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SIRT1 Activation Ameliorates Aldara-Induced Psoriasiform Phenotype and Histology in Mice

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TO THE EDITOR

Sirtuin 1 (SIRT1), an NAD^+-dependent deacetylase (Imai *et al.*, 2000), acts as a metabolic sensor that functions on both histone and non-histone proteins (Leibiger and Berggren, 2006; Li, 2013). In this study, we find that SIRT1 activation in animals could inhibit Aldara-induced psoriasiform lesions. Moreover, SIRT1–STAT3 interaction may serve as an important mechanism that underlies this anti-psoriasis process in keratinocytes.

A psoriatic mouse model (The protocols were approved by the Animal Care and Use Committee at Nanjing University) using Aldara cream (5% imiquimod, 3 M Pharmaceuticals) on shaved back skin exhibited signs of erythema, scaling, and thickening, a psoriasiform phenotype (van der Fits *et al.*, 2009; Walter *et al.*, 2013). Histologic examination showed epidermal hyperplasia and parakeratosis (Figure 1a and b). In addition, the marker for cellular proliferation Ki-67 and the marker for abnormal differentiation of keratinocytes Keratin 17 (Fu and Wang, 2012) were significantly increased in the lesion epithelia (Figure 1d and e). The manifestations closely resembled the characteristics of psoriatic pathology (Supplementary Figure S1 online).

Interestingly, the severity of the skin lesion was significantly reduced, when the mice were treated with an SIRT1 activator resveratrol, before and during Aldara administration. This resulted in smoother and thinner skins with decreased scales and erythemas, compared with the mice treated with Aldara only. To determine whether SIRT1 functioned in this process, a SIRT1 inhibitor EX527 was applied to the mice in the same manner. EX527 treatment exacerbated the psoriasiform symptoms (Figure 1a). The score of psoriasis area and the severity index showed a consistent change (Figure 1c). Histologically, skin lesions of resveratrol-treated mice showed reduced epidermal hyperplasia. In comparison, increased epidermal hyperplasia and acanthosis were observed in EX527-treated mice (Figure 1b, Supplementary Figure S2 online). The changes in Ki-67 and Keratin 17 levels in the epithelia were consistent with the histological alterations (Figure 1d and e, Supplementary Figure S3A–S3C online). Furthermore, increased $CD4^+$ immunocyte infiltration was observed in the Aldara-induced lesional skins, which resembled one of the characteristics of human psoriatic skin tissues. The infiltration of $CD4^+$ immunocytes was reduced in the resveratrol group and increased in the EX527 group (Figure 1f).

Signal transducer and activator of transcription 3 (STAT3) is a latent cytoplasmic transcription factor that regulates cell growth and differentiation in response to cytokines (Sano *et al.*, 2008). Excessive activation of STAT3

Abbreviations: Ac-STAT3, acetylated STAT3; PY-STAT3, STAT3 phosphorylated in Tyr705; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3

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has a key role in psoriasis pathogenesis (Sano *et al.*, 2005; Andres *et al.*, 2013). In our study, Aldara treatment also increased STAT3 phosphorylation in Tyr705 (PY-STAT3) in the epithelia of mouse lesion skin. We previously demonstrated that SIRT1 suppresses STAT3 phosphorylation by deacetylating STAT3 key lysine sites in hepatic

