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Ultraviolet light-induced gasdermin C expression is mediated via TRPV1/calcium/calcineurin/NFATc1 signaling

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Abstract. Gasdermin (GSDM)-C is a member of the GSDM gene family and is expressed in the epithelial cells of various tissue types, including skin. GSDMC expression is induced by ultraviolet (UV) irradiation and contributes to UV-induced matrix metalloproteinase 1 expression in human skin keratinocytes. However, how UV irradiation induces GSDMC expression remains unclear. The present study aimed to investigate the role of transient receptor potential cation channel subfamily V member 1 (TRPV1) and a calcium/calcineurin-signaling pathway in UV-induced GSDMC expression in human skin keratinocytes. Suppression of TRPV1 activity by treatment with the TRPV1 antagonists capsazepine and ruthenium red significantly reduced UV-induced GSDMC expression, whereas direct activation of TRPV1 by capsaicin, a TRPV1 agonist, increased GSDMC expression. The results demonstrated that extracellular calcium and calcineurin activity may be necessary for UV-induced GSDMC expression in HaCaT cells. In addition, UV-induced GSDMC expression was either decreased or increased following knockdown or overexpression of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), respectively. These data suggested that TRPV1 may serve an important role in the induction of GSDMC expression by UV and that UV-induced GSDMC expression may be mediated via a calcium/calcineurin/NFATc1 pathway.

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Abbreviations: UV, ultraviolet; GSDMC, gasdermin C; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; TRPV1, transient receptor potential cation channel subfamily V member 1

Key words: gasdermin C, UV, calcium, calcineurin, NFATc1, TRPV1

Introduction

Ultraviolet (UV) radiation is an important environmental factor; exposure of skin to UV irradiation may induce various kinds of skin damage, including sunburn, premature aging, cancer and inflammation. The effects of UV may be mediated by various types of cellular-level changes, including autophagy, cell cycle control and cell death, as well as molecular-level changes, including signal transduction and gene expression. UV irradiation may also serve a role as a broad activator of cell surface growth factor receptors and cytokine receptors (1,2). Activation of ligand-independent receptors stimulates various downstream signaling pathways that control the expression of a number of genes (1-3). Matrix metalloproteinases (MMPs) are group of zinc-dependent endopeptidases that degrade extracellular matrix proteins (4-6). Certain MMPs have been reported to be induced by UV irradiation, which contributes to skin damage by UV irradiation *in vivo* (2-4).

Gasdermin (GSDM)-C belongs to the Gasdermin superfamily, a novel group of genes that include GSDMA, GSDMB, GSDMC and GSDMD, as well as the Gasdermin-related genes (GSDME and pejvakin) in humans (5-7). GSDM family members were reported to be differentially expressed in the epithelial cells of various tissue types, including the skin (5,8). Previous studies have suggested that GSDMC may serve a role in the course of carcinogenesis, such as colorectal cancer cell proliferation and increased metastatic potential in malignant

melanoma cells (9-11). However, the functions of GSDMC in the skin remain poorly understood.

Our previous study reported that GSDMC is induced by UV irradiation and contributes to MMP-1 expression through the activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) pathways in human skin keratinocytes (6). However, how UV modulates GSDMC expression has remained unclear. In the present study, the signaling pathways involved in UV-induced GSDMC expression in human skin keratinocytes were examined by determining the role of transient receptor potential cation channel subfamily V member 1 (TRPV1) on UV-induced GSDMC expression. TRPV1 is a capsaicin receptor and functions as a non-selective cation channel that may lead to calcium influx (12-14). TRPV1 and GSDMC have been previously reported to serve crucial roles in UV-induced MMP-1 expression in human skin keratinocytes (6,12). However, TRPV1 activation appears to occur at relatively early time points (12), whereas GSDMC expression increases at relatively late time points, following UV irradiation (6). Therefore, whether TRPV1 may serve any role in UV-induced GSDMC expression was examined. The results demonstrated that TRPV1 serves an important role in UV-induced GSDMC expression. Through additional studies, UV-induced GSDMC expression was determined to be dependent on calcium and calcineurin; it was also demonstrated that nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) mediated UV-induced GSDMC expression.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), was purchased from Welgene, Inc. (Gyeongsan, Gyeongsangbuk, Korea). Calcium-free DMEM was obtained from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Fetal Bovine Serum (FBS) was purchased from HyClone (GE Healthcare Life Sciences, Logan, UT, USA). Keratinocyte basal medium MCDB 153 was purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany) and keratinocyte growth medium was purchased from Clonetics Corp. (San Diego, CA, USA). Antibiotics (penicillin and streptomycin) and TRIzol reagent were obtained from Thermo Fisher Scientific, Inc. Capsazepine, ruthenium red and capsaicin were purchased from Sigma-Aldrich; Merck KGaA. Expression plasmids for the wild-type (pMX-NFATc1-WT) or a constitutively active form of NFATc1 (pMSCV-NFATc1-CA) were kindly provided by Dr Hong-Hee Kim (Department of Cell and Developmental Biology, Seoul National University, Seoul, Korea).

