Terminal differentiation of keratinocytes was damaged in type 2 diabetic mice

Takeshi Takayanagi1 · Hiroyuki Hirai1 · Yohei Asada1 · Takaaki Yamada2 · Seiji Hasegawa2 · Eisuke Tomatsu1 · Yoshiteru Maeda1 · Yasumasa Yoshino1 · Izumi Hiratsuka1 · Sahoko Sekiguchi-Ueda1 · Megumi Shibata1 · Yusuke Seino1 · Yoshihisa Sugimura1 · Hirohiko Akamatsu3 · Mitsuyasu Itoh1 · Atsushi Suzuki1

Received: 11 July 2021 / Accepted: 11 March 2022 / Published online: 26 March 2022 © The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Aims Although skin manifestations are common in diabetic patients, its characteristics are poorly identified. This study explored the differentiation process of keratinocytes in type 2 diabetes mellitus (T2DM) in vivo.

Methods Back skin of T2DM model KKAy/TaJcl mice (KKAy) and C57BL/6JJcl mice (control) aged 8 and 12 weeks was used. The mRNA expression of differentiation markers of keratinocytes was measured by quantitative real-time polymerase chain reaction (qRT-PCR). The expression of each marker in situ was examined immunohistochemically.

Results KKAy mice showed hyperglycemia versus control mice. The histological findings showed increased thickness and structural impairment of epidermal tissue in KKAy mice. The qRT-PCR revealed that the expression of integrin beta 1 and keratin 14 in KKAy and control mice was identical. However, the expression of involucrin at 8 weeks, keratin 10 at 12 weeks, and filaggrin and loricrin at 8 and 12 weeks was decreased in KKAy mice. Immunohistochemical findings showed that filaggrin was markedly decreased in KKAy mice, though Ki-67 remained unchanged.

Conclusion The terminal differentiation process was impaired in the diabetic skin, while keratinocyte proliferation was preserved. Damaged terminal differentiation of keratinocytes may contribute to impairment of the skin barrier function in diabetic dermatoses.

Keywords Diabetes mellitus · Diabetic complications · Diabetic skin · Keratinocytes · Filaggrin

Introduction

Diabetes mellitus is the most common metabolic disease with severe impact on health because of major complications involving the kidneys, retinas, peripheral nerves, and atherosclerotic vascular diseases [1, 2]. It is established that skin complications also frequently develop in patients with diabetes. Approximately one-third of all patients with diabetes have some cutaneous manifestations in the course of the disease [14]. Noninfectious as well as infectious diseases have been described as diabetic skin manifestations of diabetes mellitus, including diabetic thick skin, diabetic dermopathy, calciphylaxis, and acanthosis nigricans. Moreover, skin injuries in patients with diabetes often worsen, especially when complicated with neuropathy and peripheral arterial disease. The susceptibility to skin infection in diabetes increases the possibility of hospitalization and could lead to limb amputation. However, the mechanisms of diabetic skin manifestations remain to be elucidated. Coincident with a variety of skin lesions in diabetes, the results of previous studies were controversial. Some studies showed that the epidermis of non-wounded diabetic skin becomes thinner than normal skin, whereas others showed thicker epidermis in patients with diabetes [3, 8, 20, 22, 25].

Regarding the outer skin barrier formed by keratinocytes, two obese mouse models demonstrated that the number of keratinocytes is reduced, and the epidermal structure is altered [25]. In another study, the gene expression of
keratin-associated proteins and keratin complexes at baseline was increased in diabetic mice; however, these levels decreased after injury [21]. While still a matter of debate, these reports agree at least that proliferation/differentiation derangement underlies the mechanism of diabetic skin manifestations.

The epidermis, which is the outermost layer of the skin, is separated from the underlying dermis by the basement membrane. The chief cellular component of the epidermis are keratinocytes, which proliferate within the basal cell layer, progress upwards, differentiate through different epidermal layers (i.e., the spinous layer, granular layer, and cornified layer or stratum corneum), become anucleated, and finally desquamate from the skin surface. Each stage of epidermal differentiation is characterized by the expression of specific proteins, such as integrin beta 1 (Itgb1) (basal keratinocyte marker), keratin 14 (Krt14) (basal layer marker), and involucrin (Ivl) and keratin 10 (Krt10) (spinous layer markers) [23]. Advancing knowledge on the development of the skin yielded information regarding new molecular markers, such as filaggrin (Flg) (granular and cornified layer marker) and loricrin (Lor) (cornified layer marker), which are differentiation markers and play an important role in skin barrier function [4, 27]. In the present study, we histologically investigated the non-wounded diabetic skin of model mice with type 2 diabetes (T2DM) using these molecular markers. Understanding this mechanism will help in determining molecular targets for the prevention and treatment of skin injuries in diabetes mellitus.

