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RESEARCH ARTICLE

Decreased keratinocyte Proline-Rich protein expression in cutaneous T-cell lymphoma

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Abstract

Background: Cutaneous T-cell lymphomas (CTCLs) often have skin dryness and skin barrier dysfunctions, which are also seen in atopic dermatitis (AD). Expression of keratinocyte proline-rich protein (KPRP) was decreased in the epidermis of AD, which led to barrier defects and further allergic inflammation.

Objectives: The aim of this study was to analyze KPRP expression in CTCL.

Materials & Methods: Skin samples of CTCL and healthy control were collected, and KPRP mRNA expression in the skin tissue was analyzed. We also performed immunohistochemistry of KPRP protein.

Results: Keratinocyte proline-rich protein (KPRP) mRNA expression was decreased compared with normal skin. KPRP expression was positively correlated with that of filaggrin and loricrin, and it was negatively correlated with serum interleukin-2 receptor levels. Immunohistochemistry staining showed that KPRP was detected in upper part of granular layer of epidermis in normal skin and in early-stage CTCL, but rarely detected in the advanced-stage CTCL.

Conclusion: In conclusion, we revealed the decrease in KPRP expression in CTCL.

KEYWORDS
CTCL, filaggrin, KPRP, loricrin, Th2

1 | INTRODUCTION

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of extra-nodal non-Hodgkin's lymphomas. Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common types of CTCL.[1] Many cases with CTCL, especially at an advanced stage, have skin barrier dysfunction, accompanied with pruritus, dry skin, and increased susceptibility to bacterial or viral infections, and show a T helper 2 (Th2)-dominant phenotype, characterized by increased interleukin (IL)-4, IL-5, IL-10, and IL-13 production.[2,3] Filaggrin and loricrin, representative barrier function proteins, are decreased in CTCL.[4] The expression levels of these proteins were negatively correlated to those of IL-4, thymus, and activation-regulated chemokine (TARC) expression in lesional skin, and serum soluble IL-2 receptor (sIL-2R).

It is also revealed that IL-4 and IL-13 suppress the gene expression of filaggrin, loricrin, and involucrin in normal human epithelial keratinocytes.[5,6] Collectively, Th2-dominant microenvironment in CTCL can induce decrease in barrier function molecules such as filaggrin, loricrin, and involucrin. The decrease in barrier function molecule was originally investigated in atopic dermatitis (AD). In AD patients, multiple factors, including immune dysregulation, gene mutation of barrier function proteins, deficiency of antimicrobial peptides, and skin dysbiosis, contribute to skin barrier defects.[7] CTCL and AD share these similar features, and thus, it is sometimes difficult to distinguish CTCL from AD.[8]

Keratinocyte proline-rich protein (KPRP) was first described as a new keratinocyte-specific protein with many similarities to cornified envelope proteins.[9] Human KPRP was also identified in 2005.[10] KPRP is decreased in AD patients in general, and its deficiency leads to barrier dysfunction.[11] In this article, we examined KPRP expression in CTCL and analyzed the relationship of KPRP expression to filaggrin and loricrin expression, and serum sIL-2R levels. We also performed immunohistochemistry of KPRP in CTCL and in normal skin samples.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

Ninety-two biopsy specimens were collected in our department from January 2012 to May 2020. Skin samples from cases of MF (n = 64), SS (n = 16), and normal skin adjacent to benign tumor (n = 12) were used for mRNA expression analysis. Serum levels of sIL-2R corresponding to the MF/SS samples were also acquired. Samples for immunohistochemistry were collected from each stage of MF (n = 3 or 4) and normal skin (n = 4). The medical ethics committee of the University of Tokyo approved all described studies, and the study was conducted according to the principles of the Declaration of Helsinki.

