Potential relevance of periostin in psoriasis

Lili Borbála Flink

Ph.D. Thesis

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Supervisors:
Prof. Dr. Zsuzsanna Bata-Csörgő
Dr. Renáta Bozó

Doctoral School of Clinical Medicine
Department of Dermatology and Allergology
Albert Szent-Györgyi Medical School
University of Szeged, Szeged, Hungary

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LIST OF PUBLICATIONS

Scientific paper included in this thesis

I. **Flink LB**, Ghaffarinia A, Papp BT, Varga Á, Vigh AI, Vidács DL, Kui R, Kemény L, Bata-Csörgő Z, Bozó R. Abnormal basement membrane results in increased keratinocyte-derived periostin expression in psoriasis similar to wound healing. Sci Rep. 2023 Sep 29;13(1):16386. doi: 10.1038/s41598-023-43396-0.
IF: 4.6* (Journal specialization: Scopus – Multidisciplinary, Location: D1)

II. Bozó R, **Flink LB**, Belső N, Gubán B, Széll M, Kemény L, Bata-Csörgő Z. Could basement membrane alterations, resembling micro-wounds at the dermo-epidermal junction in psoriatic non-lesional skin, make the skin susceptible to lesion formation? Exp Dermatol. 2021 Jun;30(6):765-772. doi: 10.1111/exd.14267.
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Publications not directly related to the thesis

I. Vidács DL, Veréb Z, Bozó R, **Flink LB**, Polyánka H, Németh IB, Póliska S, Papp BT, Manczinger M, Gáspár R, Mirdamadi S, Kemény L, Bata-Csörgő Z. Phenotypic plasticity of melanocytes derived from human adult skin. Pigment Cell Melanoma Res. 2022 Jan;35(1):38-51. doi: 10.1111/pcmr.13012.
IF: 4.3 (Journal specialization: Scopus – Dermatology, Location: D1)

II. Bozó R, Danis J, **Flink LB**, Vidács DL, Kemény L, Bata-Csörgő Z. Stress-Related Regulation Is Abnormal in the Psoriatic Uninvolved Skin. Life (Basel). 2021 Jun 23;11(7):599. doi: 10.3390/life11070599.
IF: 3.253 (Journal specialization: Biochemistry, Genetics and Molecular Biology (miscellaneous), Location: Q2)

III. Gémes N, Makra Z, Neuperger P, Szabó E, Balog JÁ, **Flink LB**, Kari B, Hackler L Jr, Puskás LG, Kanizsai I, Szebeni GJ. A cytotoxic survey on 2-amino-1H-imidazol based synthetic marine sponge alkaloid analogues. Drug Dev Res. 2022 Dec;83(8):1906-1922. doi: 10.1002/ddr.22006.
IF: 3.8 (Journal specialization: Scopus – Drug Discovery, Location: Q2)

IV. Ghaffarinia A, Ayaydin F, Póliska S, Manczinger M, Bolla BS, **Flink LB**, Balogh F, Veréb Z, Bozó R, Szabó K, Bata-Csörgő Z, Kemény L. Psoriatic Resolved Skin Epidermal Keratinocytes Retain Disease-Residual Transcriptomic and Epigenomic Profiles. Int J Mol Sci. 2023 Feb 25;24(5):4556. doi: 10.3390/ijms24054556.
IF: 5.6* (Journal specialization: Scopus – Medicine (miscellaneous), Location: Q1)

LIST OF ABBREVIATIONS

AC: allergic conjunctivitis

AKT: protein kinase B

ANOVA: analysis of variance

BM: basement membrane

BMI: body mass index

BSA: bovine serum albumin

COMP: cartilage oligomeric matrix protein

DAPI: 4',6-diamidino-2-phenylindole

DDT: dithiothreitol

DMEM: Dulbecco's Modified Eagle's Medium

ECM: extracellular matrix

EDTA: ethylene-diamine-tetraacetic acid

$EDA^{+}FN$: extra-domain-A containing fibronectin

ELISA: enzyme-linked immunosorbent assay

EMI: elastin microfibril interface

EMT: epithelial-mesenchymal transition

Fas1: fasciclin 1

FBS: fetal bovine serum

FN: fibronectin

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GWAS: genome-wide association studies

H: healthy

HDF: human dermal fibroblast

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

IBD: inflammatory bowel disease

IFN-γ: interferon gamma

IgG: immunoglobulin gamma

IgG1κ: immunoglobulin gamma 1 kappa

IL-4: interleukin-4

IL-12: interleukin-12

IL-13: interleukin-13

IL-17: interleukin-17

IL-22: interleukin-22

IL-23p19: interleukin-23 subunit p19

IL-23p40: interleukin-23 subunit p40

IL-24: interleukin-24

L: lesional

MMP: Matrix methalloproteinase

mRNA: messenger ribonucleic acid

NHEK: normal human epidermal keratinocye

NL: non-lesional

NK: natural killer

PASI: psoriasis area and severity index

PBS: phosphate buffered saline

PI3K: phosphoinositide 3-kinase

PL: previously-lesional

RA: rheumatoid arthritis

PMSF: phenylmethylsulfonyl fluoride

RFI: relative fluorescence intensity

RNA: ribonucleic acid

SDS: sodium dodecyl sulfate

SEM: standard error of the mean

Th1: type 1 T helper

Th17: type 17 T helper

Th22: type 22 T helper

TIMP: tissue inhibitor of matrix methalloproteinase

TNF: tumor necrosis factor

VEGF: vascular endothelial growth factor

1. INTRODUCTION

1.1. Psoriasis, a chronic inflammatory skin disease

Chronic plaque-type psoriasis is a multifactorial, mainly Th1 and Th17 pathway-mediated inflammatory skin disease, which is the most frequent type of psoriasis. Less common types are guttate, erythrodermic, and pustular psoriasis[1]. Plaque-type psoriasis is characterized by epidermal hyperplasia with salmon-pink plaques covered in silvery scales (Figure 1), massive infiltration of immune cells and altered basement membrane (BM) composition with only partially understood pathomechanism. Lesions usually appear on extensor surfaces including elbows, knees, and scalps and the lumbosacral area are also often affected[2].

To determine the regional involvement, thus the severity of psoriasis, PASI (Psoriasis Area and Severity Index) index is used. This method takes into account the severity of skin inflammation, erythaema, extent, abnormal thickening of the skin and the degree of peeling in different areas of the body[3].

Both exogenous and endogenous factors play a role in the development of the disease, for example streptococcal inflammation of the pharyngeal tonsils[4]. Physical trauma, such as sunburn and certain emotional states can also be related with the onset of disease[5]. According to studies, severe stress can also correlate with the PASI values of psoriasis patients[6]. Cigarettes, alcohol, and ultraviolet radiation can all increase the manifestation of the disease[5,7–9].

It is well established that psoriasis is also associated with other diseases, most often with psoriatic arthritis[10,11], but emerging studies suggest an association with obesity, mental disorders, cardiovascular and metabolic diseases[1,12,13], although it needs to be further investigated whether psoriasis itself is a risk factor for these diseases[1]. Genes and environmental factors play crucial roles in the development of psoriasis, and disease manifestation requires both interactions. The main risk factor for developing psoriasis is heritability, which is supported by examinations of twins. Genome-wide association studies (GWAS) have helped to identify several risk loci in the genome, which are known as psoriasis susceptible loci (PSORS) [14]. PSORS1 locus is located at the major histocompatibility complex region, and on this locus HLA-Cw6 is present, which is the most frequently mapped allele in those patients, who have early onset disease[15].

Dendritic cells, T-cells and keratinocytes, the non-professional immune cells, also play a role in the disease, moreover NK cells are also involved in the development of the lesions by releasing cytokines such as IFN-γ, TNF and IL-22, which can result in the proliferation of

keratinocytes. Dendritic cells can activate IL-17-producing T cells, Th1 cells, and Th22 cells, which can then produce IL-17, IFN-γ, TNF and IL-22, and then lead to the amplification of keratinocyte hyperproliferation and inflammation[1,16].

Recent therapies are able to induce complete resolution of the symptoms, but if treatment is suspended, symptoms may occur again very often at the same body sites, indicating that in resolved lesions a molecular scar remains[17], and epigenetic changes detected in epidermal keratinocytes of resolved skin may be responsible for the disease residual transcriptomic profile found in the same regions[18].

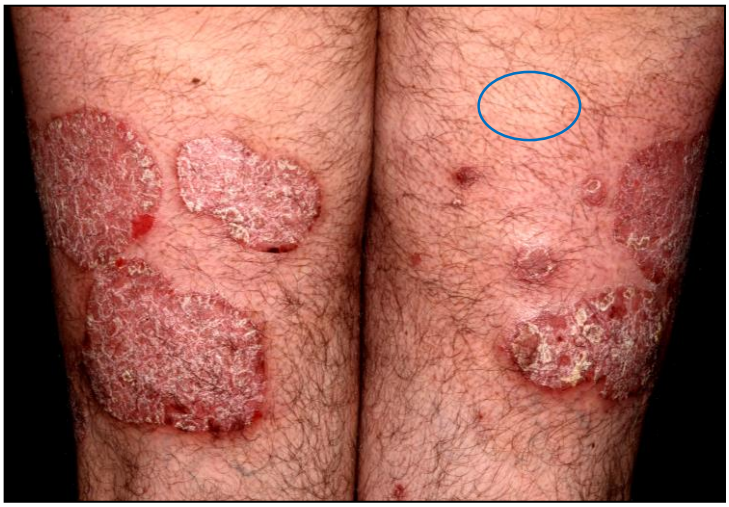

Figure 1.: Non-lesional and lesional psoriatic skin. Psoriatic lesional skin is characterized by salmon-pink plaques covered with silvery scales. Non-lesional skin is labelled with the blue circle. The picture is from the clinical archive of the Department of Dermatology and Allergology, University of Szeged.

1.2. Abnormalities in the basement membrane of psoriatic skin

Numerous data indicate that alterations of the dermal-epidermal junction region and BM zone are already present in the phenotypically healthy-looking, non-lesional psoriatic skin and it shares similarities with wound healing processes[19–22]. In the non-lesional psoriatic BM, laminin-1 is discontinuous, moreover both non-lesional and lesional skin lack the laminin-α1 chain required to maintain normal BM structure[20,22].

Furthermore, elevated expression of cartilage oligomeric matrix protein (COMP) was observed at the dermal-epidermal junction region in the non-lesional skin. COMP could interact with basal keratinocytes via α5β1-integrin through the uneven BM regions with potential anti-

proliferative effect[23]. Basal keratinocytes overexpress α5β1-integrin, the main receptor of FN, in the non-lesional psoriatic skin[24]. As opposed to normal skin both plasma and cellular forms of FN are altered in the non-lesional psoriatic skin. It has been shown that plasma FN was present around basal keratinocytes at the non-lesional dermal-epidermal junction, which could be a result of the abnormal BM. Non-lesional keratinocytes have also been reported to be more capable to produce extra-domain-A containing FN (EDA^+FN) in response to signals of activation compared to normal keratinocytes without hyperproliferation of the non-lesional epidermis[19,21,25,26]. These results suggest that despite the abnormalities at the dermal-epidermal junction region of the non-lesional skin, it shows seemingly healthy-looking phenotype suggesting the presence of compensatory mechanisms in the non-lesional skin.

1.3. The psoriatic skin resembles wound healing

The evidence that psoriasis shows a number of characteristics for healing wounds has been presented in numerous studies. The psoriatic lesional and non-lesional skin was shown to heal significantly faster than skin of healthy individuals[27]. In both wound healing and psoriasis, keratinocyte hyperproliferation, infiltration of inflammatory cells and neovascularization occur, and similirities were also observed in the expression of filaggrin, transglutaminase, involucrin, keratin-1, keratin-10, keratin-6 and keratin-16 as well[28,29]. Some antimicrobial peptides are produced not only upon injury, but in psoriasis as well[28]. Both EDA^+FN and its receptor, α5β-integrin play a key role in wound healing, where their expressions are increased, and this tendency is also present in psoriasis[24,30,31]. In normal healing wounds COMP expression is minimal, which is not the case in non-healing wounds, where COMP is overexpressed[32], similar to the psoriatic non-lesional skin. *Ex vivo* models revealed that COMP treatment decreased the proliferation rate of keratinocytes causing a delay in wound healing[23].

During the injury of the tissue, the BM is also affected[33]. In the wound bed, the BM is often not intact. Previous studies showed that in wound bed the BM is incomplete, similar to the psoriatic non-lesional skin, where in some regions the laminin layer is uneven, and EDA^+FN, α5β1-integrin and COMP show elevated expression (Figure 2). It has long been known that in psoriasis, fenestration of the BM is crucial in lesion formation[34], and soluble FN might penetrate the epidermis, developing micro-wounds[35,36].

Matrix methalloproteinases (MMPs) are also important components of wound healing. Due to their ability to remodel the ECM, MMP-2 and MMP-9 play crucial roles in wound healing, as MMP-2 is increasingly expressed at the edge of acute wounds, and in wound closure, MMP-

9 is expressed in the leading edges of migration keratinocytes[37]. MMP-2, MMP-9 and tissue inhibitor of matrix metalloproteinases (TIMP)-2 are all elevated in non-lesional psoriatic skin[38–40].

We have previously shown that the keratinocyte growth factor (KGF) and its receptor KGFR are both overexpressed in psoriasis, implying the activation of a wound healing[26]. Psoriatic non-lesional keratinocytes also express significantly higher proinflammatory IL-1 in the presence of IL-17, in contrast to normal keratinocytes, which implies an intrinsic feature of the psoriatic epithelium[41].

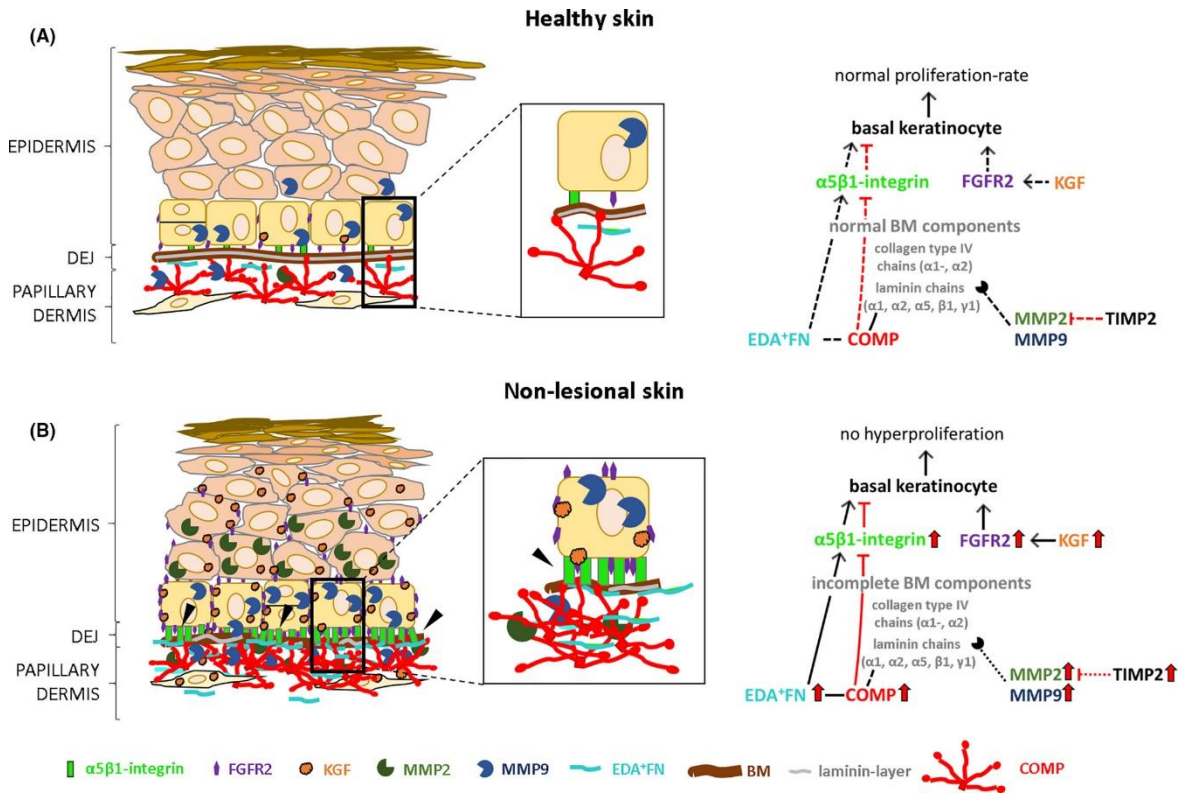

Figure 2. Micro-wounds at the dermal-epidermal junction in psoriatic non-lesional skin. Schematic representation of (A) healthy and (B) psoriatic non-lesional skin. The incompleteness of the basement membrane and elevated cartilage oligomeric matrix protein level indicates the "non-healing-like" micro-wounds at the dermal-epidermal junction region of the non-lesional skin. These sites are labelled with black arrowheads. Framed areas show enlarged regions. Straight lines indicate direct, and dashed lines indicate partial relationship between the components. Literature data indicate potential relationship between the dotted line marked components. Red arrows represent the elevated expression of the proteins. (Abbreviations: BM, basement membrane; COMP, cartilage oligomeric matrix protein; DEJ, dermal-epidermal junction; EDA^{+}FN, fibronectin splice variant containing the extra domain A; FGFR2, fibroblast growth factor receptor 2; KGF, keratinocyte growth factor; MMP2, matrix-metalloproteinase 2; MMP9, matrix-metalloproteinase 9; TIMP2, tissue inhibitor of matrix metalloproteinases 2.)

1.4. Periostin and its role in inflammatory diseases

Periostin is a 90 kDa extracellular matrix (ECM) protein, which is composed of 3 main domains; EMI, Fas1 and C-terminal[42]. The C-terminal is a hydrophilic domain that is also an alternatively spliced region, which makes periostin capable to form 11 different splice variants (Figure 3)[42,43]. In the skin periostin is mainly located in the papillary dermis and at the dermal-epidermal junction. It is expressed by both keratinocytes and fibroblasts and can bind to other ECM molecules such as type I collagen, FN and integrins, including α5β1-integrin[43,44]. It is well established that periostin plays a vital role in wound healing by maintaining tissue structure, inducing proliferation and differentiation of epithelial cells, and contributing to fibroblast activation and fibroblasts myofibroblasts transformation after transforming growth factor beta activation[45,46].

The role of periostin has been widely investigated in atopic dermatitis (AD), a skin disease with different immunopathology compared to psoriasis. Periostin has been shown to play a role in Th2 pathway-mediated inflammatory diseases, such as AD, where IL-4 and IL-13 cytokines have been reported to activate periostin production in fibroblasts (Figure 4). Periostin was shown to be elevated not only in the inflamed dermis but also in the serum of AD patients and its level correlated with disease severity suggesting that periostin is an accelerator of AD progress[47–49].

In asthma, airway epithelial cells secrete periostin induced by IL-13, which then mediates the hypersecretion of mucus and maintain eosinophilic inflammation, therefore exacerbating the asthmatic symptoms[50].

In rheumatoid arthritis, periostin is increased in the serum and is also upregulated in cells derived from the synovium and also the synovial fluid[51–53].

Furthermore, periostin was also shown to play a role in atherosclerosis, as it can cause arterial calcification and its absence protects from the disease[54,55].

The role of periostin in wound healing and other inflammatory skin diseases, such as AD, is relatively well-known[46,47,49,56]. However, its potential role in the pathogenesis of psoriasis remains undetermined.

