Nrf2 regulates epidermal keratinization under psoriatic skin inflammation

Short running title: Nrf2 regulates epidermal keratinization

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Abstract

Psoriasis is an autoinflammatory/autoimmune skin disease and the epitome of exaggerated primary inflammatory response in the surface barrier tissue. Despite the efficacy of dimethyl fumarate (DMF), an electrophilic drug for psoriasis management, there is a paucity of mechanistic evidence *in vivo*. In response to electrophiles, the KEAP1/NRF2 system mediates a myriad of cytoprotective mechanisms, including the regulation of excessive inflammatory response and epidermal differentiation. Since the psoriasiform tissue reaction comprises neutrophil infiltration and parakeratotic scaling, we hypothesized that Nrf2 not only regulates inflammatory responses but is also required for the maintenance of epidermal differentiation, a hallmark of epidermal homeostasis. By utilizing the imiquimod-induced cutaneous inflammation model, we showed an exaggerated inflammatory response and impaired epidermal differentiation in *Nrf2*$^{-/-}$ mice. DMF treatment in *Nrf2*$^{+/+}$ mice attenuated psoriasiform tissue reaction and rescued epidermal differentiation, which was not observed in *Nrf2*$^{-/-}$ mice. In accordance with the fact that psoriasis plaques form well-demarcated parakeratotic lesions in association with the psoriasiform tissue reaction, the lesional skin exhibited

reduced expression levels of *NRF2* and its downstream target genes compared with non-lesional skin. In conclusion, our results suggest that Nrf2 attenuates psoriasiform tissue reaction and underscore the mechanistic legitimacy of the electrophile-based approach for the management of psoriasis.

Introduction

From the evolutionary and adaptive perspectives of the skin, timely neutrophil recruitment in response to tissue-derived danger signals is instrumental in eradicating microorganisms on the surface [1]. However, an inflammatory response involving excessive neutrophil recruitment leads to uncontrolled production of reactive oxygen species (ROS) and autoinflammatory/autoimmune tissue destruction, a typical pathological consequence of which is psoriasis. The transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) and its cytoskeleton-associated negative regulator Kelch-like ECH-associated protein 1 (KEAP1) system constitutes a master regulator of an antioxidant/antielectrophilic response [2], and *Nrf2*-deficient ($Nrf2^{-/-}$) mice not only exhibit dysregulated innate immune responses [3, 4] but also develop autoimmune phenotypes [5]. Conversely, constitutive activation of NRF2, which can be obtained by inhibiting the negative regulator KEAP1, protected against various forms of inflammation, such as lipopolysaccharide-induced septic shock [4], acute kidney injury [6], and lethal autoimmune phenotypes, in forkhead box P3-deficient Scurfy mice [7]. In fact, *Keap1*-deficient mice suffer from hyperorthokeratosis in the forestomach that leads to

lethality [8], and Nrf2 directly transactivates the gene that encodes for loricrin (Lor) [8], a keratohyalin granule constituent that mediates cornification by cross-linking cytoskeletal proteins keratin 1/10 (K1/K10) and filaggrin (Flg) [9]. The recovery of epidermal barrier function in the absence of *Lor* requires KEAP1/NRF2 system activation [10, 11]. These lines of evidence underscore that NRF2 is a regulator of epidermal barrier function [8, 12].

Typical psoriasis plaques form well-demarcated parakeratotic lesions, and non-inflamed (non-lesional) areas exhibit orthokeratosis; these mutually exclusive modes of keratinization are hallmarks of psoriasiform inflammation and quasi-homeostasis, respectively. Moreover, dimethyl fumarate (DMF), an electrophilic reagent, has been used for the treatment of psoriasis [13, 14] and recently became an FDA-approved drug for the treatment of relapsing–remitting multiple sclerosis [15]. Therefore, we hypothesized that NRF2 not only regulates inflammatory responses but also is required for the maintenance of orthokeratosis in an inflammatory epidermal milieu. Here we show that *Nrf2*$^{-/-}$ mice exhibit an enhanced inflammatory response and parakeratosis in imiquimod (IMQ)-induced cutaneous inflammation, which is a model of the

psoriasiform tissue reaction [16].

Materials and Methods

Mice

C57BL/6 mice (8–12 weeks of age) were obtained from Charles River Laboratories Japan (Yokohama, Japan), and *Nrf2*-deficient ($Nrf2^{-/-}$) mice were from Riken Bioresource Research Center (Tsukuba, Japan) [17]. All mice were maintained under specific pathogen-free conditions at the animal facility of the University of Tsukuba, and all procedures were approved by the University of Tsukuba Ethics Committee.

IMQ-Induced Psoriasis-Like Skin Inflammation Model

Eight-week-old $Nrf2^{-/-}$ and wild-type ($Nrf2^{+/+}$) female mice with the C57BL/6 background were used. On day 0, the back skin was carefully trimmed using a mechanical trimmer (Trimmer 8655; Wahl, Sterling, IL, USA), and 60 mg/day (back, 50 mg; ear, 10 mg) of 5% IMQ cream (Mochida Pharmaceutical, Tokyo, Japan) was applied.

N-acetylcysteine Treatment

N-acetylcysteine (NAC) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The mice received either deionized water or water containing NAC (7 mg/mL; neutralized to pH 7.4 with NaOH) starting two days prior to the IMQ treatment until termination. Water containing NAC was freshly prepared every day.

DMF Treatment

DMF was purchased from FUJIFILM Wako Pure Chemical Corporation. The reagent was diluted in 200 μL of 0.1% Methocel/H_2O and administered by oral gavage starting from day 0 until termination. The mice received DMF (15 mg/kg) or the vehicle control (Methocel/H_2O) twice a day.