2.2 | Quantitative real-time reverse transcriptase-PCR

RNA was obtained from fresh and frozen skin samples with RNeasy Fibrous Tissue Mini Kit (Qiagen). Complementary DNA was synthesized using ReverTra Ace qPCR RT Master Mix (TOYOBO). Then, quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using THUNDERBIRD SYBR qPCR Mix (TOYOBO), according to the manufacture's instruction. Primers for KPRP, filaggrin, loricrin, IL-4, IL-13, GAPDH are as follows:

KPRP: forward, 5′-GCA TCA GGA CCA TGT GTG ACC-3′ and reverse, 5′-GGA CAG GGT ACA TAG TTC TGGA-3′; filaggrin: forward, 5′-GAA GAC AAG GAT CGC ACC AC-3′ and reverse, 5′-ATG GTG TCC TGA CCC TCT TG-3′; loricrin: forward, 5′-TCA TGA TGC TAC CCG AGG TTT G-3′ and reverse, 5′-CAG AAC TAG ATG CAG CCG GAG A-3′; IL-4: forward, 5′-CAC AGG CAC AAG CAG CTG AT-3′ and reverse, 5′- CTC TGG TTG GCT TCC TTC ACA -3′; IL-13: forward, 5′-CGA GAA GAC CCA GAG GAT GCT −3′ and reverse, 5′-GGG CCA CCT CGA TTT TGG -3′; GAPDH: forward, 5′-ACC CAC TCC ACC TTT GA-3′, and reverse, 5′-CAT ACC AGG AAA TGA GCT TGA CAA-3′.

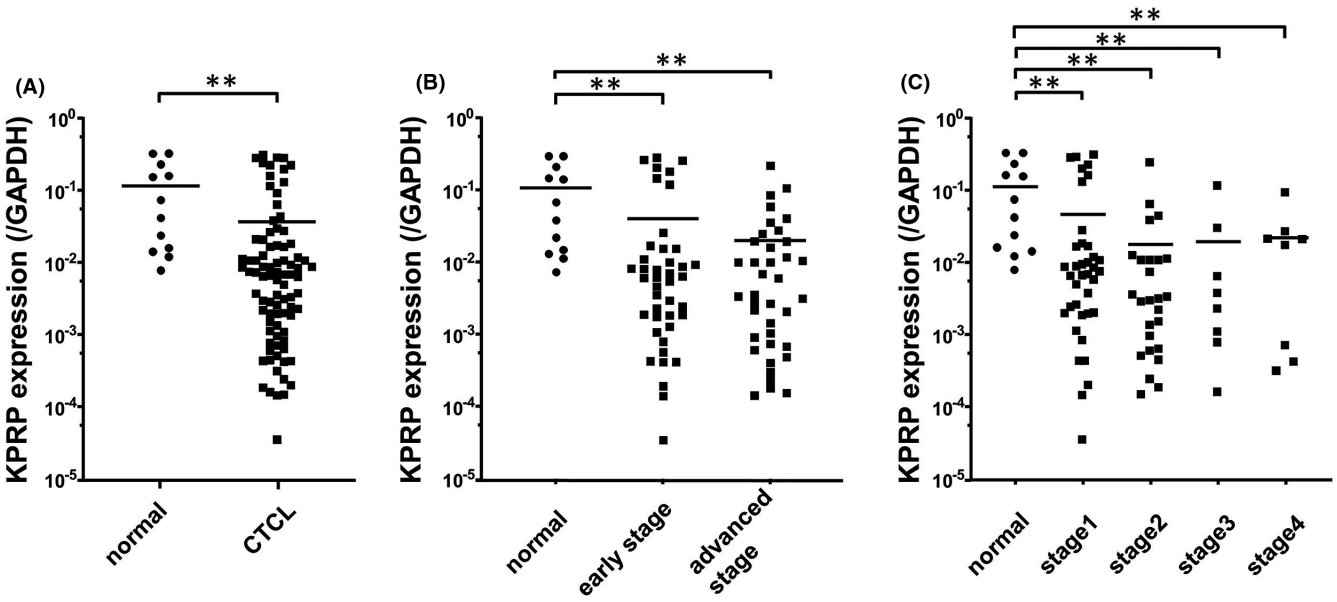

FIGURE 1 mRNA expression of KPRP in CTCL skin samples. (A) KPRP mRNA expression was decreased in CTCL skin samples compared with normal skin. (B, C) The decrease was seen both in early-stage CTCL and in advanced-stage CTCL, or in stages 1, 2, 3, or 4. The measured values from individual patients were plotted by dots. Each bar showed mean. $^{**}p < .01$

All samples were analyzed in parallel for GAPDH gene expression as an internal control. The relative expression levels of each gene were determined by the $2^{-\Delta\Delta CT}$ method.