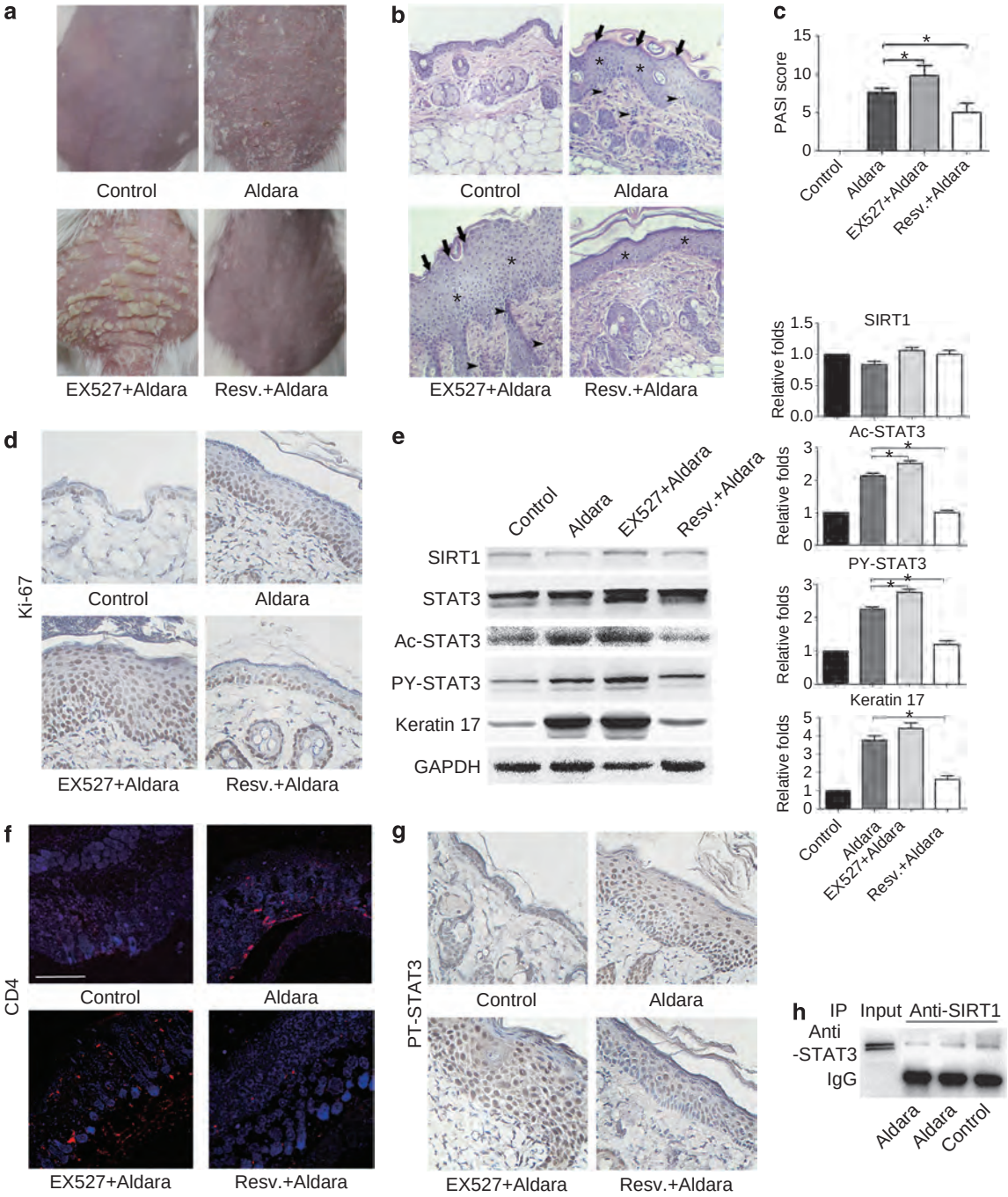

Figure 1. Sirtuin 1 activation counteracted psoriasiform pathology in Aldara-induced mouse skin. (**a**) Psoriasiform phenotype was induced by Aldara ($n=5$). (**b**). Parakeratosis (arrows), acanthosis (asterisks), and inflammatory infiltrates (black arrowheads) were revealed in H&E-stained mouse lesion skin (original magnification ×100; $n=5$). (**c**) The PASI score indicated disease severity ($n=5$). (**d**) Ki-67 immunohistochemical staining in the epithelia (original magnification ×200; $n=5$). (**e**) The relevant proteins of lesion skin were measured by western analysis. The values were normalized to GAPDH. The relative quantitation in each group ($n=5$) was expressed as fold induction of its value vs. that of control group. (**f**) $CD4^+$ cell infiltration (red fluorescence, Scale bars: 100 μm) was detected. (**g**) PY-STAT3 staining in the epithelia (original magnification ×200). (**h**) STAT3 was detected in complexes immunoprecipitated with anti-SIRT1 antibody ($n=5$). H&E, hematoxylin and eosin staining; PASI, psoriasis area and the severity index; PY-STAT3, STAT3 phosphorylated in Tyr705; STAT3, signal transducer and activator of transcription 3.