Keratinocyte culture and knockdown experiment

Human Epidermal Keratinocytes, neonatal ([HEKn] C0015C; Thermo Fisher

Scientific, Waltham, MA) were maintained in EpiLife medium (MEPI500CA; Thermo Fisher Scientific) with EpiLife Defined Growth Supplement (s0125; Thermo Fisher Scientific). Lentiviral particles encoding shRNA targeting *Keap1* mRNA were purchased from Santa Cruz Biotechnology (sc-43878-V). Lentiviral particles encoding a scrambled shRNA sequence (Scr) (sc-108080) were also purchased from the same source for use as a control. HEKn cells were transduced with these lentiviral particles in accordance with the manufacturer's instructions, and the knockdown efficacy was assessed at the mRNA level by quantitative real-time PCR. For the analysis of proliferation rate, HEKn cells of each strain were seeded into 6-well plates at a density of 5×10^4 cells/well in the growth medium described above. The next day, the medium was changed for fresh medium, and cell numbers in triplicate wells were counted by trypsinization at indicated time points.

Generation of Bone Marrow Chimera Mice

To generate bone marrow chimera mice, recipient *Nrf2*$^{+/+}$ or *Nrf2*$^{-/-}$ mice underwent 9-Gy total body irradiation on day 1. Bone marrow single-cell suspensions

from either *Nrf2*$^{+/+}$ or *Nrf2*$^{-/-}$ mice on day 0 intravenously through the tail vein, and mice that had undergone mock injection failed to survive seven days after the irradiation, validating successful bone marrow suppression. Successful generation of chimerism was determined through the genotyping of DNA derived from peripheral blood and tail snip. The mice received sterilized water acidified with hydrochloric acid for 2 months after transplantation to prevent opportunistic infection.

Collection of lesional and Non-lesional Skin of Human Psoriasis

Biopsy samples from lesions and normal-looking non-lesional skin were taken from patients with psoriasis using a 3-mm punch. Pieces of skin samples were homogenized in TRIzol reagent (15596-026; Thermo Fisher), and total RNA was isolated. Written informed consent was provided from all subjects under the approval from the Institutional Review Board at the University of Tsukuba (Tsukuba Clinical Research & Development Organization; H29-003).

Histopathology and Immunohistochemistry

The ear or back skin was fixed overnight with 10% buffered formalin. After paraffin embedding, 3-μm sections were deparaffinized and incubated overnight with primary antibodies diluted as follows after routine retrieval and blocking: anti-PCNA (0.14 μg/mL, GTX100539; GeneTex, Irvine, CA), anti-signal transducers and activator of transcription 3 (STAT3) (0.6 μg/mL, GTX104616; GeneTex), anti-phosphorylated STAT3 ([pSTAT3] 0.6 μg/mL, GTX118000; GeneTex), anti-FLG (0.2 μg/mL, PRB-417P-050; Eurogentec, Seraing, Belgium), anti-LOR (1:1000; the antibody was kindly provided by Dr. Roop) [19], anti-K5 (0.1 μg/mL, PRB-160P; BioLegend), anti-K6 (1:1000, the antibody was kindly provided by Dr. Roop) [20], anti-K10 (0.048 μg/mL, ab76318; Abcam, Cambridge, England), anti-K16 (CSB-PA002020; 1 μg/mL, Wuhan Huamei, Wuhan, China) and anti-K17 (CSB-PA002024; 1 μg/mL, Wuhan Huamei). After blocking of endogenous peroxidase with PBS containing 0.3% NaN_3 and 0.01% H_2O_2, visualization was performed using an ABC system according to the standard protocol (Vector Laboratories, Burlingame, CA), followed by light counterstaining with hematoxylin. PCNA, STAT3, and pSTAT3-positive keratinocytes were counted in high-power fields by three independent dermatologists (TO, YN, and YI) using a digital

microscope (AX80; Olympus, Tokyo, Japan) and Flovel Filing System (Flovel, Tokyo, Japan), and the positivity rate for each immunogen was subjected to statistical analysis. The expression levels of LOR FLG, K5, K6, K16 and K17 were evaluated according to the staining intensity of the positive keratinocytes as follows: Grade 1, none; Grade 2, weak; Grade 3, strong; and Grade 4, very strong. The expression level of K10 was evaluated according to the numbers of K10-negative layers as follows: Grade 1, all layers; Grade 2, 5–10 layers; Grade 3, 2–4 layers; and Grade 4, one layer, as previously described [21].

Quantitative Real-Time PCR

Pieces of full-thickness skin were homogenized in TRIzol reagent (15596018; Thermo Fisher), and total RNA was isolated. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (4368814; Thermo Fisher). Primers specific for each target with double-quenched probes were purchased from the IDT Primetime Predesigned Library (IDT, Coralville, IA; See Table below), and primers for *Gapdh* (4352339E; Thermo Fisher) were used for internal reference. All data were analyzed

using the Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher). The interpretation of quantitative real-time PCR data was conducted using the comparative Ct method (ΔΔCT method).

Statistical Analysis

Data are expressed as means ± SEM. Comparisons were performed using the unpaired *t*-test with Welch's correction (between two groups) or the two-way ANOVA (time-course experiments) using the Prism 7 software (GraphPad, La Jolla, CA). In all analysis, $p < 0.05$ was taken to indicate statistical significance.

