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Barrier function-related genes and proteins have an altered expression in acne-involved skin

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Abstract

Background: Acne vulgaris provides a unique disease setting in which a prominent skin inflammation is coupled with the overproduction of lipid-rich sebum.

Objectives: Our goal was to evaluate the expression of barrier molecules in papular acne skin samples obtained from untreated patients and compare those to the results of healthy and of papulopustular rosacea-involved ones at the mRNA and protein levels. In addition, we aimed to assess the effects of various sebum composing lipids on the expression of proteins involved in barrier formation in keratinocytes.

Methods: Available microarray data sets of papular acne and papulopustular rosacea-affected skin samples were re-analysed with a focus on epidermal barrier-related pathways. Immunohistochemistry was performed to detect barrier molecules in the interfollicular regions of human acne and healthy skin samples. Protein levels of barrier-related genes were measured by western blot in samples of HaCaT keratinocytes treated with selected lipids.

Results: Meta-analysis of whole transcriptome data sets revealed that barrier-related pathways are significantly affected in acne vulgaris skin samples. While an altered expression of key molecules in maintaining barrier functions such as filaggrin, keratin 1, involucrin, desmoglein 1, kallikrein 5 and 7, was also observed at the protein levels, our data demonstrated that sebum composing lipids may selectively modify the levels of epidermal barrier-related molecules.

Conclusions: Our results suggest that although not as prominently as in the dry papulopustular rosacea skin, the epidermal barrier in the interfollicular region may be damaged also in the lipid-rich skin samples of papular acne. Furthermore, our findings indicating diverse regulatory effects of various sebum lipids on the expression of barrier molecules in keratinocytes suggest, that they may influence the moisturization of the skin as well. Altogether, our findings could have implications in the development of sebum-modulating anti-acne therapies and even in the care of symptom-free skin.

INTRODUCTION

Acne vulgaris is a non-infectious, inflammatory disorder that localizes to the sebaceous gland-rich parts of the body such as the face, the back and the chest, affecting the majority of adolescents and young adults worldwide.[1–3] Although it is the disease of the pilosebaceous unit, acne may involve the interfollicular regions as well, reflected by the clinical findings where inflammation exceeds the openings of the follicles already in the mild forms of acne.[4] Furthermore, a characteristic feature of acne is increased sebum production, providing a unique setting wherein inflammation is not linked to skin dryness but

instead to a lipid-rich, oily skin environment. It is not yet fully understood if the epidermal barrier may be impaired in acne as well, thus, further contributing to inflammation.[5,6]

Epidermal barrier is formed by a cooperative network of physical, chemical and cellular components and serves as a defence line to prevent uncontrolled water loss, damage from chemical and physical harms as well as pathogen invasion. Regarding the integrity of the physical barrier, it is regulated by a well-defined set of molecules that are involved in filaggrin metabolism, cornified envelope formation, intercellular lipid lamellae formation, corneodesmosome organization, corneocyte desquamation and tight junction formation.[7,8] While the physical barrier consists of various junctional and associated cytoskeletal proteins of the upper epidermal regions and the stratum corneum, lipids produced both by keratinocytes and sebocytes, acids, as well as hydrolytic enzymes form the chemical barrier. The immunological/cellular aspects of the epidermal barrier should not only consider immune cells, which are known for identifying and eliminating pathogens, but also non-myeloid/lymphoid cells such as keratinocytes and sebocytes. Recent findings have shown that these cells produce barrier alarmins,[9–17] which contribute to the immune functions of the epidermal barrier.[18,19]

In this study, we aimed to evaluate the gene expression profiles of barrier-related molecules in skin samples of patients with papular acne and compare those to our previously published results from samples of healthy and papulopustular rosacea (PPR) skin, which also localizes to the sebaceous gland-rich face and in which we found an impaired barrier function associated with dryness and increased water loss.[20] We conducted additional research to examine alterations in protein levels in healthy skin samples and skin samples affected by acne. Additionally, we studied the impact of different sebum composing lipids on the levels of molecules related to barrier function in keratinocytes.

MATERIALS AND METHODS

Histological samples

Formalin-fixed paraffin-embedded (FFPE) human skin tissue samples from the Department of Dermatology, University of Debrecen (DD UD), Hungary were used after approval of the Regional and Institutional Ethics Committee (Approval ID: UD REC/IEC No. 4103-2014). The study was conducted according to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. Specimens from the scapular area of male patients with papular acne who never received systemic treatment and had no topical treatment for the previous 2 months were used for the experiments. Healthy control, region-identical skin samples were selected from the archive of the DD UD. The evaluated set contained five normal and five acne vulgaris skin samples. A detailed description of samples is indicated in Appendix S1.

Immunohistochemistry and quantification of stainings

Immunohistochemistry (IHC) was performed on 3-μm-thick tissue sections of FFPE samples according to the manufacturer's instructions. Staining intensities of digitized sections were quantified in FIJI software as described by Crowe et al.[21,22] The detailed protocol is indicated in Appendix S1.

Meta-analysis

The publicly available sequencing data[20,23] were downloaded from the relevant public database (see Data Availability Statement). Affymetrix Cel files (acne sequencing data) were downloaded from the Gene Expression Omnibus database and analysed using GeneSpring GX 14.9.1 software. RMA algorithm was used for normalization. Differentially expressed genes between conditions were determined by using Wilcoxon Mann–Whitney paired test with Benjamini–Hochberg FDR.

Downstream analysis of rosacea sequencing data was performed using StrandNGS software (version 2.8, build 230243; Strand Life Sciences). BAM files were imported into the software, and DESeq algorithm was used for normalization. Differential expression p-values were calculated using moderated t-test method. mRNA levels with corrected $p < 0.05$ were stated as significantly changed.

