ACVR1C 의 역할은 충분히 연구 되지 않고 있다.

피부는 우리 몸에서 가장 큰 기관이며, 자외선 (UV) 조사를 포함한 외부 자극으로부터 우리 몸을 보호하는 장벽 역할을 한다. 자외선은 조기 피부 노화, 염증 및 피부암을 유발할 수 있다. 연구에 따르면 자외선은 표피 지질 대사 및 표피 분화를 저해 할 수 있으며, 이러한 현상은 피부 질환과 밀접한 관련이 있다.

1 장에서는 자외선 조사로 인한 사람 피부의 표피 지질 생성이 감소하는 과정에서 ACVR1C 의 새로운 역할을 확인하였다. UV 조사는 사람 피부 및 사람 표피 각질형성세포 (NHEK)에서 ACVR1C 의 발현을 감소시킨다. 표피 트리글리 세라이드 (TG) 합성은 자외선 조사 한 NHEK 에서도 감소했으며, 이는 UV 조사 한 사람 피부에서의 경향과 일치하였다. ACVR1C 와 표피 TG 합성 사이의 관련성을 밝히기 위해, 주요 지방 생성 유전자인 sterol regulatory element binding protein-1 (SREBP1) 와 acetyl-CoA carboxylase (ACC)의 발현을 확인하였으면, 두 유전자 모두 UV 조사 한 NHEK 에서 현저하게 감소하였다. 또한, shRNA 에 의한 ACVR1C 녹다운은 UV 조사에 의해 감소 된 SREBP1 및 ACC 를 더 감소시켰다. 반대로, ACVR1C 의 과발현은 UV 에 의한 SREBP1 및 ACC 의 감소를 약화시켰다. 또한, UV 조사 한 NHEK 에서 인산화 된 SMAD2 의 감소는 ACVR1C

shRNA 로 처리 된 세포에서 더 억제되며, 최종적으로 SREBP1 및 ACC 의 감소를 야기한다.

2 장에서는 ACVR1C 가 각질형성세포 분화에 관여 함을 보여주었다. ACVR1C 의 발현은 HaCaT 각질형성세포의 칼슘에 의한 분화과정에서 증가 하였다. ACVR1C 가 각질형성세포 분화 마커를 조절하는지 밝히기 위해 세포를 ACVR1C shRNA 및 발현 벡터로 형질감염시켰다. 그러나 shRNA 에 의한 ACVR1C 녹다운은 칼슘 의해 증가된 keratin 1, involucrin 및 loricrin 과 같은 분화 마커를 더 증가시켰다. 반대로 ACVR1C 의 과발현은 증가 된 keratin 1 및 loricrin 분화마커를 약화시켰다.

결과를 요약하면, 이러한 결과는 ACVR1C 가 UV 에 의한 지질 대사 장애 및 표피 분화 이상과 관련된 피부 질환을 예방 또는 치료하는 표적이 될 수 있음을 시사하였다.

주요어: ACVR1C; 광노화; 표피 지질 형성; 표피 분화;

학번: 2015-22410