Results

Nrf2 Attenuates IMQ-Induced Skin Inflammation

Nrf2$^{-/-}$ mice were subjected to daily application of IMQ cream and exhibited significantly enhanced ear swelling compared with wild-type (*Nrf2*$^{+/+}$) mice (Fig. 1A). Accordingly, the expression levels of the psoriasis-related proinflammatory cytokine genes were significantly increased in *Nrf2*$^{-/-}$ mice compared with those in *Nrf2*$^{+/+}$ mice after IMQ treatment in the ear skin; reflecting the activation of leukocytes with dendritic cell/monocytes lineage in response to danger signals [1], *Il23* expression levels showed significance at the earlier time point, whereas significantly increase expression levels of *Il6*, *Tnf*, and *Il17* were noted at the later time point (Fig. 1C). However, the expression levels of *Il22* were not substantially changed in *Nrf2*$^{-/-}$ mice compared with those in *Nrf2*$^{+/+}$ mice (data not shown), and epidermal hyperplasia (acanthosis) was evoked to comparable degrees in both genotypes (Fig. 1B and 2A). Correspondingly, PCNA staining signals, which denote DNA replication activity, were not substantially altered between the genotypes (Fig. 2 A and B).

Nrf2 Attenuates Psoriasiform Tissue Reaction

The epidermal phenotypes in IMQ-treated back skin were analyzed. IMQ-treated *Nrf2*$^{-/-}$ back skin epidermis not only had obvious edema (spongiosis) but also exhibited prominent hypogranulosis and parakeratosis, the hallmarks of psoriasiform tissue reaction [15]. On the other hand, IMQ-treated *Nrf2*$^{+/+}$ mice retained stratum granulosum (SG) and orthokeratosis despite the presence of acanthosis (Fig. 2A). Reflecting the absence of SG, immunohistochemical analysis revealed significantly decreased expression levels of LOR, FLG, and the early keratinization marker K10, while the expression levels of basal keratin K5 and stress-induced keratins K6/K16/K17 were unchanged between the two genotypes (Fig. 2 A and B). Staining signals of the pSTAT3 protein [22], but not unphosphorylated STAT3 protein, in IMQ-treated *Nrf2*$^{-/-}$ epidermis were comparable with those in the *Nrf2*$^{+/+}$counterpart (Fig. 2 A and B).

Reversal of IMQ-Induced Skin Inflammation with the thiol antioxidant NAC

KEAP1-inhibition-mediated NRF2 activation profoundly depends on the modification of sulfhydryl groups on cysteine residues on the KEAP1 protein that are

oxidized by ROS [23], and free sulfhydryl groups were supplemented by way of oral NAC treatment. As expected, NAC treatment completely abrogated the ear swelling response in both genotypes (Fig. 3A).

DMF Attenuates Psoriasiform Tissue Reaction

To determine the therapeutic efficacy of DMF in the experiment, the mice were treated with DMF by oral gavage, as previously described [24]. DMF treatment in *Nrf2*$^{+/+}$ did not evoke ear swelling response, while *Nrf2*$^{-/-}$ mice ended up with a partial attenuation (Fig. 3B). In *Nrf2*$^{+/+}$ mice, the expression levels of *Il6*, *Tnf*, *Il17a*, and *Il23a* were significantly decreased in response to DMF treatment (Fig. 3C), but those of *Il22* were not decreased in the DMF-treated group compared to the vehicle control group (data not shown). Accordingly, although the degree of acanthosis was not affected in response to the treatment, the expression levels of *Lor* and *Flg* were significantly increased compared to those in the vehicle-treated *Nrf2*$^{+/+}$ mice, and changes in the expression levels of the inflammatory cytokines and the epidermal differentiation complex genes in response to the treatment were attenuated in *Nrf2*$^{-/-}$ mice (Fig. 3C).

Nrf2 activates antioxidative response in the epidermis

NRF2 is known to enhance keratinocyte proliferation, and its expression is enhanced in psoriatic epidermis compared with normal skin [25]. We took a knockdown approach to activate the KEAP1/NRF2 system. Knockdown of *KEAP1* using a shRNA-encoding lentiviral vector resulted in significantly increased mRNA expression levels of *NRF2* and its downstream target genes [2] NAD(P)H dehydrogenase, quinone 1 (*NQO1*), Glutathione S-Transferase Pi 1 (*GSTP1*), Heme oxygenase 1 (Hmox1), *LOR* and small proline-rich protein 2A (*SPRR2A*) (Fig. 4A). Significantly increased proliferation rates were also noted (Fig. 4B). Psoriasiform tissue reaction requires blood flow-derived neutrophils as primary effector cells [1, 16]. Given the pleiotropic role of Nrf2 to counteract inflammatory response and NRF2 activation in psoriatic epidermis, the results above suggested that keratinocyte hyperproliferation in psoriatic epidermis was a consequence of host's inherent anti-inflammatory response on the KEAP1/NRF2 system. To address the issue, bone marrow chimeras were generated, in which bone marrow cells from each genotype were transplanted into either genotype. *Nrf2*$^{-/-}$

recipients not only showed significantly increased ear swelling (Fig. 4C), but also exhibited remarkable hypogranulosis and parakeratosis compared with *Nrf2*$^{+/+}$ recipients, whereas the *Nrf2*$^{-/-}$ donor did not (Fig. 4D). This indicated that radioresistant cells such as keratinocytes or IL-17-producing dermal γδ T cells [27, 28] are responsible for the enhanced psoriasiform tissue reaction observed in *Nrf2*$^{-/-}$ mice. Collectively, these results indicated that Nrf2 activates antioxidative response in the epidermis.