Functional enrichment analysis

Functional gene enrichment test was performed with the STRING platform (version 11.5, using Reactome pathways classification system[24]). Lists of differentially expressed genes with expression fold change >1.5 at $p < 0.05$ significance level were used as input. Gene enrichment test was carried out with medium FDR stringency and using gene expression values for each gene for ranking.

Cell culture and treatment

Immortalized human HaCaT keratinocytes were cultured and treated with palmitic acid, stearic acid, oleic acid, squalene, linoleic acid or vehicle (DMSO:etOH 1:1) for 48 h as done in our previous study[25] and as described in Appendix S1.

Western blot analysis

Protein levels of selected barrier molecules after lipid treatments in HaCaT keratinocytes were measured using western blot. See the detailed protocol in Appendix S1.

Statistical evaluation

Our data did not follow the normal distribution, p-values were calculated using the non-parametric Mann–Whitney test, $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ considered statistically significant. Staining intensity values (SIVs) were calculated by two independent observers with a significant correlation (Spearman's $\rho=0.73$, $p<0.0001$). Values are presented as mean ± SEM. Data distribution were analysed by Kolmogorov–Smirnov test.

RESULTS

Functional gene enrichment analysis identified barrier-related pathways to be differentially regulated in acne vulgaris samples

As a start point for our studies, we performed meta-analysis of available gene expression data of acne lesional and non-lesional whole-tissue skin samples published by Kelhala et al.[23] Enrichment analysis of differentially expressed genes revealed that besides pathways involved in inflammatory, cell cycle and lipid metabolism processes, a set of genes could also be clustered into the category of epidermal barrier-related pathways (Table 1, see the full list for affected pathways in Table S1). According to the STRING enrichment analysis, the formation of cornified envelope and keratinization were affected significantly in acne lesional skin specimens.

Expression changes of barrier-related genes in acne samples are partially overlapping with the ones in papulopustular rosacea skin

Although prominent differences exist between acne and PPR: while acne involves teenagers, rosacea appears in the 30–40 years of life; there is a strong overproduction of sebum lipids in acne while rosacea is marked by dry skin; the associated pathogen is *Cutibacterium acnes* in acne and *Demodex folliculorum* in rosacea, and importantly, acne starts in the follicles and involves the interfollicular region only in later stages but rosacea is localized primarily interfollicularly, still the two diseases are commonly compared.[26–29] Therefore, in order to define if the detected changes are specific to acne, as a next step, we used our RNASeq data obtained previously from PPR and healthy skin samples and compared the data set with the ones by Kelhala et al.[23]

Re-analysis of the sequencing data using STRING revealed that similar barrier-related pathways are affected in acne and PPR samples such as cell junction organization, keratinization and formation of the cornified envelope (see the full list of affected pathways in PPR in Table S2) with a remarkable overlap also at the level of the contributing genes (Figure 1, Venn diagram). Using heat maps to visualize the changes in the expression levels revealed that of the 58 overlapping genes, 45 showed similar changes in acne and PPR samples and only 13 were oppositely regulated in the two diseases (Figure 1a, Box 1). Assessing the genes which showed statistically significant expression changes only in rosacea (Figure 1a, Box 2) or in acne (Figure 1a, Box 3) samples, the majority of the genes still had similar trends in the change of their expression levels in both diseases.

Further grouping the affected genes in STRING based on their contribution to barrier function such as the formation of the cornified envelope, cell junction organization and tight junction interactions, we found that although similar changes and trends in the up- and downregulation were detected in both acne and PPR samples, the changes were more prominent in the case of PPR samples. Interestingly, the group of tight junction interactions was the only one in which all the related genes were downregulated (Figure 1b), while most of the genes forming clusters such as adherens junction interactions and type I hemidesmosome assembly were differentially regulated in the two diseases. See the detailed description of gene expression changes in the legend of Figure 1.

Barrier-related molecules are altered at the protein levels in acne samples

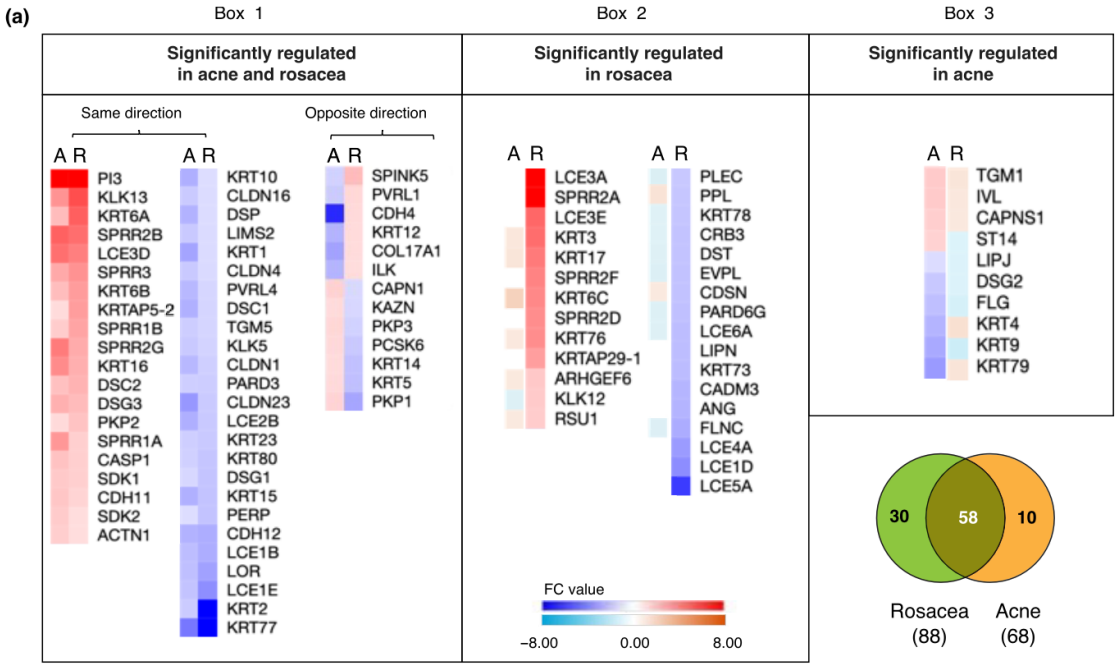
To confirm if changes in the levels of barrier-related genes could also be detected at the protein level, we performed immunohistochemical analyses of five papular acne and five healthy skin specimens obtained from matched body sites. We selected the major barrier molecules taking part in tight junction formation

