**ORIGINAL ARTICLE**

# Potential Role of Cytosolic RNA Sensor *MDA5* as an Inhibitor for Keratinocyte Differentiation in the Pathogenesis of Psoriasis

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**Background:** Psoriasis is a chronic inflammatory skin disease. The etiology of psoriasis is not fully understood, but the genetic background is considered to be the most important factor. To date, many psoriasis-related genes have been discovered, but the role of many important genes has not been well understood. **Objective:** The purpose of this study is to uncover possible roles of *MDA5* in psoriasis. **Methods:** Expression of MDA5 was investigated using immunohistochemistry. Then, *MDA5* was overexpressed in keratinocytes using a recombinant adenovirus. **Results:** As a result of immunohistochemical staining, the expression of *MDA5* was significantly increased in the epidermis of psoriasis compared to normal skin. Similarly, the expression of *MDA5* was increased in imiquimod-induced psoriasiform dermatitis model. In cultured keratinocytes, toll-like receptor 3 agonist poly(I:C) induced expression of *MDA5* at both mRNA and protein levels. When *MDA5* was overexpressed using a recombinant adenovirus, poly(I:C)-induced cytokine expression was significantly increased. Finally, *MDA5* over-expression significantly inhibited calcium-induced differentiation of keratinocytes. **Conclusion:** These results suggest that MDA5 increases in psoriasis and negatively regulates keratinocyte differentiation. **(Ann Dermatol 33(4) 339~344, 2021)**

-Keywords-
Differentiation, Keratinocytes, MDA5, Psoriasis

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## INTRODUCTION

Psoriasis is an inflammatory skin disease characterized by erythematous plaques with silvery scales[1,2]. Although the pathogenesis of psoriasis is not fully understood, much evidence supports that psoriasis is a T cell-mediated disease and closely related to dysregulated keratinocyte differentiation[3,4]. For example, interleukin (IL)-17 and IL-22 produced by T helper 17 cells mediate epidermal hyperplasia and tissue inflammation inherent in psoriasis[5]. In addition, numerous studies have shown that epidermal keratinocytes also play an important role in the pathogenesis of psoriasis. Stimulation of keratinocytes with various pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) activates innate immunity and produces inflammatory cytokines that can cause and worsen psoriasis[6-8].

In addition to dysregulation of the immune system, the genetic background is considered the most important factor causing psoriasis. For example, linkage analysis identifies distinct disease-sensitive regions on the chromosome

called PSORS1 through PSORS7[9]. In another example, a genome-wide association study (GWAS) identifies new psoriasis-sensitive loci that carry genes with recognized immune functions such as *IL28RA, REL, MDA5, ERAP1, TRAF3IP2, NFKBIA,* and *TYK2*[10].

In this study, we selected *MDA5* (melanoma differentiation-associated protein 5) and investigated its putative role in keratinocytes. MDA5 is the cytosolic RNA sensor that plays an important role in recognizing antigens that induce innate immunity[11]. Although identified as a psoriasis sensitive gene, the putative functional role of MDA5 in keratinocytes is still unclear. Our data show that MDA5 enhances the inflammatory reaction of keratinocytes, but inhibits keratinocyte differentiation.

## MATERIALS AND METHODS

### Immunohistochemistry

Human skin tissues were obtained with the donors' written consent in accordance with the ethics committee approval procedure of the Chungnam National University Hospital Institutional Review Board (IRB number: 2016-07-009). Skin tissues were fixed in 10% formaldehyde and embedded in paraffin. The paraffin-embedded sections were de-waxed, re-hydrated and incubated overnight at 4°C with anti-MDA5 antibody (Abcam, Cambridge, MA, USA). After washing, sections were incubated with peroxidase-conjugated secondary antibody (Dako, Carpinteria, CA, USA) and visualized with Chemmate envision detection kit (Dako).

### Animal test

The imiquimod-induced psoriasiform dermatitis model was created based on the previously reported method[12,13]. Male BALB/c mice, 6 to 8 weeks old, were purchased from OrientBio (Seongnam, Korea). Psoriasiform skin dermatitis was induced by topical application of 5% imiquimod cream (Aldara; Dong-A ST, Seoul, Korea) daily for 7 days. All animal experiments were approved by Chungnam National University Institutional Animal Care and Use Committee (CNU-00639).