Materials and methods

Animals and experimental design

Male T2DM model KKAY mice and their control mice (aged 4 weeks) were purchased from CLEA Japan, Inc. (Tokyo, Japan). All mice were housed at 24 °C under a 12-h light/dark cycle, maintained with ad libitum access to standard pelleted rodent chow and tap water. KKAY mice and control mice were used in experiments at 8 and 12 weeks of age. After anesthesia using isoflurane (DS Pharma Animal Health Co., Osaka, Japan), we collected blood samples from the orbital sinus. The blood samples were analyzed at an external laboratory (SRL.Inc., Tokyo, Japan). Following blood collection, we acquired full-thickness shaved skin (size: ~2 × 2 cm) from the back of each mouse. The skin samples were divided into two parts: one for histological and immunohistochemical analysis, and another for quantitative real-time polymerase chain reaction (qRT-PCR). All animal procedures and the protocol were approved by the institutional animal care and use committee and the government authorities.

Histology

The acquired shaved skin was fixed in 4% paraformaldehyde solution for 24 h. Subsequently, paraffin-embedded skin samples were cut into slices (thickness: 4 μm) using a microtome (Sakura Finetek Japan, Tokyo, Japan). The sections were stained with hematoxylin and eosin and observed through a phase-contrast microscope (Olympus BX51, Olympus Corp., Tokyo, Japan). Epidermal thickness was measured using the acquired images.

Immunohistochemistry

Paraffin-embedded sections were deparaffinized and treated with protease K (Dako Denmark A/S, Glostrup, Denmark) for antigen retrieval. The sections were immunostained with anti-Flg rabbit IgG (1:400, ab24584, Abcam, Cambridge, MA, USA) and anti-Itgb1 rat IgG (1:200, MAB1997, Millipore, Billerica, MA, USA), and probed with Alexa Fluor® 488-labeled anti-rabbit IgG (1:500, A32790, Life Technologies, Carlsbad, CA, USA) and Alexa Fluor® 594-labeled anti-rat IgG (A21209, 1:500) secondary antibodies. Alternatively, they were incubated with anti-Ivl goat IgG (1:200, SC-15227, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-Itgb1 rat IgG (1:200) and probed with Alexa Fluor® 488-labeled anti-goat IgG (A11055, 1:500) and Alexa Fluor® 594-labeled anti-mouse IgG (A21203, 1:500) secondary antibodies. Anti-Ki-67 rabbit IgG (1:200, RM-9106, Thermo Fisher Scientific, Waltham, MA, USA) and Alexa Fluor® 488-labeled anti-goat IgG (1:500) secondary antibodies were used to detect Ki-67. 4′,6-Diamidino-2-phenylindole (VECTASHIELD H-1200; Vector Laboratories, Burlingame, CA, USA) was used for nuclear staining. Stained sections were observed using a fluorescence microscope (Olympus IX71, Olympus Corp., Tokyo, Japan). For the expression level of Flg, the average fluorescence intensities of all epidermal layers were measured using an image analysis software ImageJ [24].

qRT-PCR

Skin samples were frozen in liquid nitrogen and stored at -80°C until RNA extraction. Total RNA was isolated from skin samples (size: 1 × 2 cm) using the TRizol Reagent (Invitrogen Co., Tokyo, Japan) and homogenized through TOMY Micro Smash MS-100 (TOMY SEIKO Co., Ltd., Tokyo, Japan).

cDNA was synthesized from 1 μg total RNA using a PrimeScript™ RT Master Mix (Takara Bio Inc., Kusatsu, Japan) according to the protocol provided by the manufacturer. Subsequently, the cDNA was subjected to qRT-PCR.
using the ABI PRISM7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). We used the Platinum™ SYBR™ Green qPCR SuperMix-UDG (Invitrogen Co, Tokyo, Japan) in this reaction. Relative quantification was calculated by the comparative CT method, and we evaluated the following six markers: Itgb1, Krt14, Iv1, Krt10, Flg, and Lor. PCR was performed at 95°C for 15 s and 60°C for 60 s (40 cycles). The amplification of these markers was compared with that of a housekeeping gene encoding 18S (Rn18s). The set of primers for mouse Itgb1, Krt14, Iv1, Krt10, Flg, Lor, and Rn18s were prepared as shown in Table 1.