Cell culture and treatments. An immortalized human keratinocyte cell line, HaCaT, was purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany). Primary human skin keratinocytes were cultured from foreskin of healthy donors. HaCaT cells were cultured in DMEM supplemented with glutamine (2 mM), penicillin (400 U/ml), streptomycin (50 mg/ml) and 10% FBS in a humidified 5% CO_2 atmosphere at 37˚C. Primary human skin keratinocytes were cultured in keratinocyte growth medium supplemented with penicillin (400 U/ml) and streptomycin (50 mg/ml) in a humidified 5% CO_2 atmosphere at 37˚C; cultured primary human skin keratinocytes at passages 3-4 were used.

For treatments, HaCaT cells were cultured to 80% confluence and serum-starved for 24 h in DMEM without FBS, and primary human skin keratinocytes were serum-starved for 24 h in MCDB 153. HaCaT cells and primary human skin keratinocytes were washed with phosphate-buffered saline (PBS) two times; subsequently, HaCaT cells were irradiated with UV at 60 mJ/cm^2 and primary human skin keratinocytes were irradiated with UV at 100 mJ/cm^2 in PBS. UV irradiation was performed with Philips TL 20W/12RS fluorescent sun lamps (Philips Medical Systems B.V., Eindhoven, The Netherlands) with an emission spectrum between 275 and 380 nm (peak, 310-315 nm); a Kodacel filter TA401/407 (Kodak, Rochester, NY, USA) was used to block UVC of wavelength below 290 nm. UV irradiation intensity was measured with a Model 585100 UV meter from Herbert Waldmann GmbH & Co. KG (Villingen-Schwenningen, Germany). Following UV irradiation, PBS was removed and replaced with DMEM without FBS for HaCaT cells and keratinocyte basal medium for primary human skin keratinocytes, and cells were further incubated for 24 h. When required, specific TRPV1 antagonist (capsazepine or ruthenium red) or specific TRPV1 agonist (capsaicin) was added 30 min prior to UV irradiation, and treated again with specific TRPV1 antagonist (capsazepine or ruthenium red) for 24 h following UV irradiation; calcineurin inhibitor (cyclosporine A) was added immediately following UV irradiation and cells were incubated for 24 h. To investigate the role of extracellular calcium in UV irradiation, HaCaT cells were serum-starved for 24 h and cultured in either calcium-free DMEM or calcium-containing DMEM for 30 min prior to UV irradiation. Fresh corresponding culture medium was added, and the cells were further incubated for 24 h. Each experiment was repeated three times. The medical ethical committee at Seoul National University approved the study protocol, and written informed consent was received from the guardians of participants. The study was conducted according to the Declaration of Helsinki principles.

Transfection with NFATc1 small interfering (si)RNA. For knockdown of NFATc1, cultured HaCaT cells were seeded and maintain until approximately 80% confluency, and subsequently transfected with the scrambled negative control siRNA (siNC) or a NFATc1-specific siRNA (siNFATc1; 5'-CCAAGG UCAUUUUCGUGGA-3'; Bioneer Corporation, Daejeon, Korea) at 100 pmol using Lipofectamine® 2000 Reagent (Invitrogen: Thermo Fisher Scientific, Inc.) in a humidified 5% CO_2 atmosphere at 37˚C for 6 h, according to manufacturer's instruction. The concentration of siRNA primer pairs was determined by dose response (data not shown). Following transfection, cells were serum-starved for 24 h, treated with UV and incubated for an additional 24 h. Cells were harvested for analysis of mRNA or protein. Each experiment was repeated three times.