TABLE 1 Genes linked to barrier-related pathways are significantly regulated in acne vulgaris lesional samples.

Term description	FDR	Matching genes
Formation of the cornified envelope	4.21E-07	*PKP2, KRT14, TGM1, KRT23, TGM5, PI3, CAPNS1, KRT9, KRT5, KRT6B, KRT15, DSG3, DSG1, DSG2, PKP1, ST14, DSC2, KRT16, SPRR1B, KRT2, KRT79, SPRR3, PKP3, KRT77, SPINK5, LCE1B, LOR, SPRR2G, SPRR2B, IVL, LCE1E, LCE3D, FLG, LIPJ, KAZN, KRT6A, KRT80, PERP, CAPN1, KRT4, KLK13, PCSK6*
Keratinization	8.50E-07	*PKP2, KRT14, TGM1, KRT23, TGM5, PI3, CAPNS1, KRT9, KRT5, KRT6B, KRT15, DSG3, DSG1, DSG2, PKP1, ST14, DSC2, KRT16, SPRR1B, KRT2, KRT79, SPRR3, PKP3, KRT77, SPINK5, LCE1B, LOR, SPRR2G, SPRR2B, IVL, LCE1E, LCE3D, FLG, LIPJ, KAZN, KRT6A, KRT80, PERP, KRTAP5-2, CAPN1, KRT4, KLK13, PCSK6*

Note: Functional clustering of the differentially expressed genes from the data set of Kelhala et al.[23] in which acne samples were compared to healthy skin samples revealed that in addition to inflammatory, cell cycle and lipid metabolism-related pathways (see the full list in Table S1), genes could be grouped also into clusters such as cornified envelope formation and keratinization.

Abbreviation: FDR, false discovery rate.