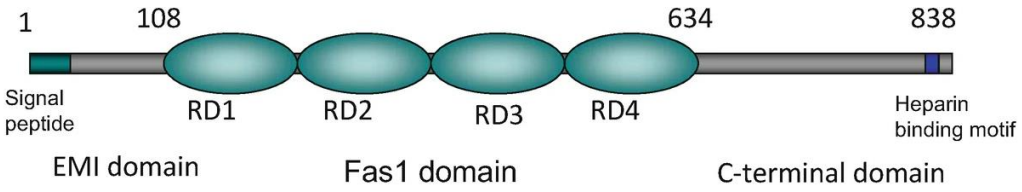

Figure 3. The structure of periostin. The EMI domain is responsible for type I collagen, FN and Notch1 biding, while the Fas1 domain contains four repeating domains, which bind integrins, tenascin-C and BMP-1. At the C-terminus, splice variants can be found, and proteolytic cleavage also occurs at this domain[42,43]. (Modified figure based on the publication of Kudo et al., 2019)

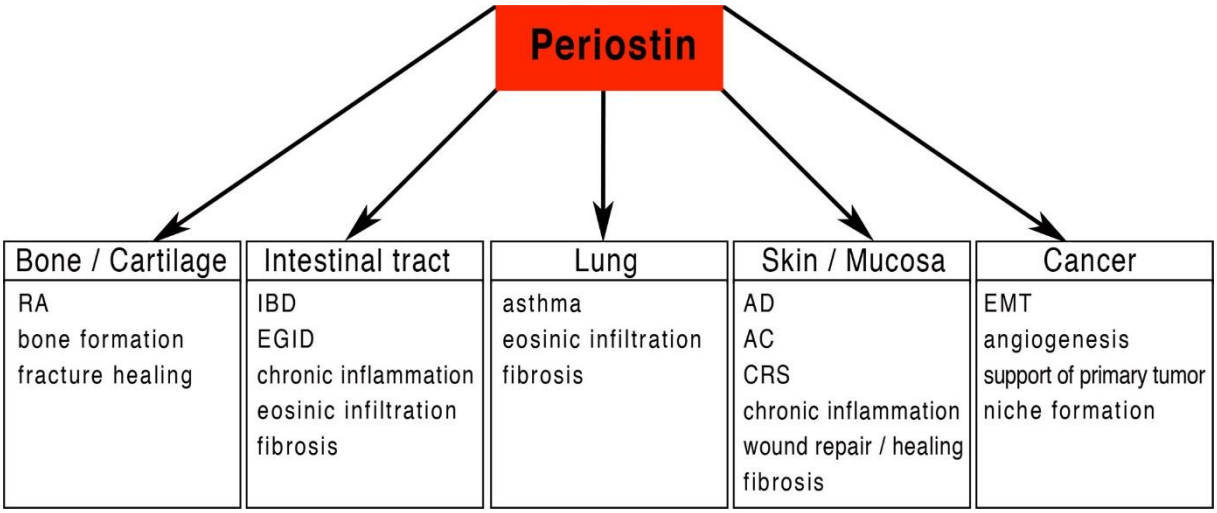

Figure 4. Implications of periostin. Periostin is involved in several biological and pathological processes, involving the attraction and proliferation of inflammatory cells throughout a number of different organs and tissues. In many tissues, periostin plays an important role in driving chronic inflammation and fibrosis[57]. (Modified figure based on the publication of Sonnenberg-Riethmacher et al., 2021)

2. AIMS

Psoriasis is an inflammatory disease, that mostly affects the skin and joints, and is mediated by Th1 and Th17 pathways. In the psoriatic non-lesional skin alterations are present, including abnormalities in the BM, which manifest in "micro-wounds" along the dermal-epidermal junction. The BM alterations include but are not limited to the overexpression of EDA^+FN, α5β1-integrin and COMP, and the unevenness of the laminin-layer.

Periostin is an ECM protein, which can interact with several molecules, including other ECM proteins such as α5β1-integrin. It is present in many tissues, also in the skin, where it is located in the dermis.

In the mainly Th2 pathway-mediated AD, periostin plays and important role, as it serves as a serum biomarker, as it is abundant in it, which correlates with the severity of the disease, and is also increased in the inflamed dermis of the AD skin. In contrast to AD, the function of periostin in psoriasis is unknown.

In this work our goal was to study the potential role of periostin in the pathomechanism of psoriasis.

We aimed to

- determine the serum periostin levels of untreated and systemically treated psoriatic patients and healthy individuals,
- examine the tissue distribution of periostin in the healthy, psoriatic non-lesional, lesional and previously-lesional skin,
- determine the protein level of periostin in healthy, psoriatic lesional and previously-lesional skin, and the mRNA expression in healthy, psoriatic non-lesional and lesional skin,
- study how different types of wounding affect the expression of periostin using *ex vivo* skin models,
- create a new *ex vivo* BM-injury skin model to examine periostin and β1-integrin expression in it, as in the psoriatic skin the BM contains "micro-wounds",
- study the potential relationship between periostin and β1-integrin in an *in vitro* wound healing model.

3. MATERIALS AND METHODS

3.1. Sample collection

In this study, we recruited patients with chronic plaque-type psoriasis and their initial Psoriasis Area Severity Index (PASI) scores were determined. Blood serum samples were collected from 105 patients in total with chronic plaque-type psoriasis and 49 healthy volunteers. The characteristics of Psoriatic patients are listed in Table 1. Untreated patients (n=41) did not receive topical therapies for 4 weeks and systemic treatments for 8 weeks before blood collection. Treated patients (n=64) received either different types of biological therapies (TNF-α inhibitors, anti-IL-12- and IL-23p40 antibody, anti-IL-17 antibody and anti-IL-23p19 antibody), or immunosuppressants (methotrexate, steroid, acitretin).

Skin punch biopsies (dia=6 mm) were collected from untreated psoriatic patients from lesional (n=4) and non-lesional (n=4, at least 6 cm from the lesion) skin areas and from healthy individuals (n=4). For the *ex vivo* wound healing models, skin biopsies were harvested from healthy volunteers (n=5). Punch biopsies were also collected from systemically treated patients from their previously-lesional, healed (n=4) skin areas. Following the rules of the Helsinki Declaration, all donors provided written informed consent before sample collection. The protocols for this study were approved by the Regional and Institutional Research Ethics Committee (HCEMM-001, 10/2020, 4702, 20 January 2020; PSO-VA0223-001, 65/2018, 4236, 19 March 2018, Szeged, Hungary; PSO-CELL-01, 90/2021, 4969, 26 April 2021, Szeged, Hungary; PSO-EDAFN-002, 34/2015, 3517, 23 February 2015, Szeged, Hungary).

Patients data	**H volunteers**	**Untreated PS patients**	**Treated PS patients**
Patients number	49	41	64
Initial median PASI	-	16.6	16.6
Initial average PASI	-	20.5	18.3
Initial PASI range	-	5.0-61.2	3.3-37.5
Genders	31 males 18 females	34 males 7 females	40 males 24 females
Median age	48	56	58
Biological therapies	-	-	43
Immunosuppressants			21
	Types of biological therapies		
TNF-α inhibitors	-	-	15
anti-IL-12- and IL-23p40 antibody	-	-	17
anti-IL-17 antibody	-	-	9
anti-IL-23p19 antibody	-	-	2
	Types of immunosuppressants		
Methotrexate	-	-	19
Steroid	-	-	1
Acitretin			1

Table 1. Clinical characteristics of psoriatic (PS) patients and healthy (H) individuals

3.2. Cell cultures

Healthy and previously-lesional psoriatic human skin biopsies were washed in Salsol A (Human Rt, Gödöllő, Hungary) containing 2% antibiotic/antimycotic solution (Sigma-Aldrich, Saint Louis, Missouri, USA). Punch biopsies were cut into small pieces and incubated in Dispase II (Roche Diagnostics, Manheim, Germany) solution overnight, then the epidermis and dermis were separated. Primary human keratinocytes were isolated from the epidermis after incubation in trypsin-EDTA solution (Sigma Aldrich, Saint Louis, Missouri, USA) for 5

minutes at 37 °C to achieve an epidermal cell suspension. Human fibroblasts were obtained from the dermis after incubation in Digestion Mix (Collagenase, Hyaluronidase, and Deoxyribonuclease, Sigma Aldrich, Saint Louis, Missouri, USA) for 2h at 37°C. The cell suspensions were filtered through 100 μm strainers (BD Falcon, San Jose, CA, USA) and pelleted by centrifugation. Primary keratinocytes were cultured in epidermal growth factor and bovine pituitary extract containing serum-free media (Gibco Keratinocyte SFM Kit; Life Technologies, Copenhagen, Denmark), while fibroblasts were grown in low glucose DMEM (Lonza Group, Basel, Switzerland) media containing 5% FBS (EuroClone, Pero, Italy). Both types of media were supplemented with 1% antibiotic/antimycotic solution (Sigma Aldrich, Saint Louis, Missouri, USA) and 1% L-glutamine (PAA Laboratories GmBH, Pasching, Austria). Cells were cultured in 75 cm^2 cell-culture flasks at 37 °C and 5% CO^2 in humidified conditions. Cell culture media were changed every 2–4 days and cells were passaged at 80% confluence. Keratinocytes were used in the third passage, fibroblasts were used in the fifth passage at 80% confluency for the experiments.

3.3. Determination of periostin in the serum

Periostin levels in the serum were measured by sandwich enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, Minnesota, USA) kits according to manufacturer's instruction.

3.4. Gene expression data analysis

To analyze periostin gene expression, GEO Profile Database (GDS4602 datasets, ID:100674764) was used, which stores publicly available microarray data from total RNA content derived from healthy (n=64), psoriatic lesional (n=58) and non-lesional (n=58) whole skin punch biopsies.

3.5. Western blot

Punch biopsies were cut and incubated in a 6 M guanidine hydrochloride (Sigma-Aldrich, Saint Louis, Missouri, USA) solution. The supernatant was collected and ethanol-based precipitation was performed, then the pellet was dissolved in 3 M urea (Sigma-Aldrich, Saint Louis, Missouri, USA). Fibroblast and keratinocyte cultures were collected in phosphate-

buffered saline and then lysed in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 150 mM potassium chloride (KCl), 1 mM magnesium chloride ($MgCl_2$), 1 mM dithiothreitol (DTT), 10% glycerol, 0.1% NP-40 (all from Sigma-Aldrich, Saint Louis, Missouri, USA) and 5% TritonX-100 (Sigma-Aldrich, Saint Louis, Missouri, USA), supplemented with 1% protease inhibitor cocktail, 1% phenylmethylsulfonyl fluoride (PMSF) and 5% of 10% sodium dodecyl sulfate (SDS) (all from Sigma-Aldrich, Saint Louis, Missouri, USA). Cell lysates and supernatants of *ex vivo* wound healing and cultured salt split models were boiled for 5 minutes with 4X loading buffer (Lonza Group, Basel, Switzerland), tissue extracts were boiled for 10 minutes with 4X loading buffer (Lonza Group, Basel, Switzerland) supplemented with β-mercaptoethanol (Sigma-Aldrich, Saint Louis, Missouri, USA) then all extracts were separated on 4–20% Mini-PROTEAN®TGX™ Precast Gels and transferred to nitrocellulose membranes (all from Bio-Rad, Hercules, California, USA), finally stained with PonceauS (Thermo Fischer Scientific, Waltham, Massachusetts, USA). Membranes were blocked with 5% non-fat milk powder containing Tris-buffered saline either supplemented with or without 1% bovine serum albumin, then incubated overnight with mouse anti-human periostin (1:500, #sc-398631, Santa Cruz Biotechnology, Dallas, Texas, USA), rabbit anti-human periostin (1:1000, #NBP1-30042, Novus Bio, Centennial, CO, USA), rabbit anti-human actin (1:2000, #A2066, Sigma-Aldrich, Saint Louis, Missouri, USA) and mouse anti-human GAPDH (1:1000, #G8795, Sigma-Aldrich, Saint Louis, Missouri, USA) either with or without membrane stripping with 100 mM glycine (Sigma-Aldrich, Saint Louis, Missouri, USA) solution. Detection was performed using horseradish peroxidase-conjugated secondary antibodies (1:2000, Southern Biotech, Birmingham, Alabama, USA) and bands were visualized by an enhanced chemiluminescent system (Bio-Rad, Hercules, California, USA) with a LI-COR C-DiGit Blot Scanner (LI-COR, Lincoln, Nebraska, USA).

3.6. Tape-stripping, *ex vivo* human skin wound healing and cultured salt-split models

For tape-stripping model (n=3), biopsies were tape-striped by adhesive tape 10 times. For the cutting-type *ex vivo* wound healing models (n=5), skin pieces were cut out of healthy skin, shaped into approximately 1 cm diameter pieces, then wounded by a 4 mm punch biopsy scalpel (Steele Supply Company, St. Joseph, MI, USA). For the cultured salt-split (n=5), 6 mm punch biopsies were incubated in 1 M NaCl (Sigma-Aldrich, Saint Louis, Missouri, USA) for 5 hours at 4 °C. All skin samples were then cultured for either 24 or 72 hours at an air–liquid interface in transwell cell culture inserts (Corning Inc., Corning, NY, USA) in 10% fetal bovine serum

(FBS, EuroClone, Pero, Italy) containing DMEM F12 (Lonza Group, Basel, Switzerland) media supplemented with 1% antibiotic/antimycotic solution (Sigma-Aldrich, Saint Louis, Missouri, USA). Samples were embedded in cryogenic solution (Thermo-Fischer Scientific, Waltham, Massachusetts, USA) for stainings, and supernatants (n=3) were collected at 0, 24 hours from *ex vivo* and 0, 24 and 72 hours from cultured salt-split models.

3.7. Tissue stainings

3.7.1. Hematoxylin eosin staining

Hematoxylin-eosin (H&E, Leica Biosystems, Wetzlar, Germany) staining was performed on tape-stripping, *ex vivo* wound healing, and cultured salt split models according to the manufacturer's instructions in a Leica ST5020 Multistainer device (Leica Biosystems, Wetzlar, Germany).

3.7.2. Immunofluorescence labeling

Frozen, 4% paraformaldehyde fixed and 0.25% TritonX-100 (Sigma Aldrich, Saint Louis, Missouri, USA) permeabilized 6 μm skin sections and were blocked with 3% normal goat serum and 1% bovine serum albumin containing (both Sigma Aldrich, Saint Louis, Missouri, USA) Tris-buffered saline. For immunolabeling mouse anti-human periostin (1:125, #sc-398631, Santa Cruz Biotechnology), and β1-integrin (1:100, #ab30394, Abcam, Cambridge, UK) were used overnight followed by Alexa Fluor 647 conjugated goat anti-mouse IgG (Life Technologies, Carlsbad, California, USA). As isotype control mouse IgG1κ (#400102, BioLegend, San Diego, California, USA) was used, 4′,6-diamidino-2-phenylindole (DAPI, 1:100, Sigma Aldrich) labeled the nuclei. Visualization, image processing and fluorescence quantification were performed by Zeiss Axio Imager Z1 microscope, ZEN 2012 Microscope Imaging software (Carl Zeiss AG, Oberkochen, Germany) and Fiji software (ImageJ, Wisconsin, USA).

3.8. *In vitro* scratch assay

Primary normal human keratinocytes were plated onto a 6-well plate at a density of 5 10^5, then 1 μg/ml β1-integrin blocking antibody (#ab30394, Abcam, Cambridge, UK, and #303004, BioLegend, San Diego, California, USA) was added 5 hours post-seeding. After 24 hours, 100% confluent cultures were scratched and cultured for 24 hours and cells were harvested for western blot analysis. The wound closure was monitored with a Zeiss Axiolab Vert.A1 microscope (Carl Zeiss AG, Oberkochen, Germany).

3.9. Statistical analysis

All data were normalized to control and were presented as mean ± standard error of the mean. Comparisons between two groups were tested for statistical significance by either one- or two-tailed two-sample *t*-test, for more than two groups Kruskal-Wallis or one-way ANOVA tests were used followed by Pairwise Wilcoxon test or Tukey's post hoc test according to the figure legends. Correlations were determined by Spearman's rank test. ***P <0.0001, **P <0.01 or *P <0.05 were considered statistically significant. Data analysis and illustration were performed either using R-Studio software (version 4.1.3 R-Studio, Boston, USA) or Prism-GraphPad 8 software (Graphpad Software Inc. version 8.0.2, San Diego, California, USA).

4. RESULTS

4.1. Serum periostin level is the highest in the systemically treated psoriatic patients and is independent of their clinical characteristics

Among serum inflammatory markers (VEGF, survivin, uPar, FN, data not shown) we found that periostin was significantly elevated in psoriatic patients, which is in agreement with previous data[47] (Figure 5a). Interestingly, among all patients, the systemically treated group showed the highest elevation in periostin serum level (Figure 5b). We did not observe significant differences between male and female patients (Figure 6a) or between younger and older patients (Figure 6b), and the Body Mass Index (BMI) did not influence on the measured periostin level (Figure 6c).

As opposed to AD, in which periostin serum levels are closely related to the severity and activity of the disease[47], in psoriasis serum periostin levels did not correlate with the severity of the disease (Figure 7a), even when we looked separately in groups of 15 and >15 Psoriasis Area Severity Index (PASI) score patients (data not shown). We compared serum periostin levels in patients on biological vs. other systemic therapies, and no significant difference was found (Figure 7b).

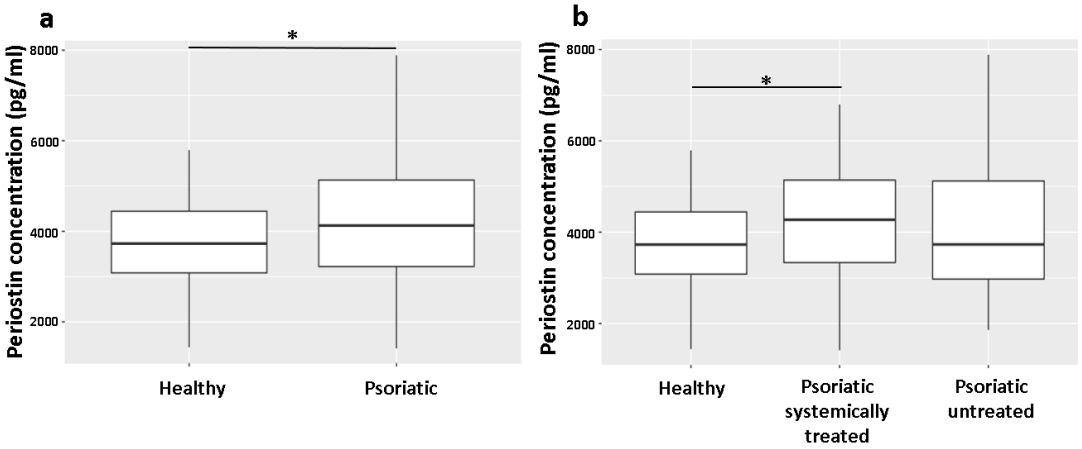

Figure 5.: Serum periostin level is elevated in psoriatic patients, especially in patients with systemic therapy. (a) Significantly elevated serum periostin levels were measured using sandwich ELISA assay in psoriatic patients (n=105) compared to healthy individuals (n=49). *P* values are determined by two-sided two-sample *t*-tests. (b) Periostin levels were measured in untreated (n=41), systemically treated psoriatic patients (n=64), and healthy individuals (n=49).