2.3 | Immunohistochemistry

Skin tissue samples were stained for KPRP. Briefly, 5 μm-thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. These sections were then stained with polyclonal antibody against human KPRP (1:200, ab122201; Abcam), followed by ABC staining (Vector Lab). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer hematoxylin was performed, according to manufactures' instructions.

2.4 | Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test for the two groups, and the significance of the differences for multiple comparisons was evaluated by the Steel test. *p*-value <.05 was considered statistically significant. Correlation coefficients were determined by using Spearman's rank correlation test. $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | The mRNA expression of KPRP in MF was decreased compared with normal skin

The mRNA expression of KPRP was determined with quantitative RT-PCR. First, CTCL skins showed less KPRP expression compared with normal skin (Figure 1A). Next, we revealed that early-stage MF and advanced-stage MF each showed decreased KPRP expression compared with normal skin (Figure 1B). Similarly, when we analyzed KPRP expression according to clinical stage, stage 1, stage 2, stage 3, and stage 4, respectively, showed lower KPRP expression than normal skins (Figure 1C).

3.2 | KPRP mRNA expression was positively correlated to that of filaggrin and loricrin, and negatively correlated to serum sIL-2R level

We next analyzed the expression of filaggrin and loricrin. The expression levels of IL-4 and IL-13 were also determined with RT-PCR. Correlations between KPRP and these proteins were analyzed (Figure 2, upper and middle). The statistically significant correlation between KPRP and filaggrin was observed, and KPRP and loricrin also had positive correlation. However, IL-4 and IL-13 lacked the correlation to KPRP (data not shown). We also found a negative correlation between KPRP and serum sIL-2R levels, which is a disease activity marker of MF (Figure 2, bottom).

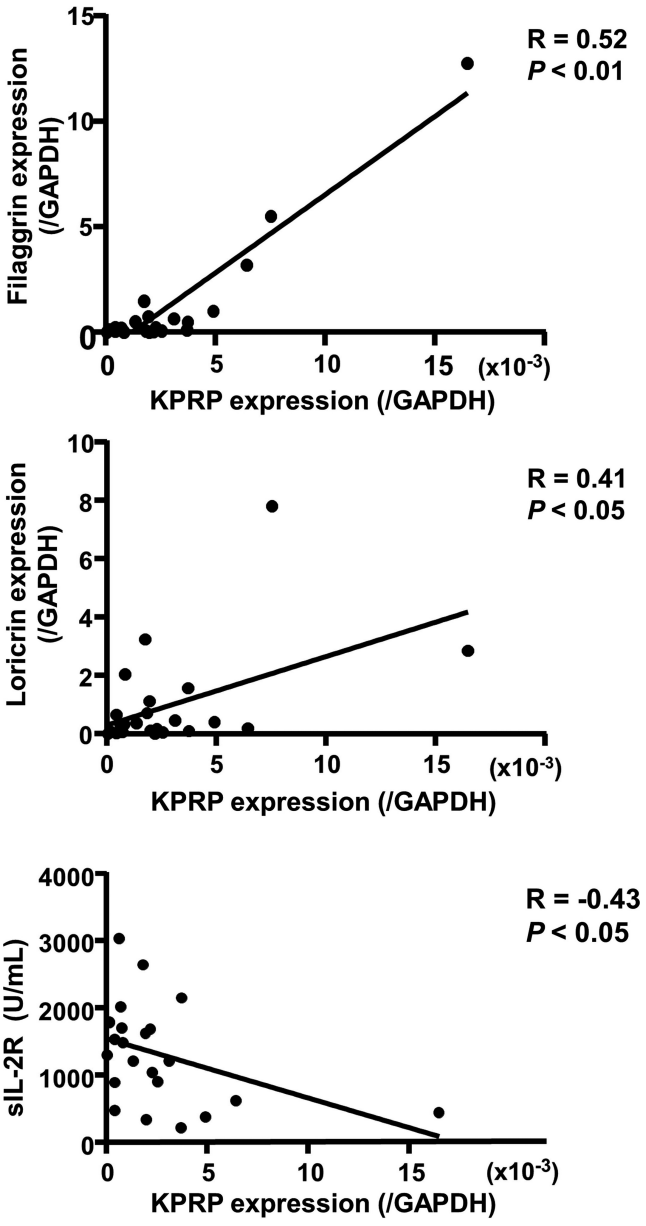

FIGURE 2 Correlations between KRPR and filaggrin, loricrin, and serum sIL-2R levels. Filaggrin and loricrin were positively correlated with KPRP expression. Serum sIL-2R level was negatively correlated with KPRP expression