Decreased NRF2 and Downstream Target Genes in Lesional Psoriasis Skin

Considering the above results showing that the activation of the KEAP1/NRF2 system attenuates psoriasiform tissue reaction, the expression levels of NRF2-target genes were analyzed in skin samples from patients with psoriasis. As expected, the mRNA expression levels of *NRF2* and its downstream targets *NQO1* [2], and *LOR* [8] as well as *FLG* were decreased significantly in the lesional area compared with the non-lesional area (Fig. 5), validating the dysregulated KEAP1/NRF2 system that leads to the persistent psoriasiform tissue reaction that is critical for psoriatic plaque generation.

Discussion

Immune therapy targeting IL-17 or its leukocyte-derived upstream cytokine IL-23 has brought a remarkable treatment efficacy and safety for psoriasis and related conditions [29]. However, despite mechanistic evidence from a mouse model [30], targeting IL-22, another IL-23 downstream cytokine that mediates epidermal hyperproliferation following IL-23-induced STAT3 activation [31], was not effective [32]. This unequivocally suggests that IL-23/IL-17 pathway primarily mediates the psoriasiform tissue reaction [16], rather than mere acanthosis. Accordingly, we have shown that DMF reversed the expression levels of the effector cytokine *Il17*, but not *Il22*, *in vivo* in an *Nrf2*-dependent manner. Given that NRF2 directly transactivates the stress-related cytokeratins K6/K17 [8, 25, 33] in response to abundant IL-17 and mediates keratinocyte hyperproliferation in lesional psoriasis skin [25], hyperkeratosis following acanthosis presumably reflect an innate cytoprotective response against the excessive psoriasiform tissue reaction in the epidermis, which depends on the KEAP/NRF2 system (Fig. 6).

As Mrowietz proposed earlier that fumaric acid esters (FAEs) including DMF interfere with cellular redox systems and inhibits nuclear factor-kappa B (NF-κB)

translocation [13], NRF2 counteracts NF-κB activation and regulates innate immune response [3]. Indeed, autoinflammatory conditions in the epidermis, such as deficiency of the IL-1 receptor antagonist/IL-36R antagonist [34] or dominant gain-of-function caspase recruitment domain-containing protein 14 [35], result in psoriasis or generalized pustular psoriasis. Moreover, dendritic cells (DCs) treated with the electrophile diethyl maleate [36] or DMF [37, 38] retain immature/anti-inflammatory phenotypes, and *Nrf2*$^{-/-}$ DCs exhibit mature/proinflammatory phenotypes [39], which in turn profoundly affect the outcome of ensuing adaptive immune responses. Therefore, our results mechanistically corroborate the legitimacy of using DMF, an electrophilic drug, for the treatment of multiple autoinflammatory/autoimmune disorders in the epidermis, including psoriasis.

With a translational approach, we have shown that NRF2 attenuates the psoriasiform tissue reaction, a primary inflammatory response in the surface barrier tissue [1], and the mechanistic legitimacy of using electrophilic drugs for the treatment of psoriasis and related conditions in which autoinflammation/autoimmunity plays significant roles. However, given the immunosuppressive side effects in association with oral FAEs treatment that have been reported increasingly [40], the electrophile-based

approach may still require cautious optimization strategy.

