Loss of sirtuin 1 (SIRT1) disrupts skin barrier integrity and sensitizes mice to epicutaneous allergen challenge

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Background: Skin barrier integrity requires a highly coordinated molecular system involving the structural protein filaggrin (FLG). Mutational loss of the skin barrier protein FLG predisposes subjects to the development of atopic dermatitis (AD).

Objective: We sought to determine the role of sirtuin 1 (SIRT1) in skin barrier function, FLG expression, and development of AD.

Methods: Skin histology of mice with skin-specific SIRT1 deletion and wild-type control animals was examined by using hematoxylin and eosin staining. Protein and mRNA abundance was analyzed by means of immunoblotting, immunohistochemistry, immunofluorescence, and RT-PCR. Serum antibody levels were assessed by means of ELISA.

Results: Here we show that FLG is regulated by the protein deacetylase SIRT1 and that SIRT1 is critical for skin barrier integrity. Epidermis-specific SIRT1 ablation causes AD-like skin lesions in mice, and mice with epidermal SIRT1 deletion are sensitive to percutaneous challenge by the protein allergen ovalbumin. In normal human keratinocytes and mouse skin SIRT1 knockdown or genetic deletion downregulates FLG, and regulation of FLG expression by SIRT1 requires the deacetylase activity of SIRT1. SIRT1 also promotes activation of the aryl hydrocarbon receptor, and the aryl hydrocarbon receptor ligand restores FLG expression in SIRT1-inhibited cells. Compared with normal human skin, SIRT1 is downregulated in both AD and non-AD lesions.

Conclusion: Our findings demonstrate a critical role of SIRT1 in skin barrier maintenance, open up new opportunities to use SIRT1 as a pharmacologic target, and might facilitate the development of mechanism-based agents for AD prevention and therapy. (J Allergy Clin Immunol 2015;135:936-45.)

Key words: *Sirtuin 1 (SIRT1), skin barrier, filaggrin*

Abbreviations used

AD:	Atopic dermatitis
AhR:	Aryl hydrocarbon receptor
ARNT:	Aryl hydrocarbon receptor nuclear translocator
BaP:	Benzo(a)pyrene
cHet:	Epidermis-specific heterozygous SIRT1 deletion
cKO:	Epidermis-specific homozygous SIRT1 deletion
CYP1B1:	Cytochrome P450 1B1, an aryl hydrocarbon receptor (AhR) target gene
EGFR:	Epidermal growth factor receptor
FLG:	Filaggrin
K10:	Keratin 10
KO:	Knockout
MEF:	Mouse embryonic fibroblast
NC:	Negative control
NF-κB:	Nuclear factor κB
NHEK:	Normal human epidermal keratinocyte
OVA:	Ovalbumin
siRNA:	Small interfering RNA
SIRT1:	Sirtuin 1
siSIRT1:	Small interfering RNA targeting SIRT1
TEWL:	Transepidermal water loss
WT:	Wild-type
XRE:	Xenobiotic response element

The skin is the essential barrier protecting against environmental insults, including infectious pathogens, chemicals, and UV radiation, and minimizing water loss from the body. As the most abundant cells forming the epidermis, keratinocytes proliferate and differentiate to form an impermeable barrier. Defects in the skin barrier have an active role in the pathogenesis of several chronic inflammatory skin diseases, including atopic dermatitis (AD).[1-4]

AD is an increasingly common pruritic inflammatory disease affecting 10% to 20% of children and 3% of adults in the United States and other developed countries.[5,6] The clinical picture evolves in stages and is frequently associated with an increased serum IgE concentration and a number of cutaneous, ocular, and other atopic disorders, such as allergic rhinitis and asthma. There is no cure for AD, and its molecular pathogenesis is still poorly understood. Although for many years AD was considered to be primarily an immunologically driven disease with a secondary barrier defect (the so-called inside-outside hypothesis), investigators hypothesized that the primary defect was in the skin barrier (the outside-inside hypothesis).[4,6] Abnormal barrier function of the skin has long been noted in patients with ichthyosis vulgaris, a common skin condition characterized by postnatal appearance of dry flaking skin, even in the absence of AD. Recent studies have disclosed a strong association between a defect in the skin barrier and the pathogenesis of AD.[6] This defect is due to a genetic loss-of-function mutation of the gene filaggrin *(FLG)*,

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which encodes the skin barrier protein profilaggrin (proFLG)/FLG.[7,8] These mutations, which are carried by up to 10% of Europeans, represent a strong genetic predisposing factor for AD, asthma, and allergies.[9]

Although *FLG* mutation carriers have a greatly increased risk of AD, AD develops in only approximately 42% of all mutation carriers.[10] This outcome implies that genetic modifiers and environmental factors are both important. Impaired barrier function increases transepidermal penetration of environmental allergens. This is supported by the finding that FLG deficiency in mice facilitates and permits increased percutaneous sensitization with protein allergens, irritants, and haptens.[11-14]

Sirtuin 1 (SIRT1), a mammalian counterpart of the yeast silent information regulator 2 (Sir2) and a protomember of the sirtuin family, is an nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase crucial for cell survival, metabolism, senescence, and stress response in several cell types and tissues.[15-19] Both histone and nonhistone targets of SIRT1 have been identified, including forkhead box O (FOXO), p53, peroxisome proliferator-activated receptor (PPAR)-gamma coactivator (PGC-1α), nuclear factor κB (NF-κB), and PPARs.[16,18,20] SIRT1 also deacetylates xeroderma pigmentosum group A protein (XPA) to regulate UV-induced DNA damage repair.[21] We have demonstrated previously that SIRT1 positively regulates UV-induced DNA damage repair by promoting xeroderma pigmentosum group C protein (XPC) expression and that it has a critical role in skin tumorigenesis and homeostasis.[22,23] In addition, SIRT1 has been found to promote differentiation of normal human keratinocytes *in vitro*,[24] suggesting a possible role in barrier function and thus the development of AD.

