Potential relevance of altered cartilage oligomeric matrix protein in psoriasis

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LIST OF PUBLICATIONS

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I. **Bozó R**, Szél E, Danis J, Gubán B, Bata-Csörgő Z, Szabó K, Kemény L, Groma G. Cartilage Oligomeric Matrix Protein Negatively Influences Keratinocyte Proliferation Via α5β1-Integrin: Potential Relevance of Altered Cartilage Oligomeric Matrix Protein Expression in Psoriasis. J Invest Dermatol. 2020 Feb 11; doi: 10.1016/j.jid.2019.12.037. [Epub ahead of print]

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Publications not directly related to the thesis

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III. Tripolszki K, Danis J, Padhi AK, Gomes J, **Bozó R**, Nagy ZF, Nagy D, Klivényi P, Engelhardt JI, Széll M. Angiogenin mutations in Hungarian patients with amyotrophic lateral sclerosis: Clinical, genetic, computational, and functional analyses. Brain Behav. 2019;9(6):e01293. doi: 10.1002/brb3.1293.

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IV. Szél E, **Bozó R**, Hunyadi-Gulyás É, Manczinger M, Szabó K, Kemény L, Bata-Csörgő, Z, Groma, G. Comprehensive Proteomic Analysis Reveals Intermediate Stage of Non-Lesional Psoriatic Skin and Points out the Importance of Proteins Outside this Trend. Sci Rep. 2019 0;9(1):11382 doi: 10.1038/s41598-019-47774-5.

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1. INTRODUCTION

1.1. Characteristics of the inflammatory skin disease, psoriasis

Plaque-type psoriasis (also known as *Psoriasis vulgaris*) is a chronic inflammatory, multifactorial, immune-mediated skin disease, characterized by dry, red, scaly patches and affects 1–3% of the global population. The pathogenesis of this complex disease is only partially understood, and currently only symptomatic treatments are available. Major characteristics of psoriatic plaques are hyperproliferation and altered differentiation of keratinocytes coupled with massive immune-cell infiltration. Secreted cytokines and chemokines are believed to key mediators of the initiation of the disease. The onset of symptoms is presumably triggered by an overreaction of the immune system. This is followed by the response of keratinocytes to cytokines by proliferation, activation and secretion of proinflammatory mediators (cytokines, chemokines, antimicrobial peptides) which help to amplify the inflammatory process. Based on age, as well as genetic and immunopathogenic characteristics, chronic plaque type psoriasis has two distinct subtypes: early and late onset psoriasis. Early onset psoriasis presents at or before 40 years of age and affects 75% of patients, while late onset psoriasis first presents after the age of 40 years and affects 25% of patients.

Genetic factors play an important role in the pathogenesis of the disease. At least fifteen psoriasis susceptible loci (PSORS) have been already discovered in the human genome. PSORS1 locus -which located at the region of major histocompatibility complex on chromosome 6- is responsible for 30-50% of genetic origin of the disease. On the PSOROS1 locus, HLA-Cw6 is the most frequently mapped allele in population with early onset of the disease. Furthermore, single nucleotide polymorphisms are discovered in fifty different regions of the human genome by genome-wide association studies, which are in connection with psoriasis. Apart from the genetic factors, environmental effects could also play role in the pathogenesis of the disease including stress, physical trauma, smoking and alcohol.

1.2. Alterations in the non-lesional psoriatic skin

Alterations in healthy looking non-lesional skin of psoriasis patients have been described, which may contribute to the development of the symptoms. In addition to hyperproliferation, altered keratinocyte differentiation and massive immune-cell infiltration, the dermal extracellular matrix (ECM) and the basement membrane (BM) are also affected in non-lesional skin of patients. Non-lesional epidermal keratinocytes have been shown to represent a “pre-

activated" state for hyperproliferation: these cells are more sensitive to stress and to proliferative signals. Alterations of the ECM that are already present in non-lesional skin also affect the cell attachment modulator fibronectin (FN), which is differentially expressed in non-lesional skin. Previously, we found that fibroblasts, as well as basal keratinocytes, express high levels of the FN splice variant that contains the extra domain A (EDA+FN) in non-lesional skin. Moreover, some integrins, including the FN-interacting α5β1-integrin, also exhibit an increased expression in keratinocytes at the dermal–epidermal junction (DEJ). The enhanced EDA+FN and α5β1-integrin production that we observed in psoriatic non-lesional skin may contribute to the induction of keratinocyte proliferation. Furthermore, at the DEJ in non-lesional skin, the laminin layer of the BM is discontinuous and the connection of keratinocytes to the BM is also altered.

