Final Report of the PD OTKA, entitled „The role of extracellular matrix proteome alterations in the pathomechanism of multifactorial inflammatory diseases"

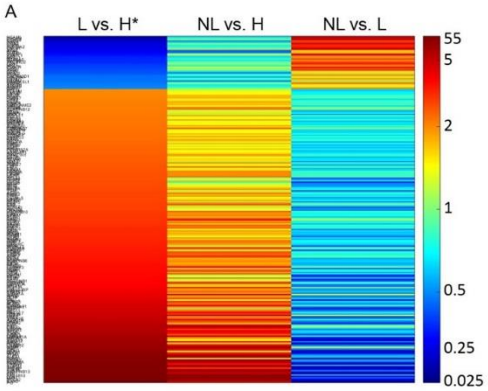
NKFI Num.: PD116992

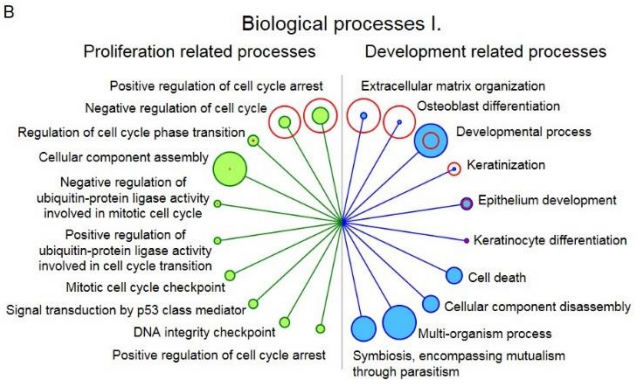
Introduction

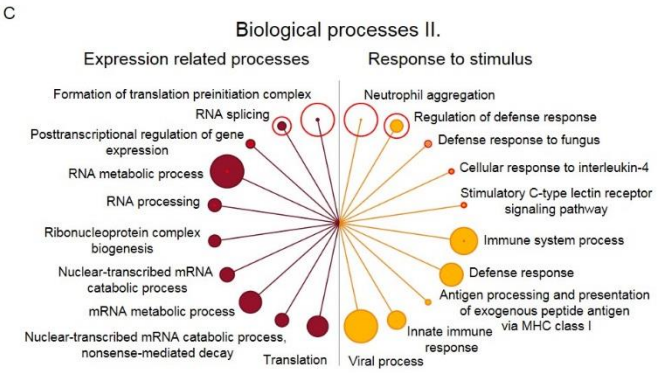
Approximately 2-3% of the world's population suffer from the immune-mediated disease psoriasis. Despite the large advances in psoriasis therapy, currently available management still only treat symptoms. We mainly but not exclusively focused on the characterization of non-lesional skin alterations, since many of these are believed to be predisposing factor leading to the manifestation of symptoms. We also aimed to gain further insight into the role of extracellular matrix (ECM) molecules in the context of pathomechanism of psoriasis, therefore a quantitative proteomic analysis was performed as a large-scale method to compare the healthy (H), psoriatic non-lesional (NL) and lesional (L) skin at the proteome level.

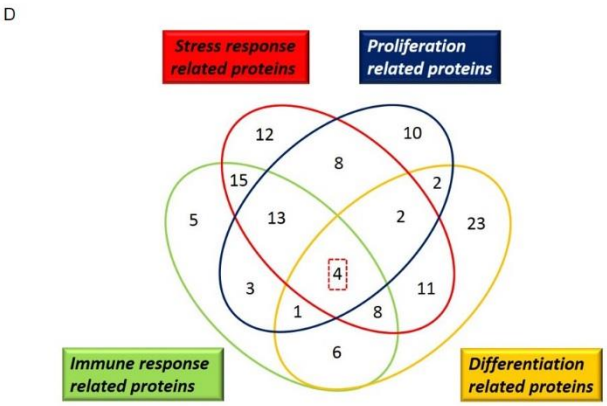
Results

In line with our commitments and aims, skin biopsies were collected from healthy individuals and psoriatic patients (NL and L). Skin samples were applied for multi-step sequential solubility-based protein extraction and gained extracts were analyzed by 2D nano LC-MSMS spectrometer for protein identification. Relative amounts of proteins of H, NL and L were compared and screened for differences in amount. As a result of our comprehensive and comparative proteomic analyses over 240 proteins were identified with differential expression in healthy and lesional skin (fig. 1.a). Biological processes for which proteins were differentially expressed in L and H were characterized by commutated literature-based analysis (fig. 1.b and c). MYBBP1A and PRKDC were identified as potential new regulators of key pathomechanism, including stress and immune response, proliferation and differentiation together with two known proteins PML and STAT1 (fig. 1.d and e).