FDR-adjusted *P* values are calculated by the Kruskal-Wallis test followed by the pairwise Wilcoxon test. Median serum periostin values are indicated by horizontal bars, the top and bottom of the box represent the lower and upper quartiles, and vertical lines show the outliners. **P* <0.05 versus healthy controls.

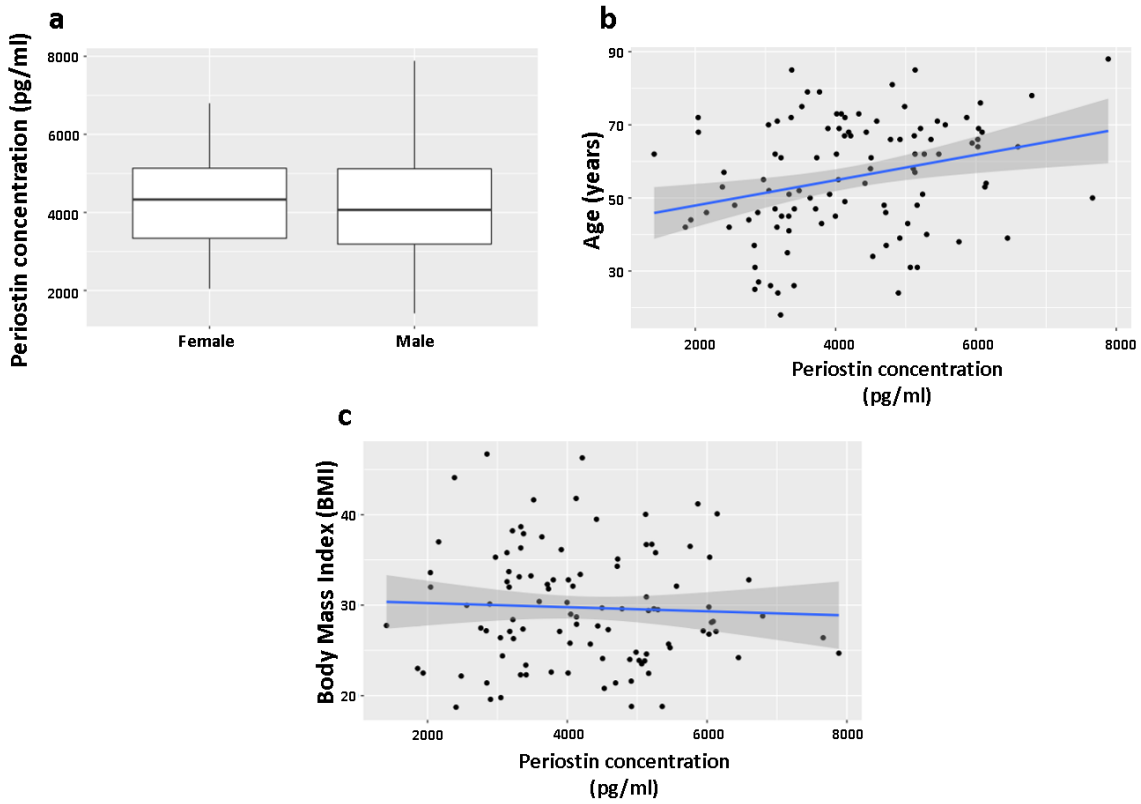

Figure 6.: Elevated serum periostin levels are independent of gender, age, and BMI in psoriatic patients. (a) Serum periostin levels in female and male psoriatic patients. *P* values are calculated by two-sided two-sample *t*-tests. (b) Correlation between serum periostin and age (n=105, r=0.291, *P*=0.003). (c) Correlation between serum periostin levels and Body Mass Index (BMI) values (n=105, r=-0.023, *P*=0.891). Correlations were determined by Spearman's rank correlation test.

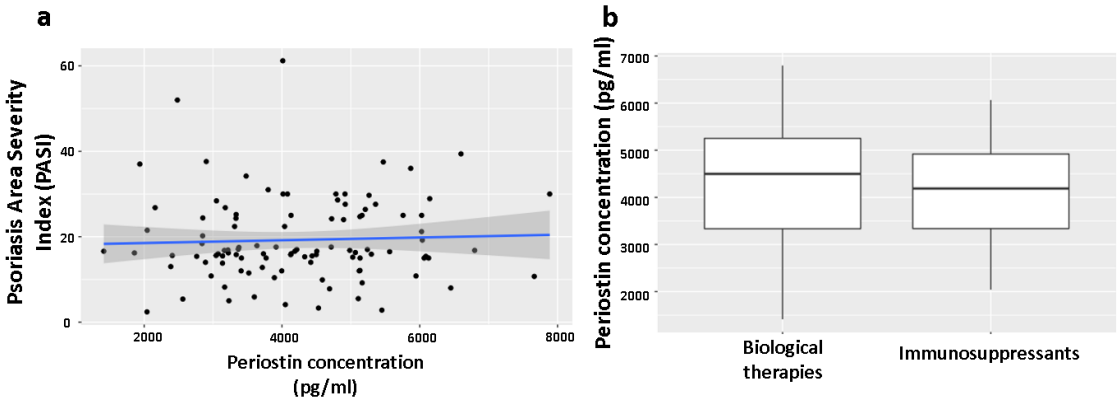

Figure 7.: Elevated serum periostin levels are independent of disease severity and the type of systemic treatment in psoriatic patients. (a) The correlation between serum periostin levels and Psoriasis Area and Severity Index (PASI) values (n=105, r=0.057, *P*=0.563). (b) The correlation was determined by Spearman's rank correlation test. Comparison of serum periostin levels in psoriatic patients treated with biological therapies and immunosuppressants. *P* values are calculated by two-sided two-sample *t*-tests.

4.2. Periostin mRNA level is decreased in the psoriatic lesional skin

Periostin mRNA levels in healthy, non-lesional, and lesional psoriatic skin were also analyzed to determine the periostin expression in the skin using data from the publicly available GEO Profile dataset. We found significantly decreased periostin mRNA expression in lesional skin compared to non-lesional and healthy skin samples (Figure 8).

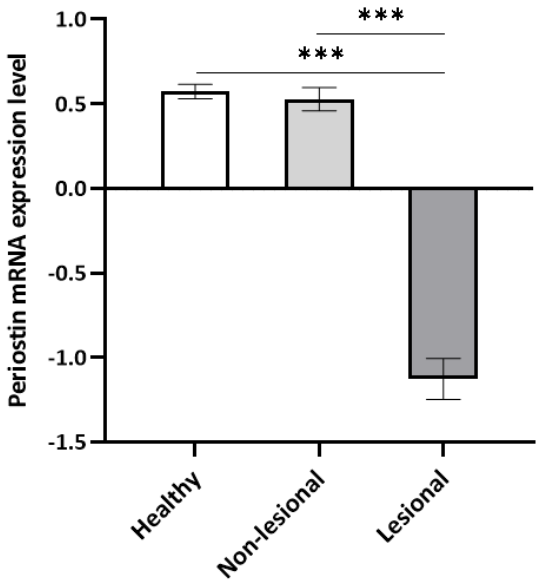

Figure 8.: Periostin mRNA expression is decreased in the lesional psoriatic skin. Periostin expression profile from the publicly available GEO Profile data was analyzed and compared with one-way ANOVA test followed by Tukey's posthoc test. H=healthy, NL=non-lesional, L=lesional. The graph shows mean±SD of healthy (n=64), non-lesional (n=58), and lesional (n=58) skin. ***:*P* <0.0001 was considered statistically significant.

4.3. Periostin expression is elevated in basal keratinocytes but not in the dermis of psoriatic skin

In normal skin, periostin is known to be localized at the papillary dermis[48]. Investigation of periostin expression in the healthy, non-lesional, and lesional skin of untreated patients as well as in the previously-lesional, healed psoriatic skin by immunofluorescence labeling revealed decreased protein levels in the dermis of lesional skin, but not in the non-lesional skin compared to healthy skin (Figure 9a). The lowest dermal periostin expression was observed in the previously-lesional skin (Figure 9d). At the same time, immunofluorescence staining also revealed a statistically significant increase in periostin expression of basal keratinocytes in the lesional and previously-lesional healed epidermis, and it was nearly significant in the non-lesional skin in contrast to healthy skin (Figure 9b,c) based on relative fluorescence intensity (RFI). With western blot analysis, we found significantly decreased periostin levels in lesional and previously-lesional protein extracts from whole skin punch biopsies versus healthy skin (Figure 10a and 10b). In previously-lesional skin, as opposed to decreased periostin at the dermal-epidermal junction, basal keratinocytes showed the highest expression (Figure 9b). Western blot analysis revealed that periostin expression of keratinocytes derived from previously-lesional psoriatic skin was increased compared to healthy cells (Figure 10c and 10d), suggesting a correlation with the immunofluorescence staining results.

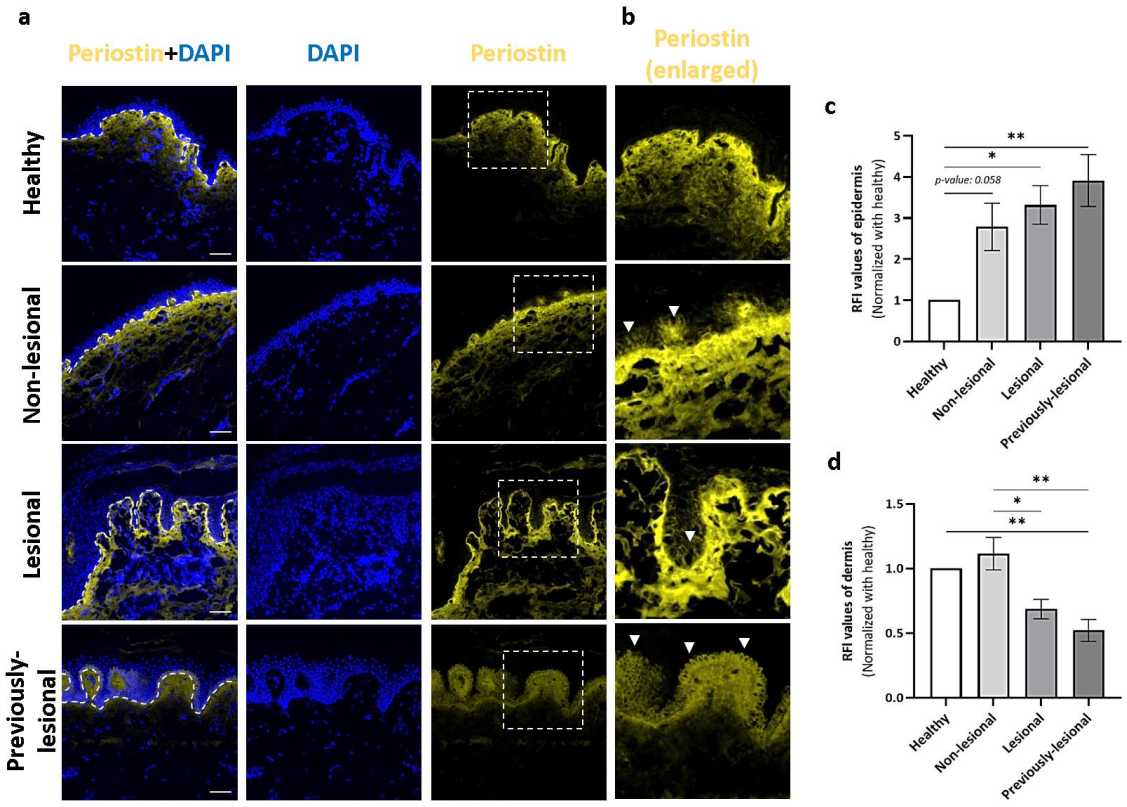

Figure 9.: Periostin expression is reduced in the lesional and previously-lesional dermis and increased in the non-lesional, lesional, and previously-lesional psoriatic epidermis. (a) Immunofluorescence staining of periostin in healthy, psoriatic non-lesional, lesional, and previously-lesional skin. Representative pictures from 4 independent donors are shown. Dotted lines highlight the border of the epidermis and dermis, dotted rectangles indicate the enlarged regions, magnification: 20x. Bar = 50 µm. (b) Periostin is present in the layer of basal keratinocytes in the non-lesional, lesional, and previously-lesional skin. Arrows indicate the positive cell layer. (c) Relative fluorescence intensity (RFI) measurement of periostin in the dermis of the healthy, non-lesional, lesional, and previously-lesional skin. (d) RFI measurement of the healthy, non-lesional, lesional, and previously-lesional epidermis. Data represent the mean±SEM (n=4). RFI values were tested for significance by using one-way ANOVA followed by Tukey's post-hoc test. *:P <0.05 and **:P <0.01 were considered significant.

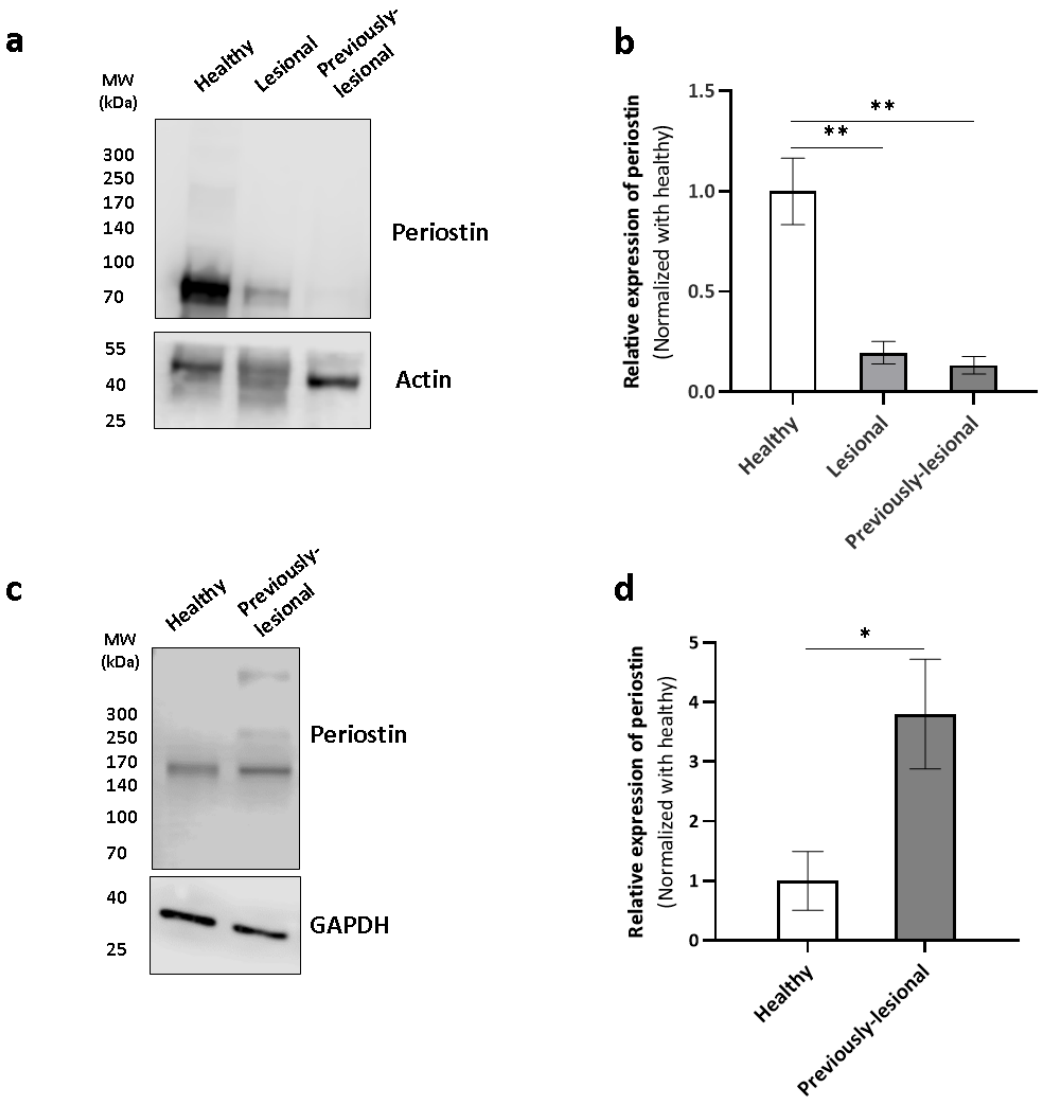

Figure 10.: Periostin is decreased in the lesional and previously-lesional psoriatic whole skin, but increased in cultured, previously-lesional psoriatic keratinocytes. (a) Western blot analysis of periostin in healthy (H), lesional (L), and previously-lesional (PL) tissue extracts and in previously-lesional and healthy primary keratinocyte lysates. Representative data from western blot analysis from 3 independent donors are shown. (b) Periostin bands were quantified by Image Studio software (LI-COR Biosciences, Lincoln, Nebraska, USA). Data were normalized to actin. The graph shows mean±SEM (n=3) of L, PL vs. H. **:P <0.01 calculated by one-way ANOVA, followed by Tukey's posthoc test. (c, d) Previously-lesional and healthy keratinocytes' periostin expressions were analyzed by western blot. GAPDH was used as loading control. Data were normalized to GAPDH. The graph shows the mean±SEM (n=3) of PL vs. H cultured keratinocytes. *P <0.05, determined by one-tailed two-sample t-test.

4.4. Periostin is produced differently in dermal fibroblasts compared to epidermal keratinocytes

Periostin is known to be expressed by both keratinocytes and fibroblasts for different molecular effects[56]. We examined how healthy cultured fibroblasts and normal human epidermal keratinocytes express periostin and we also found that both cultured cell types can produce periostin, however, the monomeric form of periostin was more characteristic of fibroblasts (Figure 11).

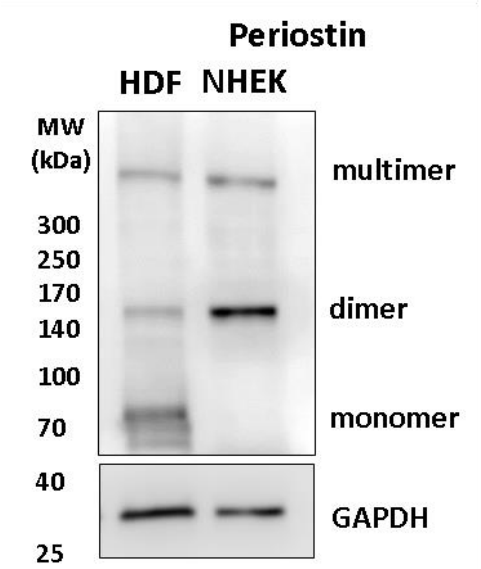

Figure 11.: Primary human keratinocytes and fibroblasts show characteristic bands of periostin. Western blot analysis of periostin in cultured normal human epidermal keratinocyte (NHEK) and human dermal fibroblast (HDF) lysates. Representative image from western blot analysis from 4 independent donors are shown. GAPDH was used as loading control.

4.5. Periostin expression is more intense in *ex vivo* wound healing- and cultured salt-split models in contrast to tape-stripping models

In order to examine the effect of different skin injuries on periostin expression, we used various wounding types using *ex vivo* skin: tape-stripping, to model barrier disruption characteristic for AD; cutting through the tissue as a classical 3D *ex vivo* wound healing model and we newly developed a cultured salt-split model, where only the BM was wounded. In the tape-stripping model, there was no obvious periostin expression in basal keratinocytes at 24- and 72 hours (Figure 12a). In the cutting model, we observed a prominent periostin expression in basal keratinocytes at the wound edges after 24 hours (Figure 12b).