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Figures

Figure 1

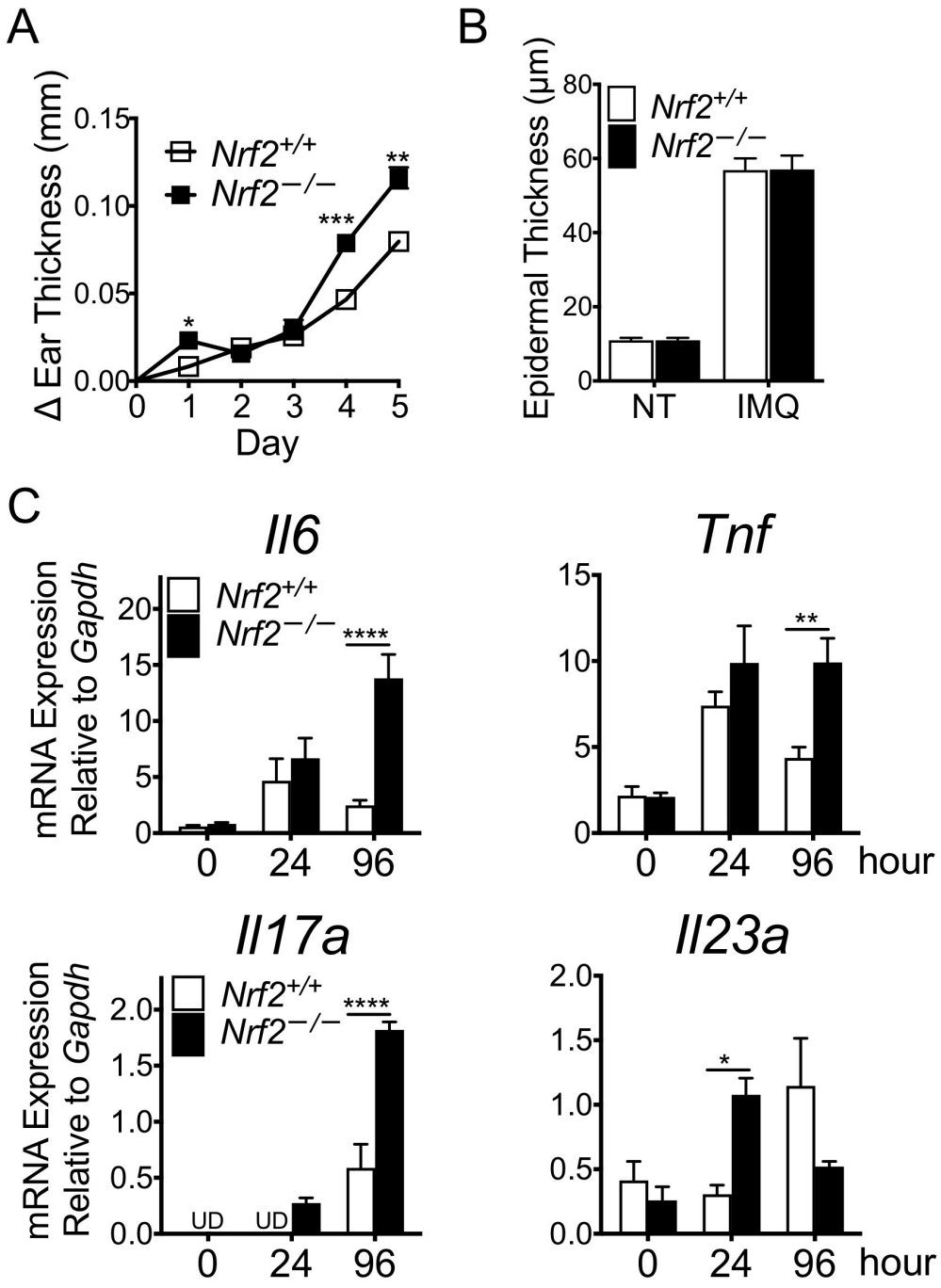


Figure 2

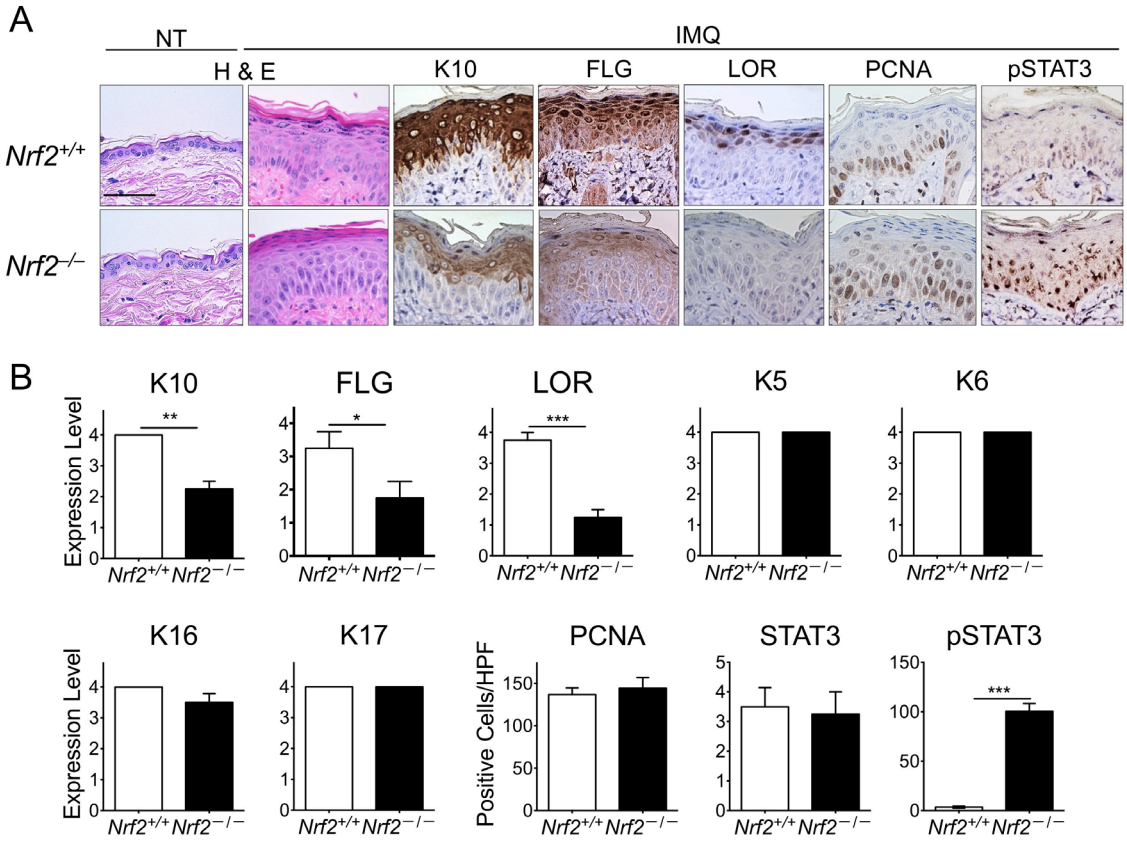

A
NT
IMQ
H & E
K10
FLG
LOR
PCNA
pSTAT3
Nrf2+/+
Nrf2−/−
B
K10
FLG
LOR
K5
K6
K16
K17
PCNA
STAT3
pSTAT3
Expression Level
Positive Cells/HPF
**
*
***
***
Nrf2+/+ Nrf2−/−

