Possible Role of Lysine Demethylase 2A in the Pathophysiology of Psoriasis

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Background: Psoriasis is a common chronic inflammatory skin disease. The development of psoriasis is dependent on many intercellular events such as innate immunity and T cell-mediated inflammation. Furthermore, genetic factors are strongly implicated in the pathophysiology of psoriasis. Although a variety of susceptible genes are identified, it is likely that many important genes remain undisclosed.

Objective: The aim of this study is to investigate the possible role of lysine demethylase 2A (KDM2A) in the pathophysiology of psoriasis.

Methods: We examined the expression of KDM2A using a well established imiquimod-induced psoriasiform dermatitis model.

Results: Immunohistochemistry analysis showed that expression of KDM2A was increased in imiquimod-induced psoriasiform dermatitis. Consistent with this result, KDM2A level was markedly increased in the epidermis of psoriatic patient. When keratinocytes were stimulated with TLR3 agonist poly(I:C), KDM2A was increased at both the mRNA and protein levels. Poly(I:C) increased the expression of psoriasis-related cytokines including tumor necrosis factor-α, interleukin-8, and CCL20, and KDM2A inhibitor daminozide enhanced the poly(I:C)-induced cytokine expression. Finally, topical co-application of imiquimod and daminozide exacerbated the imiquimod-induced psoriasiform dermatitis.

Conclusion: Together, these results suggest that KDM2A is increased to negatively regulate the inflammatory reaction of epidermal keratinocytes in psoriasis.

Keywords - Imiquimod, Keratinocytes, Lysine demethylase 2A, Polyinosinic:polycytidylic acid, Psoriasis

INTRODUCTION

Psoriasis is an inflammatory skin disease that is characterized by papules and silvery white scales, of which condition repeats deterioration and improvement. Although the precise mechanism underlying the onset of disease is not fully understood, it is thought that psoriasis is a T cell-mediated disease and closely related to abnormal keratinocyte differentiation. There are lots of immune cell infiltrates in lesional area of psoriasis, and inhibition of T cells by immunosuppressant ameliorates the disease state. The cytokines produced from T cells affect epidermal keratinocytes, thereby contributing to development of psoriasis. For example, T cell-derived interleukin (IL)-17A activates epidermal keratinocytes to produce abundant cytokines and inflammatory mediators such as IL-8 and CCL20. Also, IL-17A inhibits terminal differentiation of keratinocytes and increases cell proliferation. Another important factor is genetic background. It has been demonstrated that familial history positively correlates with the development of psoriasis and the probability of occur-
rence reaches about 70% between identical twins. Recognizing the importance of genetic background, many investigations identify plenty of susceptible genes using various experimental techniques. For example, linkage analysis identifies distinct disease susceptibility regions (PSORS1-7). In other example, a genome-wide association study (GWAS) identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. Although a number of genes linked to psoriasis have been discovered, it is likely that many of important genes remain undisclosed. We attempted to find genes related with psoriasis, and found that lysine demethylase 2A (KDM2A) might be involved in the pathogenesis of psoriasis.

KDM2A is a histone 3 lysine 36 (H3K36) demethylase, which plays many roles in biological activities such as cell division, epithelial–mesenchymal transition and cancer cell metastasis. KDM2A is known to play an important role in diseases such as cancer, but its role in inflammatory diseases such as psoriasis is not well known. In this study, we suggest that KDM2A is increased to negatively regulate the inflammatory reaction of epidermal keratinocytes.

MATERIALS AND METHODS

Animal test

The imiquimod-induced psoriasiform dermatitis model was established according to the method described previously. Male BALB/c mice at 6 to 8 weeks of age were obtained from OrientBio (Seongnam, Korea). The psoriasiform skin inflammation 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).

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 washed three times with phosphate buffered saline. Sections were incubated with primary antibody for overnight at 4°C. Sections were then incubated with peroxidase-conjugated secondary antibody (Dako, Carpinteria, CA, USA) and visualized with a Chemmate detection kit (Dako). The following primary antibodies were used: KDM2A, CXCL10 (Abcam, Cambridge, MA, USA); CD90 (R&D Systems, Minneapolis, MN, USA); β-actin (Sigma-Aldrich, St. Louis, MO, USA).