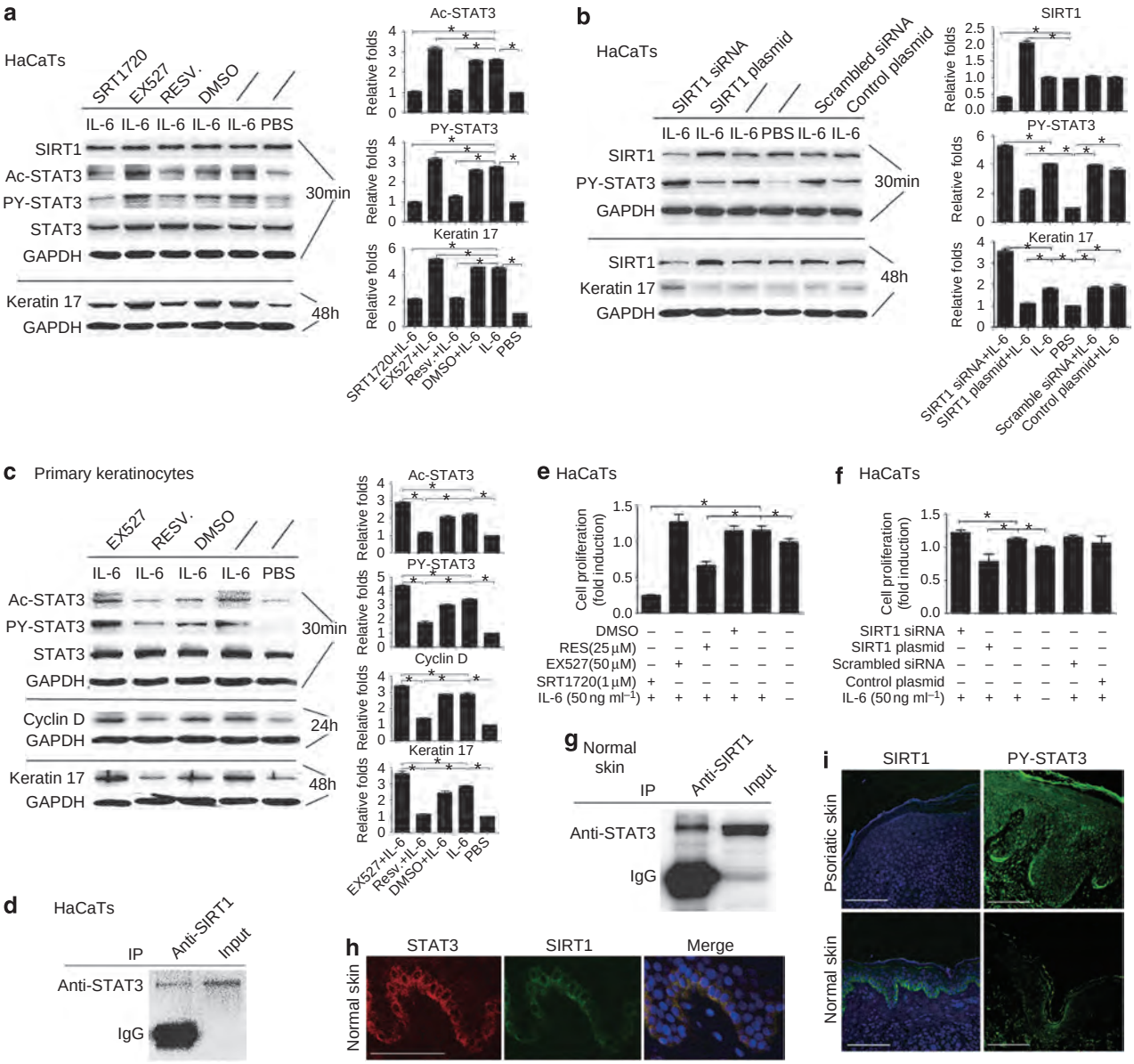

Figure 2. Sirtuin 1 counter-regulated signal transducer and activator of transcription 3 (STAT3) activation in human keratinocytes. (**a**) HaCaTs were stimulated by IL-6, following a pretreatment with SIRT1 regulators. (**b**) HaCaTs were incubated for 48 hours after transfection with SIRT1 siRNA or overexpression plasmid, before IL-6 stimulation. (**c**) Primary human keratinocytes were treated with SIRT1 regulators before IL-6 stimulation. (**d** and **g**) STAT3 was detected in complexes immunoprecipitated with anti-SIRT1 antibody from the extracts of HaCaTs (**d**) or human normal skins ($n = 5$; **g**). (**e** and **f**) Cell proliferations were measured by the MTS assay. *$P < 0.05$. (**h**) Co-labeled of STAT3 (red) and SIRT1 (green) in paraffin sections of human normal skin ($n = 5$). (**i**) SIRT1 and STAT3 staining (green) in paraffin sections of lesion skins from psoriatic patients and normal skins from healthy individuals ($n = 10$). siRNA, small interfering RNA.