Figure 3

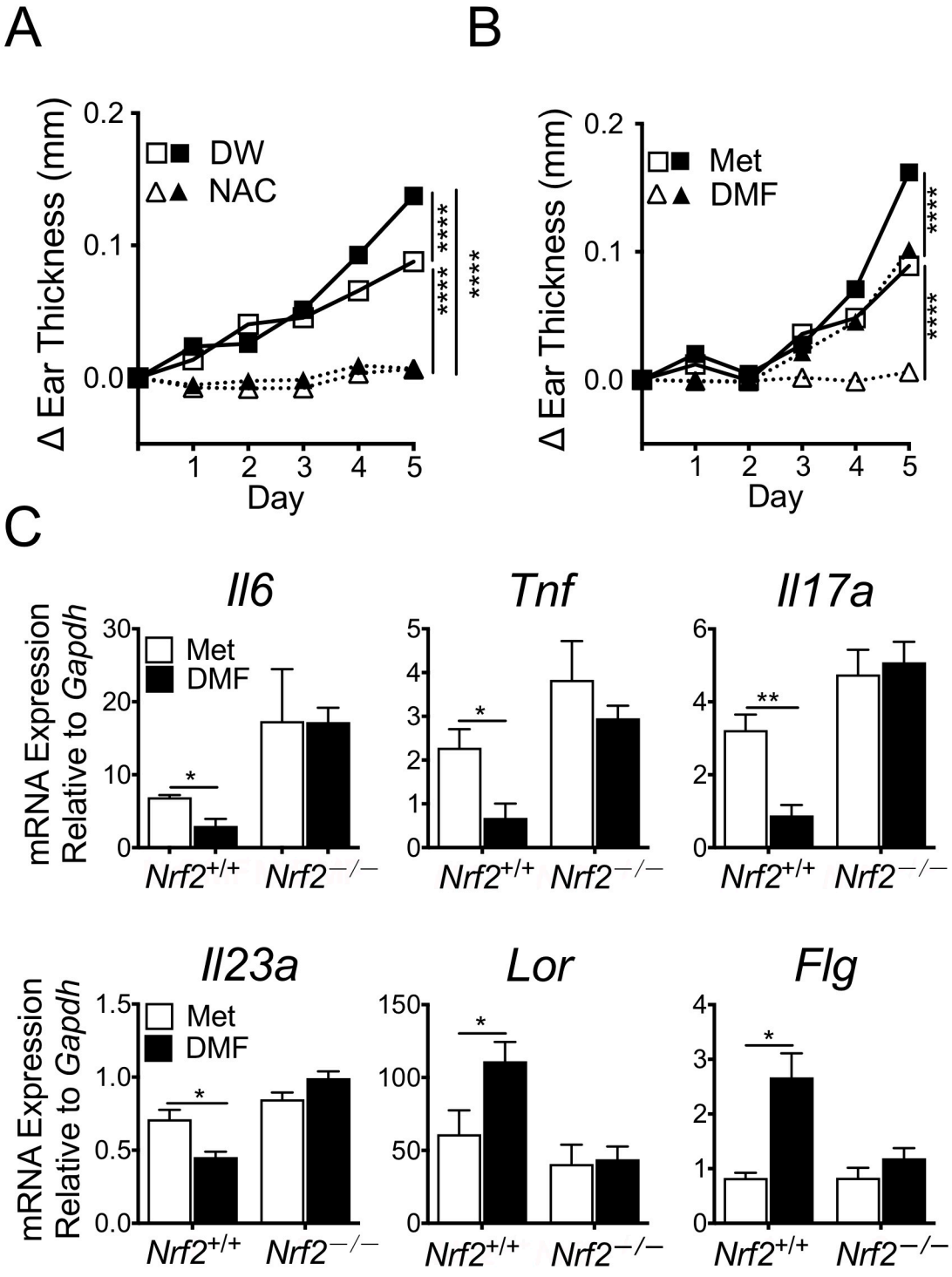

A
B
C
Δ Ear Thickness (mm)
DW
NAC
Met
DMF
Day
****
mRNA Expression Relative to Gapdh
Il6
Tnf
Il17a
Il23a
Lor
Flg
Nrf2+/+
Nrf2−/−
*
**

