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CORRESPONDENCE

KEAP1 and epidermal differentiation: Psoriatic epidermis as a model

Dear Editor,

The stratum corneum (SC) covers the body of terrestrial animals and protects against the entry of pathogens, allergens, and irritants while preventing dehydration. Epidermal layers exhibit a characteristic gradient of free sulfhydryl groups (–SH groups) allowing for adaptation to such xenobiotic insults.[1] Upon exposure to the air–liquid interface at the uppermost living epidermal layer, keratinocytes in the stratum granulosum (SG) undergo extensive disulfide (S–S) covalent linkages.[1] This biological process promotes extensive cytoskeletal cross-linkages, with a significant contribution of the major thiol-rich cell envelope protein loricrin (LOR). The transcription factor NF-E2-related factor 2 (NRF2) establishes the epidermal thiol gradient and promotes keratinization.[1–3] The cytoskeleton-associated Kelch-like erythroid cell-derived protein

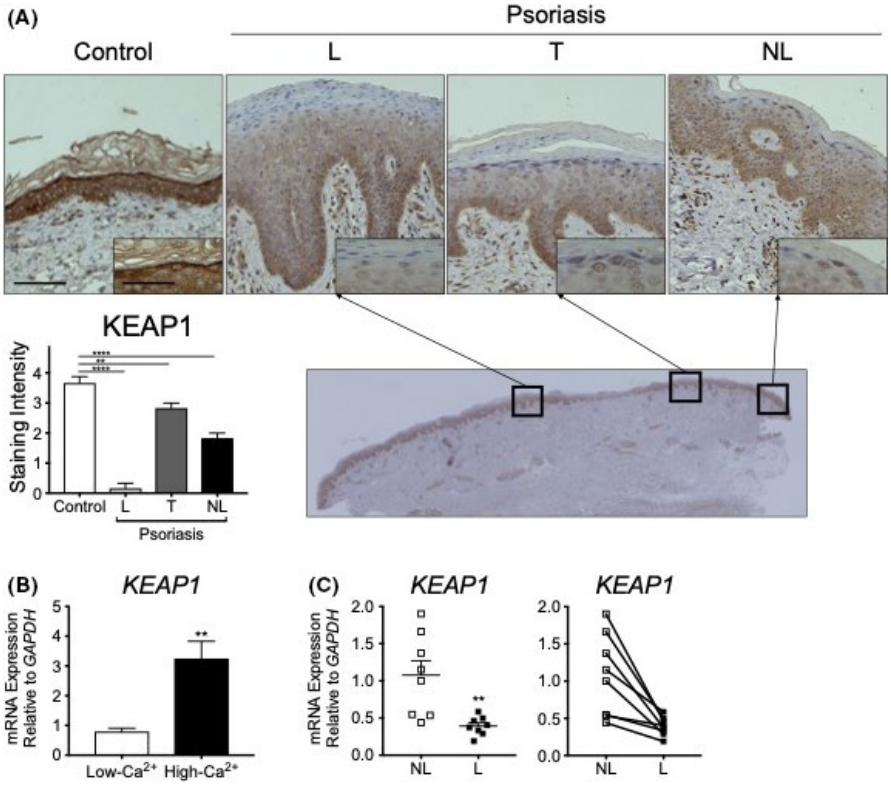

FIGURE 1 KEAP1 expression in psoriatic skins and cultured keratinocytes. (A) Representative immunohistochemical images of KEAP1 in healthy control and lesional (L), transitional (T), or nonlesional (NL) skin of psoriasis. Lesional skin of psoriasis exhibited lower KEAP1 expression level compared to that of transitional or nonlesional skin. Control skin showed strong linear KEAP1 expression in the stratum granulosum (SG). The staining intensity of the positive keratinocytes in the SG as follows: Grade 1, none; Grade 2, weak; Grade 3, strong; and Grade 4, very strong. Bar = 100 μm, 50 μm (inset). $n = 6$, $^{**}p < 0.01$, $^{****}p < 0.001$, one-way ANOVA. (B, C) *KEAP1* mRNA expression levels of cultured neonatal human epidermal keratinocytes (HEKn) or psoriatic skin. For the epidermal differentiation, HEKn was incubated under the high Ca^{2+} condition (1.2 mM) for 96 h. *KEAP1* mRNA expression correlated with epidermal differentiation. $n = 4$ or 8, $^{**}p < 0.01$, unpaired or paired *t*-test

with cap-n-collar homology-associated protein 1 (KEAP1) senses redox-disruptive stimuli *via* reactive cysteine residues and inhibits NRF2 activation.[2]