To gain further insight into the pathomechanism of psoriasis, we previously performed large-scale proteomic study in which healthy, psoriatic non-lesional and lesional skin were compared. In this study, cartilage oligomeric matrix protein (COMP) exhibited elevated expression in non-lesional skin in comparison to healthy skin.

1.3. Structure, functions and interaction partners of COMP

COMP is a non-collagenous, 524-kDa, homopentameric, glycoprotein component of the ECM. It belongs to the family of thrombospondins and often referred to as thrombospondin-5. The main functions of thrombospondins are ECM organization and remodeling. The homopentameric structure of COMP is formed via the N-terminal coiled-coil domain, resulting in a flexible molecule with a bouquet-like form that allows to interact with multiple cellular and extracellular components simultaneously.

COMP is mainly expressed in cartilage, but it also appears in tendon, ligament, synovium, vascular smooth muscle cells, cardiomyocytes, activated platelets and skin as well.

Interaction partners of COMP in the ECM are collagen type I and III and FN, among others. Interestingly, the molecules mentioned above are also known to be affected in psoriasis.

COMP modulates cellular behavior via direct interactions with cell-surface proteins, including the α5β1, α7β1 and αvβ3 members of the integrin family. α5β1-integrin modulates processes in psoriasis pathogenesis, including inflammatory responses and keratinocyte proliferation.

1.4. COMP in diseases

COMP has been associated with several diseases. Autosomal dominant mutations in the COMP gene cause skeletal disorders, such as pseudoachondroplasia and multiple epiphyseal dysplasia. The presence of COMP in serum and synovial fluid serves as a biomarker in inflammatory diseases affecting the cartilage of joints, such as rheumatic disorders including rheumatoid and psoriatic arthritis.

1.5. COMP in the skin

In healthy skin, COMP is primarily produced by fibroblasts and localizes to the papillary dermis where it is believed to take part in ECM stabilization and provide cohesion between the anchoring plaques of the upper dermis and the BM.

COMP accumulation in the dermis is elevated in various skin disorders, including localized scleroderma, wounds and exudates of patients with venous leg ulcers, in the fibrotic stroma of basal cell carcinoma and skin keloids. Although COMP accumulation in the dermis is elevated in several skin disorders, it has not been previously investigated previously in the context of psoriasis.

2. AIMS

Psoriasis is an inflammatory skin disease, where the non-lesional skin of patients contains alterations, which predispose to the development of the symptoms. These alterations affect the DEJ region, where the fibronectin splice variant, EDA+FN and α5β1-integrin showed increased expression, and the laminin layer within the BM is discontinuous.

In healthy skin, COMP is localized into the DEJ region, and believed to provide cohesion between the anchoring plaques of the upper dermis and the BM. Furthermore, it can directly interact with α5β1-integrin and fibronectin as well. Besides, in our previous proteomic study, we found elevated COMP protein level in non-lesional skin in comparison to healthy skin. Based on these previous results, we aimed to study the potential role of COMP in the pathomechanism of psoriasis.

We aimed to

- examine the tissue distribution of COMP in the healthy, psoriatic non-lesional and lesional skin.
- determine the protein level of COMP in the healthy, psoriatic non-lesional and lesional skin.