Statistical analysis

We analyzed the results with the Welch test. For all analyses, p < 0.05 denoted statistically significant differences and all data are expressed as mean ± standard deviation. All statistical analyses were performed using the R software version 3.0.2 (http://cran.r-project.org/).

Results

Diabetic KKAy mice showed significantly higher blood glucose concentration compared with age-matched control mice: 118.0 ± 28.3 mg/dL in diabetic mice vs. 81.7 ± 23.8 mg/dL in control mice at 8 weeks (p = 0.03), and 176.4 ± 85.8 mg/dL in diabetic mice vs. 114.4 ± 81.0 mg/dL in control mice at 12 weeks (p = 0.01). These results were consistent with those of previous reports on the characteristic features of KKAy mice [13].

Histological analysis

The histological findings showed significant thickening and structural impairment of epidermal tissue in KKAy mice at 12 weeks (Fig. 1). Basal cells were regularly aligned in the basal layer in both KKAy and control mice. Increased layers of the stratum corneum were found in KKAy mice. There was no infiltration of inflammatory cells or increase in capillary numbers. These findings indicate that diabetic skin was impaired either in the proliferation process or differentiation process.

mRNA expression of differentiation markers

We evaluated the mRNA expression of differentiation markers representative of epidermal layers to examine the impaired process. The qRT-PCR documented that the expression of Itgb1 and Krt14 in diabetic mice was not significantly different from that of control mice. However, the mRNA expression of Iv1 (8 weeks of age), Krt10 (12 weeks of age), and Flg and Lor (8 and 12 weeks of age) in diabetic mice was significantly decreased compared with that measured in the control mice (Fig. 2).

Immunohistochemistry

Immunohistochemistry showed that Flg staining was coarse and sparse, which suggested the defective cornified envelope formation. However, the epidermal thickness of the Flg-stained area was increased in KKAy mice versus control mice. In contrast, Itgb1 and Ki-67 staining was not different between these mice (Figs. 3, 4).

Discussion

Diabetes mellitus is complicated by a variety of cutaneous manifestations, including xerosis and foot ulcers [14]. Nevertheless, the precise mechanisms through which pathological and physiological changes in dermal tissues develop have not yet been defined. In the present study, we showed that the differentiation process of keratinocytes in T2DM model KKAy mice was impaired; however, the proliferation process of basal keratinocyte in these mice was normal.

We used KKAy mice, which were developed from KK mice by introducing the lethal yellow obese gene (Ay). The heterozygous KKAy mice become severely obese, hyperglycemic, and hyperinsulinemic at approximately the age of 8 weeks. The KKAy mouse is regarded as a suitable
model for exploring the mechanisms of obesity-induced T2DM [7]. The KKAy mice in our study showed T2DM phenotypes, including hyperglycemia, hypertriglyceridemia, hypercholesteremia, and elevated liver enzymes. The histological findings revealed that the epidermal thickness was increased in KKAy mice versus control mice, especially in the granular cell layer. It has been reported that aged skin was associated with thinning of the epidermis, decreased proliferation, and increased apoptosis below granular layer [12]. We used the mice at age 8 and 12 weeks, as aging process could modify the effect of metabolic disorder in our mouse model.

Our experiments demonstrated a reduced expression of Flg and Lor, which are the main components of the epidermal differentiation complex. Especially Flg is a critically important, multifunctional protein required for the normal biogenesis and physiology of the stratum corneum [23]. Flg is a base, histidine-rich, and insoluble protein with molecular mass of 35–37 kDa. The name refers to its ability to aggregate keratin filaments (Flg = filament aggregation protein). Flg is produced from profilaggrin, a precursor included in keratohyalin granules in the granular layer. Together with keratin, they constitute approximately 80–90% of protein ingredients in the epidermis. Flg is responsible for the proper
formation of the cornified layer in the skin, which protects from loss of moisture and forms a barrier for allergens, toxins, and microbes [6]. Clinically, the risk of skin infections in patients with diabetes mellitus increases [16]. Although diabetic neuropathy and angiopathy play an important role in infection, abrogation of the skin barrier function can increase susceptibility to skin infection. In fact, Flg mutations are associated with recurrent skin infections in patients with atopic dermatitis [5].