3.3 | Decreased KPRP expression was observed in MF skin, particularly in advanced-stage MF

Immunohistochemistry of KPRP was performed using lesional skin of patch, plaque, and tumor of MF, SS, and normal skin. KPRP was detected in upper part of granular layer of epidermis in normal skin, patch MF to some measure, and a little in plaque MF, but it was rarely detected in tumor MF or SS skin (Figure 3). This result was generally consistent with mRNA levels quantified by real-time PCR.

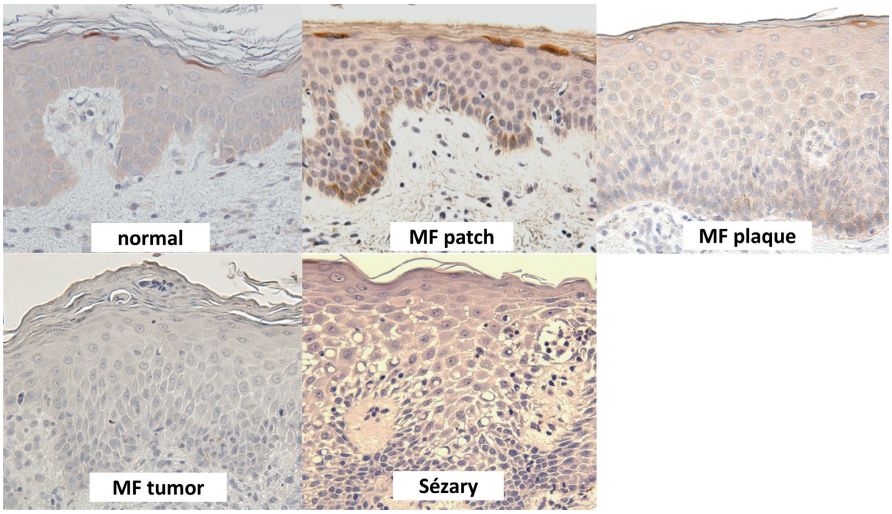

FIGURE 3 Immunohistochemical staining for KPRP. Representative pictures of normal skin, patch MF, and plaque MF are shown on upper panel. Tumor MF and Sézary syndrome are shown in lower panel. Little KPRP staining was seen in tumor MF and Sézary syndrome samples. Scale bar = 50 μm. MF, Mycosis fungoides

4 | DISCUSSION

This study revealed that KPRP expression was decreased in CTCL lesional skin compared with normal skin. Especially, KPRP was decreased in advanced-stage CTCL.

Keratinocyte proline-rich protein is one of barrier-related proteins with many similarities to cornified envelop proteins and is localized to the upper granular layer of epidermis. Its gene is located in the q21 region of chromosome 1, close to *filaggrin*, *involucrin*, *loricrin*, and S100 family.[9,10] KPRP is decreased in AD patients and its deficiency leads to barrier dysfunction.[11] In AD, KPRP expression is correlated to loricrin expression, and it also tends to correlate to filaggrin expression though no statistical significance is seen.[11] A single nucleotide polymorphism in human *KPRP* gene is detected in AD patients, which contributes AD development.[11] Likewise, gene deficiency in AD patients was first reported for *filaggrin*, and now, some barrier-related genes are discovered as sensitive genes for AD. However, environmental factors and epigenetic modulation are also important to AD development. As noted above, Th2 cytokine downregulates barrier-related molecules resulting in further inflammation. Also, it is known that many of CTCL patients show Th2 cytokine dominant phenotype. IL-4 is reported to be abundantly expressed by lesional skin of CTCL.[12] Therefore, although no gene deficiency of barrier-related protein in CTCL is reported, these findings suggest that KPRP would be also decreased in CTCL due to downregulation by IL-4 and IL-13, just like filaggrin or loricrin. However, microenvironment of skin lesions in AD and in CTCL may not be totally the same. Some cornified envelop protein expression profile in CTCL is reportedly different from that in AD.[13] In CTCL, our study showed that KPRP and filaggrin, as well as KPRP and loricrin, had statistically significant positive correlations. These results were partially compatible with our previous results regarding KPRP in AD patients.[11]

In addition, we also showed negative correlations between KPRP expression and serum sIL-2R levels. Filaggrin and loricrin are downregulated in advanced-stage CTCL.[4] Since patients with advanced-stage CTCL tend to have high sIL-2R levels, our findings are compatible with previous findings.[14] We also analyzed the correlations between KPRP and IL-4 and IL-13, but they did not reach statistical significance probably because of sample sizes.