- determine COMP expression at the mRNA level in healthy and non-lesional cultured fibroblasts.
- study whether COMP accumulation allows interaction with basal keratinocytes through the discontinuous BM comparing the healthy and non-lesional skin.
- further study the DEJ region and examine, whether COMP accumulation allows interaction with a potential interaction partner, fibronectin splice variant EDA+ FN - which is known to influence the behavior of the keratinocytes-, comparing the healthy and non-lesional skin.
- examine whether COMP can affect the behavior of keratinocytes *in vitro* and *ex vivo.*

3. MATERIALS AND METHODS

3.1. Skin samples and ethics

Skin punch biopsies (d=6 mm) were collected from healthy volunteers (n=10; age 18–70 years), and from psoriatic patients with moderate-to-severe chronic plaque-type psoriasis from lesional (n=13) and non-lesional skin areas (n=13; minimum of 6 cm from lesional region; age 18–70 years). Tissue collection was obtained after written informed consent, in accordance with the rules of the Helsinki Declaration. The study was confirmed by the Human Investigation Review Board of the University of Szeged (PSO-EDAFN-002, 34/2015, 3517, 23 February 2015, Szeged, Hungary; PSO-ECMPR-002 IF-562-5/2016 and; 157/2015-SZTE, 3638, 21 September 2015, Hungary).

3.2. Protein isolation and western blot analysis

For preparation of tissue protein extracts, skin biopsies (healthy, psoriatic non-lesional and psoriatic lesional) were cut into small pieces with a razor blade. 6 M guanidine hydrochloride solution was used as an extraction buffer for 24 h at 4°C under continuous agitation. Protein extracts (25 μg) were separated on a 4–20% gradient SDS polyacrylamide gel under reducing or non-reducing conditions. Proteins were transferred to a nitrocellulose membrane and blocked in 5% non-fat milk powder containing Tris-buffered saline (TBS) for 60 min at room temperature (RT). Membranes were incubated for overnight at 4°C with anti-human COMP and actin primary antibodies. Subsequently, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 60 min at RT. Signal was visualized with Clarity Max Western ECL Substrate on a C-digit blot scanner.

3.3. Fluorescence microscopic analysis

Biopsies were frozen in a cryogenic matrix or were paraffin embedded and were subsequently cut into 5 μm sections. For fixation and permeabilization, 4% paraformaldehyde followed by 0.25% TritonX-100 or commercially available staining buffer set were used. For blocking, TBS containing 1% bovine serum albumin and 1% normal goat serum (NGS) was used, and for frozen samples, which were digested with chondroitinase ABC, 10% fetal bovine serum (FBS) and 5% NGS were applied. Samples were incubated with the following anti-human primary antibodies: COMP, β1-integrin, actin, Ki67, keratin-17 (KRT17), laminin alpha-1 (LAMA1) and fibronectin (EDA+FN). Isotype controls were the following: rabbit polyclonal IgG and mouse IgG1 antibodies. As secondary antibodies, Alexa Fluor (AF) 546 conjugated anti-rabbit IgG and AF 647 conjugated anti-mouse IgG were used. Nuclei were visualized with 4′,6-diamidino-2-phenylindole (DAPI) staining. Zeiss LSM 880 or Zeiss Axio Imager Z1 microscopes were used for visualization.

Pearson's correlation coefficient, R, was calculated using ImageJ/Fiji software.

3.4. RNA isolation and real-time RT-PCR

Total RNA was isolated from primary fibroblasts from healthy and psoriatic non-lesional skin cultured in 75 cm^2 cell culture flasks and collected at the fifth passage using TRI-Reagent as described by the manufacturer. After the RNA isolation cDNA synthesis was performed, followed by real-time RT-PCR using COMP and 18S rRNA primers.

3.5. Cell cultures and examination of cellular properties

3.5.1. Cell cultures

Primary dermal fibroblasts were isolated from healthy and psoriatic non-lesional skin biopsies, and normal human epidermal keratinocytes (NHEKs) were isolated from healthy skin samples. Keratinocytes were obtained from the epidermal part and the cells were then grown in keratinocyte serum-free medium supplemented with 1% antibiotic/antimycotic solution (AB/AM), brain pituitary extract and epidermal growth factor. Fibroblasts were obtained from the dermal part and were cultured in Dulbecco's Modified Eagle Medium (DMEM) 1 g/l glucose, supplemented with 5% FBS, 1% AB/AM and 1% L-glutamine. The human immortalized keratinocyte cell line HPV-KER was also used for our experiments. Culture conditions of HPV-KER cells are the same as NHEK cells. Each cell type was cultured at 37°C in a humidified atmosphere with 5% v/v CO2.

