Potential relevance of periostin in psoriasis

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Ph.D. Thesis

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LIST OF PUBLICATIONS

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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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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. Plaque-type psoriasis is characterized by epidermal hyperplasia with salmon-pink plaques covered in silvery scales, massive infiltration of immune cells and altered basement membrane (BM) composition with only partially understood pathomechanism. Both exogenous and endogenous factors play a role in the development of the disease. It is well established that psoriasis is also associated with other diseases, most often with psoriatic arthritis, but emerging studies suggest an association with obesity, mental disorders, cardiovascular and metabolic diseases. Genes and environmental factors play crucial roles in the development of psoriasis, and disease manifestation requires both interactions. Genome-wide association studies (GWAS) have helped to identify several risk loci in the genome, which are known as psoriasis susceptible loci (PSORS). Dendritic cells, T-cells and keratinocytes, the non-classic immune cells, also play a role in the disease, moreover NK cells are also involved in the development of the lesions. 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. 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, and epigenetic changes detected in epidermal keratinocytes of resolved skin may be responsible for the disease residual transcriptomic profile found in the same regions.

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. 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. 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. Basal keratinocytes overexpress α5β1-integrin, the main receptor of FN, in the non-lesional psoriatic skin. 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. 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. 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. Some antimicrobial peptides are produced not only upon injury, but in psoriasis as well. Both EDA^+FN and its receptor, α5β1-integrin play a key role in wound healing, where their expressions are increased, and this tendency is also present in psoriasis. In normal healing wounds COMP expression is minimal, which is not the case in non-healing wounds, where COMP is overexpressed, 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.

During injury of the tissue, the BM is also affected. 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. It has long been known that in psoriasis, fenestration of the BM is crucial in lesion formation, and soluble FN might penetrate the epidermis, developing micro-wounds.

Matrix methalloproteinases (MMPs) are also important components of wound healing. It has been shown that MMP-2, MMP-9 and tissue inhibitor of matrix metalloproteinases (TIMP)-2 are all elevated in non-lesional psoriatic skin. We have previously shown that the keratinocyte growth factor (KGF) and its receptor KGFR are both overexpressed, implying the activation of a wound healing. Psoriatic non-lesional keratinocytes also express significantly higher proinflammatory IL-1 in the presence of IL-17.

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. 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. It is well established that periostin plays a vital role in wound healing. The role of periostin has been widely investigated in atopic dermatitis (AD), 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 AD, where IL-4 and IL-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 AD patients and its level correlated with disease severity suggesting that periostin is an accelerator of AD progress.

In asthma, airway epithelial cells secrete periostin induced by IL-13, which exacerbates the asthmatic symptoms. In rheumatoid arthritis, periostin is increased in the serum and is also upregulated in cells derived from the synovium and also the synovial fluid. Furthermore, periostin was also shown to play a role in atherosclerosis, as it can cause arterial calcification and its absence protects from the disease.

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.

Based on these previous results, we aimed 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. 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, or immunosuppressants. 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).

3.2. Cell cultures

Healthy and previously-lesional psoriatic human skin biopsies were used for keratinocyte and fibroblast isolation. Primary keratinocytes were cultured in epidermal growth factor and bovine pituitary extract containing serum-free media, while fibroblasts were grown in low glucose DMEM media containing 5% FBS. Both types of media were supplemented with 1%

antibiotic/antimycotic solution and 1% L-glutamine. 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 solution. The supernatant was collected and ethanol-based precipitation was performed, then the pellet was dissolved in 3 M urea. Fibroblast and keratinocyte cultures were collected in phosphate-buffered saline and then lysed. Cell lysates and supernatants of *ex vivo* wound healing and cultured salt split models were boiled with 4X loading buffer, tissue extracts were boiled minutes with 4X loading buffer supplemented with β-mercaptoethanol then all extracts were separated on 4–20% Mini-PROTEAN®TGX™ Precast Gels and transferred to nitrocellulose membranes. 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, rabbit anti-human periostin, rabbit anti-human actin and mouse anti-human GAPDH either with or without membrane stripping with 100 mM glycine solution. Detection was performed using horseradish peroxidase-conjugated secondary antibodies and bands were visualized by an enhanced chemiluminescent system with a LI-COR C-DiGit Blot Scanner.

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. For the cultured salt-split model (n=5), 6 mm punch biopsies were incubated in 1 M NaCl 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 in 10% fetal bovine serum containing DMEM F12 media supplemented with 1% antibiotic/antimycotic solution. Samples were embedded in cryogenic solution 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 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.

3.7.2. Immunofluorescence labeling

Frozen, 4% paraformaldehyde fixed and 0.25% TritonX-100 permeabilized 6 μm skin sections and were blocked with 3% normal goat serum and 1% bovine serum albumin containing Tris-buffered saline. For immunolabeling mouse anti-human periostin, and β1-integrin were used overnight followed by Alexa Fluor 647 conjugated goat anti-mouse IgG. As isotype control mouse IgG1κ was used, 4′,6-diamidino-2-phenylindole labeled the nuclei. Visualization, image processing and fluorescence quantification were performed by Zeiss Axio Imager Z1 microscope, ZEN 2012 Microscope Imaging software and Fiji software.

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 was added 5 hours post-seeding. After 24 hours, 100% confluent cultures were scratched with a pipette tip 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.

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 or Prism-GraphPad 8 software.

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. Interestingly, among all patients, the systemically treated group showed the highest elevation in periostin serum level. We did not observe significant differences between male and female patients or between younger and older patients, and the Body Mass Index (BMI) did not influence on the measured periostin level.

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

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.

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. 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. The lowest dermal periostin expression was observed in the previously-lesional skin. 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 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. In previously-lesional skin, as opposed to decreased periostin at the dermal-epidermal junction, basal keratinocytes showed the highest

expression. Western blot analysis revealed that periostin expression of keratinocytes derived from previously-lesional psoriatic skin was increased compared to healthy cells.

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

Periostin is known to be expressed by both keratinocytes and fibroblasts to different molecular effects. 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.

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. In the cutting model, we observed a prominent periostin expression in basal keratinocytes at the wound edges after 24 hours.

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. 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.

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. 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.

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. Western blot analysis revealed that periostin production was reduced in keratinocytes due to blocking β1-integrin compared to unblocked control. These results indicate that β1-integrin is needed for proper wound healing and it contributes to the induction of periostin.

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. Periostin is involved in different inflammatory conditions such as asthma, atherosclerosis, rheumatoid arthritis, and other skin diseases, such as AD. 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. 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, 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. 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. 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. IL-13 activates IL-24 in keratinocytes in a periostin-dependent way causing filaggrin downregulation, which results in an epithelial barrier dysfunction in AD.

Several studies have described that both non-lesional and lesional psoriatic skin show similarities with wound repair processes, 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. It has been described that in mouse skin upon wounding, periostin was expressed by migrating keratinocytes. 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, 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, 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. 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. 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. 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. 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.

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 data. 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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