Cultured salt-split samples, the models for BM injury, revealed increased periostin expression in basal keratinocytes after 72 hours compared to 24 hours' samples post-injury (Figure 12c). Since we observed increased expression of periostin by basal keratinocytes, we collected supernatant at 0 and 24 hours post-wounding from the cutting model as well as from the cultured salt-split model at 0, 24, and 72 hours post-wounding. We found similarly increased elevated periostin levels in the supernatant to what we observed in basal keratinocytes at 24 and 72 hours upon cutting or salt-split. (Figure 12d,e).

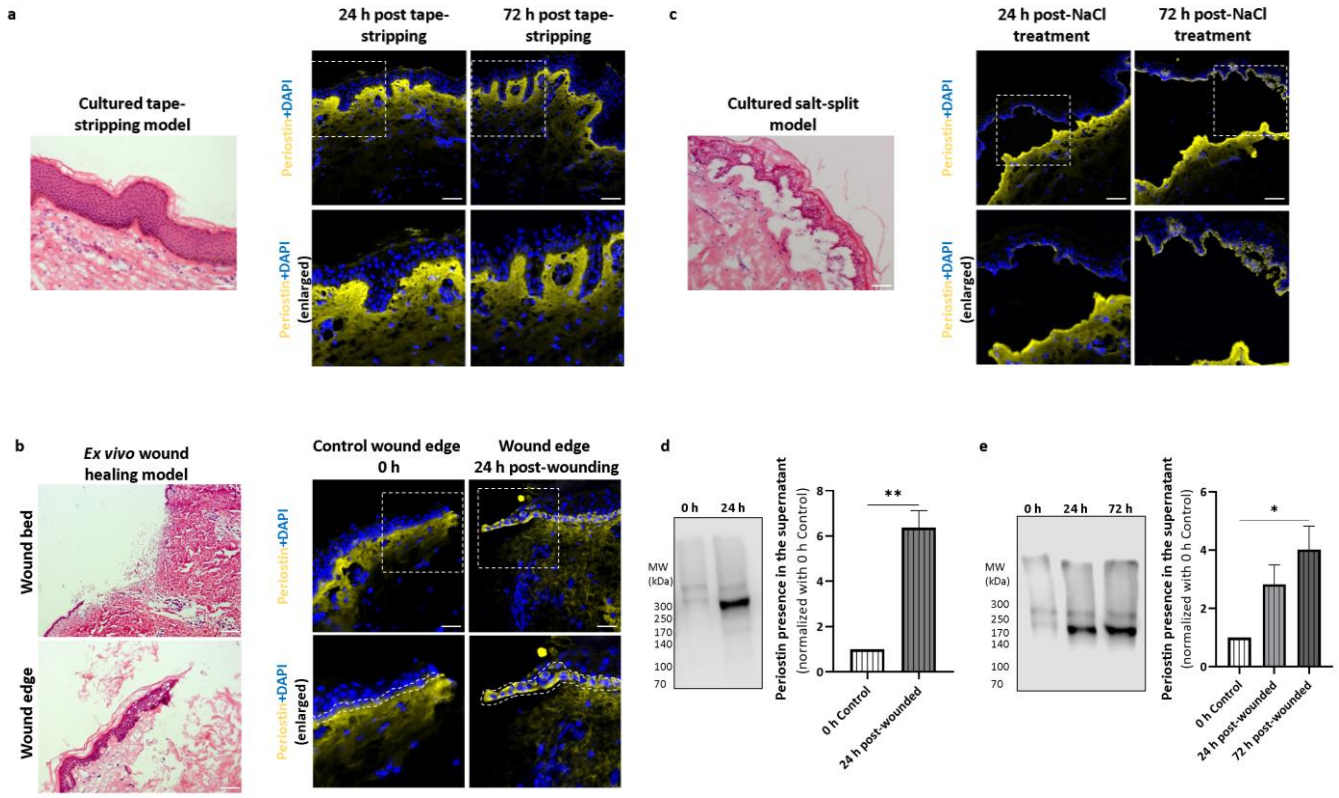

Figure 12.: Periostin expression of basal keratinocytes and the presence of periostin in the supernatant are increased in cutting-type wound healing and cultured salt-split models but not in tape-stripping models. Representative images of hematoxylin-eosin and periostin immunofluorescence staining (a) in tape-stripping models at 24 and 72 hours post-treatment (n=3), (b) in cutting-type *ex vivo* skin wound healing models at 0 and 24 hours post wounding (n=5, basal keratinocytes are highlighted by dotted lines, magnification in the hematoxylin-eosin stained wound bed: 2,5x, bar = 250 μm), and (c) in cultured salt-split basement membrane injury models at 24 and 72 hours post-injury (n=5). Dotted rectangles indicate all the enlarged regions, magnification: 20x. Bar = 50 μm. The graphs show mean±SEM (n=3) of the periostin presence in the supernatant of the (d) *ex vivo* wound healing at 0 and 24 hours, and (e) cultured salt-split models at 0, 24 and 72 hours using western blot analysis. Data were normalized to 0 hour control samples, $^{**}P < 0.01$, determined by one-tailed two-sample *t*-test; $^{*}P < 0.05$, determined by one-way ANOVA followed by Tukey's posthoc test**.** Band intensities of periostin were quantitated with Image Studio software (LI-COR Biosciences, Lincoln, NE). Representative blots are shown.

4.6. Parallel with increased periostin, β1-integrin expression is also increased in basal keratinocytes in *ex vivo* cultured and salt split wound healing models

Our previous data suggested a crucial role of β1-integrin in the stabilization of the epidermis upon BM disruption in the psoriatic non-lesional skin[23]. Examining whether β1-integrin on basal keratinocytes can perceive injuries and potentially contribute to the BM injury induced increased expression of periostin, immunofluorescence staining was performed on the cultured cutting-type and salt-split *ex vivo* models. Similar to periostin, we also detected increased β1-integrin expression by basal keratinocytes at 24 hours post-wounding in the cutting-type as well as in the cultured salt-split models after 72 hours compared to 24 hours (Figure 13a,b).

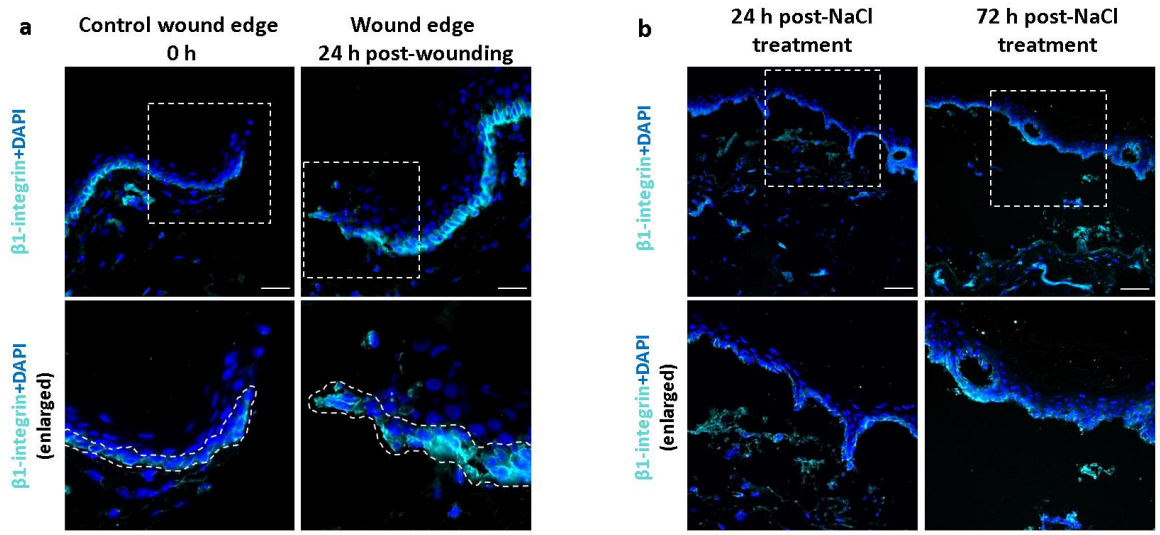

Figure 13.: β1-integrin expression of basal keratinocytes is increased in *ex vivo* wound healing- and cultured salt-split models. Immunofluorescence labeling of β1-integrin in (a) cutting-type *ex vivo* wound healing models at 0 and 24 hours (n=5, basal keratinocytes are highlighted by dotted lines), and (b) in cultured salt-split models (n=3) at 24 and 72 hours. Dotted rectangles indicate all the enlarged regions, magnification: 20x. Bar = 50 μm.

4.7. Periostin expression is reduced upon blocking β1-integrin in normal human epidermal keratinocytes

To investigate whether β1-integrin could mediate the expression of periostin, β1-integrin-blocking was applied in *in vitro* scratch wound healing assay using normal human keratinocytes. Blocking β1-integrin resulted in delayed closure of the wounds compared to unblocked normal keratinocytes (Figure 14a). Western blot analysis revealed that periostin production was reduced in keratinocytes due to blocking β1-integrin compared to unblocked control (Figure 14b,c). These results indicate that β1-integrin is needed for proper wound healing and it contributes to the induction of periostin.

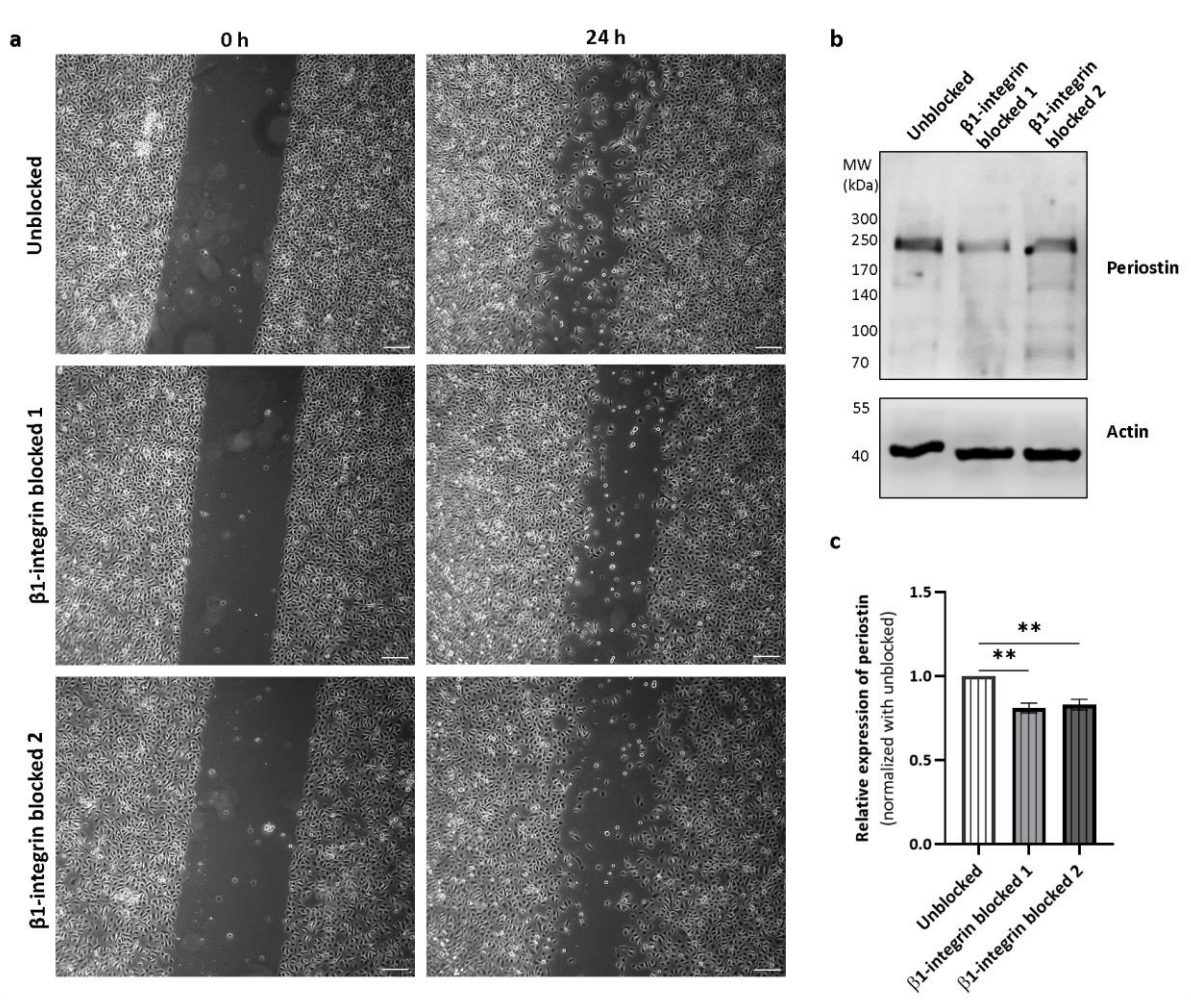

Figure 14.: β1-integrin blocking on normal human epidermal keratinocytes resulted in delayed wound healing and reduced periostin expression. (a) Representative images of *in vitro* scratch assay on primary normal human keratinocytes combined with β1-integrin blocking with two different antibodies at 0 and 24 hours post wounding (n=3, magnification: 5x. Bar = 200 µm). (b) Representative blot of periostin in keratinocyte lysates from *in vitro* scratch assays (n=3). (c) The graph shows the mean±SEM (n=3) of the periostin lysates. Actin was used as a loading control. Band intensities of periostin were quantitated with Image Studio software (LI-COR Biosciences, Lincoln, NE) and presented as fold changes normalized to actin. ***P* <0.01, determined by one-way ANOVA followed by Tukey post hoc test.

5. DISCUSSION

Increasing evidence suggests that psoriasis not only affects the skin but can be considered as a systemic inflammatory disease with abnormalities present in the circulation[58]. Periostin is involved in different inflammatory conditions such as asthma, atherosclerosis, rheumatoid arthritis, and other skin diseases, such as AD[47,50,54,55,59]. Increased serum periostin is detected in patients with AD and psoriasis, but its level is the highest in AD and correlates with disease severity. As symptoms of AD improve, serum periostin level decreases to normal level[47]. We also found significantly elevated serum periostin in psoriatic patients compared to healthy individuals, but in contrast to patients with AD, interestingly, its level was the highest in systemically treated patients. Although analysis of serum periostin levels and age of psoriatic patients showed a tendentious correlation, we did not find any statistically significant association with other clinical characteristics of the patients. Similar to our observation in psoriasis, in rheumatoid arthritis, patients in remission had higher periostin serum levels compared to healthy individuals[51], suggesting a relationship between serum periostin levels and improvement of the symptoms.

In AD, inflammation is characterized by enhanced fibroblast proliferation with an increased number of thickened collagen fibers, BM thickening, and elevated production of ECM proteins proteins, including periostin[48]. Similar tissue alterations are present in other Th2 pathway-mediated disorders such as asthma, in which serum periostin reflects inflammation activity, thus, it serves as a biomarker of acute flare of the disease[60]. As opposed to skin of AD we found periostin positivity in epidermal keratinocytes of lesional and even in non-lesional skin compared to healthy skin, at the same time, in the lesional psoriatic skin of untreated patients periostin distribution was decreased at the dermal-epidermal junction, which was confirmed by the mRNA expression and western blot analysis. Since the most prominent serum periostin expression was detected in the systemically treated psoriatic patients, we also analyzed periostin expression of previously-lesional healed skin. The previously-lesional skin showed an overall reduced periostin expression in the dermis, but basal keratinocytes showed the most prominent periostin positivity in the epidermis. Previously-lesional keratinocytes compared to healthy cells significantly overexpressed periostin in *in vitro* cultures, suggesting an activated state of the cells. In western blot analysis of periostin in cultured primary normal human keratinocytes and fibroblasts, we observed that the monomeric form of periostin was more characteristic for fibroblasts. Moreover, fibroblasts-derived monomeric periostin form was similar to what we detected in the whole tissue extracts by western blot analysis suggesting fibroblast contribution

to the whole skin periostin content. IL-4 and IL-13 can stimulate fibroblasts' periostin production, and periostin expression is elevated in the lesional dermis of patients with AD, but expression changes in epidermal keratinocytes cannot be detected[49]. IL-13 activates IL-24 in keratinocytes in a periostin-dependent way causing filaggrin downregulation, which results in an epithelial barrier dysfunction in AD[61].

Several studies have described that both non-lesional and lesional psoriatic skin show similarities with wound repair processes[62], and activation of keratinocytes is well-known during wound healing. A "pre-activated" state for hyperproliferation of keratinocytes has also been reported in the non-lesional skin[63]. It has been described that in mouse skin upon wounding, periostin was expressed by migrating keratinocytes[56]. Since BM abnormalities at the dermal-epidermal junction are characteristic alterations in psoriasis, and micro-wounds can be found along the BM, already in the non-lesional psoriatic skin, moreover wound-healing like changes are induced in keratinocytes[20–22,62], we created the cultured salt-split model, to mimic skin with BM injuries. Increased periostin expression in keratinocytes in the cultured salt-split model indicates that induction of periostin depends on injuries localized at the dermal-epidermal junction. This wound-healing like process is also present in the non-lesional and lesional skin, and the healing process in the previously-lesional, resolved skin can remain switched on and be strengthened as a result of the therapy. However, further studies are needed to determine how long lasting these changes are in the healed skin. The elevated periostin level in the supernatant of our *ex vivo* and cultured salt-split wound models indicate that activation of basal keratinocytes leads to the release of cell-produced periostin, which could partially explain the elevated serum periostin levels we detected in the systemically treated psoriatic patients. Since previous animal studies have shown that periostin promotes arterial calcification and its deletion protects against atherosclerosis[54,55], the increased serum periostin could play a role in the systemic inflammation described in psoriasis patients.

In AD the epidermal barrier injury is localized to the upper layer of keratinocytes, therefore we decided to use a tape-stripping type of injury to model the AD skin. In this model periostin expression in keratinocytes was not induced, indicating that a surface barrier epidermal damage does not induce periostin expression in keratinocytes. The abnormal BM structure of psoriatic skin can be sensed by integrins. Abnormal BM structures and injuries that affect the BM result in α5β1-integrin overexpression by keratinocytes[64]. We previously reported that β1-integrin and cartilage oligomeric matrix protein could interact in the psoriatic non-lesional skin due to the disrupted laminin-layer[23]. β1-integrin blocking resulted in suppression of periostin expression in our scratch model indicating that β1-integrin can mediate periostin production upon

wounding in keratinocytes. PI3K/AKT is the main regulator of periostin expression and growth factors, transforming growth factor beta, and integrins can also activate periostin expression via this pathway[65]. Although, we did not examine the exact mechanism for how β1-integrin can induce periostin production, further experiments could reveal that in basal keratinocytes when BM- injury occurs, β1-integrin could influence through the PI3K/AKT pathway periostin expression. Taken together, abnormal BM-induced periostin expression of basal keratinocytes can be mediated by β1-integrin, which can act as a sensor of BM injuries.

Finally, this is the first study, which describes the elevated periostin expression in psoriatic keratinocytes, which could potentially contribute to the increased serum periostin detected in this disease. In contrast to lesional skin of AD, where Th2-type cytokines stimulate fibroblasts to increase periostin production, in psoriatic skin basal keratinocytes play a key role in enhanced periostin production. Our results suggest that basal keratinocytes are in an activated state in the non-lesional, lesional, and even more so in the previously-lesional psoriatic epidermis and they show a stable wound healing-like phenotype with the overexpression of periostin reflecting the abnormal BM. β1-integrin, also overexpressed in the cells, contributes to enhanced periostin production (Figure 15). Our results also demonstrate how tissue resident cells could be differentially activated by distinct spatial changes in tissue. The abnormal BM-induced wound healing as a potential compensatory mechanism is initiated already in the non-lesional skin, it is present in the lesion, and it can be amplified as a result of the therapy and remain active in the healed skin.