### Cell culture

Keratinocytes were isolated from donated skin tissue and immortalized using a retrovirus carrying Simian virus 40 large T antigen. Immortalized keratinocytes were cultured in keratinocyte-serum free medium supplemented with bovine pituitary extract (BPE) and recombinant human epidermal growth factor (rhEGF) (Life Technologies Corporation, Grand Island, NY, USA). When the cells reached about 70% confluence, they were exchanged with MCDB153 medium (Welgene, Gyeongsan, Korea) supplemented with BPE and rhEGF. After incubation overnight, cells were treated with 1 $\mu$g/ml poly(I:C) (InvivoGene, San Diego, CA, USA).

### Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated and cDNA was prepared using Moloney murine leukemia virus reverse transcriptase (MMLV-RTase; Elpis Biotech, Daejeon, Korea). A portion of cDNA was taken and used for the PCR reaction. For quantitative real-time PCR, SYBR Green mixture (Elpis Biotech, Daejeon, Korea) was used for the amplification reaction. The sequences of primers were as follows; MDA5, 5'-CTGCTGCAGAAAACAATGGA and 5'-TGCCCATGTTG CTGTTATGT; tumor necrosis factor (TNF)-$\alpha$, 5'-CTCCTTC AGACACCCTCAACCT and 5'-CGACCCTAAGCCCCCAA TT; IL-6, 5'-CTGCGCAGCTTTAAGGAGTTC and 5'-CCAT GCTACATTTGCCGAAGA; IL-8, 5'-CCTTTCCACCCCAAA TTTATCA and 5'-TTTCTGTGTTGGCGCAGTGT; C-C motif chemokine ligand 20 (CCL20), 5'-CCACCTCTGCGGCG AAT and 5'-TGTGTATCCAAGACAGCAGTCAAA; involucrin, 5'-CCACTGGCTCCACTTATTTCG and 5'-GGACAGA GTCAAGTTCACAGATGAG; filaggrin, 5'-CGAAGGAGCC AAAAATATAAAACAG and 5'-GATGTGCTAGCCCTGATG TTGA; keratin1, 5'-GGCTATGACCCTGCTTTGTTCT and 5'-TCATGTGGGTGGTGGTCACT; SPRR1A, 5'-GCCTCTGC GTAAGGCTGAAC and 5'-TGCACCCGAGCAACAAGAA; GAPDH, 5'-TGCACCACCAACTGCTTAGC and 5'-GGCA TGGACTGTGGTCATGAG.

### Western blot

Cellular protein was extracted using cell lysis buffer (Intron, Daejeon, Korea). The protein was separated on sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes (Pall Corporation, Port Washington, NY, USA). After blocking with 5% skim milk, the membrane was sequentially incubated with the primary antibody and peroxidase-conjugated secondary antibody. The bands were visualized using enhanced chemiluminescence (Intron). The following primary antibodies were used: MDA5 (Abcam); involucrin, filaggrin (Santa Cruz Biotechnology, Santa Cruz, CA, USA); $\beta$-Actin (Sigma-Aldrich, St. Louis, MO, USA).

### Adenovirus creation

Full-coding fragment of MDA5 cDNA was amplified by PCR with primer sets: 5'-ATGTCGAATGGGTATTCCAC and 5'-CTAATCCTCATCACTAAATA. The MDA5 cDNA was subcloned into pENTR/CMV vector, and the replication-incompetent adenovirus was generated using the Virapower adenovirus expression system (Life Technologies Corporation) according to the manufacturer's protocol.

## Statistical analysis

Data were evaluated statistically by one-way ANOVA or Student's t-test using IBM SPSS software ver. 22.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set to $p < 0.05$.