3.5.2. Real-time, label-free cellular analysis of HPV-KER cells using the xCELLigence system

xCELLigence is a real-time, impedance measurement-based cellular analysis system, where dimensionless Cell Index (CI) value is calculated. This system was used to investigate the effect of the COMP protein on keratinocytes. HPV-KER cells were plated at a density of 10,000 cells/well in uncoated 96-well E-plates or wells that were coated with low-concentration (1 μg/ml) or high-concentration (10 μg/ml) recombinant human COMP protein (rhCOMP). Impedance measurement was performed in every 15 min for 140 h, and a dimension-free CI value was calculated for every time point.

3.5.3. Cell-proliferation assay

To investigate the effect of COMP on keratinocyte proliferation, a bromodeoxyuridine/5-bromo-2'-deoxyuridine (BrdU) cell-proliferation colorimetric ELISA assay was performed. HPV-KER and NHEK cells were plated at a density of 10,000 cells/well in 96-well plates that were uncoated or coated with low- (1 μg/ml) or high-concentration (10 μg/ml) rhCOMP protein, in three technical replicates. For the blocking of α5- and β1-integrin, the following primary antibodies (1 μg antibody for 10^6 cells) were used: mouse anti-human α5-integrin antibody, mouse anti-human β1-integrin. Goat anti-human COMP antibody (1 μg antibody for 10 μg/ml rhCOMP protein) was applied to block the COMP protein. Integrin- and COMP-blocking was applied to cells grown on uncoated plates or plates coated with 10 μg/ml rhCOMP protein. BrdU assay was performed at 24 and 72 hours after blocking, according to the manufacturer's instructions.

3.5.4. Further investigation of keratinocyte cell-proliferation

To further investigate the effect of COMP on NHEK cell's proliferation, Ki67 immunofluorescent staining was applied, using integrin and COMP-blocking as described above. Cells were plated at a density of 20,000 cells/well in 8-well chamber slides that were uncoated or coated with high-concentration (10 μg/ml) rhCOMP, in three biological replicates. Ki67 positive cells were counted on three randomly selected areas per group, and statistical analysis was performed.

3.6. *Ex vivo* skin wound-healing assay

Healthy skin samples were collected for the *ex vivo* organotypic wound healing assay. Approximately 1 cm diameter skin pieces were cut and mildly wounded in the middle using a

4 mm punch biopsy blade. Wounded skin samples and unwounded control samples were incubated for 72 hours at an air–liquid interface on the upper part of transwell cell culture inserts. The dermal part was in contact with DMEM F12 culture media supplemented with 10% FBS and 1% AB/AM. The middle of the wounds was treated for 72 hours with high-concentration (10 μg/ml) rhCOMP diluted in phosphate buffered saline (PBS) or PBS only as a control. Samples were fixed in formalin and embedded in paraffin for immunofluorescent staining. To determine the rate of proliferation, 50 cells on each wound edge were counted and the proportion of Ki67 positive cells was determined. Re-epithelization of untreated, control (where only PBS was administered), and COMP-treated wounds were assessed by measuring the area using the ImageJ software.

3.7. Hematoxylin eosin staining and light microscopic analysis

To visualize the tissue structure of *ex vivo* wound model samples, hematoxylin-eosin staining was performed according to the manufacturer's instructions in a Leica ST5020 Multistainer device. The stained samples were visualized with a Nikon eclipse TS100 microscope.

3.8. Statistical analysis

For comparing only two groups, two-tailed Student t test was performed. One-way analysis of variance (ANOVA) with Tukey post hoc test was used to compare more than two groups. Differences were considered statistically significant at **P<0.01, *P<0.05.

4. RESULTS

4.1. COMP level is elevated in psoriatic non-lesional skin

Non-lesional skin carries several known alterations of the ECM in the papillary dermis. Since COMP has previously been reported to also be present in the papillary dermis, COMP-protein accumulation was characterized in non-lesional and lesional skin from psoriasis patients and skin from healthy individuals. COMP protein was detected with western blot analysis under reducing and non-reducing conditions. Under reducing conditions, we detected elevated COMP monomer and fragment levels in psoriatic non-lesional protein extracts, compared to healthy skin.