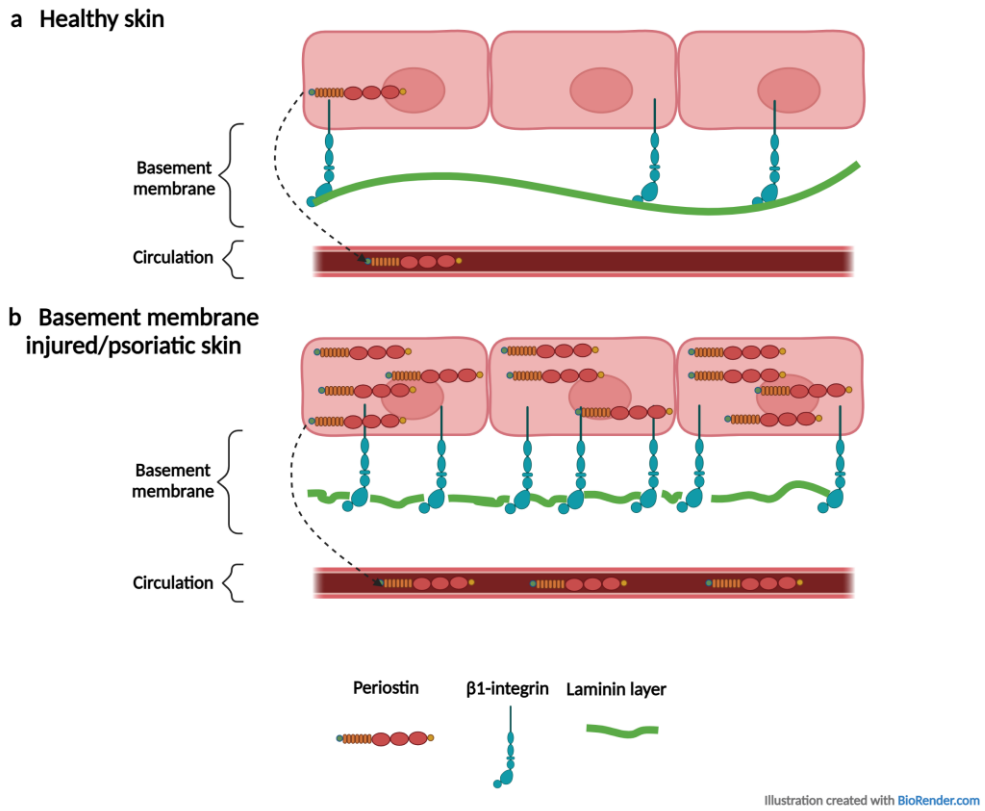

Figure 15.: Abnormal basement membrane can induce periostin expression of basal keratinocytes, and β1-integrin can potentially act as a sensor of the basement membrane injuries and potentially contribute to the increased periostin expression. Schematic representation of (a) healthy and (b) basement membrane injured, psoriatic non-lesional, lesional, and previously-lesional skin. In healthy skin, the basement membrane is intact, the laminin-layer is continuous and periostin and β1-integrin are normally expressed. In the basement membrane injured, cultured salt model, and psoriatic skin, the basement membrane is discontinuous, including the laminin-layer, β1-integrin overexpressed for providing better stabilization for the cells, and periostin expression is also induced. The periostin production of these activated keratinocytes may also contribute to the elevated serum periostin levels in psoriatic patients. The illustration was created with BioRender.com.

SUMMARY

Psoriasis is a Th1 and Th17 pathway-mediated inflammatory skin disease, where abnormalities in the BM are present, in the non-lesional skin as well. These BM alterations include the increased expression of $EDA^{+}FN$, α5β1-integrin and COMP, and the discontinuous laminin-layer. Periostin is present in many tissues, including the skin, where it is located in the dermis, and it can interact with several other ECM molecules, such as α5β1-integrin.

In this study, we show that serum and basal keratinocyte periostin expression is elevated in psoriasis. Moreover, in the serum of systemically treated patients was the highest periostin concentration. The elevated serum levels were independent of the patients' clinical characteristics.

Immunofluorescent staining revealed increased periostin expression in the layer of basal keratinocytes in the psoriatic lesional, non-lesional, and especially in the previously-lesional skin, in contrast to healthy skin. However, in the dermis, decreased periostin expression was detected in the lesional, non-lesional and previously-lesional skin as well.

Our different *ex vivo* skin wound healing models, particularly our BM injury model, revealed increased periostin expression in basal keratinocytes and increased presence of periostin in the supernatant upon healing. We also found that besides periostin, β1-integrin expression was similarly elevated in basal keratinocytes in our models, and by blocking it in our *in vitro* scratch assay led to a decrease in periostin, so the increased periostin expression was likely mediated by β1-integrin. These results indicate the role of periostin in the wound healing phenotype of psoriatic basal keratinocytes, which may be the result of BM abnormalities found in psoriatic skin.

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Co-author certification

I, myself as a corresponding author of the following publication(s) declare that the authors have no conflict of interest, and Lili Borbála Flink Ph.D. candidate had significant contribution to the jointly published research(es). The results discussed in her thesis were not used and not intended to be used in any other qualification process for obtaining a PhD degree.

DR. RENÁTA BOZÓ

14 / 11 / 2023 ..... Renáta Bozó

date author

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Abnormal basement membrane results in increased keratinocyte-derived periostin expression in psoriasis similar to wound healing

Lili Borbála Flink[1,2✉], Ameneh Ghaffarinia[2], Benjamin Tamás Papp[1], Ákos Varga[1], András István Vigh[1], Dániel László Vidács[1], Róbert Kui[1], Lajos Kemény[1,2,3], Zsuzsanna Bata-Csörgő[1,2,3] & Renáta Bozó[1,2]

The psoriatic skin resembles wound healing, and it shows abnormalities at the basement membrane (BM), also in the non-lesional skin. Fibroblast-derived dermal periostin has well-known functions in wound healing and Th2-mediated diseases, such as atopic dermatitis. Here we show that serum periostin level was elevated in psoriatic patients, remarkably in the systemically treated ones. Obvious periostin positivity was detected in basal keratinocytes of the non-lesional, lesional, and previously-lesional psoriatic vs. healthy skin. Ex vivo skin models were generated to examine how different skin injuries affect periostin expression during wound healing. Our newly developed cultured salt-split model demonstrated that BM-injury induced periostin expression in basal keratinocytes, and periostin levels in the supernatant were also increased upon healing. In wound healing models, β1-integrin expression was similarly induced. β1-integrin blocking caused reduced periostin expression in in vitro scratch assay, indicating that β1-integrin can mediate periostin production. In contrast to atopic dermatitis, psoriatic basal keratinocytes are in an activated state and show a stable wound healing-like phenotype with the overexpression of periostin. This abnormal BM-induced wound healing as a potential compensatory mechanism can be initiated already in the non-lesional skin present in the lesion and keratinocytes can remain activated in the healed skin.

Chronic plaque-type psoriasis is a multifactorial, mainly Th1 and Th17 pathway-mediated inflammatory skin disease, which is the most frequent type of psoriasis[1] with characteristic red, scaly patches. It is characterized by epidermal hyperplasia, massive infiltration of immune cells and altered basement membrane (BM) composition with only partially understood pathomechanism. Numerous data indicate that alterations of the dermal–epidermal junction region and BM zone are already present in the phenotypically healthy-looking, non-lesional psoriatic skin[2–5]. It is well established that psoriasis is also associated with other diseases, most often with psoriatic arthritis[6,7], but emerging studies suggest an association with obesity, mental disorders, cardiovascular and metabolic diseases[1,8,9], although it needs to be further investigated whether psoriasis itself is a risk factor for these diseases[1]. Genes and environmental factors play crucial roles in the development of psoriasis, and disease manifestation requires both interactions[1].

Recent therapies are able to induce complete resolution of the symptoms, but if treatment is suspended, symptoms may occur again very often at the same body sites, indicating that in resolved lesions a molecular scar remains[10], and epigenetic changes detected in epidermal keratinocytes of resolved skin may be responsible for the disease residual transcriptomic profile found in the same regions[11].

Periostin is an extracellular matrix component, in the skin it is mainly located in the papillary dermis and at the dermal–epidermal junction. It is well established that periostin plays a vital role in wound healing by

[1]Department of Dermatology and Allergology, Albert Szent-Györgyi Medical School, University of Szeged, Korányi Street 6, Szeged 6720, Hungary. [2]HCEMM-USZ Skin Research Group, University of Szeged, Szeged 6720, Hungary. [3]HUN-REN–SZTE Dermatological Research Group, Hungarian Research Network, Szeged 6720, Hungary. ✉email: flink.lili.borbala@med.u-szeged.hu

maintaining tissue structure, inducing proliferation and differentiation of epithelial cells, and contributing to fibroblast activation and fibroblasts myofibroblasts transformation after transforming growth factor beta activation[12,13].

The role of periostin has been widely investigated in atopic dermatitis, a skin disease with very different immunopathology compared to psoriasis. Periostin has been shown to play a role in Th2 pathway-mediated inflammatory diseases, such as atopic dermatitis, where interleukin-4 and interleukin-13 cytokines have been reported to activate periostin production in fibroblasts. Periostin was shown to be elevated not only in the inflamed dermis but also in the serum of atopic dermatitis patients and its level correlated with disease severity suggesting that periostin is an accelerator of atopic dermatitis progress[14–16].

The role of periostin in wound healing and other inflammatory skin diseases, such as atopic dermatitis, is relatively well-known[13,14,16,17]. However, its potential role in the pathogenesis of psoriasis remains undetermined.

Here we show that serum and basal keratinocyte periostin expression is elevated in psoriasis. Our different ex vivo skin wound healing models, particularly our BM injury model, revealed increased periostin expression in basal keratinocytes and increased presence of periostin in the supernatant upon healing. We also found that besides periostin, β1-integrin expression was similarly elevated in basal keratinocytes in our models and the increased periostin expression was likely mediated by β1-integrin. These results indicate the role of periostin in the wound healing phenotype of psoriatic basal keratinocytes, which may be the result of BM abnormalities found in psoriatic skin.

Results

Serum periostin is increased in psoriatic patients

Among serum inflammatory markers (VEGF, survivin, uPar, fibronectin, data not shown) we found that periostin was significantly elevated in psoriatic patients, which is in agreement with previous data[14] (Fig. 1a). Interestingly, among all patients, the systemically treated group showed the highest elevation in periostin serum level (Fig. 1b). We did not observe significant differences between male and female patients (Supplementary Fig. S1a) or between younger and older patients (Supplementary Fig. S1b), and the Body Mass Index (BMI) did not influence on the measured periostin level (Supplementary Fig. S1c).

As opposed to atopic dermatitis, in which periostin serum levels are closely related to the severity and activity of the disease[14], in psoriasis serum periostin levels did not correlate with the severity of the disease (Supplementary Fig. S2a), even when we looked separately in groups of 15 and > 15 Psoriasis Area Severity Index (PASI) score patients (data not shown). We compared serum periostin levels in patients on biological vs. other systemic therapies, and no significant difference was found (Supplementary Fig. S2b). Periostin mRNA levels in healthy, non-lesional, and lesional psoriatic skin were also analyzed to determine the periostin expression in the skin using data from the publicly available GEO Profile dataset. We found significantly decreased periostin mRNA expression in lesional skin compared to non-lesional and healthy skin samples (Supplementary Fig. S3).

Periostin expression is elevated in basal keratinocytes but not in the dermis of psoriatic skin

In normal skin, periostin is known to be localized at the papillary dermis[15]. Investigation of periostin expression in the healthy, non-lesional, and lesional skin of untreated patients as well as in the previously-lesional, healed psoriatic skin by immunofluorescence labeling revealed decreased protein levels in the dermis of lesional skin, but not in the non-lesional skin compared to healthy skin (Fig. 2a). The lowest dermal periostin expression

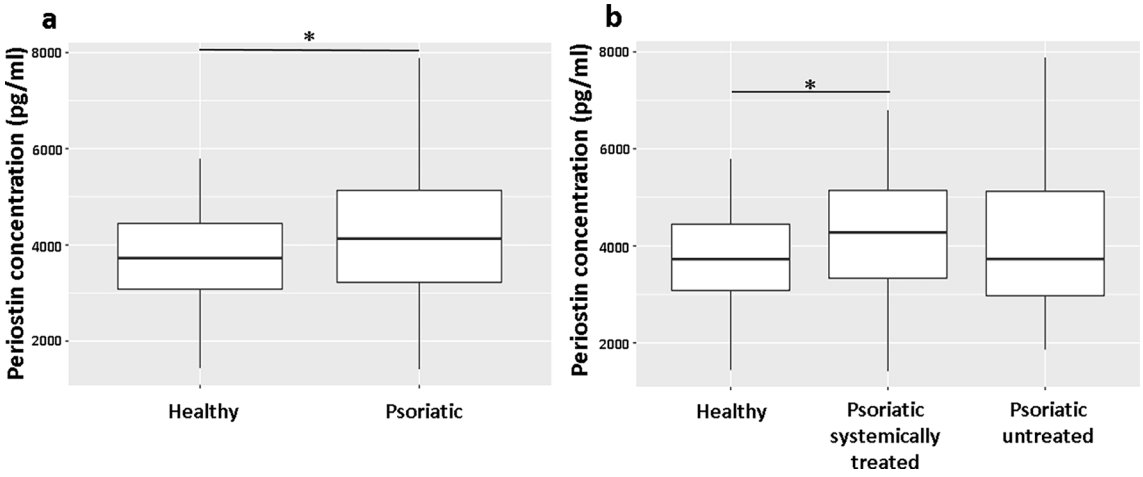

Figure 1. Serum periostin level is elevated in psoriatic patients, especially in patients with systemic therapy. (**a**) Significantly elevated serum periostin levels were measured using sandwich ELISA assay in psoriatic patients (n = 105) compared to healthy individuals (n = 49). *P* values are determined by two-sided two-sample *t*-tests. (**b**) Periostin levels were measured in untreated (n = 41), systemically treated psoriatic patients (n = 64), and healthy individuals (n = 49). FDR-adjusted *P* values are calculated by the Kruskal–Wallis test followed by the pairwise Wilcoxon test. Median serum periostin values are indicated by horizontal bars, the top and bottom of the box represent the lower and upper quartiles, and vertical lines show the outliners. $^{*}P < 0.05$ versus healthy controls.

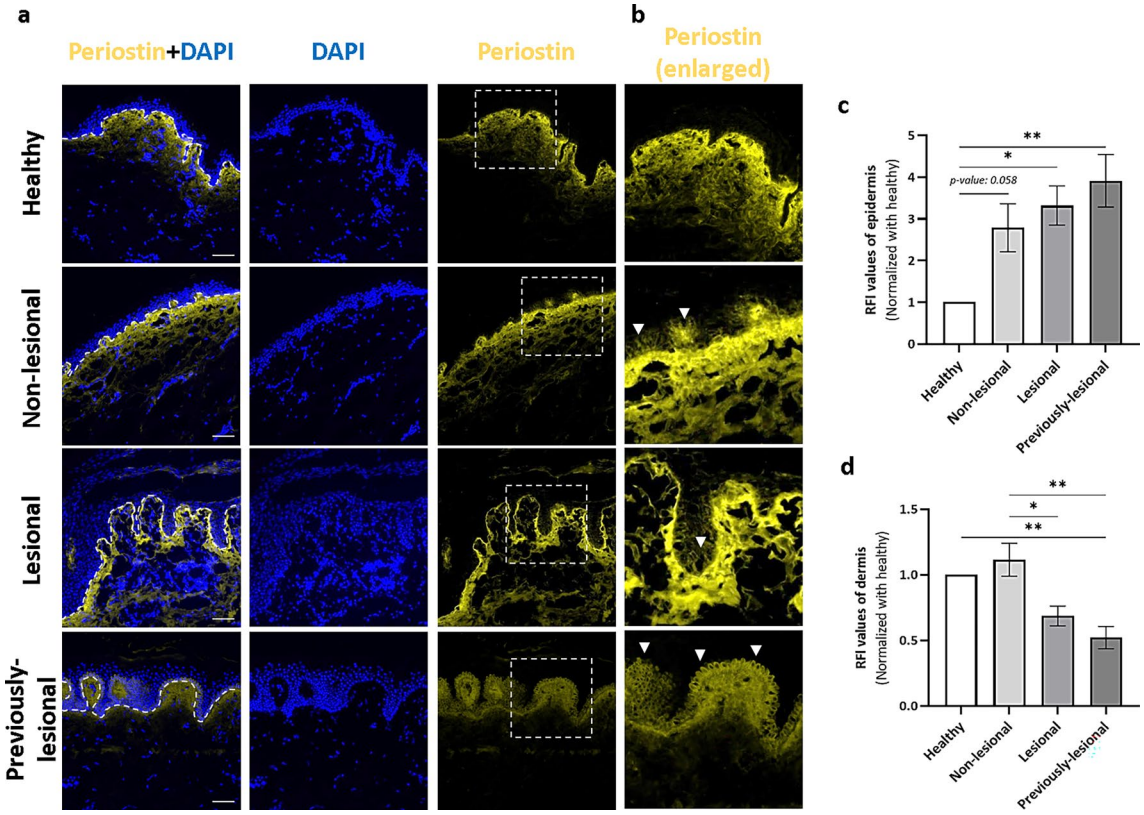

Figure 2. Periostin expression is reduced in the lesional and previously-lesional dermis and increased in the non-lesional, lesional, and previously-lesional psoriatic epidermis. (**a**) Immunofluorescence staining of periostin in healthy, psoriatic non-lesional, lesional, and previously-lesional skin. Representative pictures from 4 independent donors are shown. Dotted lines highlight the border of the epidermis and dermis, dotted rectangles indicate the enlarged regions, magnification: 20x. Bar = 50 µm. (**b**) Periostin is present in the layer of basal keratinocytes in the non-lesional, lesional, and previously-lesional skin. Arrows indicate the positive cell layer. (**c**) Relative fluorescence intensity (RFI) measurement of periostin in the dermis of the healthy, non-lesional, lesional, and previously-lesional skin. (**d**) RFI measurement of the healthy, non-lesional, lesional, and previously-lesional epidermis. Data represent the mean ± SEM (n = 4). RFI values were tested for significance by using one-way ANOVA followed by Tukey's post-hoc test. $^{*}P < 0.05$ and $^{**}P < 0.01$ were considered significant.

was observed in the previously-lesional skin (Fig. 2d). At the same time, immunofluorescence staining also revealed a statistically significant increase in periostin expression of basal keratinocytes in the lesional and previously-lesional healed epidermis, and it was nearly significant in the non-lesional skin in contrast to healthy skin (Fig. 2b,c) based on relative fluorescence intensity (RFI). With western blot analysis, we found significantly decreased periostin levels in lesional and previously-lesional protein extracts from whole skin punch biopsies versus healthy skin (Supplementary Fig. S4a and S4b). In previously-lesional skin, as opposed to decreased periostin at the dermal–epidermal junction, basal keratinocytes showed the highest expression (Fig. 2b). Western blot analysis revealed that periostin expression of keratinocytes derived from previously-lesional psoriatic skin was increased compared to healthy cells (Supplementary Fig. S4c and S4d), suggesting a correlation with the immunofluorescence staining results. Periostin is known to be expressed by both keratinocytes and fibroblasts to different molecular effects[17]. We examined how healthy cultured fibroblasts and normal human epidermal keratinocytes express periostin and we also found that both cultured cell types can produce periostin, however, the monomeric form of periostin was more characteristic of fibroblasts (Supplementary Fig. S5).