## RESULTS

To investigate the expression of *MDA5*, immunohistochemical staining was performed on normal and psoriasis lesion skin tissues. The expression of *MDA5* was not well observed in normal skin, but was significantly increased in the epidermis of psoriasis lesion skin (Fig. 1A). To further investigate the expression of *MDA5* in psoriasis, we used a well-established imiquimod-induced psoriasiform dermatitis model[12,13]. Consistent with previous reports, topical application of imiquimod increased epidermal thickness and inflammatory cell infiltration. The increase in epidermal thickness reached a peak after about 5 days of application of imiquimod, and gradually decreased, returning to an almost normal level after about 2 weeks. Similarly, the expression of *MDA5* in the epidermis began to increase after imiquimod application, showing the highest level around 5 days and then gradually decreasing (Fig. 1B).

To investigate the expression of *MDA5* during the innate immune response of keratinocytes, we used a poly(I:C)-induced keratinocyte inflammation model. Poly(I:C) is a synthetic analog of double-stranded RNA and induces a keratinocyte innate immune response by activating toll-like receptor 3, which reflects to some extent the state of keratinocytes in psoriasis[7,14]. As a result of treating poly(I:C) on keratinocytes, it was confirmed that the expression of *MDA5* increased at the mRNA and protein levels (Fig. 2). These results are consistent with previously reported data[15], suggesting that MDA5 may play a role in the pathogenesis of psoriasis.

To study the possible role of MDA5 in keratinocytes, we produced a recombinant adenovirus capable of overexpressing *MDA5*. After transduction of adenovirus into keratinocytes, it was confirmed that the expression of *MDA5* was increased at the mRNA and protein levels (Fig. 3A). We then investigated the effect of *MDA5* overexpression on keratinocyte innate immunity. After transduction of adenovirus, we treated keratinocytes with poly(I:C) and examined the expression of the psoriasis-related cytokine genes. In the control group transduced with adenovirus expressing LacZ, poly(I:C) increased the expression of inflammatory cytokines such as *TNF-α*, *IL-6*, *IL-8*, and *CCL20*. When *MDA5* was overexpressed, poly(I:C)-induced
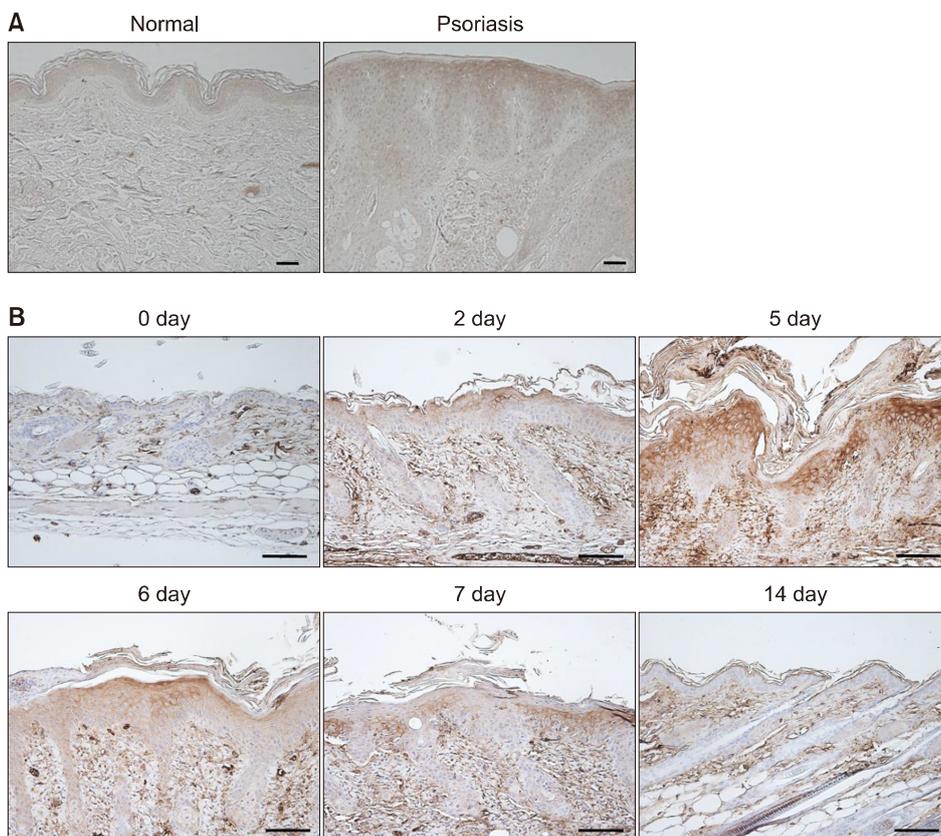