Transfection with mammalian NFATc1 overexpression vector. For overexpression of NFATc1, cultured HaCaT cells were seeded and maintain until approximately 80% confluency, and subsequently transfected with the control empty vector or the mammalian expression vectors containing either pMX-NFATc1-WT or pMSCV-NFATc1-CA using Lipofectamine 2000 Reagent (Invitrogen; Thermo Fisher

Scientific, Inc.) at 2 μg in a humidified 5% CO_2 atmosphere at 37˚C for 6 h, according to the manufacturer's protocol. The concentration of vectors was determined by dose response (data not shown). Following transfection, cells were serum-starved for 24 h, treated with UV and incubated for an additional 24 h. Cells were harvested for analysis of mRNA or protein. Each experiment was repeated three times.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from HaCaT cells at ~70% confluency using the TRIzol method, according to the manufacturer's protocol. The quality of isolated RNA samples were measured by electrophoresis in 1% agarose gels (data not shown). Total RNA (1 μg) was used in a 20 μl reaction for first-strand cDNA synthesis using First Strand cDNA Synthesis Kit (MBI Fermentas; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. cDNA was subjected to amplification reactions using a 7500 Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR *Premix Ex Taq,* Perfect Real-time (Takara Bio, Inc., Otsu, Japan), according to the manufacturer's protocol, with the following primer pairs: 36B4, forward 5'-TGGGCTCCAAGCAGATGC-3', reverse 5'-GGCTTCGCT GGCTCCCAC-3'; GSDMC, forward 5'-TGCTCCCTCGAG TTTCAAAT-3', reverse 5'-GGCTCTGGATCCAACAGT TT-3'. PCR thermocycling conditions were as follows: 50˚C for 2 min and 95˚C for 2 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. Relative mRNA expression levels were normalized to 36B4 and relative expression levels of the target gene were calculated using the $2^{-\Delta\Delta Cq}$ method (15). Each experiment was repeated three times.

Western blotting. Western blot analysis was performed by extracting proteins from HaCaT cells and primary human skin keratinocytes at ~70% confluency using Radioimmunoprecipitation Assay Lysis Buffer (EMD Millipore, Billerica, MA, USA) mixed with protease inhibitor mixture (Roche Applied Science, Penzberg, Germany) and phosphatase inhibitor mixture (Sigma-Aldrich; Merck KGaA). Cell lysates were centrifuged at 13,500 x g at 4˚C for 15 min, and supernatants were collected. The total cell extract protein concentration was quantified by the Bicinchoninic Acid assay reagent (Sigma-Aldrich; Merck KGaA). Equal amounts of protein, 20 μg per well, were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Roche Applied Science). Following blocking for 1 h in 5% skim milk diluted with Tris-buffered saline containing 0.1% Tween-20, the membranes were incubated overnight with primary antibodies (1:1,000) at 4˚C with rabbit polyclonal antibody against GSDMC (cat. no. STJ93220; St. John's Laboratory, London, United Kingdom), mouse monoclonal antibody against NFATc1 (7A6; cat. no. sc-7294; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and goat polyclonal antibody against β-actin (I-19; cat. no. sc-1616; Santa Cruz Biotechnology, Inc.); β-actin was used as a loading control. The membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit (sc-2004), goat anti-mouse (sc-2005) or mouse anti-goat (sc-2354) immunoglobulin G (Santa Cruz Biotechnology, Santa Cruz, CA) as secondary antibodies (1:5,000) for 1 h in room temperature. Immunoreactive bands were visualized using the Enhanced Chemiluminescence Detection System (Thermo Fisher Scientific, Inc.). Signal intensity was measured by ImageJ software version 1.51w (National Institutes of Health, Bethesda, MD). Protein expression levels were normalized to β-actin. Each experiment was repeated three times.

Statistical analysis. Significance was determined using analysis of variance followed by Tukey's multiple comparison test. Data are presented as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

TRPV1 serves an important role in UV-induced GSDMC expression in human skin keratinocytes. A number of previous studies have reported that TRPV1 or GSDMC serve important roles in UV-induced MMP-1 expression (6,12,13). However, TRPV1 activation is induced at relatively early time points (12), whereas GSDMC expression is induced at relatively late time points, following UV irradiation (6). Therefore, whether early activation of TRPV1 served a role in late induction of GSDMC expression following UV irradiation was examined. Serum-starved HaCaT cells were pre-treated with different TRPV1 inhibitors, either capsazepine (a specific TRPV1 antagonist) or ruthenium red (a non-selective TRPV1 antagonist), irradiated with UV and subsequently treated again with capsazepine or ruthenium red. Cells cultured with either TRPV1 inhibitor exhibited a reduction in UV-induced expression of GSDMC in a dose-dependent manner (Fig. 1A and B). Furthermore, to confirm whether TRPV1 was involved in UV-induced GSDMC expression, HaCaT cells were treated with capsaicin (a specific TRPV1 agonist) (16-18) and GSDMC expression was examined. The results demonstrated that capsaicin treatment increased GSDMC expression in a dose-dependent manner (Fig. 1C). In addition, whether the induction of GSDMC expression in primary human skin keratinocytes had similar effects s those obtained for HaCaT cells was examined; similarly, capsazepine and ruthenium red treatments inhibited UV-induced expression of GSDMC (Fig. 1D and E), whereas capsaicin treatment increased GSDMC expression (Fig. 1F) in primary human skin keratinocytes in a dose-dependent manner. Taken together, these results suggested that TRPV1 may serve a crucial role in UV-induced GSDMC expression in human skin keratinocytes.