Periostin expression is more intense in ex vivo wound healing- and cultured salt-split models in contrast to tape-stripping models

In order to examine the effect of different skin injuries on periostin expression, we used various wounding types using ex vivo skin: tape-stripping, to model barrier disruption characteristic for atopic dermatitis; cutting through the tissue as a classical 3D ex vivo wound healing model and we newly developed a cultured salt-split model, where only the BM was wounded. In the tape-stripping model, there was no obvious periostin expression in basal keratinocytes at 24- and 72 h (Fig. 3a). In the cutting model, we observed a prominent periostin expression in basal keratinocytes at the wound edges after 24 h (Fig. 3b).

Cultured salt-split samples, the models for BM injury, revealed increased periostin expression in basal keratinocytes after 72 h compared to 24 h' samples post-injury (Fig. 3c). Since we observed increased expression of periostin by basal keratinocytes, we collected supernatant at 0 and 24 h post-wounding from the cutting model

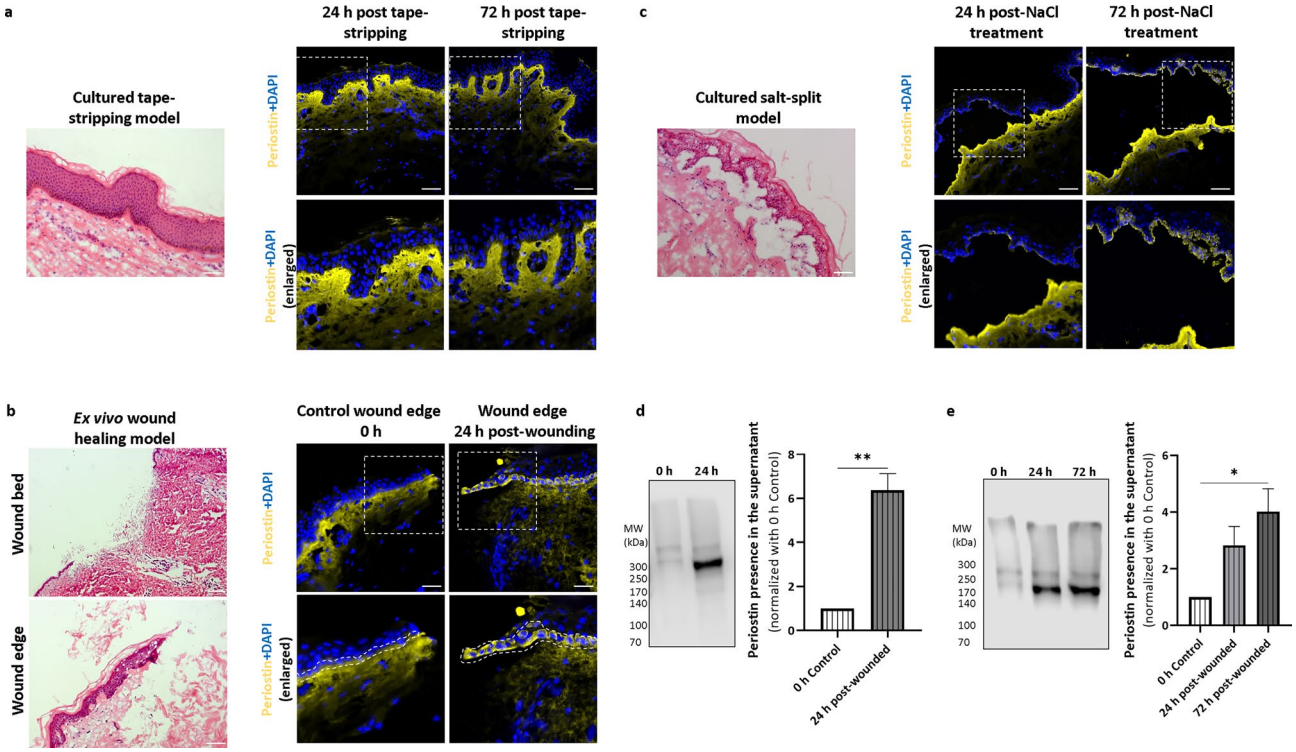

Figure 3. Periostin expression of basal keratinocytes and the presence of periostin in the supernatant are increased in cutting-type wound healing and cultured salt-split models but not in tape-stripping models. Representative images of hematoxylin–eosin and periostin immunofluorescence staining (**a**) in tape-stripping models at 24 and 72 h post-treatment (n = 3), (**b**) in cutting-type ex vivo skin wound healing models at 0 and 24 h post wounding (n = 5, basal keratinocytes are highlighted by dotted lines, magnification in the hematoxylin–eosin stained wound bed: 2,5x, bar = 250 µm), and (**c**) in cultured salt-split basement membrane injury models at 24 and 72 h post-injury (n = 5). Dotted rectangles indicate all the enlarged regions, magnification: × 20. Bar = 50 µm. The graphs show mean ± SEM (n = 3) of the periostin presence in the supernatant of the (**d**) ex vivo wound healing at 0 and 24 h, and (**e**) cultured salt-split models at 0, 24 and 72 h using western blot analysis. Data were normalized to 0 h control samples, $^{**}P < 0.01$, determined by one-tailed two-sample *t*-test; $^{*}P < 0.05$, determined by one-way ANOVA followed by Tukey's posthoc test**.** Band intensities of periostin were quantitated with Image Studio software (LI-COR Biosciences, Lincoln, NE). Representative blots are shown.

as well as from the cultured salt-split model at 0, 24, and 72 h post-wounding. We found similarly increased elevated periostin levels in the supernatant to what we observed in basal keratinocytes at 24 and 72 h upon cutting or salt-split. (Fig. 3d,e).

Parallel with increased periostin, β1-integrin expression is also increased in basal keratinocytes in ex vivo cultured and salt split wound healing models

Our previous data suggested a crucial role of β1-integrin in the stabilization of the epidermis upon BM disruption in the psoriatic non-lesional skin[18]. Examining whether β1-integrin on basal keratinocytes can perceive injuries and potentially contribute to the BM-injury induced increased expression of periostin, immunofluorescence staining was performed on the cultured cutting-type and salt-split ex vivo models. Similar to periostin, we also detected increased β1-integrin expression by basal keratinocytes at 24 h post-wounding in the cutting-type as well as in the cultured salt-split models after 72 h compared to 24 h (Fig. 4a,b).

Periostin expression is reduced upon blocking β1-integrin in normal human epidermal keratinocytes

To investigate whether β1-integrin could mediate the expression of periostin, β1-integrin-blocking was applied in in vitro scratch wound healing assay using normal human keratinocytes. Blocking β1-integrin resulted in delayed closure of the wounds compared to unblocked normal keratinocytes (Fig. 5a). Western blot analysis revealed that periostin production was reduced in keratinocytes due to blocking β1-integrin compared to unblocked control (Fig. 5b,c). These results indicate that β1-integrin is needed for proper wound healing and it contributes to the induction of periostin.

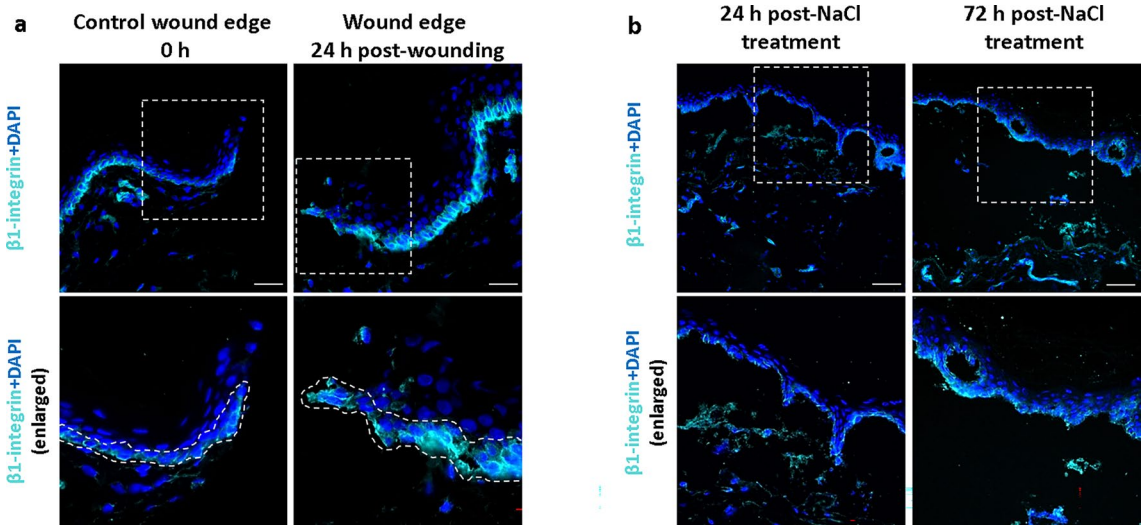

Figure 4. β1-integrin expression of basal keratinocytes is increased in ex vivo wound healing- and cultured salt-split models. Immunofluorescence labeling of β1-integrin in (**a**) cutting-type ex vivo wound healing models at 0 and 24 h (n = 5, basal keratinocytes are highlighted by dotted lines), and (**b**) in cultured salt-split models (n = 3) at 24 and 72 h. Dotted rectangles indicate all the enlarged regions, magnification: × 20. Bar = 50 µm.

Discussion

Increasing evidence suggests that psoriasis not only affects the skin but can be considered as a systemic inflammatory disease with abnormalities present in the circulation[19]. Periostin is involved in different inflammatory conditions such as asthma, atherosclerosis, rheumatoid arthritis, and other skin diseases, such as atopic dermatitis[14,20–23]. Increased serum periostin is detected in patients with atopic dermatitis and psoriasis, but its level is the highest in atopic dermatitis and correlates with disease severity. As symptoms of atopic dermatitis improve, serum periostin level decreases to normal level[14]. We also found significantly elevated serum periostin in psoriatic patients compared to healthy individuals, but in contrast to patients with atopic dermatitis, interestingly, its level was the highest in systemically treated patients. Although analysis of serum periostin levels and age of psoriatic patients showed a tendentious correlation, we did not find any statistically significant association with other clinical characteristics of the patients. Similar to our observation in psoriasis, in rheumatoid arthritis, patients in remission had higher periostin serum levels compared to healthy individuals[24], suggesting a relationship between serum periostin levels and improvement of the symptoms.

In atopic dermatitis, inflammation is characterized by enhanced fibroblast proliferation with an increased number of thickened collagen fibers, BM thickening, and elevated production of extracellular matrix proteins proteins, including periostin[15]. Similar tissue alterations are present in other Th2 pathway-mediated disorders such as asthma, in which serum periostin reflects inflammation activity, thus, it serves as a biomarker of acute flare of the disease[25]. As opposed to skin of atopic dermatitis we found periostin positivity in epidermal keratinocytes of lesional and even in non-lesional skin compared to healthy skin, at the same time, in the lesional psoriatic skin of untreated patients periostin distribution was decreased at the dermal–epidermal junction, which was confirmed by the mRNA expression and western blot analysis. Since the most prominent serum periostin expression was detected in the systemically treated psoriatic patients, we also analyzed periostin expression of previously-lesional healed skin. The previously-lesional skin showed an overall reduced periostin expression in the dermis, but basal keratinocytes showed the most prominent periostin positivity in the epidermis. Previously-lesional keratinocytes compared to healthy cells significantly overexpressed periostin in in vitro cultures, suggesting an activated state of the cells. In western blot analysis of periostin in cultured primary normal human keratinocytes and fibroblasts, we observed that the monomeric form of periostin was more characteristic for fibroblasts. Moreover, fibroblasts-derived monomeric periostin form was similar to what we detected in the whole tissue extracts by western blot analysis suggesting fibroblast contribution to the whole skin periostin content. Interleukin-4 and interleukin-13 can stimulate fibroblasts' periostin production, and periostin expression is elevated in the lesional dermis of patients with atopic dermatitis, but expression changes in epidermal keratinocytes cannot be detected[16]. Interleukin-13 activates interleukin-24 in keratinocytes in a periostin-dependent way causing filaggrin downregulation, which results in an epithelial barrier dysfunction in atopic dermatitis[26].

Several studies have described that both non-lesional and lesional psoriatic skin show similarities with wound repair processes[27], and activation of keratinocytes is well-known during wound healing. A "pre-activated" state for hyperproliferation of keratinocytes has also been reported in the non-lesional skin[28]. It has been described that in mouse skin upon wounding, periostin was expressed by migrating keratinocytes[17]. Since BM abnormalities at the dermal–epidermal junction are characteristic alterations in psoriasis, and micro-wounds can be found along the BM, already in the non-lesional psoriatic skin, moreover wound-healing like changes are induced in keratinocytes[3–5,27], we created the cultured salt-split model, to mimic skin with BM injuries. Increased periostin expression in keratinocytes in the cultured salt-split model indicates that induction of periostin depends on injuries localized at the dermal–epidermal junction. This wound-healing like process is also present in the

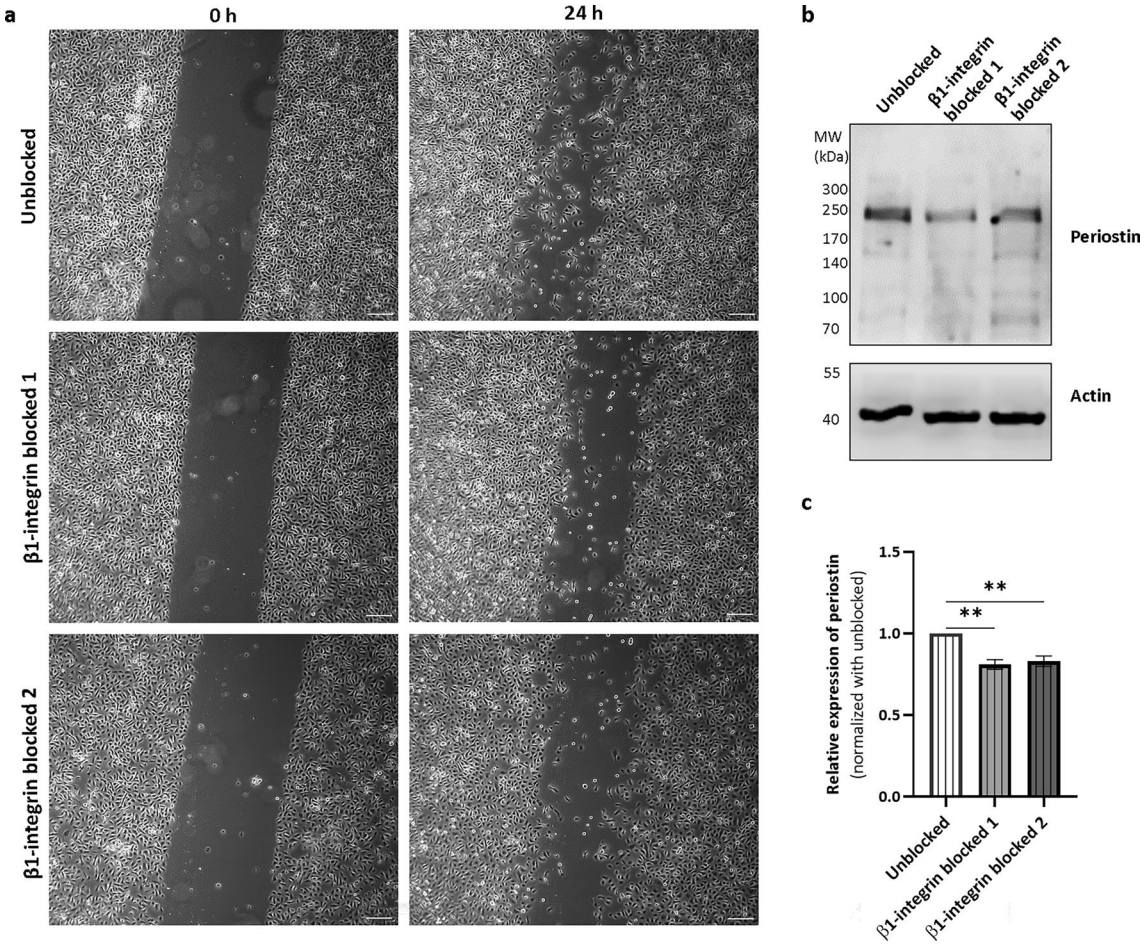

Figure 5. β1-integrin blocking on normal human epidermal keratinocytes resulted in delayed wound healing and reduced periostin expression. (**a**) Representative images of in vitro scratch assay on primary normal human keratinocytes combined with β1-integrin blocking with two different antibodies at 0 and 24 h post wounding (n = 3, magnification: × 5. Bar = 200 µm). (**b**) Representative blot of periostin in keratinocyte lysates from in vitro scratch assays (n = 3). (**c**) The graph shows the mean ± SEM (n = 3) of the periostin lysates. Actin was used as a loading control. Band intensities of periostin were quantitated with Image Studio software (LI-COR Biosciences, Lincoln, NE) and presented as fold changes normalized to actin. $^{**}P < 0.01$, determined by one-way ANOVA followed by Tukey post hoc test.

non-lesional and lesional skin, and the healing process in the previously-lesional, resolved skin can remain switched on and be strengthened as a result of the therapy. However, further studies are needed to determine how long lasting these changes are in the healed skin. The elevated periostin level in the supernatant of our ex vivo and cultured salt-split wound models indicate that activation of basal keratinocytes leads to the release of cell-produced periostin, which could partially explain the elevated serum periostin levels we detected in the systemically treated psoriatic patients. Since previous animal studies have shown that periostin promotes arterial calcification and its deletion protects against atherosclerosis[21,22], the increased serum periostin could play a role in the systemic inflammation described in psoriasis patients.

In atopic dermatitis the epidermal barrier injury is localized to the upper layer of keratinocytes, therefore we decided to use a tape-stripping type of injury to model the atopic dermatitis skin. In this model periostin expression in keratinocytes was not induced, indicating that a surface barrier epidermal damage does not induce periostin expression in keratinocytes. The abnormal BM structure of psoriatic skin can be sensed by integrins. Abnormal BM structures and injuries that affect the BM result in α5β1-integrin overexpression by keratinocytes[29]. We previously reported that β1-integrin and cartilage oligomeric matrix protein could interact in the psoriatic non-lesional skin due to the disrupted laminin layer[18]. β1-integrin blocking resulted in suppression of periostin expression in our scratch model indicating that β1-integrin can mediate periostin production upon wounding. PI3K/AKT is the main regulator of periostin expression and growth factors, transforming growth factor beta, and integrins can also activate periostin expression via this pathway[30]. Although, we did not examine the exact mechanism for how β1-integrin can induce periostin production, further experiments could reveal that in basal keratinocytes when basement membrane injury occurs, β1-integrin could influence through the PI3K/AKT pathway periostin expression. Taken together, abnormal BM-induced periostin expression of basal keratinocytes can be mediated by β1-integrin, which can act as a sensor of BM injuries.