**Fig. 1.** (A) Expression of MDA5 in psoriasis. Skin specimens were obtained from normal volunteer and psoriatic patient. MDA5 level was significantly increased in the epidermis of psoriatic patient. Bar=100 $\mu$m. (B) Expression of MDA5 in imiquimod-induced psoriasiform dermatitis. BALB/c mice were topically applied with 5% imiquimod cream (Aldara) daily for 7 days. After final application of imiquimod cream, mice were untreated for 7 days. Skin specimens were obtained at the indicated time points and stained with anti-MDA5 antibody. MDA5 level was increased after imiquimod treatment and then decreased to baseline level in a time-dependent manner. Bar=100 $\mu$m. Sections of skin tissues were incubated with anti-MDA5 antibody, then visualized using peroxidase/3,3'-diaminobenzidine tetrahydrochloride salt (DAB) detection kit (200×).

cytokine expression was significantly enhanced (Fig. 3B). These results indicate that MDA5 has an effect of promoting innate immunity of keratinocytes.

In psoriasis, keratinocyte proliferation increases while cell differentiation does not occur normally[3,4]. In order to examine the effect of MDA5 on keratinocyte differentiation, we overexpressed MDA5 and then treated with calcium, the most well-known keratinocyte differentiation inducer. In control adenovirus (Ad/LacZ)-transduced cells, calcium

increased mRNA levels of differentiation markers, including *involucrin*, *filaggrin*, *keratin 1*, and *SPRR1A*. In contrast, mRNA expression of the differentiation marker was significantly suppressed by MDA5 overexpression (Fig. 4A). Consistent with these results, protein levels of involucrin and filaggrin were significantly inhibited by *MDA5* overexpression (Fig. 4B). These results suggest that MDA5 acts as a negative regulator for keratinocyte differentiation.

## DISCUSSION

Psoriasis is a skin disease associated with T-cells, but recent studies have shown that epidermal keratinocytes also play an important role in the pathogenesis of psoriasis. In particular, it has been found that the innate immune response of keratinocytes induced by PAMPs or DAMPs may be the primary event of psoriasis development[2]. Among several substances that can induce innate immunity of keratinocytes, double-stranded RNA is released from damaged cells and then acts as DAMP or can be provided as PAMP by viral infection[16]. Pathogenic double-stranded RNA binds to the cytoplasmic sensor MDA5 and induces oligomerization of MDA5. This activated
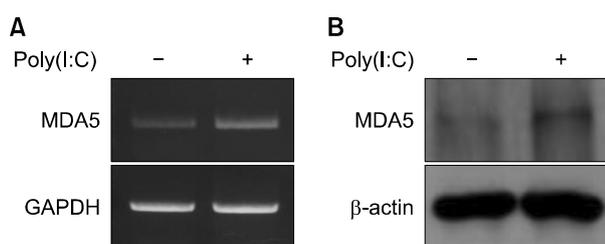