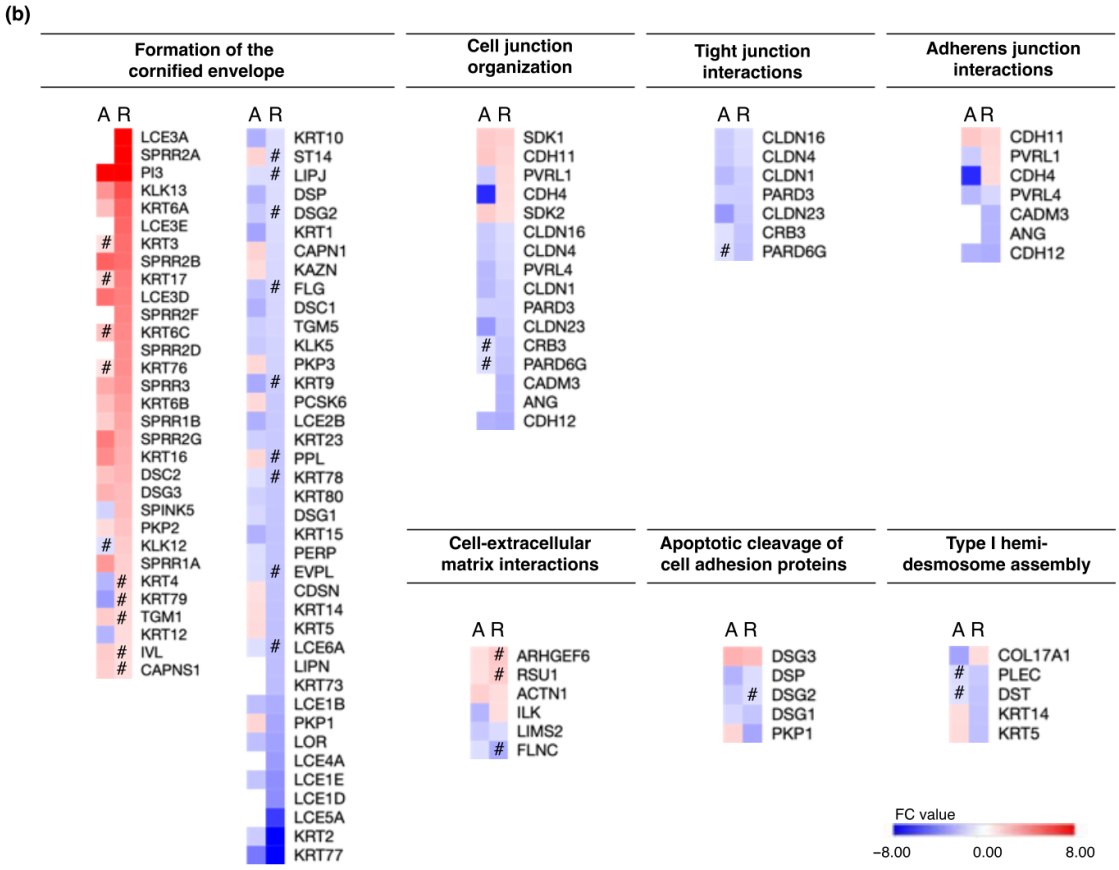


FIGURE 1 Expression changes of significantly regulated genes involved in epidermal barrier functions in acne and in PPR skin samples. (a) Heat map visualization showing that 45 of the 58 overlapping genes from the Venn diagram had significant gene expression changes (vs. relevant control) in the same direction, of which keratinization-related (SPRR1A,-1B,-2B,-2G,-3 and LCE3D) and cell adhesion molecule-coding (DSC2, DSG3, PKP2, SDK1,-2, CDH11 and ACTN1) genes showed a significant increase, while another group of keratinization-related (KRT1,-2,-10,-15,-23,-77,-80, TGM5, LCE2B, LCE1B, LOR and LCE1E) and cell adhesion-related (CLDN1,-4,-16,-23, DSP, LIMS2, PVRL4, DSC1, PARD3, DSG1, PERP and CDH12) genes showed significant downregulation. Thirteen genes showed opposite expression changes in acne and rosacea samples, of which the majority are involved in the regulation of cell–cell adhesion (SPINK5, PVRL1, CDH4, COL17A1, ILK, KAZN, PKP3 and PKP1) and cytoskeletal remodelling (KRT12, CAPN1 and KRT14), while PCSK6 is involved in the regulation of lipid metabolism (Box 1). The expression levels of genes coding cell–cell adhesion molecules (e.g. DST, EVPL and CDSN) and genes involved in the keratinization process (e.g. SPRR2A,-2D,-2F, LCE3E, KRT3,-17 and LIPN) were only changed in rosacea samples (Box 2). While the expression levels of IVL increased in acne but were not affected significantly in rosacea samples, TGM1 and FLG significantly decreased in acne (Box 3). (b) Heat maps generated based on the functional clusters by using the STRING platform. Genes involved in the regulation of tight junctions (CLDN1,-4,-16,-23; PARD3,-6G; CRB3) showed downregulation in both acne and rosacea samples, while the genes that are involved in the formation of the cornified envelope, cell junction organization, adherens junction interactions, cell–extracellular matrix interactions, apoptotic cleavage of cell adhesion proteins and type I hemidesmosome assembly showed up- and downregulation as well. Expression values were determined by RNASeq analysis, normalized to region-matched healthy skin samples, colour intensities are defined by the fold change values. The red-blue colour scale represents significantly regulated genes, and the orange-cyan blue colour scale and # signs represent statistically not significant gene expression values ($p > 0.05$). Heat maps were generated with Morpheus platform (https://software.broadinstitute.org/morpheus). A, acne; R, rosacea.

(claudin-1 [CLDN1] and occludin [OCLN]) and composing the stratum corneum (cornified envelope formation, desmosome organization and corneocyte desquamation) that are crucial in maintaining the water barrier of the skin.

While a slight, statistically not significant elevation was observed in the case of CLDN1 (p:0.1), no differences were found in OCLN staining intensities between acne and control samples. Although the overall staining intensity of CLDN1 does not differ statistically between healthy control and acne-prone skin, the staining patterns are quite different. While in normal skin, the immune positivity localizes the uppermost layers of the epidermis, in acne-involved skin, the protein is detectable from basal to granular layers with a clearly visible perinuclear accumulation, and completely missing from the stratum corneum.

When performing stainings against proteins involved in cornified envelope formation, intensities of filaggrin (FLG) and keratin 1 (KRT1) were decreased significantly (SIV 30.6 vs. control 58.4, p:0.01 for FLG, and 67 vs. 93.3, p:0.02 for KRT1), while of involucrin (IVL) showed a significant increase in acne samples (SIV 89 vs. 50.1, p:0.0002). We found a tendentious but statistically not significant increase in transglutaminase 5 (TGM5) expression in acne samples (SIV 51.1 vs. 39.3 compared to control, p:0.07) and no differences in loricrin (LOR) stainings between acne and control samples. In parallel with the differences in intensity levels, notable changes were seen in the patterns of FLG and IVL stainings. In comparison with healthy skin samples, FLG-positive cells were seen in a wider, 3–4 granular cell layers in acne samples, in a non-continuous manner. Regarding IVL protein stainings in acne samples, all suprabasal cell layers showed strong positivity, while in control samples, IVL was detectable with increasing intensity from the upper spinous layers to the stratum corneum.

Stainings against corneodesmosin (CDSN) and desmoglein 1 (DSG) proteins which are involved in desmosome organization revealed a significant decrease in the protein expression of DSG1 (SIV 36 vs. 57.7, p:0.007) and no differences in that of CDSN in acne samples.

Regarding proteins contributing to corneocyte desquamation, stainings against kallikrein 5 (KLK5) and kallikrein 7 (KLK7) showed a significant decrease in acne samples (KLK5 SIV 45.8 vs. 66.7, p:0.007; KLK7 SIV 33.2 vs. 54.9, p:0.0001). The pattern of KLK7 staining was remarkably changed in acne samples: in control samples, the staining followed an increasing intensity from suprabasal layers to stratum corneum, while the staining was irregular in acne samples, whereas staining intensities were weaker in the granular layers compared to lower or upper epidermal layers.

Immunohistochemical stainings and staining intensity values of control and papular acne samples of each protein are shown in Figure 2 and Figure S1.

