Terminal Differentiation of Keratinocytes Was Damaged In Type 2 Diabetic Mice

Takeshi Takayanagi

Fujita Health University: Fujita Ika Daigaku

Hiroyuki Hirai

Fujita Health University: Fujita Ika Daigaku

Yohei Asada

Fujita Health University: Fujita Ika Daigaku

Takaaki Yamada

Nippon Menard Cosmetics, Research Laboratories

Seiji Hasegawa

Nippon Menard Cosmetics, Research Laboratories

Eisuke Tomatsu

Fujita Health University: Fujita Ika Daigaku

Yoshiteru Maeda

Fujita Health University: Fujita Ika Daigaku

Yasumasa Yoshino

Fujita Health University: Fujita Ika Daigaku

Izumi Hiratsuka

Fujita Health University: Fujita Ika Daigaku

Sahoko Sekiguchi-Ueda

Fujita Health University: Fujita Ika Daigaku

Megumi Shibata

Fujita Health University: Fujita Ika Daigaku

Yusuke Seino

Fujita Health University: Fujita Ika Daigaku

Yoshihisa Sugimura

Fujita Health University: Fujita Ika Daigaku

Hirohiko Akamatsu

Fujita Health University: Fujita Ika Daigaku

Mitsuyasu Itoh

Fujita Health University: Fujita Ika Daigaku

Atsushi Suzuki (✉ aslapin@fujita-hu.ac.jp)

Fujita Health University: Fujita Ika Daigaku <https://orcid.org/0000-0003-0807-199X>

Research Article

Keywords: diabetes mellitus, diabetic complications, diabetic skin, keratinocytes, filaggrin

Posted Date: July 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-706566/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Molecular Biology Reports on March 26th, 2022. See the published version at <https://doi.org/10.1007/s11033-022-07367-4>.

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 w, keratin 10 at 12 w, and filaggrin and loricrin at 8 and 12 w was decreased in KKAY mice. Immunohistochemical findings showed that filaggrin was markedly decreased in KKAY mice, though Ki-67 remained unchanged in control mice.

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.

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 [13]. 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 [18][8][20][16][3] .

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 [20]. 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 [17]. 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 prickle 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) (stem cell marker), keratin 14 (Krt14) (basal layer marker), and involucrin (Ivl) and keratin 10 (Krt10) (prickle layer markers) [19]. 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][21]. 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 hr. 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 proteinase K (Dako Denmark A/S, Glostrup, Denmark) for antigen retrieval. The sections were immunostained with anti-Flg rabbit IgG (1:400, Abcam, Cambridge, MA, USA) and anti-Itgb1 rat IgG (1:200, Millipore, Billerica, MA, USA), and probed with Alexa Fluor® 488-labeled anti-rabbit IgG (1:500, Life Technologies, Carlsbad, CA, USA) and Alexa Fluor® 594-labeled anti-rat IgG (1:500) secondary antibodies. Alternatively, they were incubated with anti-lvl goat IgG (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-Itgb1 rat IgG (1:200), and probed with Alexa Fluor® 488-labeled anti-goat IgG (1:500) and Alexa Fluor® 594-labeled anti-mouse IgG (1:500) secondary antibodies. Anti-Ki-67 rabbit IgG (1:200, 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).

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, lvl, Krt10, Flg, and Lor. PCR was performed at 95°C for 15 sec and 60°C for 60 sec (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, lvl, Krt10, Flg, Lor, and Rn18s were prepared as shown in Table 1.

Table1 Primers used in this study.

Symbol	Gene	Forward	Reverse
Rn18s	18S ribosomal RNA	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG
Itbg1	integrin beta 1	CTGATTGGCTGGAGGAATGT	TGAGCAATTGAAGGATAATCATAG
Krt14	keratin 14	AGGACGCCCACCTTTCATCT	TGCACATCCATGACCTTGGT
Ivl	involucrin	GCAACCAACTCCACATCCTACA	CATGTTTGGGAAAGCCCTTCT
Krt10	keratin 10	GCCTACCTAAAGAAGAACCACGAA	GCAGCGTTCATTTCCACATTC
Flg	filaggrin	TGCATCTCCAGGTATATCGTTTCTT	GAAGTATCACCCAAATGGAAAAACC
Lor	loricrin	ACTCATCTTCCCTGGTGCTTCA	GGAGTGGGCTGCTTTTTCTG

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 $\pm$ 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 [12].

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 *Ivl* (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.

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 (Fig. 3, 4).

Discussion

Diabetes mellitus is complicated by a variety of cutaneous manifestations, including xerosis and foot ulcers [13]. 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 stem cells 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, hypercholesterolemia, 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.

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 [19]. *Flg* is a basic, 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 [14]. 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 stem cell 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 [15]. As to obese T2DM model mice, dysfunctional $\gamma\delta$ T cells contributed to the impaired keratinocyte homeostasis and decreased in thickness of epidermis [20]. 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). 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.

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.

Declarations

Acknowledgement

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.

Conflict of interest

TY and SH are employees of Nippon Menard Cosmetics Co., Ltd.

Ethics 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 “Archives of Dermatological Research”.

Availability of data and material

Not applicable

Code availability

Not applicable

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 cornified envelope: A model of cell death in the skin. *Nat Rev Mol Cell Biol* 6:328–340. <https://doi.org/10.1038/nrm1619>
7. Chatzigeorgiou 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 fang, Lin W dong, Lu S liang, 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](https://doi.org/10.1016/S0140-6736(05)67700-8)
11. Furrer 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](https://doi.org/10.1016/s0733-8635(02)00018-9)
12. 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>
13. Lima AL, Illing T, Schliemann S, Elsner 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>
14. 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>

15. Okano J, Kojima H, Katagi M, et al (2016) Hyperglycemia induces skin barrier dysfunctions with impairment of epidermal integrity in non-wounded skin of type 1 diabetic mice. PLoS One 11:1–22. <https://doi.org/10.1371/journal.pone.0166215>
16. 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>
17. 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>
18. 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>
19. 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>
20. Taylor KR, Costanzo AE, Jameson JM (2011) Dysfunctional $\gamma\delta$ T Cells Contribute to Impaired Keratinocyte Homeostasis in Mouse Models of Obesity. J Invest Dermatol 131:2409–18. <https://doi.org/10.1038/jid.2011.241>
21. Yoneda K, Nakagawa T, Lawrence OT, et al (2012) Interaction of the profilaggrin 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>

Figures

Figure 1

Skin morphology and thickness of the epidermis. (a) Hematoxylin and eosin staining showed alignment of basal cells in the back skin of control and KKAY mice. This alignment was regular in each mouse at 8 and 12 weeks of age. (b) The thickness of the epidermis did not show a significant difference between the control (white bar) and KKAY (black bar) mice at 8 and 12 weeks of age. (* $P < 0.05$) Data represent means $\pm$ S.D. of six animals.

Figure 2

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) (stem cell marker), (b) keratin 14 (Krt14) (basal layer marker), (c) keratin10 (Krt10) and (d) involucrin (Ivl) (prickle layer markers), (e) loricrin (Lor) and (f) filaggrin (Flg) (granular layer markers). (*P<0.05) Data represent means $\pm$ S.D. of six animals.

Figure 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 $\pm$ S.D. of six animals.

Figure 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. Flg (granular layer marker) (green), Itgb1 (stem cell marker) (red), and 4',6-diamidino-2-phenylindole (blue) for nuclear staining