**Fig. 2.** Expression of MDA5 in human keratinocytes. (A) Keratinocytes were treated with 1 μg/ml poly(I:C) for 2 hours, and then mRNA level was measured by reverse transcription-polymerase chain reaction. (B) Cells were treated with poly(I:C) for 24 hours and then cellular extract was obtained. The protein level of MDA5 was examined by Western blot. Poly(I:C) increased MDA5 at both mRNA and protein levels.
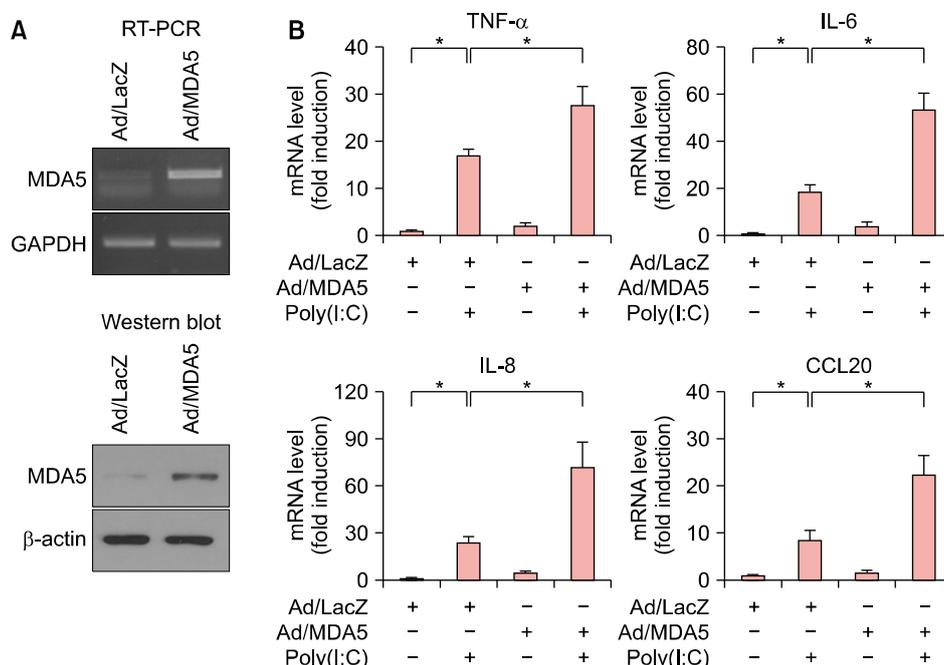


**Fig. 3.** Effect of MDA5 overexpression on poly(I:C)-induced cytokine expression. (A) Keratinocytes were transduced with adenovirus expressing MDA5. Expression of MDA5 was increased at both mRNA and protein levels by transduction of adenovirus. Adenovirus expressing LacZ was used as negative control. (B) After overexpression of MDA5, cells were treated with poly(I:C) for 2 hours. The mRNA level was determined by quantitative reverse transcription-polymerase chain reaction (RT-PCR). Poly(I:C)-induced cytokine expression was significantly increased by overexpression of MDA5. Data are expressed as fold induction. The mean values (±standard deviation) are averages of triplicate measurements. Ad: adenovirus, CCL: C-C motif chemokine ligand, TNF: tumor necrosis factor, IL: interleukin. *Statistically significant ($p < 0.05$).
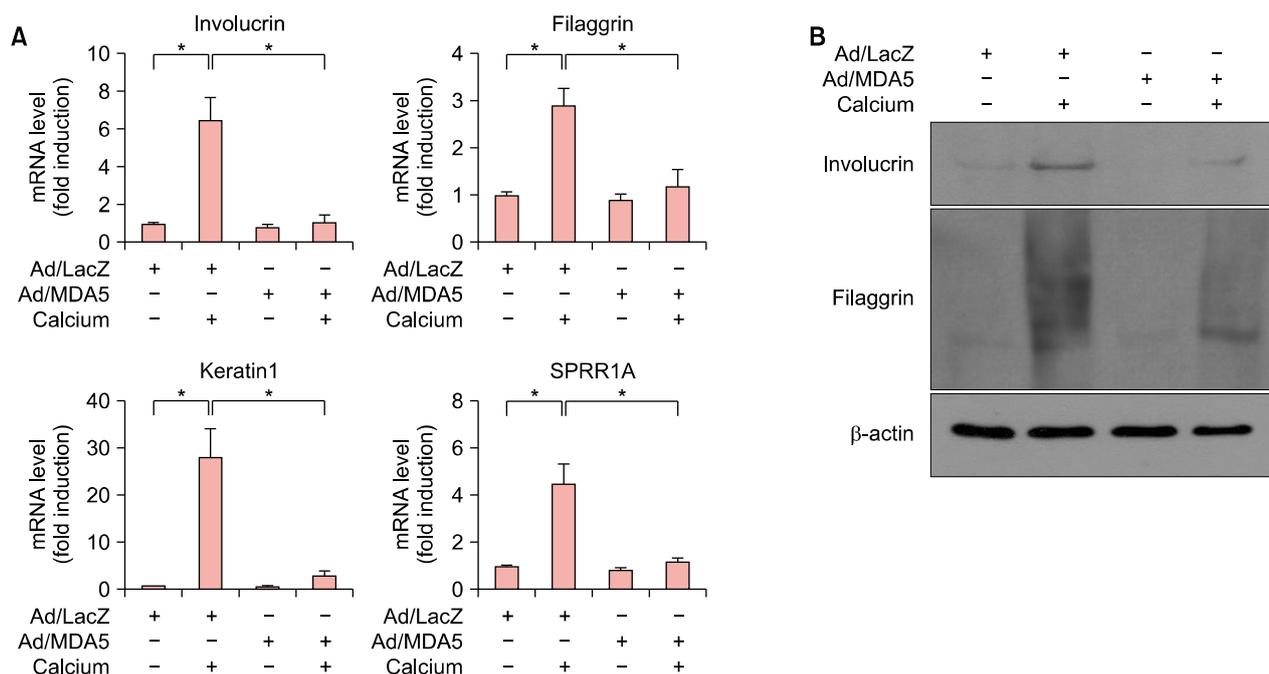
**Fig. 4.** Effect of MDA5 on keratinocyte differentiation. (A) Keratinocytes were transduced with adenovirus expressing MDA5. After overexpression of MDA5, cells were treated with 1.2 mM calcium for 5 days. The mRNA levels of the differentiation markers were determined by quantitative real-time polymerase chain reaction. Data are expressed as fold induction. The mean values (±standard deviation) are averages of triplicate measurements. (B) The protein levels of involucrin and filaggrin were examined by Western blot. Calcium-induced differentiation of keratinocytes was significantly inhibited by MDA5 overexpression. Ad: adenovirus. *Statistically significant ($p < 0.05$).