UV-induced GSDMC expression is calcium-dependent in HaCaT cells. TRPV1 acts as a non-selective cation channel and the activation of TRPV1 leads to calcium influx (12-14). As TRPV1 may be involved in UV-induced GSDMC expression, the role of extracellular calcium on UV-induced GSDMC expression was examined. Serum-starved HaCaT cells were pre-incubated in either calcium-containing DMEM or calcium-free DMEM, irradiated with UV and further incubated for 24 h in the corresponding media. UV-induced GSDMC mRNA and protein expression levels were notably reduced in calcium-free DMEM, compared with expression in calcium-containing DMEM (Fig. 2A and B). However, calcium supplementation into calcium-free DMEM led to increased

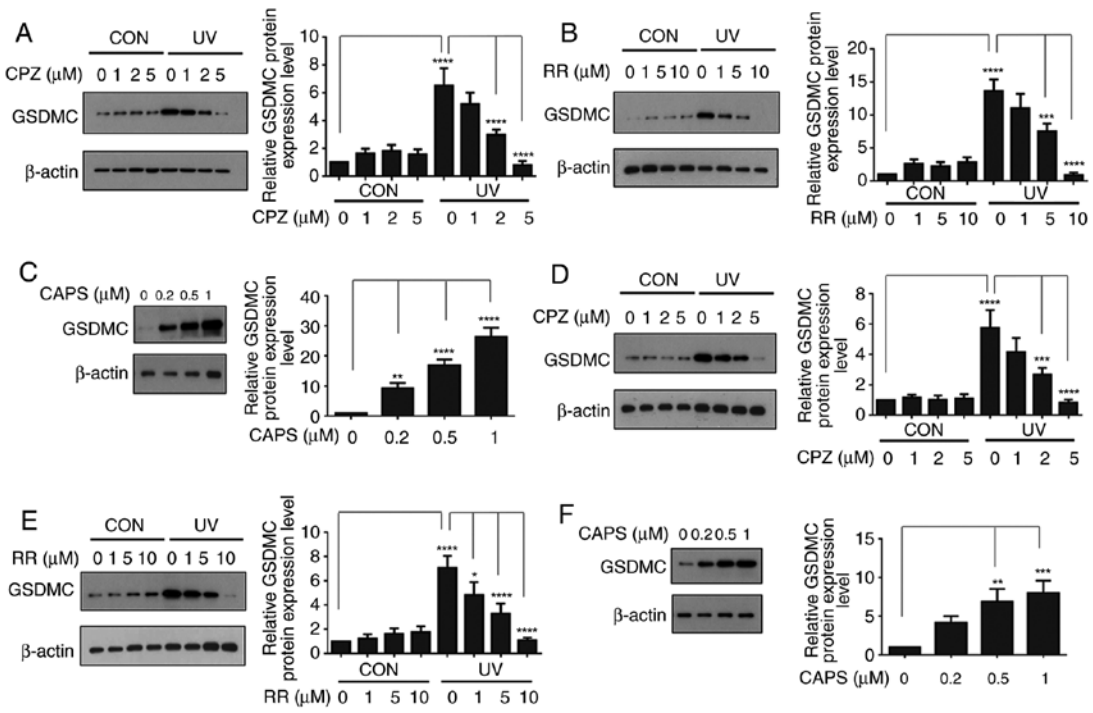

Figure 1. TRPV1 serves an important role in UV-induced GSDMC expression in human skin keratinocytes. (A and B) HaCaT cells were serum-starved for 24 h. Following pre-treatment with (A) CPZ or (B) RR for 30 min, cells were irradiated with UV and fresh media containing the corresponding inhibitor were added and cells were incubated for an additional 24 h. (C) HaCaT cells were serum-starved for 24 h and treated with CAPS at the various concentrations for 24 h. (D and E) Primary human skin keratinocytes were serum starved for 24 h. Following pretreatment with (D) CPZ or (E) RR for 30 min, cells were irradiated with UV and fresh media containing the corresponding inhibitor were added and cells were incubated for an additional 24 h. (F) Primary human skin keratinocytes were serum-starved for 24 h and treated with CAPS at the various concentrations for 24 h. GSDMC protein expression was analyzed by western blotting and relative protein levels were quantified by ImageJ software; β-actin was used as a loading control. Data are presented as the mean ± standard deviation; n=3; *P<0.05, **P< 0.01, ***P<0.001 and ****P<0.0001. CAPS, capsaicin; CPZ, capsazepine; CON, control non-UV irradiated; GSDMC, gasdermin C; RR, ruthenium red; UV, ultraviolet irradiated.