Sebum composing lipids may selectively regulate the levels of barrier function-related proteins in keratinocytes

To test the regulatory role of various sebum composing lipids on barrier function-related proteins, that were found to be differentially expressed in papular acne-involved skin samples compared to healthy ones, we performed western blot analysis of HaCaT keratinocytes treated with selected sebum composing lipids or vehicle for 48 h. The selected lipids for the study were stearic and palmitic acid, which have pro-inflammatory characteristics,[30,31] oleic acid and linoleic acid, which have anti-inflammatory properties[32,33] and squalene which is heavily present in sebum and associated with comedogenesis.[34]

The tested lipids affected two molecules involved in cornified envelope formation significantly: linoleic acid caused a decrease in KRT1 levels, and treatments with palmitic and stearic acids significantly decreased the protein levels of IVL. Squalene caused a slight increase in FLG protein levels, while linoleic acid a tendentious decrease in TGM5 levels. Treatment with palmitic acid caused a tendentious decrease in the levels of the tight junction formation-related OCLN. Regarding desmosome organization palmitic, stearic and oleic acids caused a slight decrease in DSG1 levels.

In the protein levels of corneocyte desquamation-related molecules, squalene and palmitic acid caused a slight increase in KLK5, while stearic acid caused a slight decrease in KLK7 levels.

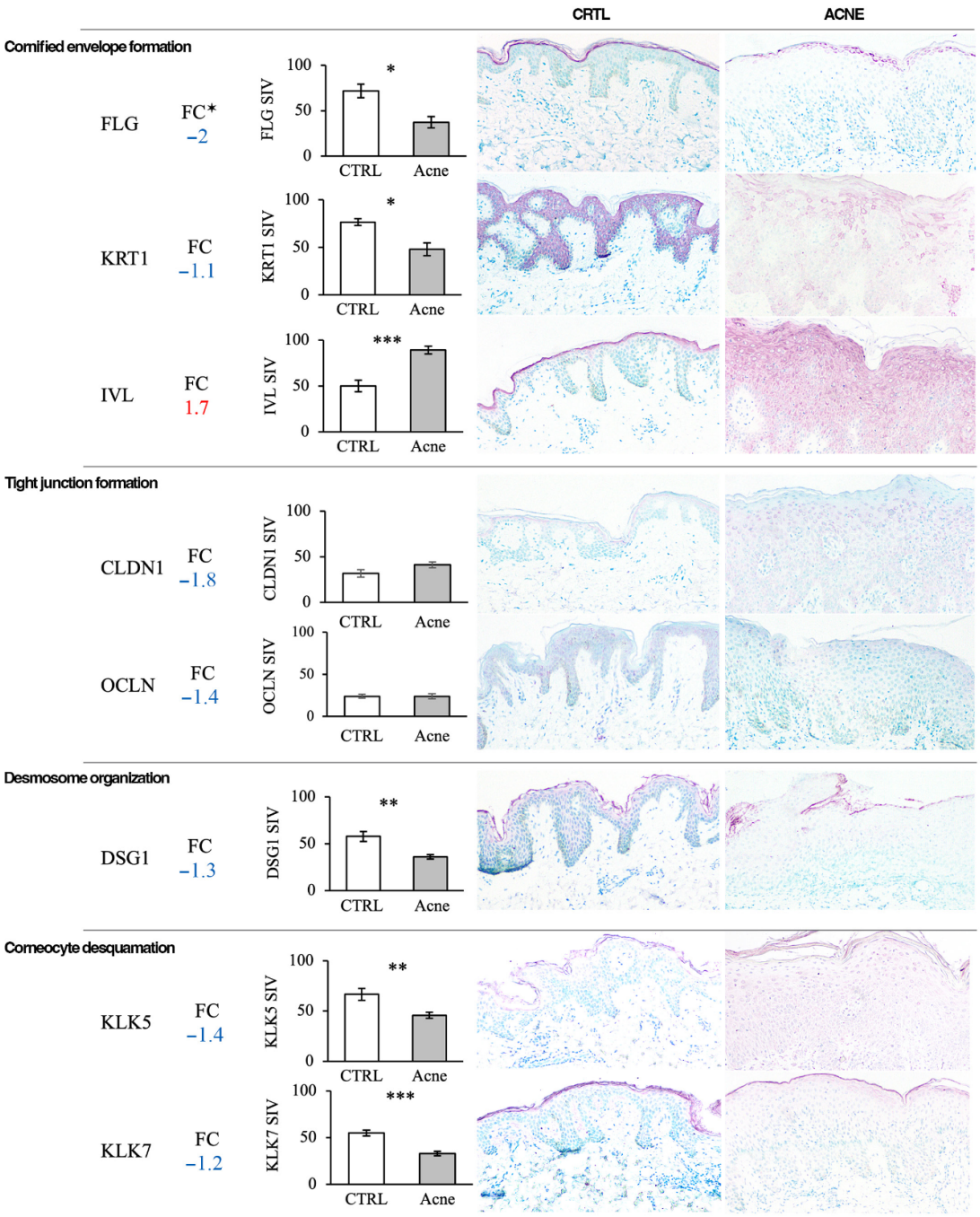


FIGURE 2 Immunohistochemical stainings confirm differences in the expression of barrier-related proteins between region-matched healthy (CTRL) and papular acne (ACNE) skin samples. A significant increase was detected in staining intensity of involucrin (IVL) in acne vulgaris-affected skin samples, while staining intensity of filaggrin (FLG), keratin 1 (KRT1), desmoglein 1 (DSG1), kallikrein 5 (KLK5) and kallikrein 7 (KLK7) was significantly decreased for the same samples. Staining intensities of claudin 1 (CLDN1) and occludin (OCLN) were not changed significantly. Compared to healthy skin, a remarkable change in staining patterns of FLG, IVL and KLK5 was observed. Note the thickened, hyperproliferative epidermis in the interfollicular regions of acne samples. Immunohistochemical staining using Vector VIP chromogen (red) with methylene green background staining. FC: fold change, as revealed by the re-analysis of the data set by Kelhala et al.[23] SIV, staining intensity value. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, p-values were determined using non-parametric Mann–Whitney test. Representative images are shown from five healthy, region-matched control (CTRL) and five acne vulgaris (ACNE) samples, original magnification ×100.[23]

Protein level changes of epidermal barrier-related molecules after treatment with sebum composing lipids are shown in Figure 3.