MDA5 interacts with mitochondrial antiviral-signaling proteins (MAVS), which in turn activates interferon regulatory factor (IRF) 3 and 7. The activated IRF3 and IRF7 form dimer and then translocate to the nucleus to promote the expression of type I interferon genes[17]. In addition to antiviral responses, MDA5 has been reported to affect inflammatory cytokine expression such as *TNF-α*, *IL-6*, and *IL-8* in keratinocytes[18]. Consistent with these findings, our study also showed that the inflammatory response of keratinocytes by poly(I:C) was promoted when MDA5 was overexpressed. Together with GWAS data, these results suggest that MDA5 play a role in the pathogenesis of psoriasis.

It is known that keratinocyte proliferation is increased while differentiation is dysregulated in psoriasis[18]. Various inflammatory cytokines are known to affect keratinocyte differentiation. For example, IL-36γ or IL-17 interferes with keratinocyte differentiation[19]. Based on these facts, there is a possibility that the cytoplasmic RNA sensor MDA5 can affect keratinocyte differentiation. Indeed, in our study, calcium-induced keratinocyte differentiation was significantly inhibited by *MDA5* overexpression, suggesting that MDA5 contributes to dysregulation of keratinocyte differentiation. The mechanism by which MDA5 affects

keratinocyte differentiation is not well understood. Interestingly, it has been reported that Notch1, a keratinocyte differentiation factor, negatively regulates IRF3 and IRF7. Conversely, when the *Notch1* gene was deleted, *IRF3* gene expression was up-regulated, and expression of the keratinocyte proliferation factor *p63* was increased[20]. These results suggest that IRF3 and IRF7 act as factors that inhibit keratinocyte differentiation. Therefore, when the IRF3 and IRF7 signaling systems are activated by MDA5, keratinocyte differentiation can be negatively controlled. The exact molecular mechanism needs to be further studied.

In summary, we demonstrated that MDA5 was increased in the epidermis of psoriasis, and that MDA5 enhances the inflammatory response of keratinocytes while inhibiting keratinocyte differentiation. Our data provide clues as a basis for further research on how MDA5 affects the pathogenesis of psoriasis.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

## FUNDING SOURCE

## DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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