GSDMC mRNA and protein expression levels following UV irradiation in a dose-dependent manner (Fig. 2C and D). These results indicated that UV-induced GSDMC expression may be calcium-dependent.

Calcineurin pathway serves a crucial role in UV-induced GSDMC expression in HaCaT cells. Calcineurin is a calcium and calmodulin-dependent serine/threonine protein phosphatase (19,20). As the present results indicated that UV-induced GSDMC expression may be calcium-dependent, whether calcineurin was involved in UV-induced GSDMC expression was examined. HaCaT cells were irradiated with UV and subsequently treated with cyclosporine A (a calcineurin inhibitor) at the various concentrations (0, 5, 10 or 20 μM) for 24 h. UV-induced GSDMC mRNA and protein expression levels were notably inhibited by cyclosporine A in a dose-dependent manner, in control non-UV-irradiated cells and in UV-irradiated cells (Fig. 3A and B). These results indicated that the calcineurin pathway may serve an important role in UV-induced GSDMC expression.

UV-induced GSDMC expression is mediated by NFATc1 in HaCaT cells. Whether NFATc1 was involved in UV-induced GSDMC expression was examined, as calcineurin is known to activate the NFATc family members by dephosphorylating them, and NFATc1 was reported to be expressed in HaCaT cells (21-24). HaCaT cells were transfected with either siNC or siNFATc1, serum-starved for 24 h, treated with UV and further incubated for 24 h. NFATc1 protein expression levels were increased by UV irradiation in the siNC- and in the siNFATc1-treated cells (Fig. 4A), which was consistent with previous reports indicting that UV induces NFATc1 expression (25). However, the knockdown of NFATc1 expression notably reduced the basal and the UV-induced levels of GSDMC protein and mRNA expression (Fig. 4A and B). In addition, HaCaT cells were transfected with a control empty vector or with either a mammalian expression vectors containing a wild-type or a constitutively active form of NFATc1 gene. The vector-transfected HaCaT cells were serum-starved for 24 h, treated with UV and further incubated for 24 h. Overexpression of the wild-type of NFATc1 notably increased UV-induced GSDMC expression and the overexpression of a constitutively active form of NFATc1 also notably increased both basal levels and UV-induced levels of GSDMC mRNA and protein expression (Fig. 4C and D). Taken together, these results indicated that UV-induced GSDMC expression may be mediated through NFATc1 in HaCaT cells.

Discussion

UV radiation is a major environmental factor that affects human health. The skin is the largest organ of the body, through which humans interact with their environment. Consequently, skin is frequently exposed to UV radiation. Exposure of skin to UV is