Optimum healing of a cutaneous wound is impaired in diabetes mellitus. The healing process of a wound consists of a complex coordination: formation of granulation tissues, differentiation of keratinocytes, and epithelialization [10]. Immunohistological findings suggest that the thickness of the granular cell layer increased; however, the intensity of the immunofluorescence of Flg staining was weak and sparse in our diabetic model mouse. These results indicate that the keratinocyte differentiation process and epithelial cornification were retarded in diabetic model mouse, and the mechanism was pivotal in delayed wound healing of diabetic skin. Interestingly, Ki-67 staining showed that basal keratinocyte proliferation was normal in this study. Based on the recent progress in cell-based regenerative medicine, the use of stem cells carries great potential for wound healing. Many types of adult stem cells have been used in clinical trials for wound healing; however, none have been approved for clinical use thus far [9]. The development of diabetes-induced impairment of glucose metabolism and insulin resistance in the epidermis damages keratinocyte differentiation, which could lower the success rate of stem cell transplantation. For the successful application of cell-based therapy, it is important to restore the impaired keratinocyte differentiation.

Concerning the thickness of epidermis, several researchers showed the opposite conclusion. Type 1 diabetic model rats induced by streptozotocin showed decreased thickness and infiltration of inflammatory cells [8], and type 1 diabetic model mouse induced by the same procedure showed a decrease in number of epidermal cells, impaired skin barrier function, and decreased cell proliferation [19]. As to obese T2DM model mice, dysfunctional γ6 T cells contributed to the impaired keratinocyte homeostasis and decreased in thickness of epidermis [25]. In the present study, we found increased layers of stratum corneum while mRNA of differentiation markers decreased in the skin of KKAy mice. In MafB-deficient mice, epidermal differentiation was partially impaired and the cornified layer was thinner than in wild-type mice [15]. On the contrary, cyclooxygenase-2 (COX-2) transgenic mice showed disturbed epidermal differentiation accompanied by hyperplasia in the scale epidermis, reflecting both an increase of cell number and hyperkeratosis [18]. Lipid peroxidation derivative increased in the skin of KKAy obese mice [17], and lipid peroxidation could induce the expression of COX-2 in several types of cells [26]. In the Cox2 transgenic mice, there was a strong hyperproliferative epidermis with Ki67 overexpression in supra basal layers, while in wild type epidermis, proliferating cells were located almost exclusively in the basal layer [18]. The total number of proliferating cells in epidermis of Cox2 transgenic mice was comparable to that of wild type. They suggested that hyperkeratosis of transgenic scale epidermis resulted from a decelerated desquamation of cornified cells rather than an accelerated cornification. The thickening of stratum corneum in KKAy mice could also result from the delayed desquamation. Therefore, it might be possible that our findings are, at least in part, due to increase of COX-2 expression by lipid peroxidation in KKAy mice. Further investigation would be required to explore precise mechanism of impairment of epidermal differentiation in diabetes.

![Fig. 2](https://example.com/fig2.png)

Relative mRNA expression of epidermal layer markers in control (white bar) and KKAy (black bar) mice at 8 and 12 weeks of age, analyzed by quantitative real-time polymerase chain reaction. **a** Integrin beta 1 (Itgb1) (basal keratinocyte marker), **b** keratin 14 (Krt14) (basal layer marker), **c** keratin10 (Krt10) and **d** involucrin (Ivl) (spinous layer markers), **e** loricrin (Lor) and **f** filaggrin (Flg) (granular layer markers) (*P<0.05). Data represent means±S.D. of six animals.
Our diabetic mouse model, an obesity-based T2DM model, showed thicker epidermis in this study. T2DM is genetically heterogeneous and diabetic skin complications are multifactorial. Our model appears valuable for evaluating the skin-thickening type of diabetic complications (e.g., diabetic thick skin, diabetic hand syndrome, and diabetic scleroderma) [11].