4.2. COMP deposition is altered in the psoriatic skin

Subsequently, the distribution of COMP in tissues was analyzed using immunofluorescence staining. In line with previous reports, COMP was detected in the papillary dermis of healthy

skin. In psoriatic non-lesional samples, COMP deposition extended deeper into the dermis and formed a more even and continuous layer than observed in healthy samples. In contrast, COMP deposition in lesional skin extended to the upper part of the reticular dermis and exhibited a discontinuously scattered distribution.

4.3. COMP mRNA expression is elevated in non-lesional fibroblasts

Because skin fibroblasts are the major producers of COMP protein, we examined the mRNA expression of COMP in primary dermal fibroblasts derived from healthy and psoriatic non-lesional skin and detected elevated COMP mRNA expression in non-lesional fibroblasts.

4.4. COMP co-localization with β1-integrin of basal keratinocytes and EDA+FN is increased and with LAMA1 is decreased in non-lesional psoriatic skin

COMP is known to interact with several members of the integrin cell-surface receptor family, including α5β1-integrin, whose expression increases together with EDA+FN in non-lesional skin, possibly due to damaged BM. To investigate the possible interactions of COMP with proteins in the DEJ that have been altered, confocal microscopic analysis with dual immunofluorescence staining was applied and consecutive sections of the appropriate area were analyzed.

To determine whether COMP accumulation at the DEJ allows interaction with basal epidermal keratinocytes, COMP and β1-integrin co-immunofluorescence staining was applied. In the papillary dermis, COMP staining partially co-localized with the β1-integrin from basal keratinocytes in healthy and non-lesional skin. However, the co-localization of the two proteins was most prominent in psoriatic non-lesional skin.

LAMA1 is a component of the BM laminin layer, which is fragmented and occasionally completely missing in non-lesional psoriatic skin. Therefore, to examine whether the damaged BM of non-lesional skin allows the interaction of COMP and β1-integrin, LAMA1–COMP dual immunostaining was performed. In non-lesional skin, COMP–β1-integrin double-positive regions exhibited a discontinuous LAMA1 staining pattern and the co-occurrence of COMP and LAMA1 was significantly lower in non-lesional skin compared to healthy skin.

In addition, fibronectin has also been reported to interact with COMP; therefore, confocal microscopic analysis was also applied to COMP and EDA+FN. In psoriatic non-lesional skin, in which co-localization of COMP and β1-integrin was apparent, partial colocalization of

COMP and EDA+FN was observed, and the intensity of colocalization was significantly higher in non-lesional skin relative to healthy skin.

4.5. COMP negatively influences keratinocyte proliferation *in vitro*

To investigate whether the possible interaction between COMP and β1-integrin influences keratinocyte cellular behavior, we first performed an impedance measurement-based, real-time cellular analysis of the HPV-KER immortalized keratinocyte cell line. When the culturing plate was pre-coated with rhCOMP, cells exhibited reduced CI values in a manner that was dependent on COMP concentration compared to cells grown on uncoated surfaces. CI is influenced by changes in cell proliferation, viability, morphology and adhesion. To investigate whether the proliferation rate of HPV-KER cells was affected, a BrdU cell proliferation assay was performed. Pre-coating the surface with a high concentration (10 μg/ml) of rhCOMP resulted in significantly reduced proliferation rates at 24 and 72 hours, compared to cells grown on an uncoated surface. Cell proliferation of primary NHEK cells was also reduced when the surface was coated with 10 μg/ml rhCOMP.

4.6. COMP negatively influences keratinocyte proliferation via α5β1-integrin *in vitro*

To test whether integrins mediate the observed negative effect of COMP on cell proliferation, blocking experiments using anti-α5 and anti-β1-integrin polyclonal antibodies were performed. Blocking of either α5- or β1-integrin subunit in cells grown on a surface pre-coated with 10 μg/ml rhCOMP abolished the negative effect of COMP on HPV-KER proliferation, whereas blocking either α5- or β1-integrin alone had no negative effect on these cells. Similarly, the negative effect of COMP on the proliferation rate of primary NHEK cells could also be abolished by blocking COMP, α5- or β1-integrin, as determined with the BrdU assay and Ki67 immunofluorescent staining.