DISCUSSION

Addressing tissue-specific changes in acne samples at the level of gene expression has already been performed, using whole-tissue sequencing. Functional clustering of the differentially expressed genes revealed a specific immune composition of acne-related inflammation driven by Th17 cells,[23] however, the potential impairment of the permeability barrier was not assessed. Therefore, in this study, we re-analysed the same data set with a special focus on the important permeability barrier-related terms (e.g. tight junction formation and keratinization), while in order to define the extent of the barrier damage at the level of gene expression, we compared the changes with the ones that our research group found in PPR,[20] a disease also localizing to sebaceous gland-rich skin areas of the body and therefore commonly used for comparisons with acne.

Besides showing that a set of genes encoding barrier-related proteins were altered in acne samples, our results, that when the gene expression profiles of whole-tissue acne samples from the work of Kelhala[23] and from the analyses of

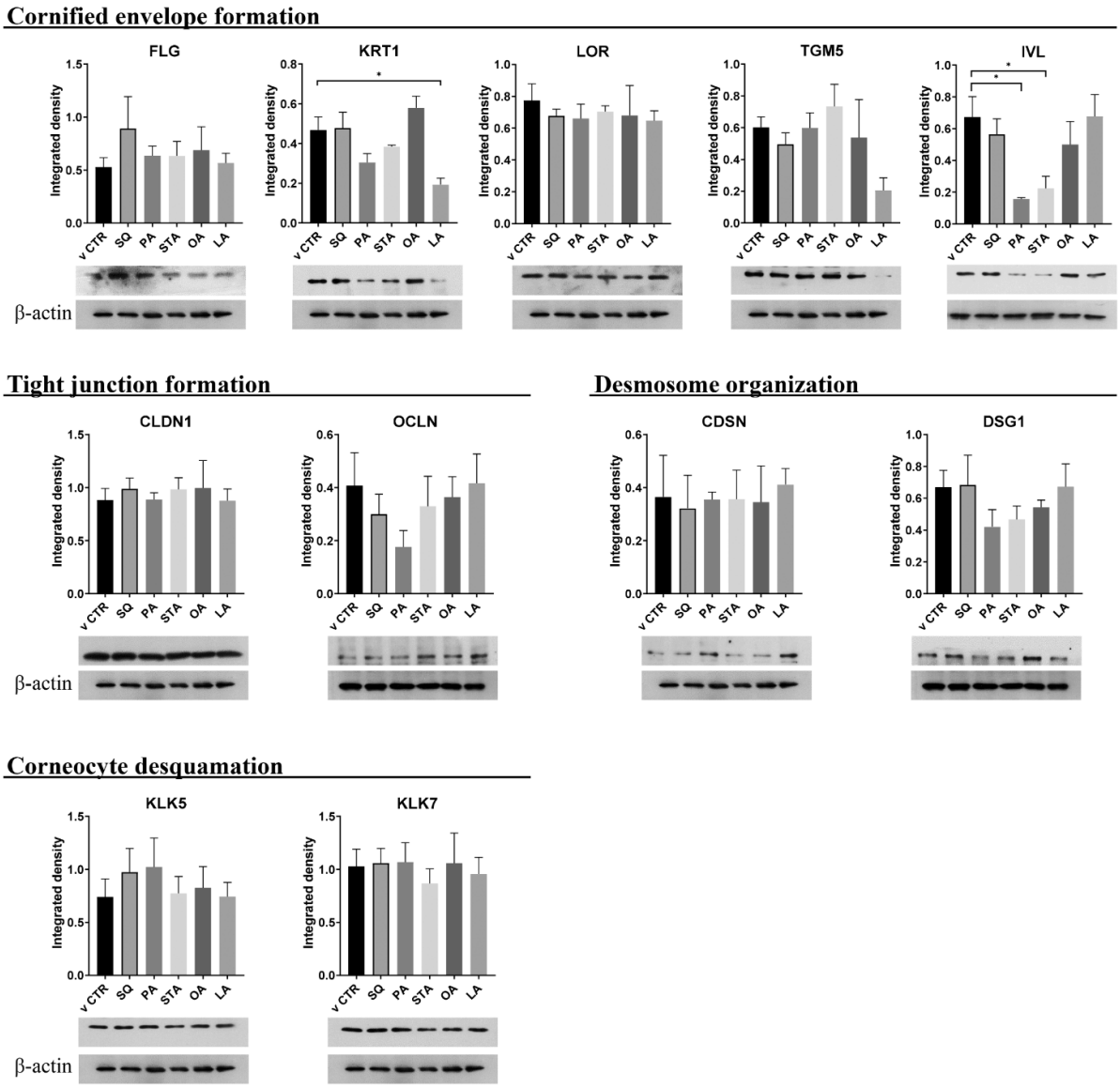


FIGURE 3 Sebum composing lipids may significantly alter the protein levels of cornified envelope formation-related molecules in keratinocytes. Changes in the levels of selected barrier-related proteins as determined by western blot in HaCaT keratinocytes treated with sebum composing lipids as described in the Appendix S1. LA, linoleic acid; OA, oleic acid; PA, palmitic acid; SQ, squalene; STA, stearic acid. Experiments were performed in triplicates; western blot images show representative photographs of a single experiment. *$p < 0.05$.

our previous findings on rosacea skin[20] were compared, we found a higher number of the barrier-related genes to be significantly regulated in rosacea samples. Interestingly, there was a large set of genes that showed similar changes in the two diseases: a significant decrease was seen in the mRNA levels of barrier structure molecules and junctional proteins such as KRT1, KRT10, LOR, TGM5, CLDNs, DSG1 and DSC1. Nevertheless, genes were also identified with opposite expression profiles. Among them, the type II (basic) KRT5 and the type I (acidic) KRT14 are the characteristic KRTs of the basal, proliferating keratinocyte layer. The different KRT5/14 profile of acne and PPR raises the possibility of a distinct proliferation programme of the keratinocytes in the two diseases even though their keratinocytes' differentiation programme showed similar deterioration.