Cell culture

The keratinocytes were isolated from the epidermis and immortalized by the transduction of retrovirus harboring Simian virus 40 large T antigen (SV40Tag). Immortalized keratinocytes were maintained in keratinocyte-serum free medium (K-SFM) supplemented with bovine pituitary extract (BPE) and recombinant human epidermal growth factor (rhEGF) (Life Technologies Corporation, Grand Island, NY, USA). When cells reached about 70% confluency, culture medium was changed with MCD1B13 (Wegene, Gyeongsan, Korea) supplemented with BPE and rhEGF. After overnight incubation, cells were treated with 1 μg/ml of poly(I:C) (Invivogene, San Diego, CA, USA). Dami-nozide was purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide (DMSO).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was purified and cDNA was prepared using moloney-murine leukaemia virus reverse transcriptase (MMLV-RTase; Elpis Biotech, Daejeon, Korea). Aliquot of cDNA was used in PCR reaction. For quantitative real-time PCR, SYBR Green mixture was used in amplification reaction. The sequence of primers were as follows; KDM2A, 5’-CGGAATTTCTTTTGGGTCAA and 5’-AAGCTGTAGACCTGGGCTACA; tumor necrosis factor (TNF)-α, 5’-TCCTCTCAAGACCCCTCACCT and 5’-AGGTCATGCAAATT; CD20, 5’-CCACCTCTGCGAGTCTAAGC and 5’-TGTGTATCCAAGACAGCAGTCAAA; GAPDH, 5’-TGCACCACCAACTGCTTAGC and 5’-GGCATGGACTGTGGTCATGAG.

Western blot

Cellular protein was prepared using a cell lysis buffer (Intron, Daejeon, Korea). Sample was run onto sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose membranes (Pall Corporation, Port Washington, NY, USA). After blocking with 5% skim milk, the membrane was incubated with primary antibody and peroxidase-conjugated secondary antibody sequentially. The Western band was visualized using enhanced chemiluminescence (Intron).

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 at p < 0.05.
RESULTS

Imiquimod is an adenosine analogue that belongs to a class of products known as immune response modifiers, and is widely used for the treatment of skin diseases including genital wart, basal cell carcinoma and actinic keratosis. Common side effect of imiquimod cream is the provocation of localized psoriasis at the site of application, and this imiquimod-induced psoriasis can be recapitulated in the animal model. We first examined the expression of KDM2A using the imiquimod-induced psoriasiform dermatitis model. Consistent with previous reports, imiquimod induced psoriasiform dermatitis, evidenced by increased epidermal thickness and immune cell infiltrates. The epidermal thickness reached maximum at around 5 day of application, then declined and returned to basal level at 2 weeks. The expression of KDM2A was increased in the epidermis after treatment of imiquimod, which also showed maximum level at around 5 day of application and then declined (Fig. 1).

We next examined the expression of KDM2A in human skin specimens. The expression of KDM2A was weakly detected in the epidermis of normal skin, whereas KDM2A expression was markedly increased in lesional area of psoriatic skin (Fig. 2A). To further investigate the putative involvement of KDM2A, we examined its expression in cultured keratinocytes. We treated keratinocytes with poly(I:C), a synthetic analogue of double-stranded RNA, that induces innate immune response in a TLR3-dependent manner, reflecting the condition of psoriatic keratino-

Fig. 1. Expression of lysine demethylase 2A (KDM2A) in the 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 non-treated for 7 days. Skin specimens were obtained at the indicated time points, and stained with anti-KDM2A antibody. KDM2A level was increased after imiquimod treatment and then declined to basal level in a time-dependent manner. Bar=100 μm.

Fig. 2. Expression of KDM2A in human keratinocytes. (A) Skin specimens were obtained from normal volunteer and psoriatic patient. KDM2A level was significantly increased in the epidermis of psoriatic patient. Bar=100 μm. (B) Keratinocytes were treated with 1 μg/ml poly(I:C) for 2 hours and then mRNA level was determined by RT-PCR. For Western blot, cells were treated with poly(I:C) for 24 hours and then cellular extract was obtained. Poly(I:C) increased KDM2A at both the mRNA and protein levels. KDM2A: lysine demethylase 2A, RT-PCR: reverse transcription-polymerase chain reaction.
Treatment with poly(I:C) led to an increase of KDM2A at both the mRNA and protein levels (Fig. 2B). These results suggest that KDM2A may have a role in the pathology of psoriasis.