Figure 4

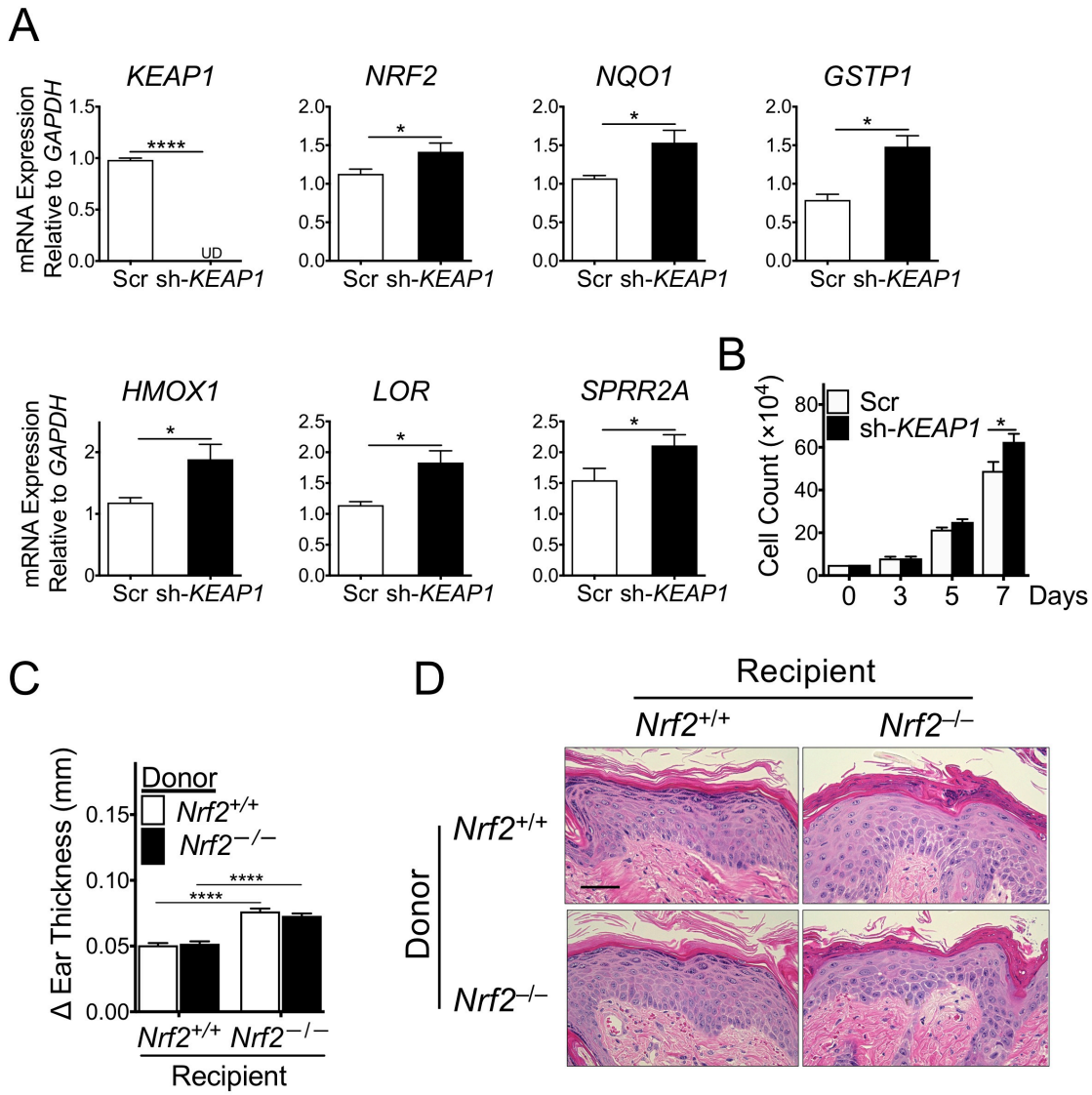

A
KEAP1
NRF2
NQO1
GSTP1
mRNA Expression Relative to GAPDH
Scr sh-KEAP1
UD
HMOX1
LOR
SPRR2A
B
Cell Count (×10^4)
Scr
sh-KEAP1
Days
C
Δ Ear Thickness (mm)
Donor
Nrf2+/+
Nrf2−/−
Recipient
D
Recipient
Nrf2+/+
Nrf2−/−
Donor

Figure 5

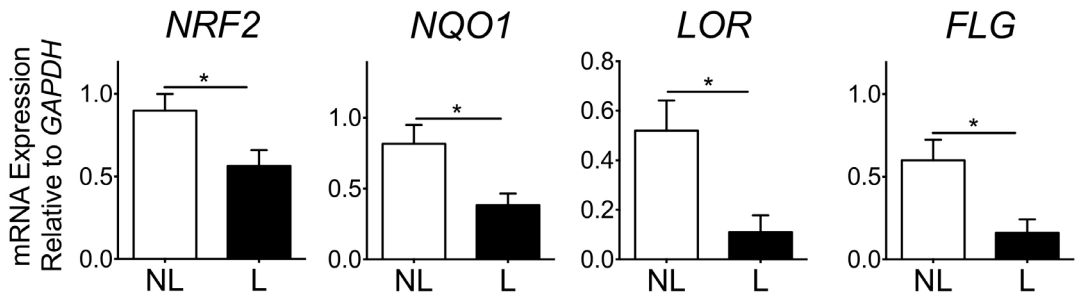
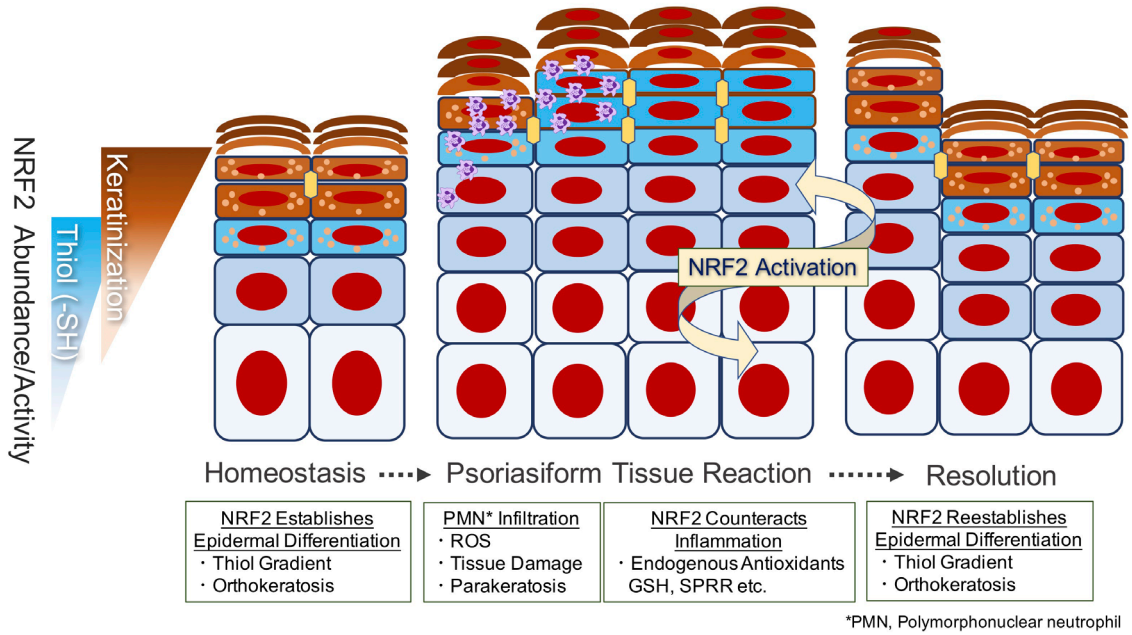