To be able to compare the barrier-related changes in the two diseases and considering that in acne the involved follicles form a unique and diverse structure[35] in which the barrier properties are mostly dependent on tight junctions, in our works addressing the protein levels we focused specifically on the interfollicular keratinocytes. Importantly, in line with the findings of Knaggs et al.,[4] we also observed thickened, hyperproliferative epidermis in the interfollicular regions of acne samples, which further supported the relevance of our studies on the interfollicular epidermis. Assessing the acne-specific changes and how keratinocyte differentiation may be affected, we first measured the expression of FLG, which assembles keratin filaments into tight bundles, thus promoting the collapse of the corneocytes into a characteristic flat shape[18] in the final stages of keratinocyte differentiation. Although Kurokawa et al.[5] reported increased FLG expression of follicular keratinocytes in acne vulgaris samples, according to our data FLG is present in a significantly lower amount in the interfollicular keratinocytes in acne samples compared to healthy skin just as we observed a significant decrease in the KRT1 levels. For this, a possible explanation may be the differences between the gene and protein expression profiles of follicular and interfollicular keratinocytes in acne (just as in healthy) skin which definitely needs further studies.

The cornified cell envelope is a protein/lipid polymer structure formed below the cytoplasmic membrane and consists of a protein envelope and a lipid envelope. The protein envelope contributes to the biomechanical properties of the CE as a result of cross-linking of structural proteins, including loricrin, involucrin, trichohyalin and to the class of small proline-rich proteins by transglutaminases.[7,36,37] Among these molecules, we detected a significant elevation of IVL and a tendentious elevation of TGM5 levels in acne samples. In addition to the significant increase in IVL staining intensity, a notable change was observed in the pattern of IVL stainings as all suprabasal layers showed strong positivity. Since IVL is an early marker of differentiation, this phenomenon suggests an immature epidermal cornification, which interestingly, was also observed in psoriatic skin.[38]

Corneocytes are held together by corneodesmosomes whose structures mediate the strong intercellular cohesion in the cornified layers that is crucial for the physical and chemical barrier function of the epidermis.[7,8,39] Through our protein-level studies, we were able to support the data of Kelhala et al. that the desmosome structures may be impaired in acne samples. Specifically, we observed a significant decrease in DSG1 expression in acne lesional skin, which is consistent with their previous observation of reduced DSG1 expression at the mRNA level. Concerning the formation of tight junctions, Kelhala et al.'s data indicate that mRNA levels of CLDNs were notably reduced in acne samples, but there were no significant variations observed at the protein level.

Corneocyte desquamation is the process by which corneocytes are constantly shed at the superficial layer of SC and is essential to maintain SC homeostasis. It is mainly regulated by a pH-dependent proteolytic cascade of kallikrein-related peptidases, such as KLK5, KLK7 and KLK14.[40] We found that corneocyte desquamation may also be affected in acne samples, as KLK5 and KLK7 protein levels were significantly decreased in our samples. In addition to the decreased staining intensities, an altered staining pattern was also seen in KLK7 labelling: while in control samples, the staining showed a gradient from suprabasal layers to stratum corneum, the staining was irregular in acne samples and the intensity was weaker in granular layer compared to lower or upper epidermal layers. This phenomenon raises the possibility of a changing pH gradient in acne lesional skin. The schematic representation of the above-mentioned proteins' expression patterns in acne lesional and healthy epidermis are shown in Figure 4.

To address the underlying mechanisms of the observed epidermal barrier damage in acne lesions, the altered cytokine as well as lipid profiles should be taken into account. Regarding proteins, the activation of the Th17 axis results in the induction of a series of cytokines (IL-1β, IL-6, TGF-β and IL23p19), chemokines (TNF-α, IL-8, CSF2, and CCL20), Th1 markers (IL12p40, CXCR3, T-bet and IFN-γ) and IL-17-related antimicrobial peptides (S100A7, S100A9, lipocalin, hBD2, hBD3 and hCAP18),[23,41] which molecules may have an impact on barrier-related genes' expression. Indeed, the data of Nograles et al.[42] support this theory as IL-17 and IFN-γ treatment caused significant downregulation of FLG, KRTs, CLDN1, DSGs and DSCs in primary human keratinocytes. In addition, Bruewer et al.[43] demonstrated that the pro-inflammatory cytokines IFN-γ and TNF-α can disrupt the properties of the epithelial barrier, while a recent study conducted by Bolla et al.[44] suggests that *Cutibacterium acnes* might impact the skin barrier properties by activating innate immune responses and inducing inflammation. However, on the other hand, sebum lipids are also potential candidates as reflected in the term sebaceous immunobiology.[45,46] It covers both their antimicrobial properties as well as their regulatory role on various cell types of the skin such as keratinocytes and immune cells. Previously, we showed that palmitic and linoleic acids were the only lipids of the tested ones to induce the expression of TSLP