To investigate the possible role of KDM2A, we treated keratinocytes with KDM2A-specific inhibitor daminozide.
Poly(I:C) increased the expression of psoriasis-related cytokines including TNF-α, IL-8, and CCL20. When keratinocytes were co-treated with daminozide, cytokine expression was significantly increased compared to poly(I:C)-only treated-group (Fig. 3). These results suggest that KDM2A may negatively regulate the inflammatory reaction of epidermal keratinocytes.

We further examined the effect of daminozide using the imiquimod-induced psoriasiform dermatitis model. When daminozide was solely applied, there was no obvious sign of inflammation. By contrast, imiquimod markedly increased the epidermal thickness, together with increase of immune cell infiltrates. Interestingly, co-treatment with imiquimod and daminozide exacerbated the skin inflammation compared to imiquimod only treated-group, in terms of increase of CXCL10 in the epidermis and CD90-positive innate lymphoid cells (Fig. 4). These results support the idea that KDM2A may have a negative role on skin inflammation.

**DISCUSSION**

Psoriasis is a complex skin disease, of which occurrence is linked to many susceptible genes. Although a variety of important genes and their functions are identified, it is still unknown how many genes are involved in the pathophysiology of psoriasis. In this study, we demonstrated that KDM2A was increased in psoriasis and it might have a negative role on the inflammatory reaction of keratinocytes.

KDM2A was originally identified as an F-box protein involved in SCF ubiquitin-protein ligase complex. KDM2A contains F-box, JmjC domain and CXXC zinc finger domain, and shows the H3K36 demethylase activity. It has been recognized that methylation of H3K36 plays an important role in chromatin remodeling and linked to transcriptionally active state. Thus, it is thought that histone modifier KDM2A is associated with transcriptional silencing. For example, KDM2A represses the transcription of ribosomal RNA (rRNA) in a demethylase activity-dependent manner. In other example, KDM2A transcriptionally represses the histone deacetylase 3 (HDAC3) gene by removing methyl groups from H3K36 at the HDAC3 promoter in non-small cell lung cancer (NSCLC) cells. In addition, KDM2A inhibits the expression of tet-eleven translocation 2 (TET2) to promote DNA methylation and silencing of tumor suppressor genes in breast cancer. Since aberrant histone modification is frequently linked to cancer, it is thought that KDM2A plays a role in tumorigenesis. However, the role of KDM2A in inflammatory reaction has not been well elucidated. Interestingly, Lu et al. have identified KDM2A as a negative regulator of NF-κB using the validation-based insertional mutagenesis. When cells are stimulated with cytokines such as TNF-α and IL-1β, the p65 subunit of NF-κB is methylated at K218 and K221 positions by lysine methylase NSD1. These methylations are required for the activation of most p65-dependent genes. By contrast, KDM2A demethylates the p65 at K218 and K221 positions, leading to the inhibition of NF-κB activity. Furthermore, expression of KDM2A is driven by NF-κB activation, revealing a negative regulatory feedback loop. Since NF-κB is a central player in inflammation, it can be speculated that KDM2A plays a role in the regulation of inflammatory reaction. In our study, KDM2A was increased in epidermal keratinocytes of psoriatic patient and experimental animal model. Especially in the imiquimod-induced psoriasiform dermatitis model, the expression of KDM2A reached maximum level at the highest inflammatory phase, and then declined during the recovery phase. Furthermore, inhibition of KDM2A activity enhanced the poly(I:C)-induced inflammatory reaction of keratinocytes. Finally, topical application of KDM2A inhibitor exacerbated the imiquimod-induced psoriasiform dermatitis. Taken together, these results suggest that KDM2A is increased to prevent the prolonged inflammatory reaction of keratinocytes by suppressing NF-κB activity in psoriasis. Since it has been well described that activation of NF-κB pathway in turn induces the expression of negative regulators for NF-κB activity, it is suggestive that KDM2A is one of feedback regulators to dampen excessive inflammatory reaction. The precise role of KDM2A in psoriasis should be investigated further.

In summary, we demonstrated that KDM2A was increased in the epidermis of psoriasis, and that inhibition of KDM2A activity exacerbated skin inflammation. Our results suggest that KDM2A may exert its effect as a negative regulator in the inflammatory reaction of keratinocytes.

**CONFLICTS OF INTEREST**

The authors have nothing to disclose.

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**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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