Finally, this is the first study, which describes the elevated periostin expression in psoriatic keratinocytes, which could potentially contribute to the increased serum periostin detected in this disease. In contrast to lesional skin of atopic dermatitis, where Th2-type cytokines stimulate fibroblasts to increase periostin production, in psoriatic skin basal keratinocytes play a key role in enhanced periostin production. Our results suggest that basal keratinocytes are in an activated state in the non-lesional, lesional, and even more so in the previously-lesional psoriatic epidermis and they show a stable wound healing-like phenotype with the overexpression of periostin reflecting the abnormal BM. β1-integrin, also overexpressed in the cells, contributes to enhanced periostin production (Fig. 6). Our results also demonstrate how tissue resident cells could be differentially activated by distinct spatial changes in tissue. The abnormal BM-induced wound healing as a potential compensatory mechanism is initiated already in the non-lesional skin, it is present in the lesion, and it can be amplified as a result of the therapy and remain active in the healed skin.

Materials and methods

Blood, skin samples and ethics

In this study, we recruited patients with chronic plaque-type psoriasis and their initial Psoriasis Area Severity Index (PASI) scores were determined. Blood serum samples were collected from 105 patients in total with chronic plaque-type psoriasis and 49 healthy volunteers. The characteristics of Psoriatic patients are listed in Table 1. Untreated patients (n = 41) did not receive topical therapies for 4 weeks and systemic treatments for 8 weeks before blood collection. Treated patients (n = 64) received either different types of biological therapies (TNF-α inhibitors, anti-IL-12- and IL-23p40 antibody, anti-IL-17 antibody and anti-IL-23p19 antibody), or immunosuppressants (methotrexate, steroid, acitretin). Initial PASI values were determined.

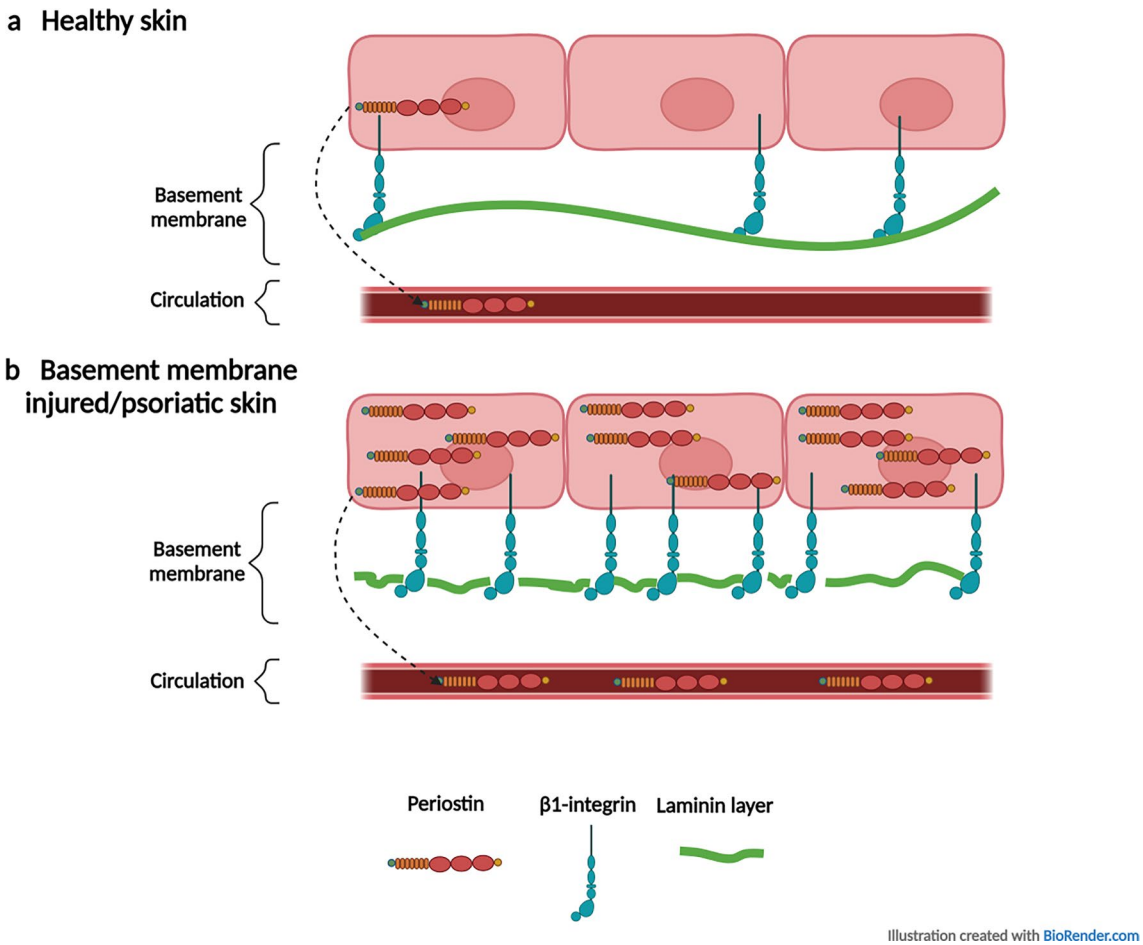

Figure 6. Abnormal basement membrane can induce periostin expression of basal keratinocytes, and β1-integrin can potentially act as a sensor of the basement membrane injuries and potentially contribute to the increased periostin expression. Schematic representation of (**a**) healthy and (**b**) basement membrane injured, psoriatic non-lesional, lesional, and previously-lesional skin. In healthy skin, the basement membrane is intact, the laminin layer is continuous and periostin and β1-integrin are normally expressed. In the basement membrane injured, cultured salt model, and psoriatic skin, the basement membrane is discontinuous, including the laminin layer, β1-integrin overexpressed for providing better stabilization for the cells, and periostin expression is also induced. The periostin production of these activated keratinocytes may also contribute to the elevated serum periostin levels in psoriatic patients. The illustration was created with BioRender.com.

Patients data	H volunteers	Untreated PS patients	Treated PS patients
Patients number	49	41	64
Initial median PASI	–	16.6	16.6
Initial average PASI	–	20.5	18.3
Initial PASI range	–	5.0–61.2	3.3–37.5
Genders	31 males	34 males	40 males
	18 females	7 females	24 females
Median age	48	56	58
Biological therapies	–	–	43
Immunosuppressants			21
Types of biological therapies			
TNF-α inhibitors	–	–	15
anti-IL-12- and IL-23p40 antibody	–	–	17
anti-IL-17 antibody	–	–	9
anti-IL-23p19 antibody	–	–	2
Types of immunosuppressants			
Methotrexate	–	–	19
Steroid	–	–	1
Acitretin			1

Table 1. Clinical characteristics of psoriatic (PS) patients and healthy (H) individuals.

Skin punch biopsies (dia = 6 mm) were collected from untreated psoriatic patients from lesional (n = 4) and non-lesional (n = 4, at least 6 cm from the lesion) skin areas and from healthy individuals (n = 4). Punch biopsies were also collected from systemically treated patients from their previously-lesional, healed (n = 4) skin areas. Following the rules of the Helsinki Declaration, all donors provided written informed consent before sample collection. The protocols for this study were approved by the Regional and Institutional Research Ethics Committee (HCEMM-001, 10/2020, 4702, 20 January 2020; PSO-VA0223-001, 65/2018, 4236, 19 March 2018, Szeged, Hungary; PSO-CELL-01, 90/2021, 4969, 26 April 2021, Szeged, Hungary; PSO-EDAFN-002, 34/2015, 3517, 23 February 2015, Szeged, Hungary).

Immunofluorescence labeling

Frozen, 4% paraformaldehyde fixed and 0.25% TritonX-100 (Sigma Aldrich, Saint Louis, Missouri, USA) permeabilized 6 µm skin sections and were blocked with 3% normal goat serum and 1% bovine serum albumin containing (both Sigma Aldrich, Saint Louis, Missouri, USA) Tris-buffered saline. For immunolabeling mouse anti-human periostin (1:125, #sc-398631, Santa Cruz Biotechnology), and β1-integrin (1:100, #ab30394, Abcam, Cambridge, UK) were used overnight followed by Alexa Fluor 647 conjugated goat anti-mouse IgG (Life Technologies, Carlsbad, California, USA). As isotype control mouse IgG1κ (#400102, BioLegend, San Diego, California, USA) was used, 4′,6-diamidino-2-phenylindole (DAPI, 1:100, Sigma Aldrich) labeled the nuclei. Visualization, image processing and fluorescence quantification were performed by Zeiss Axio Imager Z1 microscope, ZEN 2012 Microscope Imaging software (Carl Zeiss AG, Oberkochen, Germany) and Fiji software (ImageJ, Wisconsin, USA).

Determination of periostin in the serum

Periostin levels in the serum were measured by sandwich enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, Minnesota, USA) kits according to manufacturer's instruction.

Tape-stripping, ex vivo human skin wound healing and cultured salt-split models

For tape-stripping model (n = 3), biopsies were tape-striped by adhesive tape 10 times. For the cutting-type ex vivo wound healing models (n = 5), skin pieces were cut out of healthy skin, shaped into approximately 1 cm diameter pieces, then wounded by a 4 mm punch biopsy scalpel (Steele Supply Company, St. Joseph, MI, USA). For the cultured salt-split (n = 5), 6 mm punch biopsies were incubated in 1 M NaCl (Sigma-Aldrich, Saint Louis, Missouri, USA) for 5 h at 4 °C. All skin samples were then cultured for either 24 or 72 h at an air–liquid interface in transwell cell culture inserts (Corning Inc., Corning, NY, USA) in 10% fetal bovine serum (FBS, EuroClone, Pero, Italy) containing DMEM F12 (Lonza Group, Basel, Switzerland) media supplemented with 1% antibiotic/antimycotic solution (Sigma-Aldrich, Saint Louis, Missouri, USA). Samples were embedded in cryogenic solution (Thermo-Fischer Scientific, Waltham, Massachusetts, USA) for stainings, and supernatants (n = 3) were collected at 0, 24 h from ex vivo and 0, 24 and 72 h from cultured salt-split models.

In vitro scratch assay

Primary normal human keratinocytes were plated onto a 6-well plate at a density of 5 10^5, then 1 µg/ml β1-integrin blocking antibody (#ab30394, Abcam, Cambridge, UK, and #303004, BioLegend, San Diego,

California, USA) was added 5 h post-seeding. After 24 h, 100% confluent cultures were scratched and cultured for 24 h and cells were harvested for western blot analysis. The wound closure was monitored with a Zeiss Axiolab Vert.A1 microscope (Carl Zeiss AG, Oberkochen, Germany).

Further methods

More detailed information on the materials and methods is presented in the Supplementary Data.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Conceptualization: L.B.F., Z.B.C. and R.B. Experimental design: L.B.F., R.B. and Z.B.C. Investigation: L.B.F., R.B., B.T.P., Á.V., A.I.V., R.K. and D.L.V. Formal analysis: L.B.F., R.B., B.T.P. and A.G. Writing—Original Draft Preparation: L.F.B. and R.B. Writing—Review and Editing: Z.B.C. and R.B. Supervision: L.K., Z.B.C. and R.B.

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Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to L.B.F.

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VIEWPOINT

Could basement membrane alterations, resembling micro-wounds at the dermo-epidermal junction in psoriatic non-lesional skin, make the skin susceptible to lesion formation?

Renáta Bozó[1,2] | Lili Borbála Flink[1] | Nóra Belső[1,2] | Barbara Gubán[1] | Márta Széll[3,4] | Lajos Kemény[1,2,3] | Zsuzsanna Bata-Csörgő[1,2,3]

[1]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary

[2]HCEMM-SZTE Skin Research Group, Szeged, Hungary

[3]MTA-SZTE Dermatological Research Group, Szeged, Hungary

[4]Department of Medical Genetics, University of Szeged, Szeged, Hungary

Correspondence
Renáta Bozó, Department of Dermatology and Allergology, University of Szeged, Korányi Fasor 6, Szeged, H-6720 Hungary.
Email: bozo.renata@med.u-szeged.hu

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Abstract

Current data suggest that tissue microenvironment control immune functions. Therefore, understanding the tissue environment in which immune activation occurs will enhance our capability to interfere with abnormal immune pathology. Here, we argue that studying the constitutively abnormal functions of clinically uninvolved psoriatic skin in patients with plaque type psoriasis is very important to better understand psoriasis pathobiology, because non-lesional skin provides the tissue environment in which the psoriatic lesion develops. A key question in psoriasis is what initiates the abnormal, uncontrolled immune activation in the first place and the answer may lie in the skin. In light of this concept, we summarize abnormalities at the dermal-epidermal junction region which shows a special "non-healing-like" micro-wound phenotype in the psoriatic non-lesional skin that may act as a crucial susceptibility factor in the development of the disease.

KEYWORDS
basement membrane, dermal-epidermal junction, non-lesional skin, psoriasis, wound healing

1 | INTRODUCTION

Psoriasis is a complex inflammatory skin disease, which is mainly characterized by keratinocyte hyperproliferation and massive immune cell infiltration, primarily affecting the skin[1] with a wide-ranging clinical presentation.[2] However, not only the skin can show differences between patients, but some patients have arthritis[3,4] while others may have psoriasis-related other organ involvements.[5] Risk in developing the disease is based on multiple genetic and environmental factors which differ in individuals and in different populations.[6] Pustular psoriasis, the acute systemic generalized form, the chronic localized palmoplantar pustulosis and the acrodermatitis continua of Hallopeau represent a distinct group and have been linked to interleukin (IL)-36 receptor antagonist (IL36RN) mutations. Mutations were also found in that group in a gene encoding the adaptor protein 1 complex subunit (AP-1 complex subunit sigma-3, AP1S3) and a keratinocyte nuclear factor κB adaptor protein (the caspase recruitment domain-containing protein 14, CARD14).[7] Guttate psoriasis is

associated with streptococcal pharyngitis and HLA-Cw6, the most strongly associated psoriasis susceptibility allele.[8] Psoriasis studies, including our own studies, usually focus on the most frequent, dominant plaque type psoriasis phenotype. An inherently abnormal immune regulation, both in innate as well as in adaptive responses, undoubtedly plays a central role in disease pathogenesis.[9] However, it is also clear that the immune pathology is strongly influenced by the tissue environment.[10,11]

An increasing body of evidence suggests that not only the lesional skin of patients is affected in psoriasis, but the seemingly healthy-looking non-lesional skin of patients contains disease-specific alterations as well. In 1995, Kadunce and Krueger suggested that in psoriasis the entire skin has the capacity to develop lesions, and control of expression is linked to a complex interaction of epidermal, dermal and immune cells. They considered psoriatic keratinocytes to have an inherent capacity for hyperproliferation and aberrant differentiation and advised to consider not only cytokines and growth factors, but also regulators of transcription, translation and modifications of cytokines and growth factors.[12] Data suggest that the psoriatic non-lesional skin could represent an intermediate stage between the healthy and lesional skin. Alterations in the non-lesional skin can reveal predisposing factors for lesion development and specific structural changes, or factors which help in the maintenance of the non-lesional stage.[13]

2 | INFLAMMATION IN THE NON-LESIONAL PSORIATIC SKIN

The number of several immune cell types are elevated in non-lesional skin, and the inducible T-cell co-stimulator, ICOS, a T-cell activation marker, is also detected.[14] The characteristically sharp edges of psoriatic plaques are indicative of a very local, tissue-, if not cell-dependent effect. Cytokine signalling plays a key role in the pathomechanism of psoriasis. In psoriatic plaques, Th17 cytokines (mainly the IL-17A) stimulate the production of antimicrobial peptides which activate inflammatory cells, thus sustaining the inflamed state in the psoriatic skin. A large-scale study highlights the psoriasis specificity of IL-17A signature, the importance of cathelicidin (LL37) and an increase in foetal and other protein abundance, suggesting degradation deficiency.[15] The presence of circulating LL37-specific T cells significantly correlates with disease activity.[16] Dermal $CD3^{+}\gamma\delta$ T cells are the major source of IL-17A, and their number is increased in psoriatic skin.[17–19] Meta-analysis on three gene array studies showed enhanced expression of IL-17 and IL-17 signature genes in non-lesional epidermis compared with normal. The transcription factor C/EBPδ also showed elevated expression[14] and it is believed to mediate the IL-17 signalling and it could enhance the sensitivity of non-lesional keratinocytes to IL-17 stimuli.[20] Elevated expression of IL-22 in non-lesional versus healthy skin is associated with an upregulation of antimicrobial peptides.[14] In psoriasis, interferon-γ (IFNγ) is known to take part in the immune induction of the keratinocyte cell cycle progression.[21] In the above-mentioned gene array, the expression level of IFNγ and the IFNγ-related genes also showed higher expression in non-lesional versus healthy skin. It is also interesting to note that some genes, which are known to mediate keratinocyte differentiation processes, are also upregulated in the non-lesional skin.[14] Furthermore, proinflammatory mediators, namely IL-1α and IL-1β, have been reported to show lower expression level in non-lesional skin.[22] According to a recent study, in the clinically resolved lesions epidermal tissue, resident memory T cells are retained in the tissue and can produce cytokines indicating a site-specific T cell–driven disease memory.[23] Besides alterations in the resolved psoriatic skin, epidermal resident T cells in never-lesional skin from patients with psoriasis can trigger psoriasiform tissue responses.[24] These changes in the non-lesional skin affect the professional and non-professional immune cells, and the expression levels of the different cytokines suggest an altered inflammatory state of the clinically asymptomatic skin.