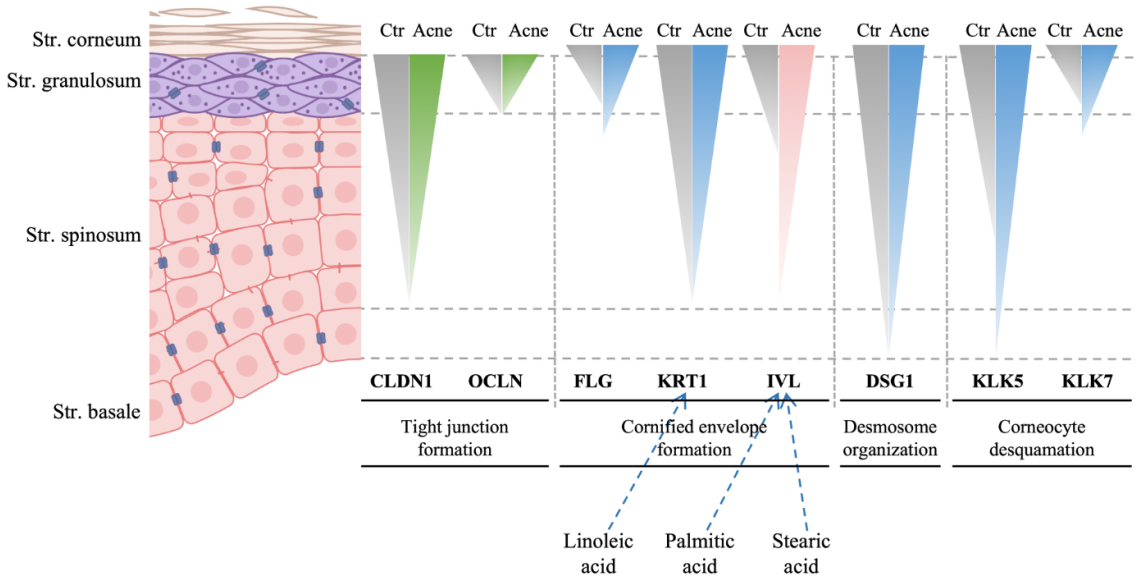


FIGURE 4 Overview of the expression patterns of epidermal barrier-related proteins in the epidermis of papular acne and healthy skin. No significant changes were observed in staining intensities of tight junction organizing proteins (CLDN1, OCLN). Proteins involved in desmosome organization (DSG1) and corneocyte desquamation (KLK5, KLK7) were present with lower staining intensity in papular acne samples. A prominent change was found in the expression patterns of IVL and KLK5: compared to healthy skin, these two molecules are expressed in a wider epidermal area, in all suprabasal layers. Among molecules involved in CE formation, lower staining intensities of FLG and KRT1 and higher intensity of IVL were found in acne samples. On the basis of our cell line experiments, treatments with linoleic acid, palmitic acid and stearic acid may cause a significant decrease in the protein levels of KRT1 and IVL. Grey: expression pattern in healthy skin samples, blue: lower staining intensities compared to healthy skin, red: higher staining intensities compared to healthy skin and green: not changed compared to healthy skin. Blue dashed arrows: downregulating effects of the used sebum lipids based on our cell line experiments.

in keratinocytes, while each lipid modulated macrophage functions and cytokine profiles differentially.[25,47] Based on these biological differences between sebum composing lipids, it is inevitable to assume that not only the amount but also a change in the composition of sebum may have (patho)physiological relevance in acne as it was previously raised also by others.[48–53] This work, by showing that the tested sebum composing lipids could have the potential to differentially alter the barrier molecules at the protein level, such as the observed prominent decrease in the protein levels of KRT1 and IVL in response to linoleic acid, palmitic acid and stearic acid treatment. This observation may provide additional evidence that an alteration in the ratio of sebum lipids could contribute to the development and progression of the disease, as suggested by previous research studies,[48,52,53] potentially by affecting the skin's barrier function. Although in addition to previous results obtained from in vitro differentiated macrophages and sebocyte cell line cultures,[47,54] we showed that sebum composing lipids may alter the functions also of keratinocytes, still it would be oversimplified to call any of these lipids 'good' or 'bad'. Keeping in mind the known limitations that arise from working on cell cultures, it is however reasonable to speculate that the modification of sebum composition may be a therapeutic target in acne also in regard to restore barrier functions.

In summary, although a few reports showed that epidermal barrier functions may be impaired in acne patients,[5,6] our study, which re-analysed the available whole-tissue sequencing data of acne samples and evaluated barrier molecules at the mRNA and protein levels, is the first to deliver a comprehensive analysis at the molecular level. By comparing the changes with the ones observed in samples of PPR, we also confirmed that despite being rich in lipids, papular acne skin also exerts an impaired barrier in the interfollicular region, which may partially be explained by our findings that sebum composing lipids could selectively alter the levels of barrier function-related molecules in keratinocytes. Translating these results into clinical practice, we propose that besides sebo-suppression,[55–59] which itself is a damaging stimulus as marked by increased TEWL and skin irritation,[56–59] in order to achieve better improvement and patient compliance, the application of barrier-improving agents is needed not only in parallel with acne therapy such as isotretinoin, but already prior to that.

AUTHOR CONTRIBUTIONS

Katalin Dull, Dániel Törőcsik and Andrea Szegedi conceived the experiments; Katalin Dull, Kinga Lénárt and Szilárd Póliska performed the experiments; Katalin Dull, Eri Uchiyama and Zoltán Hendrik evaluated IHC stainings; Katalin Dull and Zsolt Dajnoki performed pathway analyses; Katalin Dull, Dániel Törőcsik, Andrea Szegedi and Zsolt Dajnoki analysed the results and wrote the article. All authors reviewed the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

No large data sets were generated during this study. Re-analysed large data sets are available under accession numbers GSE53795[23] (NCBI GEO database), PRJNA421246 and PRJNA592080[20] (Sequence Read Archive database).

ETHICS STATEMENT

This study protocol was approved by the Regional and Institutional Ethics Committee (Approval ID: UD REC/IEC No. 4103-2014). The study was conducted according to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in this study. The patients in this article have given written informed consent to publication of their case details.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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