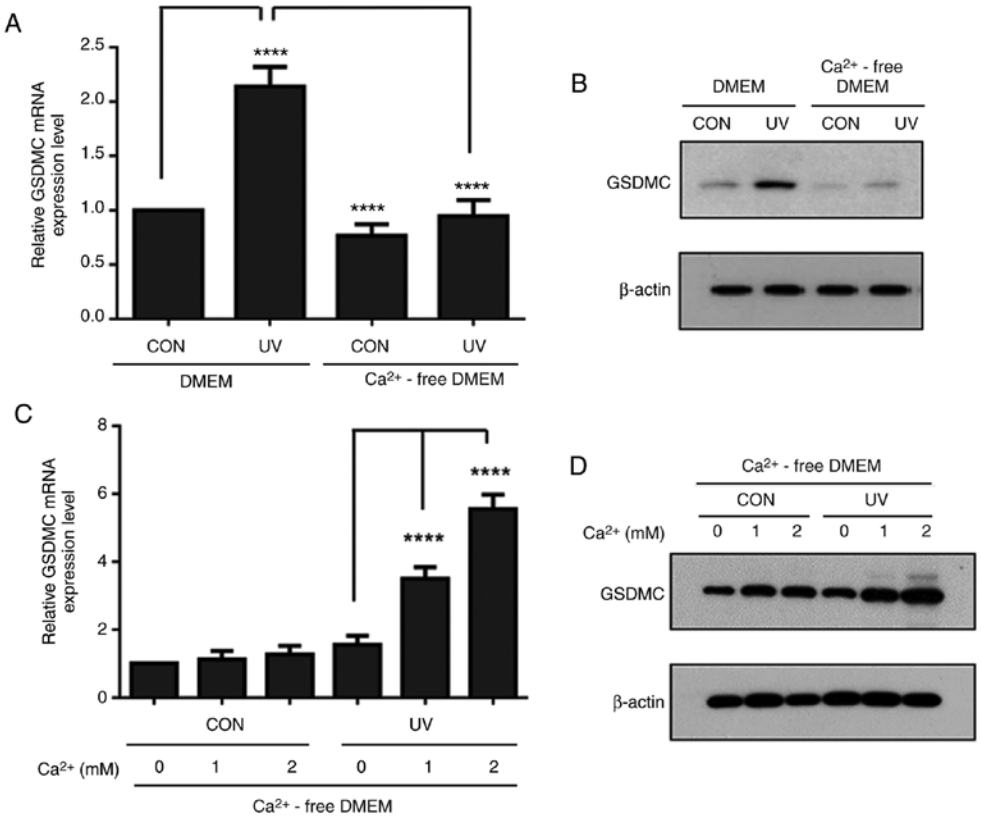

Figure 2. UV-induced GSDMC expression is calcium-dependent in HaCaT cells. (A and B) HaCaT cells were serum-starved, pre-incubated in either calcium-containing DMEM or calcium-free DMEM, irradiated with UV and incubated for an additions 24 h in fresh calcium-containing DMEM or calcium-free DMEM. (C and D) Alternatively, calcium was added at various concentrations in calcium-free DMEM prior to UV irradiation. Fresh corresponding culture medium containing calcium was added and the cells were further incubated for 24 h. GSDMC mRNA expression levels were analyzed by reverse transcription-quantitative polymerase chain reaction; relative mRNA expressions levels were normalized to 36B4. Data are presented as the mean ± standard deviation; n=3; ****P<0.0001. GSDMC protein expression levels were analyzed by western blotting; β-actin was used as a loading control; n=3. CON, control non-UV irradiated; DMEM, Dulbecco's modified Eagle's medium; GSDMC, gasdermin C; UV, ultraviolet irradiated.

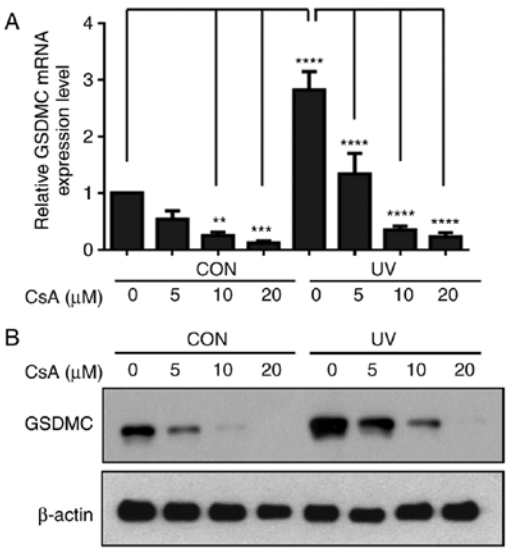

Figure 3. Calcineurin pathway serves a crucial role in UV-induced GSDMC expression in HaCaT cells. (A and B) Serum-starved HaCaT cells were irradiated with UV, incubated with CsA for 24 h. (A) GSDMC mRNA expression levels were analyzed by reverse transcription-quantitative polymerase chain reaction; relative mRNA expression levels were normalized to 36B4. Data are presented as the mean ± standard deviation; n=3; **P<0.01, ***P<0.001 and ****P<0.0001. (B) GSDMC protein expression levels were analyzed by western blotting; β-actin was used as a loading control; n=3. CON, control non-UV irradiated; CsA, Cyclosporin A; GSDMC, gasdermin C; UV, ultraviolet irradiated.

known to induce a variety of skin damages, including premature skin aging and skin carcinogenesis, through multiple and complex molecular signaling pathways (1-3,14). To advance the development of strategies and reagents for the prevention or treatment of skin damage, it is important to understand UV-induced skin damage at the molecular level and to elucidate how UV regulates the expression of a number of genes, including MMPs, such as MMP-1, MMP-3 and MMP-9, which have been reported to be important factors for the destruction of extracellular matrix proteins in the skin (26-28). MMP-1 is a major protease responsible for initiating cleavage within the central triple helix of native collagen fibrils, typically types I and III, in the skin (28). Our previous study reported that GSDMC expression is increased by UV irradiation, which contributes to the induction of MMP-1 expression through the activation of ERK and JNK pathways in human skin keratinocytes (6). However, how UV affects the regulation of GSDMC expression has not been studied yet.