The external oxidative milieu promotes the recovery of the epidermal barrier in *Lor*-deficient mice,[4,5] while *Keap1*-deficient mice exhibit uncontrolled orthohyperkeratosis and overexpression of *Lor*.[2] These observations suggest that the thiol-rich KEAP1 protein regulates keratinization in the SG. Therefore, we hypothesized that the KEAP1 is expressed at the SG and regulates excessive keratinization, which is a hallmark of psoriatic plaques.

To test this hypothesis, we determined the expression profile of KEAP1 in the epidermis. We divided psoriasis sections into three segments based on histological characteristics: lesional, transitional, or nonlesional areas. KEAP1 expression in healthy control epidermis looked concentrated in the SG but lower in the hypogranulotic lesional areas (Figure 1A). Notably, hypergranulotic SG in the transitional area showed enhanced KEAP1 expression compared to other areas in the psoriatic epidermis (Figure 1A). Next, the expression profile of KEAP1 was analyzed in cultured neonatal human epidermal keratinocytes (HEKn). Because the Ca^{2+} gradient is lost in lesional psoriatic epidermis,[6] the comparison of KEAP1 expression levels between proliferating (0.06 mM) and differentiating (1.2 mM) culture conditions could be similar to the comparison of its levels between lesional and nonlesional psoriatic areas. The *KEAP1* mRNA expression levels were significantly higher in differentiating HEKn than in proliferating HEKn (Figure 1B). Correspondingly, *KEAP1* mRNA expression levels were significantly lower in the lesional area than in the nonlesional area (Figure 1C). These results suggest that the induction of KEAP1 expression is hardwired into the epidermal differentiation program.

In line with a previous study,[7] KEAP1 protein expression was also observed around the basal layer. This might reflect robust sulfur turnover of the proliferative layer, although enhanced thiol-rich protein synthesis is a characteristic of the transitional layer between the SG and the SC.[2] Alternatively, this might be due to some post-translational modification of KEAP1, which requires further investigation. Thus, we conclude that aberrant epidermal differentiation perturbs the KEAP1/NRF2 system in lesional areas. Reactive oxygen species-mediated oxidative stress could contribute to the pathogenesis of psoriasis, and antioxidant strategies have been proven to be beneficial.[3] Consistent with these observations, our results suggest that KEAP1 supresses hyperkeratinization, which could be a consequence of prolonged psoriasiform tissue reaction.[3] These new insights contribute to a better understanding of the tissue-protective machinery of the skin.[1–3,5]

1 | DECLARATIONS

Approval of the research protocol: All procedures were approved by the Tsukuba University Hospital Ethics Committee.

Informed Consent: Patients diagnosed at Tsukuba University Hospital were included in this study with written informed consent.

Registry and the Registration No: N/A.

Animal Studies: N/A.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest. Dr Manabu Fujimoto is the Editor in Chief for the Journal of Cutaneous Immunology and Allergy. Management of the peer review process, and all editorial decision-making, for this article was undertaken by an Associate Editor.

Tatsuya Ogawa[1]
Yosuke Ishitsuka[1,2]
Manabu Fujimoto[1,2]
Toshifumi Nomura[1]

[1]*Department of Dermatology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*
[2]*Department of Dermatology, Graduate School of Medicine, Osaka University, Suita, Japan*

Correspondence
Yosuke Ishitsuka, Department of Dermatology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
Email: ishitsuka@derma.med.osaka-u.ac.jp

ORCID

Tatsuya Ogawa https://orcid.org/0000-0003-1124-4572
Manabu Fujimoto https://orcid.org/0000-0002-3062-4872

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