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Gasdermin C is induced by ultraviolet light and contributes to MMP-1 expression via activation of ERK and JNK pathways

Novi Kusumaningrum[a,b,c,d,1], Dong Hun Lee[a,b,c,d], Hyun-Sun Yoon[b,c,d,f], Yeon Kyung Kim[b,c,d], Chi-Hyun Park[b,c,d,*], Jin Ho Chung[a,b,c,d,e,*]

[a] Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea
[b] Department of Dermatology, Seoul National University College of Medicine, Seoul, Republic of Korea
[c] Institute of Human-Environment Interface Biology, Medical Research Center, Seoul National University, Seoul, Republic of Korea
[d] Laboratory of Cutaneous Aging Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea
[e] Institute on Aging, Seoul National University, Seoul, Republic of Korea
[f] Department of Dermatology, Seoul National University Boramae Hospital, Seoul, Republic of Korea

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ABSTRACT

Background: Ultraviolet (UV) radiation plays important roles in various skin diseases including premature aging and cancer. UV has been shown to regulate the expressions of many genes including matrix metalloproteinases (MMPs). Gasdermin C (GSDMC) belongs to Gasdermin family and is known to be expressed in the epithelial cells of many tissues including the skin. However, the functions of GSDMC remain poorly understood.
Objective: We aimed to investigate the role of GSDMC in UV-induced MMP-1, MMP-3, and MMP-9 expressions in human skin keratinocytes.
Methods: Primary human skin keratinocytes and an immortalized human skin keratinocyte cell line (HaCaT cells) were irradiated with UV. Knockdown and overexpression of GSDMC were performed to study the effect of GSDMC. The mRNA and protein levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) and western blotting, respectively.
Results: We found that GSDMC expression is increased by UV irradiation in human skin keratinocytes. Further studies showed that GSDMC expression is increased at relatively late time points after UV irradiation and that this GSDMC induction plays important roles in the expressions of MMP-1, but not of MMP-3 and MMP-9, and the activations of ERK and JNK induced by UV. In addition, we found that overexpression of GSDMC increases the MMP-1 expression and the activities of ERK and JNK and that GSDMC-induced MMP-1 expression is suppressed by inhibition of ERK or JNK activities.
Conclusions: Our results suggest that GSDMC is increased by UV radiation and contributes to UV-induced MMP-1 expression through the activation of ERK and JNK pathways.

1. Introduction

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent endopeptidases that are able to degrade extracellular matrix proteins [1–3]. MMP family members can be categorized into four different subfamilies: collagenases, stromelysins, gelatinases, and membrane-type MMPs [3–5]. MMPs play important roles in various physiological and pathological processes including developmental morphogenesis, tissue repair, skin aging, and tumor invasion. Expressions of some MMPs are regulated by diverse extracellular stimuli including UV, proinflammatory cytokines, and growth factors [4,6–9]. MMP-1, MMP-3, and MMP-9 are suggested to be important mediators of tissue damage by UV irradiation in human skin *in vivo* [7,10].

Gasdermin (GSDM) superfamily consists of Gasdermin family genes (GSDMA, GSDMB, GSDMC and GSDMD in humans) and Gasdermin-related genes (DFNA5, DFNB59 in humans) [11]. GSDM family members were shown to be detected in the epithelium of various tissue types in a highly tissue-specific manner and

Abbreviations: UV, ultraviolet; MMP, matrix metalloproteinases; GSDMC, Gasdermin C; ERK, extracellular signal–regulated kinase; JNK, c-Jun N-terminal kinase.

* Corresponding authors at: Department of Dermatology, Seoul National University Hospital, 101 Daehak–ro, Jongno–gu, Seoul 110–744, Republic of Korea.
E-mail addresses: chppark@hotmail.com (C.-H. Park), jhchung@snu.ac.kr (J.H. Chung).
[1] Current address: Department of Dermatology, Diponegoro University, Semarang, Indonesia.

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suggested to have differentiation-status specific roles [12–14]. Gasdermin C (GSDMC) was known as melanoma-derived leucine zipper-containing extranuclear factor (MLZE) [14,15]. Several studies have suggested that GSDMC may be involved in the course of tumorigenesis such as acquisition of metastatic potential in melanoma cells or promotion of cell proliferation in colorectal carcinogenesis and that GSDMC may function as an oncogene [15,16].

Ultraviolet (UV) radiation is one of the major environmental factors that influence living creatures. Skin is the largest human organ and frequently exposed to UV irradiation. Exposure of skin to UV irradiation is known to induce various effects including vitamin D synthesis, pigmentation, inflammation, sunburn, premature aging, and carcinogenesis. These effects of UV are mediated by a number of changes at molecular or cellular levels such as signal transduction, gene expression, cell cycle control, and apoptosis [1,17–20].