The present study aimed to identify the signaling pathways that may be involved in UV-induced GSDMC expression in HaCaT immortalized human keratinocyte cell line and in primary human skin keratinocytes. TRPV1 and GSDMC were reported to serve important roles in UV-induced MMP-1 expression in human skin keratinocytes; however, TRPV1 activation occurs at relatively early time points, whereas GSDMC expression increases at relatively late time points, following UV

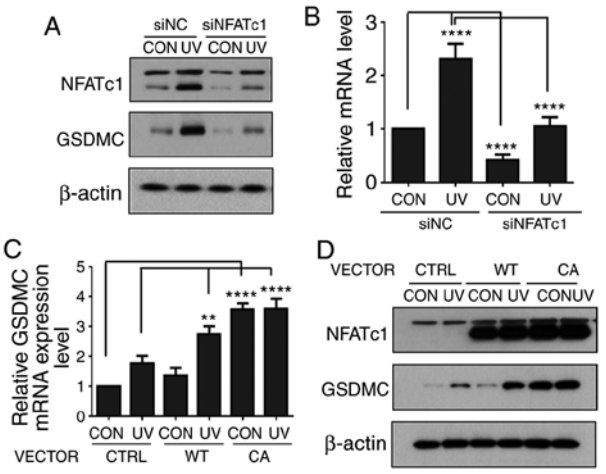

Figure 4. UV-induced GSDMC expression is mediated through NFATc1 in HaCaT cells. (A and B) HaCaT cells were transfected with siNC or siNFATc1, serum-starved, irradiated UV and harvested at 24 h following UV irradiation. (A) NFATc1 and GSDMC protein expression levels were analyzed by western blotting; β-actin was used as a loading control; n=3. (B) GSDMC mRNA expression levels were analyzed by reverse transcription-quantitative polymerase chain reaction. (C and D) HaCaT cells were transfected with the CTRL empty vector, NFATc1-WT or NFATc1-CA overexpression vectors. The cells were serum-starved, irradiated with UV and harvested at 24 h following UV irradiation. (C) GSDMC mRNA expression levels were analyzed by reverse transcription-quantitative polymerase chain reaction. (D) NFATc1 and GSDMC protein expression level were analyzed by western blotting; β-actin was used as a loading control; n=3. mRNA expression data were normalized to 36B4 and are presented mean ± standard deviation; n=3; **P<0.01 and ****P<0.0001. CA, constitutively active NFATc1 expression vector; CON, control non-UV irradiated; CTRL, empty vector control; GSDMC, gasdermin C; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; si, small interfering RNA; siNC, negative control siRNA; UV, ultraviolet irradiated; WT, wild-type NFATc1 expression vector.

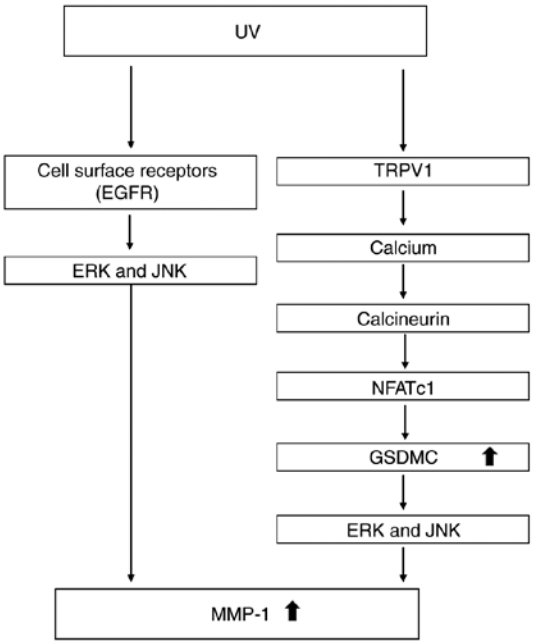

Figure 5. Schematic model of the signaling pathways involved in UV-induced GSDMC and MMP-1 expression. UV-induced expression of MMP-1 may be regulated through various factors and signaling pathways. The activation of cell surface receptors, including EGFR, by UV induces signal transduction cascades and activates various signaling pathways, such as ERK and JNK pathways, that are known to be important for MMP-1 expression. In addition to these cell surface receptors, TRPV1 is also be activated by UV, which turns on the calcium/calcineurin/NFATc1 signaling pathway and leads to increased GSDMC expression. The increase of GSDMC expression activates ERK and JNK pathways, ultimately inducing MMP-1 expression. EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; GSDMC, gasdermin C; JNK, c-Jun N-terminal kinase; MMP-1, matrix metalloproteinase 1; NFATc1, nuclear factor of activated T-cells, cytoplasmic 1; TRPV1, transient receptor potential cation channel subfamily V member 1; UV, ultraviolet irradiation.