Although genetic *FLG* mutations are strongly associated with the risk of AD, 2 independent studies have shown that both carriers and noncarriers of *FLG* mutations have childhood AD. In one study of patients with AD, 90% of whom were white, only 26.7% of patients with AD carried *FLG* mutations.[8] In Irish children with AD, approximately 47% of patients carried at least 1 null *FLG* mutation.[7] These findings indicate that more than half of all patients with AD do not carry *FLG* mutations. One implication is that nongenetic loss of proFLG/FLG, including downregulation of *FLG* expression, might play an important role. *FLG* can be downregulated by disrupting the fine balance of the complex regulation of *FLG* promoter activity during epidermal differentiation.[9] However, the mechanisms that regulate FLG expression remain poorly understood.

Here we show that epidermal SIRT1 deletion downregulates FLG and sensitizes mouse skin to the epicutaneous allergen-induced response. SIRT1 regulates FLG expression through AhR. Our findings demonstrate a new role of SIRT1 in skin barrier function and shed light on the molecular mechanisms for maintaining the skin barrier and preventing inflammatory skin diseases.

METHODS

Human normal, AD, and non-AD skin

All human specimens were studied after approval by the University of Chicago Institutional Review Board. Formalin-fixed paraffin-embedded tissue blocks were obtained from archives in the tissue bank of the Section of Dermatology, Department of Medicine, University of Chicago. Normal skin (sun protected), AD, and non-AD (spongiotic dermatitis without a history of atopy) samples were used for immunohistochemical analysis of SIRT1 protein levels. The SIRT1 staining intensity was scored blindly by 2 independent investigators as 0 (negative), 1 (low), 2 (medium), or 3 (high), as in our previous studies.[22,25]

Animal treatments

All animal resources were approved by the University of Chicago Institutional Animal Care and Use Committee. Floxed mice carrying the *Sirt1* allele (Jackson Laboratory, Bar Harbor, Me) were bred with mice expressing Cre recombinase driven by the K14 promoter (Jackson Laboratory) to generate skin keratinocyte-specific heterozygous SIRT1 deletion (cHet) and epidermis-specific homozygous SIRT1 deletion (cKO) mice with a B6 background, as described previously.[23] Mice were backcrossed with SKH1 female mice at least 5 times to generate wild-type (WT) and cKO mice in the SKH1 hairless background.[23] Mice were housed 5 animals per cage, and there was no evidence of dorsal wounds caused by fighting. Female mice ($n = 15$ for each group) were kept for 24 months for observation of survival and aging-related phenotypes.

Cutaneous treatment with ovalbumin

SIRT1 WT and cKO mice with an SKH-1 background were treated with ovalbumin (OVA; Sigma Chemicals, St Louis, Mo) by means of 3 cycles of daily cutaneous exposure to OVA for 5 consecutive days, as described previously.[11] OVA (fraction V, Sigma) was prepared in PBS (Dulbecco PBS, Sigma) at 1 mg/mL. Mice were restrained, and 50 μL of OVA solution or 50 μL of PBS was applied to the dorsal skin and allowed to air dry.

Measurement of transepidermal water loss

Transepidermal water loss (TEWL) was measured on the dorsal skin of shaved mice with a DPM9003 device (Nova Technology, East Hanover, NJ), as described previously.[26] Measurements were performed at room temperature, and results were recorded. Three readings from the dorsal skin were taken on each mouse and averaged.

Microarray gene expression

RNA was extracted from normal human epidermal keratinocytes (NHEKs) transfected with NC/small interfering RNA targeting SIRT1 (siSIRT1) with the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Total RNA concentration and purity were determined with NanoDrop (Thermo Scientific, Waltham, Mass), and total RNA integrity was confirmed with the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, Calif). The RNA samples were processed with an Affymetrix GeneChip microarray (Affymetrix, Santa Clara, Calif) at the Functional Genomics Core Facility of the University of Chicago.

Cell culture and small interfering RNA/plasmid transfection

SIRT1 WT and knockout (KO) mouse embryonic fibroblast (MEF) cells (a gift from Dr Xiaoling Li, National Institute of Environmental Health Sciences/National Institutes of Health) were maintained in a monolayer culture in 95% air/5% CO_2 at 37°C in Dulbecco modified Eagle medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, Calif). MEF cells were cultured for fewer than 20 passages. NHEKs were obtained from Clonetics (Lonza, Allendale, NJ) and cultured in KGM Gold BulletKit medium (Clonetics, Lonza), according to the manufacturer's instructions. NHEKs were cultured for fewer than 4 passages. Cells were transfected with negative control (NC) or small interfering RNA (siRNA; ON-TARGETplus SMARTpool; Dharmacon, Pittsburgh, Pa) targeting SIRT1 by using the Amaxa Nucleofector (Lonza, Basel, Switzerland), according to the manufacturer's instructions, as described previously.[22,23]

Immunohistochemistry and immunofluorescence

Immunohistochemical staining of SIRT1, keratin 10 (K10), loricrin, and FLG was performed in human skin and dermatitis specimens. SIRT1 levels were determined by using the alkaline phosphatase–anti-alkaline phosphatase method in which the substrate staining (red) is easily distinguishable from