3 | SIGNS OF TISSUE STRESS IN PSORIATIC NON-LESIONAL SKIN

One of the first indications of the altered stress response of the non-lesional skin is the Koebner phenomenon, which means that psoriatic lesions can develop on non-lesional skin after various injuries.[25] Tape stripping is one standard method to induce mechanical stress on the epidermis which results in enhanced proliferation rate [26] and overexpression of transforming growth factor (TGF) alpha in the non-lesional vs. healthy epidermis.[27]

Previously, we compared the expression profiles of non-lesional psoriatic and healthy epidermis, and we identified a long non-coding RNA induced by stress which we named Psoriasis Susceptibility-Related RNA gene induced by stress (PRINS). In our studies, tissue samples are taken from the trunk of healthy and plaque type psoriatic individuals. Interestingly, PRINS is highly expressed in non-lesional psoriatic tissue, but not in healthy or psoriatic lesional epidermis.[28] Later it was shown that PRINS can downregulate inflammation due to destabilization of IL-6 and the chemokine ligand 5 (CCL-5, RANTES) messenger RNAs.[29] Moreover, PRINS can act as an apoptotic function regulator by influencing interferon alpha inducible protein 6 (G1P3) gene expression[30] and by interacting with miR-491-5p[31] and nucleophosmin.[32] Our data indicated that similar to other known stress induced factors,[33] PRINS had a protective effect on cells. Enhanced PRINS production by non-lesional keratinocytes can alter the stress response of the non-lesional epidermis thereby contributing to the pathogenesis of psoriasis.[34]

Further evidence that demonstrates the altered stress/inflammatory state of the non-lesional skin is the elevated expression of the psoriasis-associated danger signal induced caspase recruitment domain family member 18 (CARD18), which has a negative role in keratinocyte inflammatory signalling.[35] Bioinformatic comparison of the gene expression profile of psoriatic non-lesional and healthy epidermis exposed to the same lymphokines suggested altered regulation of cell morphology, development, cell death and

metabolism of small molecules and lipids in psoriatic non-lesional epidermis. Data suggest that differential regulation of IL-23A and IL-1β genes in keratinocytes of psoriatic patients are important in lesion development.[36]

4 | ABNORMALITIES AT THE DERMAL-EPIDERMAL JUNCTION (DEJ) REGION OF PSORIATIC NON-LESIONAL SKIN

Cell-cell and cell-extracellular matrix contact abnormalities, such as the basement membrane (BM) abnormality at the DEJ, can result in cell-extrinsic stress and defense responses, that is a tissue level response, and although it involves innate immune responses, it is not equivalent to the classical acute inflammatory response (para-inflammation).[37] Para-inflammation is an adaptive response to stress and malfunction in tissues that develops for restoration of homeostasis. It is an innate response that can evolve into classic inflammation due to additional or persistent stress or malfunction. Para-inflammation relies mainly on tissue resident macrophages, and indeed activated macrophages was observed in non-lesional psoriatic tissue.[38]

Altered integrin-mediated adhesion of psoriatic keratinocytes can be seen not only in the lesional but also in non-lesional skin. The polarized distribution of α2β1, α3β1 and α6β4 integrins is lost, the β1 integrin subunit is localized to the basal surface of basal keratinocytes and is in contact with the BM, while β4 integrin is detected all around basal and suprabasal keratinocytes.[39] Furthermore, keratinocytes in non-lesional epidermis overexpress the main fibronectin (FN) receptor α5β1 integrin compared with normal skin.[39,40]

In the BM of non-lesional psoriatic skin, discontinuous and uneven laminin-1 distribution was observed.[41] Compared with normal skin, both non-lesional and lesional skin lack the expression of laminin-α1 and there is evidence that without α-chain, there is no distinct BM formation.[42] In another study, gaps, folding and reduplication were described with collagen type-IV α1, α2 chains and laminin-α2, α5, β1 and γ1-chains.[43] A synthetic C16 peptide (KAFDITYVRLKF) that deputizes the functional domain of the laminin-γ1-chain was shown to have an anti-inflammatory effect, including leucocyte infiltration reduction, in a rat acute allergic encephalomyelitis model suggesting an important role of the BM proteins in inflammation.[44]

Two major forms of FN exist, a soluble, found in the serum and synthetized by hepatocytes, and the so-called cellular form, synthetized by fibroblasts, epithelial cells and others.[45] We found that as opposed to normal skin, in psoriatic non-lesional skin, there was serum FN present around basal keratinocytes, which could be explained by a leaky BM.[40] In vivo, the alternatively spliced, extra domain A containing FN (EDA$^+$FN) variant is poorly expressed in adult tissue; however, it is overexpressed in developing embryos, in tumors (it is also called oncofoetal fibronectin), and in highly proliferating tissues, such as wounds. The exact regulation of EDA$^+$FN splicing is not completely clear, but TGFβ1 is known to enhance EDA$^+$FN production in wounded tissue.[46] We have evidence that keratinocytes are able to produce FN and its EDA$^+$ isoform, and psoriatic non-lesional keratinocytes are more readily capable of EDA$^+$FN production in response to signals of activation compared with normal cells.[47] We recently also reported that signal transducer and activator of transcription 1 (STAT1) negatively regulate both FN and EDA$^+$FN expression in healthy fibroblasts and this regulation is compromised in fibroblasts derived from non-lesional psoriatic dermis. We observed that in the non-lesional skin, STAT1 activation was absent in tissues far away from lesions.[48] This is in line with known alterations of psoriatic keratinocytes in the IFN—interferon regulatory factor-1—STAT1—suppressor of cytokine signalling-1 regulatory pathway.[49,50] Recombinant EDA$^+$FN, but not other recombinant FN domains, is known to activate human Toll-like receptor-4 (TLR4) in human embryonic kidney 293 cells. EDA stimulation of TLR4 was dependent upon co-expression of MD-2, a TLR4 accessory protein.[51] This indicates a mechanism by which EDA-containing FN fragments promote an innate inflammatory response.

At the DEJ, α5β1 integrin can mediate FN-mediated proliferative signals and can help in the cell adhesion process as well.[39,50] In addition, together with EDA$^+$FN, α5β1 integrin is overexpressed by basal keratinocytes, when the BM is disrupted, for example at psoriatic non-lesional skin sites or in wounds[52] and binding of α5β1 integrin to EDA$^+$FN is a provisional anchoring mechanism.[53] The recently discovered short laminin peptide C16, representing the laminin-γ1 functional domain, targets the α5β1 integrin and could block the association of α5β1 integrin to FN, thus suppressing FN-mediated proliferative, cytoskeletal, and inflammatory responses in HaCat keratinocytes. Furthermore, in imiquimod-induced psoriasis-like mouse model, C16 could reduce epidermal hyperproliferation and immune cell infiltration.[44]

Recent evidence indicates further abnormalities at the DEJ in psoriatic non-lesional skin suggesting a key role of this site in the pathomechanism of psoriasis. We have previously described that the cartilage oligomeric matrix protein (COMP) is also overexpressed in the psoriatic non-lesional skin.[54] COMP is a structural component of the healthy skin, and it provides the cohesion between the anchoring plaques of the upper dermis and the BM.[55] We found that COMP formed a continuous layer below basal keratinocytes in non-lesional skin and could interact with the α5β1 integrin and EDA$^+$FN due to the disrupted distribution of the laminin-α1. In addition, COMP helps in the maintenance of the non-lesional state by suppressing the proliferation of keratinocytes via interaction with α5β1 integrin despite the overexpression of EDA$^+$FN. COMP may also be involved in influencing keratinocyte proliferation through its direct interaction with EDA$^+$FN.[54]

Matrix metalloproteinases (MMPs) play an important role in the stability and homeostasis of the extracellular matrix (ECM).[56] MMP-2 has the ability to modify the ECM and the BM, as it contributes to cell migration and tissue remodelling and has several substrates including type IV collagen, laminin-1 and FN.[57,58] Both MMP-2 and tissue inhibitor of matrix metalloproteinases (TIMP)-2 are elevated in non-lesional psoriatic skin.[59] MMP-9 also showed higher expression in non-lesional versus healthy skin,[14] and it shares similar functions with MMP-2.[56]

5 | PSORIATIC NON-LESIONAL SKIN EXHIBITS CHANGES THAT RESEMBLE WOUND HEALING

Several studies have provided evidence that psoriasis demonstrates many characteristics of wound repair. Both lesional and non-lesional skin of individuals with psoriasis show significantly faster healing than skin of healthy individuals.[60] Hyperproliferation of keratinocytes, infiltration of inflammatory cells and neovascularization are common processes both in wound repair and in psoriasis. Further similarities are in filaggrin, transglutaminase and involucrin expression in both conditions. Antimicrobial peptides and specifically the antimicrobial protein REG3A, which is largely responsible for keratinocyte proliferation and differentiation after skin injury, are expressed not only following skin injury, but also in psoriasis.[61] Keratin-1 and keratin-10 show reduced, while keratin-6 and keratin-16 show increased expression in both conditions.[62]

Mechanisms similar to the wound healing process can also be observed in the psoriatic non-lesional skin. The EDA^+FN and its receptor, the $\alpha5\beta1$ integrin, is overexpressed not only in psoriasis, but also in wounded tissue.[63–65] The level of COMP in normal healing wounds is minimal; however, in chronic non-healing wounds, such as venous leg ulcer, it is overexpressed,[66] similar to its overexpression in non-lesional skin. High dose recombinant human COMP protein treatment results in a delay in the wound healing in an *ex vivo* wound healing model.[54] The BM is also affected in regeneration of the tissue following injury.[67] In the wound bed, the BM is often not intact, similar to areas of non-lesional skin where the laminin layer within the BM is discontinuous and the EDA^+FN, $\alpha5\beta1$ integrin and COMP show elevated expression. It has long been known that fenestration of the BM is part of the lesion formation process in psoriasis[68,69] and a soluble form of FN could infiltrate into the epidermis evolving a micro-wound stage.[70,71]

MMPs are also important factors in wound healing; however, they can negatively affect healing if they are not present in the appropriate amount.[72,73] MMP-2 and MMP-9 play a crucial role in wound healing due to their ability to remodel the ECM. The MMP-2 expression is linked with the expression of laminin-332 and increased keratinocyte migration at the edge of acute wounds, while MMP-9 is expressed at the leading edges of migrating keratinocytes during wound closure.[74] During wound healing, MMP-2 and the pro-MMP-2 activator MT1-MMP[59] create a fragment that triggers cell migration by cleaving the gamma-2 chain of laminin-332.[74] In chronic skin wounds, or non-healing wounds, the balance between MMPs and TIMPs is disrupted, which results in a delayed or absent wound closure. In chronic wounds the level of TIMPs are decreased, while the activity of MMPs are upregulated and this imbalance influences the turnover of the ECM.[72] The induction of MMP-2 and MMP-9 occurs via the $\alpha3$ chain of laminin-5.[75] In contrast, elevated MMP-9 is able to suppress the mobility of fibroblasts and keratinocytes[76,77] and this phenomenon can also lead to a delayed re-epithelization.[78]

Recently, we showed that in the psoriatic non-lesional skin, there is an overexpression of keratinocyte growth factor (KGF) and its receptor KGFR (also known as FGFR2), indicating the activation of a wound healing process. Tape stripping of the skin did not lead to any obvious changes in $\alpha5$ integrin, EDA^+FN, KGF or KGFR expression or distribution at 24 and 48 h after tape stripping in non-lesional skin, while in the healthy controls a slight increase in all protein expression was detected based on immunostaining.[48] KGF is known for its effect on keratinocyte proliferation and differentiation, and since it is not present in normal skin, but is induced in wound healing, it is regarded as a major player in the wound healing processes of epithelial tissues.[79] KGF in the skin is produced mainly by fibroblasts, but through the release of IL-1, keratinocytes induce KGF expression and release in fibroblasts.[80] In an *in vitro* co-culture composed of keratinocytes and fibroblasts, Jun and JunB antagonistically regulated the synthesis of fibroblast-derived KGF and GM-CSF.[81] Psoriatic keratinocytes derived from non-lesional epidermis produce significantly higher proinflammatory IL-1 in the presence of IL-17, compared with healthy skin keratinocytes, indicating an intrinsic feature of psoriasis epithelium.[22]

6 | BULLOUS PEMPHIGOID AND PSORIASIS

The coexistence of autoimmune bullous diseases, particularly the anti-laminin-$\gamma1$ (p200) pemphigoid, has been well documented in psoriasis.[82] Autoimmune diseases are characterized by autoantibodies, and autoreactive T cells specific for self-antigens. How exactly they contribute to any given pathological process is not always clear, and some may not play any role in disease pathology, they may only reflect abnormal processes taking place in tissues.[83]

Data indicate that protein complexes that are formed when cells are under stress could possess immunological features that can modulate the host's immune response and induce autoantibody production. In the psoriatic non-lesional tissue, abnormal BM proteins may expose novel epitopes to immune cells and this, together with other immune activating factors, can induce autoantibody formation.[84] It is presently not completely understood how much autoimmunity contributes to psoriasis, and although no particular autoantibody could be linked to the disease so far, one cannot rule out the possibility that autoantibodies may participate in the maintenance of chronic inflammation. Homology between the streptococcal M-protein and keratin 17 raises the possibility of molecular mimicry taking place.[85] Indeed, cross-reactive CD8+ T cells can be detected in patients with psoriasis, especially in patients with HLA-Cw*0602.[86] Circulating LL37-specific T cells correlates with disease activity [16] and ADAMTS-like protein 5 was also proposed in psoriasis as an autoantigen that can contribute to the pathogenesis.[87]

7 | CONCLUSIONS AND PERSPECTIVES

Here, we propose that the seemingly normal looking skin of psoriatic patients represents a balanced, “non-healing-like” micro-wounded

skin phenotype mainly due to its structural abnormalities (Figure 1). Environmental factors (infection, wounding etc.) can easily tip the tissue out of its abnormal, but still visibly balanced state and induce an overt wound reaction (lesional skin). The keratinocyte proliferation rate in the clinically uninvolved epidermis of psoriatic patients is almost the same, as in the healthy epidermis[26] despite the overexpression of KGF and its receptor,[48] but, the psoriatic non-lesional keratinocytes are more sensitive to stress[36] and proliferative signals[21] and the non-lesional skin shows a special para-inflammation state.[37] An intact DEJ region below the basal keratinocytes is crucial to the functional integrity of the skin. Abnormalities of the BM zone within the DEJ will have an effect both on the dermis and the epidermis.[67]

In the psoriatic non-lesional skin, the laminin layer within the BM is disrupted,[41–43] EDA$^+$FN is overexpressed[47,88] and the expression of its receptor, the α5β1 integrin, on the surface of basal keratinocytes is also elevated without any observed hyperproliferation.[39,40] COMP could be one component, which despite these alterations can restrain the proliferation of basal keratinocytes and help to maintain the uninvolved, non-hyperproliferative phenotype of the non-lesional psoriatic skin.[54] MMPs and their inhibitors may play an essential role in the regulation of the ECM proteins in the DEJ region.[56] MMP-2 and MMP-9 overexpression in non-lesional psoriatic skin[14,59] suggest a potential connection with the altered laminin layer within the BM.

Wound healing on surfaces in direct contact with the external environment is tied to innate immune functions, because an intact barrier is essential for defending the body against microbes and other damaging environmental factors. Barrier tissues are highly equipped with numerous machineries to respond to noxa, and as suggested by Matzinger et al., they control immune functions.[89] Barrier dysfunction is not unique to psoriasis; other chronic inflammatory skin diseases are known to have various structural defects in the epidermis. These structural abnormalities may be inherent to the tissue (e.g., mutations in the gene encoding

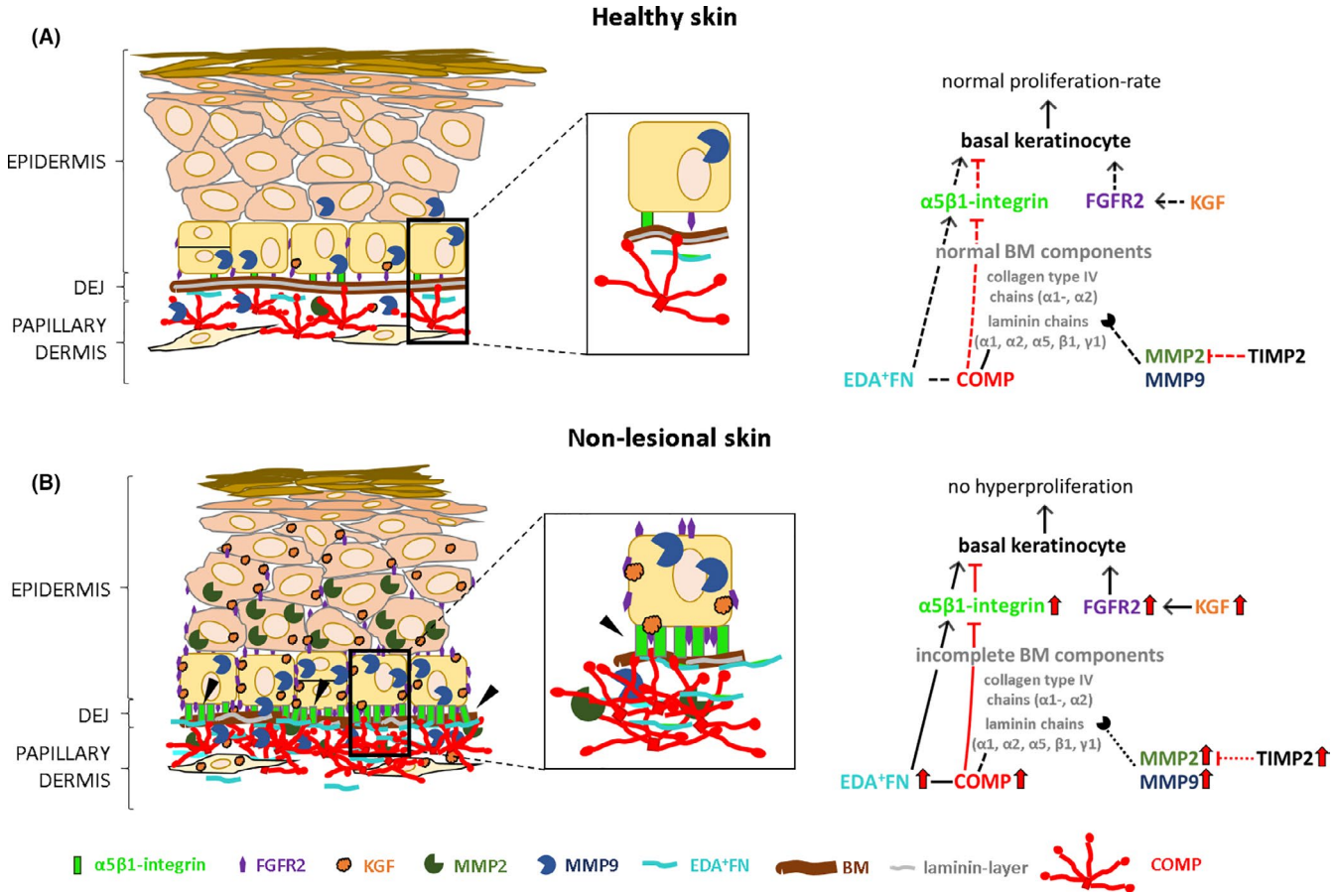

FIGURE 1 Micro-wounds at the dermal-epidermal junction in psoriatic non-lesional skin. Schematic representation of (A) healthy and (B) psoriatic non-lesional skin. The incompleteness of the basement membrane and elevated cartilage oligomeric matrix protein level indicates the "non-healing-like" micro-wounds at the dermal-epidermal junction region of the non-lesional skin. These sites are labelled with black arrowheads. Framed areas show enlarged regions. Straight lines indicate direct, and dashed lines indicate partial relationship between the components. Literature data indicate potential relationship between the dotted line marked components. Red arrows represent the elevated expression of the proteins. (Abbreviations: BM, basement membrane; COMP, cartilage oligomeric matrix protein; DEJ, dermal-epidermal junction; EDA$^+$FN, fibronectin splice variant containing the extra domain A; FGFR2, fibroblast growth factor receptor 2; KGF, keratinocyte growth factor; MMP2, matrix-metalloproteinase 2; MMP9, matrix-metalloproteinase 9; TIMP2, tissue inhibitor of matrix metalloproteinases 2.)

filaggrin in atopic dermatitis)[90] or induced by inflammation (e.g., lichen planus or drug induced reactions where basal keratinocytes are destroyed by cytotoxic T cells).[91] The nature of the barrier dysfunction (e.g., abnormal differentiation of keratinocytes, tight junction, desmosomal, hemi-desmosomal deficiency) and the nature of the dominant inflammation characterize the disease. For example, data suggest that atopic dermatitis is primarily an IL-13 dominant disease, while in psoriasis the dominant immune reaction is polarized towards the IL-17 response.[92] In the chronic phase of inflammation common features of the wound healing immune response may be seen, such as in atopic dermatitis, where in the chronic phase of the disease a prominent activation of skin barrier repair signature can be detected in transcriptomic profiling using RNA-sequencing.[93]

Understanding the contribution of the tissue factors in the pathomechanism of psoriasis will be essential to design therapies that give longer term relief from the disease than presently available treatments.

If, as indicated by our current knowledge, psoriasis is indeed genetically determined, the non-lesional tissue is the place to look for the primary effects of the inherent susceptibility factors. In future studies of the non-lesional psoriatic tissue, it will be important to better characterize the investigated tissue samples. HLA-Cw*0602 association, previous lesional versus never lesional status and severity of the disease based on PASI scores may influence the structural changes and its importance in disease pathophysiology.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

ZBC and RB have made substantial contributions to conception. ZBC, RB, LBF, NB and BG involved in drafting the manuscript. ZBC, MS and LK involved in revising critically for important intellectual content and given final approval of the version to be published. All authors have read and approved the final manuscript.

ORCID

Renáta Bozó https://orcid.org/0000-0003-4242-2474

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