irradiation (6,12). Therefore, it was hypothesized that TRPV1 may serve a role in UV-induced GSDMC expression. The results indicated that inhibition of TRPV1 activity suppressed UV-induced GSDMC expression. In addition, direct activation of TRPV1 by capsaicin increases GSDMC expression. These results indicated that TRPV1 may serve an important role in GSDMC expression. It has been reported that TRPV1 acts as a non-selective cation channel and that the activation of TRPV1 may lead to calcium influx (12-14). As the results from the present study indicated that TRPV1 may be involved in UV-induced GSDMC expression, the role of extracellular calcium on UV-induced GSDMC expression was examined and it was demonstrated that UV-induced GSDMC expression was calcium-dependent. Calcium is known to modulate several proteins, such as calcium-binding protein calmodulin, kinases and phosphatases (21,29,30). Previous studies reported that calcineurin is a calcium and calmodulin-dependent serine/threonine protein phosphatase (19,20). The present study results demonstrated that UV-induced GSDMC expression was calcium-dependent; therefore, whether calcineurin may be involved in UV-induced GSDMC expression was investigated. The data revealed that the calcineurin pathway may serve an important role in UV-induced GSDMC expression.

Calcineurin is known to activate NFATc family members by dephosphorylating them (21-24). A previous study demonstrated that UV is a strong inducer for NFATc1 transactivation and that UV induces NFATc1 by activating calcium/calcineurin pathway in skin (31). UV induces transcriptional activity and nuclear translocation of NFATc1 in human skin keratinocytes (32). Furthermore, UV is known to enhance NFATc1 binding activity to DNA. NFATc1 binds DNA cooperatively with other transcription factors to increase transcription of certain genes (33). These results indicated that NFATc1 may be activated by UV in human skin keratinocytes. Therefore, the present study aimed to determine whether NFATc1 was involved in UV-induced GSDMC expression. The results demonstrated that UV-induced GSDMC expression may be mediated through NFATc1. Taken together, the present study results indicated that the TRPV1/calcium/calcineurin/NFATc1 signaling pathway may be involved in UV-induced GSDMC expression in human skin keratinocytes.

The present study findings may help us to not only identify the molecular mechanisms involved in UV-induced GSDMC expression, but also to better understand the signaling pathways involved in UV-induced MMP-1 expression (Fig. 5). The induction of MMP-1 expression by UV may be regulated by various factors and signaling pathways. The activation of cell surface receptors including EGFR by UV induces signal transduction cascades and activates various signaling pathways, such as ERK and JNK, that are known to be important for MMP-1 expression (34,35). In addition, TRPV1 may also be activated by UV and serves a crucial role in UV-induced

MMP-1 expression (12,13). Even though the mechanism through which TRPV1 is activated by UV remains unclear, it has been reported that Src kinase mediates UV-induced TRPV1 trafficking from a vesicle inside cytoplasm to cell membrane within 15 min following UV irradiation, which was suggested to be an important step in TRPV1 activation (36). The activation of TRPV1 by UV turns on the calcium/calcineurin/NFATc1 signaling pathway, which increases GSDMC expression. The increase of GSDMC expression activates ERK and JNK pathways, leading to the induction of MMP-1 expression.

In addition, the involvement of the EGFR pathway in UV-induced GSDMC expression in HaCaT cells was also examined in the present study; however, the inhibition of EGFR by the chemical EGFR inhibitor (AG1478) did not affect UV-mediated GSDMC induction (data not shown). These results indicated that UV-induced GSDMC expression may occur independent of the EGFR pathway, and may be through the TRPV1 pathway. However, it has been reported that EGFR serves a critical role in UV-induced MMP-1 expression (2,26,37). Therefore, these previous and present results further support our hypothesis (Fig. 5), which suggested that several signaling pathways are involved in UV-induced MMP-1 expression.

In conclusion, TRPV1 may serve an important role in the induction of GSDMC expression by UV and that UV-induced GSDMC expression is mediated via the calcium/calcineurin/NFATc1 signaling pathway. These results may help us to better understand UV-induced signal transduction pathways and associated gene expression at the molecular level.

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Availability of data and material

The data sets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

NK and CHP designed the study and performed the experiments. DHL was involved in drafting the article and in the analysis of the data. HSY provided the preliminary data. CHP and JHC had full access to all the data and take full responsibility for the integrity of data and the accuracy of data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The medical ethical committee at Seoul National University approved the study protocol, and all the guardians of participants gave their written informed consent. The study was conducted according to the Declaration of Helsinki principles.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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