NRF2
NQO1
LOR
FLG
mRNA Expression
Relative to GAPDH
1.0
0.5
0.0
0.8
0.6
0.4
0.2
NL
L
*

Figure 6


NRF2 Abundance/Activity
Keratinization
Thiol (-SH)
NRF2 Activation
Homeostasis
Psoriasiform Tissue Reaction
Resolution
NRF2 Establishes Epidermal Differentiation
・Thiol Gradient
・Orthokeratosis
PMN* Infiltration
・ROS
・Tissue Damage
・Parakeratosis
NRF2 Counteracts Inflammation
・Endogenous Antioxidants GSH, SPRR etc.
NRF2 Reestablishes Epidermal Differentiation
・Thiol Gradient
・Orthokeratosis
*PMN, Polymorphonuclear neutrophil

Table

Predesigned qPCR assay (Integrated DNA Technologies, Coralville, IA)	
Gene	Assay ID
Il6	Mm.PT.58.10005566
Tnf	Mm.PT.58.12575861
Il23a	Mm.PT.58.10594618.g
Il17a	Mm.PT.58.6531092
Flg	Mm.PT.58.29195892
Lor	Mm.PT.58.12882060
KEAP1	Hs.PT.58.1898764
NRF2	Hs.PT.58.28159373
NQO1	Hs.PT.58.2697277
GSTP1	Hs.PT.58.406347
HMOX1	Hs.PT.58.45340055
LOR	Hs.PT.58.27761511
SPRR2A	Hs.PT.58.19341995
FLG	Hs.PT.58.24292320.g

Legends

Figure 1

(A) Ear thickness of *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice treated with 5% IMQ cream for five consecutive days. *Nrf2*$^{-/-}$ mice, closed square; *Nrf2*$^{+/+}$ mice, open square; $n = 6$, $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. (B) Epidermal thickness of non-treated (NT) or IMQ-treated back skin on day 5. The thickness was measured by calculating the distance between the basal layer and the top of the granular layer.

(C) mRNA expression levels of psoriasis-related cytokine genes at indicated time points in the ear skin. UD, undetected; $n = 6$, $*p < 0.05$, $**p < 0.01$, $****p < 0.001$.

Figure 2

(A) Histological images of non-treated (NT) or IMQ-treated back skin for five consecutive days. Representative images of hematoxylin and eosin staining (H & E) or immunohistochemistry for K10, Filaggrin (FLG), Loricrin (LOR), PCNA and pSTAT 3 on IMQ-treated back skin are shown. Bar = 50 μm. (B) Protein expression levels as determined by the number of negative layers (K10), staining intensity (LOR, FLG, K5,

and K6; staining intensity in the epidermis), or positivity rate (PCNA, STAT3, and pSTAT3; positive keratinocytes per HPF). $n = 4$, $*p < 0.05$, $**p < 0.01$, $***p < 0.005$

Figure 3

(A) Ear thickness of *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice treated with 5% IMQ cream for five consecutive days with intervention of either deionized water (DW) or NAC. Closed triangle, NAC-treated *Nrf2*$^{-/-}$ mice; open triangle, NAC-treated *Nrf2*$^{+/+}$ mice; closed square, control-treated *Nrf2*$^{-/-}$ mice; open square, control-treated *Nrf2*$^{+/+}$ mice; $n = 6$, $****p < 0.001$

(B) Ear thickness of *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice treated with 5% IMQ cream for five consecutive days with intervention of either vehicle (Methocel [Met]) or DMF. Closed triangle, DMF-treated *Nrf2*$^{-/-}$ mice; open triangle, DMF-treated *Nrf2*$^{+/+}$ mice; closed square, veihcle-treated *Nrf2*$^{-/-}$ mice; open square, vehicle-treated *Nrf2*$^{+/+}$ mice; $n = 6$, $****p < 0.001$

(C) mRNA expression levels of psoriasis-related cytokine and epidermal differentiation complex genes on day 4. $n = 4$, $*p < 0.05$, $**p < 0.01$

Figure 4

(A) HEKn cells were cultured under proliferative conditions (0.06 mM Ca^{2+}), and *KEAP1* knockdown experiments were performed to activate the KEAP1/NRF2 system. Efficient knockdown was validated at the mRNA level, and *KEAP1* knockdown enhanced *NRF2* and downstream target genes expression levels. Results are from at least three independent experiments. UD, undetected; $n = 4$, $*p < 0.05$, $****p < 0.001$

(B) Proliferation assay was performed to analyze the effect of *KEAP1* knockdown-mediated *NRF2* overexpression. Results are from at least three independent experiments. $n = 6$, $*p < 0.05$

(C) (D) $Nrf2^{-/-}$ or $Nrf2^{+/+}$ mice underwent 9-Gy total body irradiation and received bone marrow single-cell suspensions from $Nrf2^{-/-}$ or $Nrf2^{+/+}$ mice. Two months after transplantation, each chimera mouse was treated with 5% IMQ cream for five consecutive days. (C) Ear thickness of each group on day 5 is shown. $n = 6$, $****p < 0.001$ (D) Representative images of hematoxylin and eosin staining are shown. Bar = 50 μm.

Figure 5

Expression levels of *NRF2*, *NQO1*, *LOR*, and *FLG* in lesional (L) or non-lesional (NL) skin of psoriasis patients at the mRNA levels. $n = 8$, $*p < 0.05$

Figure 6

A schematic representation of the role of the KEAP1/NRF2 system that regulates epidermal homeostasis during the psoriasiform tissue reaction. *PMN: polymorphonuclear neutrophil