4.7. COMP has a negative effect on skin wound healing by attenuating keratinocyte proliferation and by compromising keratinocyte migration and activation in *ex vivo* wound models

To further study the effect of COMP on keratinocytes, an *ex vivo* three-dimensional skin wound-healing model was applied. Standardized wounded skin samples, with or without rhCOMP treatment, and unwounded controls were examined at 72 hours after wounding. Immunofluorescent staining revealed COMP-localization on the dermal surface of the injured region in COMP-treated wounds, whereas COMP was not detected at the injured region of

untreated wounds 72 hours after treatment. By applying Ki67 immunofluorescent staining to detect proliferating cells, we found a markedly reduced number of Ki67 positive cells in COMP-treated wounds, indicating a decreased rate of proliferation.

Cell migration processes at the wound edge during the closure of injuries require dynamic reorganization of the actin cytoskeleton in the keratinocytes located close to wound margins. To visualize these cells, immunofluorescence staining for actin was applied. In wounds not exposed to COMP, we found that keratinocytes exhibited high levels of actin expression at wound edges, while actin expression at wound edges was markedly decreased in COMP-treated wounds, indicating that actin expression was compromised, possibly resulting in a reduction of active cell migration.

KRT17 expression is known to be induced in keratinocytes at wound edges during healing. Therefore, we performed KRT17 immunofluorescence staining to further investigate the effect of COMP in the *ex vivo* wound healing model. In rhCOMP-treated wounds, KRT17 expression and re-epithelization were reduced and restricted to a smaller proportion of keratinocytes, compared to untreated control wounds. This suggests that the presence of COMP compromised keratinocyte activation.

5. DISCUSSION

In psoriasis, the non-lesional skin contains ECM alterations compared to healthy skin. COMP is localized to the papillary dermis of healthy skin and, through its interactions with type XII and XIV collagens, contributes to the stabilization of the DEJ. We found that, in psoriatic non-lesional skin, COMP is localized to the papillary dermis and, in contrast to healthy skin, it forms a continuous, more compact, linear layer beneath the basal keratinocytes. Apart from this altered localization, COMP expression was also elevated in dermal fibroblasts from psoriatic samples.

Psoriatic non-lesional skin is more sensitive to stress, and abnormalities at the DEJ and the BM are believed to be important in the development of the disease. Interruption of the BM may allow ECM components, normally located directly below the BM, to come in direct contact with basal keratinocytes. COMP reportedly binds directly to the extracellular domain of β1-integrin of both cardiomyocytes and cardiac fibroblasts, resulting in the stabilization of β1-integrin by preventing its degradation and, subsequently, improving cellular survival. In

cartilage, COMP mediates chondrocyte attachment and stabilization partially via α5β1-integrin. Our confocal microscopic analysis revealed a partial co-localization of papillary dermal COMP and β1-integrin in basal keratinocytes, which indicates the possibility of a direct interaction between these two proteins *in vivo*. In non-lesional skin, the α5-integrin subunit is overexpressed in the basal layer of the epidermis, in contrast to healthy skin, where it is present at low levels or completely missing. Our findings are in line with this observation, as COMP and β1-integrin strongly co-localize in psoriatic non-lesional epidermis and expression of both are upregulated in non-lesional skin. Moreover, the BM is partially discontinuous in psoriatic non-lesional skin, allowing direct interaction. The possibility of this interaction is supported by our finding that, in areas where COMP and β1-integrin were found to have strong co-localization in psoriatic non-lesional skin, the expression of LAMA1, a member of the BM, is reduced or completely absent.

COMP also interacts with α7β1- and αvβ3-integrins. Of these proteins, only α7β1 contains a β1-integrin subunit. There is currently no information available about α7β1-integrin expression in basal keratinocytes. Thus, we assumed that, if COMP exhibits a strong interaction with β1-integrin, its α-subunit is likely to be α5-integrin. In addition to binding to α5β1-integrin, a fibronectin receptor, COMP might also bind to the fibronectin protein itself. Furthermore, α5β1-integrin–associated fibronectin and EDA+FN are known to play roles in psoriasis pathogenesis. Enriched expression of α5β1-integrin and EDA+FN in non-lesional skin are thought to be due to the incompleteness of the laminin layer. Our confocal microscopic analysis revealed partial co-localization of COMP and EDA+FN in non-lesional skin. These results suggest that, in addition to interacting with EDA+FN, COMP may also affect basal keratinocytes via interactions with both the EDA+FN and its receptor, α5β1-integrin.