gluconeogenesis in mice (Nie *et al.*, 2009). Therefore, we asked whether this mechanism is involved in the epithelia in the Aldara-induced psoriasis model. We found that Aldara treatment decreased SIRT1 expression and upregulated acetylated STAT3 (Ac-STAT3), as well as PY-STAT3, in the lesion skin of mice. Furthermore, the epithelia of skin lesion in resveratrol-treated mice showed a significant decrease in Ac-STAT3 and PY-STAT3, indicating a downregulation of STAT3 activity in keratinocytes. By contrast, EX527 treatment upregulated acetylation and phosphorylation of STAT3 (Figure 1e and g, Supplementary Figure S3D online). Both resveratrol and EX527 act to regulate SIRT1 function by modulating its deacetylation enzyme activity (Howitz *et al.*, 2003; Napper *et al.*, 2005). Consistently, no changes in SIRT1 expression were detected after resveratrol and EX527 treatment in this study. To test the association of STAT3 with SIRT1 in the keratinocytes, the extracts of mice skin epithelia were analyzed through co-immunoprecipita-

tion. STAT3 was detected in complexes immunoprecipitated with anti-SIRT1 antibody. However, the binding of STAT3 to SIRT1 was significantly reduced in the Aldara-induced psoriatic lesions (Figure 1h, Supplementary Figure S3E online). Overall, these observations suggest that SIRT1 counteracts the pathologic effect of Aldara, presumably through its deacetylation of STAT3 in keratinocytes.

Next, we studied the role of SIRT1 in human keratinocytes *in vitro*. First, the cytokine-induced STAT3 acetylation and phosphorylation in the HaCaT cells were significantly reduced in response to pretreatment of SIRT1 activators, resveratrol, and SRT1720. Conversely, administration with SIRT1 inhibitor EX527 enhanced STAT3 acetylation and phosphorylation (Figure 2a, Supplementary Figure S4A online). These results were confirmed by silencing or overexpressing SIRT1 (Figure 2b), which suggests that SIRT1 negatively regulates STAT3 activation. Moreover, direct interaction between SIRT1 and STAT3 was detected (Figure 2d, Supplementary Figure S4B online).

The effect of SIRT1 on STAT3-dependent keratinocyte proliferation and differentiation in HaCaT cells were further determined. As expected, IL-6- and IL-22-induced cell proliferation was inhibited by SIRT1 activation, as well as its ectopic expression (Figure 2e and f, Supplementary Figure S4C–S4D online). Keratin 17, a target of STAT3 (Supplementary Figure S4E online) (Shi *et al.*, 2011), was significantly reduced by SIRT1 activation but enhanced by SIRT1 inhibition (Figure 2a and b). These observations were further confirmed in the primary human keratinocytes (Supplementary Figure S5 online). Resveratrol treatment downregulated STAT3 acetylation and phosphorylation and suppressed the expression of Keratin 17 and cyclin D. By contrast, EX527 acted in the opposite direction (Figure 2c).

Moreover, we confirmed SIRT1–STAT3 interaction in the human skin by co-immunoprecipitation. The immunofluorescent study revealed the co-localization between SIRT1 and STAT3 mainly in the basal keratinocytes of epithelium in normal human skin (Figure 2g and h). A significant reduction in SIRT1 in the epithelia was observed in the skin lesions from psoriatic patients, in comparison with the normal skin from healthy individuals. Conversely, an increase in PY-STAT3 was detected in psoriatic skin lesions (Figure 2i), which is in agreement with the previous report (Sano *et al.*, 2005).

These results suggest that SIRT1 disruption has an important role in Aldara-induced psoriasiform lesion in mice. However, whether the development of pathologic manifestation of psoriasiform lesion results from SIRT1 disruption in keratinocytes only or involves other cells like immunocytes remains uclear. Moreover, the other functions of resveratrol, such as its antioxidation effect, may not be ruled out in ameliorating the Aldara-induced psoriasiform phenotype in mice. Collectively, our study substantiated SIRT1 as a crucial regulator for the development of the Aldara-induced psoriasiform lesion and may provide a new therapeutic target for human psoriasis disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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