5 | CONCLUSION

In conclusion, we showed that KPRP expression was decreased in CTCL as well as in AD skin, especially in advanced-stage of CTCL. This may be one cause of skin barrier deficiency in CTCL.

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None.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare. Dr. Shinichi Sato is a member of the Journal of Cutaneous Immunology and Allergy Editorial Board. Management of the peer review process, and all editorial decision making, for this article, was undertaken by Editor in Chief.

DECLARATION SECTION

Approval of the research protocol: 0695-17.
Informed consent: All the patients provided informed written consent to participate in this study.
Registry and the Registration No. of the study/trial: N/A.
Animal Studies: N/A.

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REFERENCES

1. Girardi M, Heald PW, Wilson LD. The pathogenesis of mycosis fungoides. N Engl J Med. 2004;350:1978–88.
2. Vowels BR, Lessin SR, Cassin M, Jaworsky C, Benoit B, Wolfe JT, et al. Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. J Invest Dermatol. 1994;103:669–73.

3. Geskin LJ, Viragova S, Stolz DB, Fuschiotti P. Interleukin-13 is overexpressed in cutaneous T-cell lymphoma cells and regulates their proliferation. Blood. 2015;125:2798–805.
4. Suga H, Sugaya M, Miyagaki T, Ohmatsu H, Kawaguchi M, Takahashi N, et al. Skin barrier dysfunction and low antimicrobial peptide expression in cutaneous T-cell lymphoma. Clin Cancer Res. 2014;20:4339–48.
5. Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. Clin Immunol. 2008;126:332–7.
6. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2009;124:R7–12.
7. Furue M. Regulation of Filaggrin, Loricrin, and Involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: pathogenic implications in atopic dermatitis. Int J Mol Sci. 2020;21:5382.
8. Miyagaki T, Sugaya M. Erythrodermic cutaneous T-cell lymphoma: how to differentiate this rare disease from atopic dermatitis. J Dermatol Sci. 2011;64:1–6.
9. Kong W, Longaker MT, Lorenz HP. Molecular cloning and expression of keratinocyte proline-rich protein, a novel squamous epithelial marker isolated during skin development. J Biol Chem. 2003;278:22781–6.
10. Lee WH, Jang S, Lee JS, Lee Y, Seo EY, You KH, et al. Molecular cloning and expression of human keratinocyte proline-rich protein (hKPRP), an epidermal marker isolated from calcium-induced differentiating keratinocytes. J Invest Dermatol. 2005;125:995–1000.
11. Suga H, Oka T, Sugaya M, Sato Y, Ishii T, Nishida H, et al. Keratinocyte Proline-rich protein deficiency in atopic dermatitis leads to barrier disruption. J Invest Dermatol. 2019;139:1867–75.
12. Miyagaki T, Sugaya M, Fujita H, Ohmatsu H, Kakinuma T, Kadono T, et al. Eotaxins and CCR3 interaction regulates the Th2 environment of cutaneous T-cell lymphoma. J Invest Dermatol. 2010;130:2304–11.
13. Trzeciak M, Olszewska B, Sakowicz-Burkiewicz M, Sokołowska-Wojdyło M, Jankau J, Nowicki RJ, et al. Expression profiles of genes encoding Cornified envelope proteins in atopic dermatitis and cutaneous T-cell lymphomas. Nutrients. 2020;12:862.
14. Neish C, Charlry M, Jegasothy B, Tharp M, Deng JS. Proliferation cell nuclear antigen and soluble interleukin 2 receptor levels in cutaneous T cell lymphoma: correlation with advanced clinical diseases. J Dermatol Sci. 1994;8:11–7.

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