Keratinocyte behavior is influenced by ECM proteins through interactions with different cell surface integrins, and connection of basal keratinocytes to the altered BM could enhance proliferation. We analyzed the biological relevance of the interaction of COMP and β1-integrin in basal keratinocytes using HPV-KER and NHEK cells *in vitro*. We found that the presence of COMP resulted in reduced keratinocyte proliferation in both cell types and that this affect was reversible by blocking COMP with a specific antibody.

We also analyzed whether the observed negative effect of COMP on keratinocyte proliferation involves interaction with α5β1-integrin. By partially blocking the function of the

β1- or α5-integrin subunits with specific antibodies, the negative effect of COMP on cell proliferation was abolished, suggesting that the negative influence of COMP on keratinocyte proliferation involves α5β1-integrin.

Our *in vitro* findings were also validated in an *ex vivo* wound model: exogenous COMP treatment delayed healing of artificial wounds, and this affect was coupled with reduced keratinocyte proliferation and compromised actin expression, both important aspects of wound healing. In addition, keratinocyte KRT17 expression, considered a hallmark of normal wound healing, was also decreased in the presence of COMP. These results suggest that COMP has a negative influence on *ex vivo* wound healing. Interestingly, in normally healing wounds of healthy donors, COMP was hardly detectable when re-epithelialization was complete. Similarly, in psoriatic lesions, in which keratinocyte proliferation is abnormally increased, COMP was found to be discontinuous or completely absent from the papillary dermis. Although there are no data regarding keratinocyte proliferation or migration in wounds of COMP-deficient mice, in human non-healing wounds, such as venous leg ulcers, the level of COMP is reported to be highly elevated. Our findings are in agreement with this observation.

In conclusion, our study shows that COMP is present at an elevated level in the papillary dermis of non-lesional psoriatic skin and that it possibly reduces keratinocyte proliferation via the α5β1-integrin. These aspects of COMP contribute to the maintenance of the non-lesional, non-hyperproliferative state of psoriatic non-lesional epidermis, despite the overexpression of EDA+FN and α5-integrin. Similar interactions may also take place in other skin diseases in which non-healing wounds are coupled with massive COMP accumulation.

6. SUMMARY

In psoriasis, the non-lesional skin contains alterations, which affect the DEJ region, where the laminin-layer within the BM is discontinuous, as well as the fibronectin splice variant EDA+FN and the α5β1-integrin showed increased expression and could be responsible for keratinocyte hyperproliferation. COMP can modulate cellular behavior via cell-surface receptors. Furthermore, it is part of the papillary dermis of healthy skin, but its expression has not yet been studied in psoriatic skin.

In this study, we found that COMP extended deeper into the dermis and formed a more continuous layer in psoriatic non-lesional skin compared to healthy skin, while in psoriatic lesions, COMP showed a partially discontinuous deposition at the DEJ. Co-localization of COMP was increased together with basal keratinocyte β1-integrin and EDA+FN and decreased with LAMA1 in non-lesional psoriatic skin. In *in vitro* models, the presence of exogenous COMP decreased the proliferation-rate of keratinocytes and this proliferation suppressing effect was diminished by the blocking of the α5β1-integrin.

Finally, our results suggest that interacting with α5β1-integrin of non-lesional basal keratinocytes through the discontinuous LAMA1 in psoriatic non-lesional skin, COMP may contribute to restrain the proliferation rate of non-lesional basal keratinocytes, thus maintaining the non-lesional, non-hyperproliferative state of psoriatic non-lesional epidermis despite the overexpression of EDA+FN and α5β1-integrin. The antiproliferative effect of COMP is likely to be relevant to other skin diseases in which chronic non-healing wounds are coupled with massive COMP accumulation.

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