There were several limitations in this study. Firstly, we used only one type of diabetic model mice (KKAy mice) at 8 and 12 weeks old. This mouse model is an obesity-induced T2DM model with polygenic background. Hence, the skin manifestations are modified by hyperglycemia and other clinical features, such as dyslipidemia. These comorbidities are common in T2DM and medical treatment of these metabolic disorders will clarify the respective influence on skin manifestations. Nevertheless, we should pay attention to the possible involvement of other factors affecting the skin barrier function. Secondly, we cannot exclude the involvement of other types of cells, such as T lymphocytes, in the histological changes observed in this study. Thirdly, although we demonstrated the natural course of skin damage in diabetic model mice, we did not show the efficacy of treatment against diabetes on the differentiation process in this model. Fourthly, we could not measure skin barrier function directly by such as transdermal water loss test, because their body

---

**Fig. 3** Proliferation of basal cells in the epidermis of control (white bar) and KKAy (black bar) mice at 8 and 12 weeks of age, assessed by immunohistochemical staining of Ki-67. a Immunohistochemical staining of Ki-67 (green) and 4',6-diamidino-2-phenylindole stain (blue). b The percentage of Ki-67-positive cells in the basal cell layer of the epidermis. Data represent means ± S.D. of six animals.
hair disturbed the precise measurement without damage by shaving. Fifthly, we could not clarify the mechanism of impairment of differentiation process including possible involvement of Cox2 in this paper.

In conclusion, our results suggest that the terminal differentiation process was impaired in the diabetic skin, while keratinocyte proliferation was preserved. The expression of important differentiation markers (i.e., Flg and Lor) was decreased in diabetic skin. Therefore, intervention which modulates the differentiation of keratinocytes can be a therapeutic target for diabetic skin complications.

**Fig. 4** Structural comparison of the epidermal layers of control and KKAy mice, assessed by immunohistochemical staining of filaggrin (Flg) and integrin beta 1 (Itgb1) at 8 and 12 weeks of age. For the expression level of Flg, the average fluorescence intensities of all epidermal layers of control (white bar) and KKAy (black bar) were measured using an image analysis software Image J (*P < 0.05, **P < 0.01). Data represent means ± S.D. of six animals. Flg (granular layer marker) (green), Itgb1 (basal keratinocyte marker) (red), and 4',6-diamidino-2-phenylindole (blue) for nuclear staining.

**Acknowledgements** We give special thanks to Sayaka Nomura for excellent technical assistance. This study was supported by grants-in-aid from Fujita Health University.

**Funding** This study was supported by grants-in-aid from Fujita Health University to AS.

**Data availability** Not applicable.

**Code availability** Not applicable.
Declarations

Conflict of interest TY and SH are employees of Nippon Menard Cosmetics Co., Ltd. Other authors do not have any financial or non-financial conflict of interest.

Ethical approval The mice were handled ethically according to the Regulations for the Management of Laboratory Animals at Fujita Health University. The experimental protocol for the ethical use of these animals was approved by the Animal Care and Use Committee at Fujita Health University (Permit No.: M0271).

Consent to participate All the authors have made a significant contribution to this manuscript, have seen and approved the final manuscript.

Consent for publication All the authors have agreed to its submission to the “Molecular Biology Reports”.

References

1. American Diabetes Association (2019) Cardiovascular disease and risk management: standards of medical care in diabetes-2019. Diabetes Care 42:S103–S123. https://doi.org/10.2337/dc19S010

2. American Diabetes Association (2019) Microvascular complications and foot care: standards of medical care in diabetes-2019. Diabetes Care 42:S124–S138. https://doi.org/10.2337/dc19-s011

3. Bertheim U, Engström-Laurent A, Hofer PÅ et al (2002) Loss of hyaluronan in the basement membrane zone of the skin correlates to the degree of stiff hands in diabetic patients. Acta Derm Venereol 82:329–334. https://doi.org/10.1080/000155502320624041

4. Brown SJ, McLean WHI (2012) One remarkable molecule: Filaggrin. J Invest Dermatol 132:751–762. https://doi.org/10.1038/jid.2011.393

5. Cai SCS, Chen H, Koh WP et al (2012) Filaggrin mutations are associated with recurrent skin infection in Singaporean Chinese patients with atopic dermatitis. Br J Dermatol 166:200–203. https://doi.org/10.1111/j.1365-2133.2011.10541.x

6. Candi E, Schmidt R, Melino G (2005) The confined envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6:328–340. https://doi.org/10.1038/nrm1619

7. Chatzigieorgiou A, Halapas A, Kalafatakis K, Kamper EF (2009) The use of animal models in the study of diabetes mellitus. In Vivo (Brooklyn) 23:245–258

8. Chen X, Lin W, Lu S et al (2010) Mechanistic study of endogenous skin lesions in diabetic rats. Exp Dermatol 19:1088–1095. https://doi.org/10.1111/j.1600-0625.2010.01137.x