We have been studying to understand UV-induced skin damage and skin aging at the molecular levels and to elucidate how UV regulates the expressions of various MMPs in mouse or human skin in vivo and skin cells [21,22], since MMPs are known to be important factors for degradation of extracellular matrix proteins [1–3]. While we were investigating the genes whose expressions are changed by UV irradiation in hairless mouse skin using microarray, we found that the expression of GSDMC was significantly increased by UV irradiation (data not shown). Even though the expression of GSDMC in the skin has been demonstrated, the function of GSDMC in the skin remains unclear. Then, we decided to study the role of GSDMC in UV-induced expressions of MMPs in human skin cells. To do this, we first tried to examine whether GSDMC expression is regulated by UV irradiation and then to elucidate the role of GSDMC in UV-induced expressions of MMP-1, MMP-3, and MMP-9 and the molecular mechanisms involved in the process. We found that the expression of GSDMC is increased by UV irradiation in human skin keratinocytes and that this GSDMC induction contributes to the increase of MMP-1 expression specifically via activation of ERK and JNK pathways.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Welgene (Gyeongsan, South Korea). Fetal Bovine Serum (FBS) was obtained from Hyclone (Logan, UT). Keratinocyte basal medium (MCDB153) and keratinocyte growth medium were purchased from Sigma-Aldrich, (St. Louis, MO) and Clonetics (San Diego, CA), respectively. Antibiotics were obtained from Life Technologies (Rockville, MD). The MEK inhibitor PD98059 and the JNK inhibitor SP600125 were purchased from Calbiochem (San Diego, CA).

2.2. Cell culture and UV irradiation

An immortalized human keratinocyte cell line, HaCaT, and primary human skin keratinocytes established by outgrowth from foreskin biopsies of healthy donors were used. HaCaT cells were cultured in DMEM supplemented with penicillin (400 U/mL), streptomycin (50 mg/mL), and 10% FBS in a humidified 5% CO2 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% CO2 atmosphere at 37 °C. Cultured primary human skin keratinocytes at passages 3–4 were used for the experiments. For UV irradiation, Philips TL 20W/12RS fluorescent sun lamps (Philips, Eindhoven, Netherlands) with emission spectrum between 275 and 380 nm (peak, 310–315 nm) were used as the UV source. The power output distribution of the UV emission spectrum was 25.3% UVA1 (320–340 nm), 11.2% UVA2 (340–380 nm), 53.3% UVB (290–320 nm), and 10.2% UVC (275–290 nm). A Kodacel filter (TA401/407; Kodak, Rochester, NY) was used to block UVC ($<$ 290 nm). Before treatment, HaCaT cells were serum-starved for 48 h in DMEM containing 0.25% FBS, while primary human skin keratinocytes were serum-starved for 24 h in keratinocyte basal medium (MCDB153). HaCaT cells and primary human skin keratinocytes were washed with PBS twice and irradiated with UV (60 and 100 mJ/cm2 respectively) in PBS. After UV irradiation, PBS was removed and replaced with DMEM without FBS for HaCaT cells and keratinocyte basal medium for primary human skin keratinocytes. Then cells were further incubated for the indicated times. For checking cell viability, cells were irradiated with UV (20, 40, 60, 80 and 100 mJ/cm2 for HaCaT cells, and 60, 80, 100, 120, and 140 mJ/cm2 for primary human skin keratinocytes). Then cell viability was determined at 48 h after UV irradiation using EZ-CYTOX cell viability assay kit (Daeil Bio, Suwon, South Korea) according to the manufacturer's instructions. Briefly, HaCaT cells and primary human skin keratinocytes were treated with EZ-CYTOX reagent at 48 h after UV irradiation, and cultured media were collected at 30 min after treatment. Then, the absorbance at 450/650 nm of the collected media was determined by microplate reader (Molecular Devices, Sunnyvale, CA).

2.3. Knockdown of GSDMC (Transfection with small interfering RNA (siRNA))

For knockdown of GSDMC, HaCaT cells or primary human skin keratinocytes were seeded and transfected with the negative control siRNA (scrambled siRNA) or the GSDMC-specific siRNA (Bioneer, Daejeon, Korea) using Lipofectamine® 2000 Reagent (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. After transfection, cells were serum-starved for 48 h (HaCaT cells) or 24 h (primary human skin keratinocytes), treated with UV, and then harvested at 24 and 48 h for analysis of mRNA or protein.

2.4. Overexpression of GSDMC (Transfection with mammalian expression vector)

For overexpression of GSDMC, HaCaT cells or primary human skin keratinocytes were seeded and transfected with the control mammalian expression vector or the human GSDMC-containing mammalian expression vector using Lipofectamine® 2000 Reagent (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. The plasmid for human GSDMC (RC223817) was purchased from ORIGENE (Rockville, MD). After transfection, cells were serum-starved for 48 h and then harvested for analysis of mRNA or protein.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from skin samples and cells using RNAiso Plus (Takara Bio, Shiga, Japan) and the same amount of RNA per each sample was used for the first-strand cDNA synthesis using First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. To estimate mRNA expression levels, quantitative real-time PCR was performed on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA) using SYBR *Premix Ex Taq*™ (Perfect (Perfect Real Time) (Takara Bio, Shiga, Japan) according to the manufacturer's instructions, with the following primer pairs: 36B4 (forward,5′-TGGGCTCCAAGCAGATGC-3′; reverse, 5′-GGCTTCGCTGGCTCCCAC-3′), GSDMC (forward,5′-TGCTCCCTCGAGTTTCAAAT-3′; reverse,5′-GGCTCTGGATCCAACAGTTT-3′), MMP-1 (forward,5′-ATTCTACTGATATCGGGG

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