9. Dash BC, Xu Z, Lin L et al (2018) Stem cells and engineered scaffolds for regenerative wound healing. Bioengineering 5:1–19. https://doi.org/10.3390/bioengineering5010023

10. Falanga V (2005) Wound healing and its impairment in the diabetic foot. Lancet 366:1736–1743. https://doi.org/10.1016/S0140-6736(05)67700-8

11. Ferringer T, Miller III F (2002) Cutaneous manifestations of diabetes mellitus. Dermatol Clin 20:483–492. https://doi.org/10.1016/s0733-8635(02)00018-9

12. Gilhar A, Ullmann Y, Karry R et al (2004) Ageing in human epidermis: the role of apoptosis, Fas and telomerase. Br J Dermatol 150:56–63. https://doi.org/10.1111/j.1365-2133.2004.05715.x

13. Li C, Hou S, Liu S et al (2017) The albumin-exendin-4 recombinant protein E2HSA improves glycemic control and β-cell function in spontaneous diabetic KKAY mice. BMC Pharmacol Toxicol 18:1–9. https://doi.org/10.1186/s40360-017-0143-8

14. Lima AL, Illing T, Schlieemann S, Elsnner P (2017) Cutaneous manifestations of diabetes mellitus: a review. Am J Clin Dermatol 18:541–553. https://doi.org/10.1007/s40257-017-0275-z

15. Miyai M, Hamada M, Moriguchi T et al (2016) Transcription factor MafB coordinates epidermal keratinocyte differentiation. J Invest Dermatol 136:1848–1857. https://doi.org/10.1016/j.jid.2016.05.088

16. Muller LMAJ, Gorter KJ, Hak E et al (2005) Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 41:281–288. https://doi.org/10.1086/431587

17. Nakai K, Yoneda K, Ishihara Y et al (2011) Lipid peroxidation-induced VEGF expression in the skin of KKAY obese mice. Exp Dermatol 20:388–393. https://doi.org/10.1111/j.1600-0625.2010.01223.x

18. Neufang G, Furstenberger G, Heidt M et al (2001) Abnormal differentiation of epidermis in transgenic mice constitutively expressing cyclo-oxygenase-2 in skin. Proc Natl Acad Sci U S A 98:7629–7634. https://doi.org/10.1073/pnas.121574098

19. Okano J, Kojima H, Katagi M et al (2016) Hyperglycemia induces skin barrier dysfunctions with impairment of epithelial integrity in non-wounded skin of type 1 diabetic mice. PLoS ONE 11:1–22. https://doi.org/10.1371/journal.pone.0166215

20. Park HY, Kim JH, Jung M et al (2011) A long-standing hyperglycaemic condition impairs skin barrier by accelerating skin ageing process. Exp Dermatol 20:969–974. https://doi.org/10.1111/j.1600-0625.2011.01364.x

21. Rodgers KE, Ellefson DD, Espinoza T et al (2006) Expression of intracellular filament, collagen, and collagenase genes in diabetic and normal skin after injury. Wound Repair Regen 14:298–305. https://doi.org/10.1111/j.1743-6109.2006.00124.x

22. Sakai S, Endo Y, Ozawa N et al (2003) Characteristics of the epidermis and stratum corneum of hairless mice with experimentally induced diabetes mellitus. J Invest Dermatol 120:79–85. https://doi.org/10.1046/j.1523-1747.2003.12006.x

23. Sandilands A, Sutherland C, Irvine AD, McLean WHI (2009) Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 122:1285–1294. https://doi.org/10.1242/jcs.033969

24. Schneider CA, Rashand WS, Elliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675. https://doi.org/10.1038/nmeth.2089

25. Taylor KR, Costanzo AE, Jameson JM (2011) Dysfunctional γ6 T cells contribute to impaired keratinocyte homeostasis in mouse models of obesity. J Invest Dermatol 131:2409–2418. https://doi.org/10.1038/jid.2011.241

26. Uchida K (2017) HNE as an inducer of COX-2. Free Radic Biol Med 111:169–172. https://doi.org/10.1016/j.freeradbiomed.2017.02.004

27. Yoneda K, Nakagawa T, Lawrence OT et al (2012) Interaction of proflaigin N-terminal domain with loricrin in human cultured keratinocytes and epidermis. J Invest Dermatol 132:1206–1214. https://doi.org/10.1038/jid.2011.460

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.