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Protein name	Gene ID of protein	Association with psoriasis
Myb-Binding Protein 1A	MYBBP1A	novel
Protein PML	PML	known
DNA-Dependent Protein Kinase Catalytic Subunit	PRKDC	novel
Signal Transducer And Activator Of Transcription 1	STAT1	known

Figure 1. Characterization of altered protein expression of lesional (L) skin compared to healthy (H) skin.

Heatmap of relative expression of proteins differentially expressed in L and H skin (a, left column), and their expression in non-lesional (NL) and L skin (a, middle column) and NL and H skin (a, right column) (a). Biological processes for which proteins were differentially expressed in L and H are listed. The top ten processes are depicted for proliferation (b left, green circles), development (b right, blue circles), expression (c left, filled red circles) and response to stimulus (c right, orange circles). False detection rate (FDR) values are indicated with unfilled red circles around the filled circles for the various biological processes. The size of each circle is proportional to FDR values (unfilled circles) or to the number of proteins (filled circles). Four proteins differentially expressed in H and L skin are believed to participate in all four mechanisms of stress, immune response, proliferation and differentiation (d) and are listed in (e). (*Significant difference in relative protein expression at least by two-fold in L and H comparison).

Strikingly, 79.9% of the proteins that were differentially expressed in L and H skin exhibited expression levels in non-lesional skin that were within twofold of the levels observed in healthy and lesional skin, suggesting that non-lesional skin represents an

intermediate stage. Proteins outside this trend were categorized into three groups: I. proteins in non-lesional skin exhibiting expression similar to lesional skin, which might be predisposing factors (i.e., CSE1L, GART, MYO18A and UGDH); II. proteins that were differentially expressed in non-lesional and lesional skin but not in healthy and lesional skin, which might be non-lesional characteristic alteration (i.e., CHCHD6, CHMP5, FLOT2, ITGA7, LEMD2, NOP56, PLVAP and RRAS); and III. proteins with contrasting differential expression in non-lesional and lesional skin compared to healthy skin, which might contribute to maintaining the non-lesional state (i.e., ITGA7, ITGA8, PLVAP, PSAPL1, SMARCA5 and XP32). Finally, proteins differentially expressed in lesions may indicate increased sensitivity to stimuli and peripheral nervous system alterations. These major findings are summarized in a manuscript published in SCIENTIFIC REPORTS (D1), entitled "Comprehensive Proteomic Analysis Revised Intermediate Psoriatic Skin Outside This Trend" (doi: 10.1038/s41598-019-47774-5, https://doi.org/10.1038/s41598-019-47774-5. GG: senior (last), corresponding and executive author). Contributions of GG in the study: conceived the study; supervised the project (with other authors); designed the experiments and performed the experiments (with other authors); analyzed the proteomic data (with other authors); and wrote the manuscript (with other authors).

The transcription factor STAT1, also identified in our proteomic study with altered amounts, is believed to be a central regulator in psoriasis. However, less is known about its expression and deposition in the non-lesional skin. Therefore, we decided to investigate it further. We found that STAT1 is in an active state in both healthy and in psoriatic lesional skin, in contrast to the NL skin where it is mainly inactive, and that STAT1 activation correlates with the PASI (psoriasis area and severity index) of psoriatic patients. The results of this study and are new findings regarding STAT1 are summerized in a paper published with the title “Abnormal STAT1 activation in psoriasis” (BÔRGYÓGYÁSZATI ÉS VENEROLÓGIAI SZEMLE, DOI: 10.7188/bvsz.2016.92.1.3; GG: co-author).

Proliferation, differentiation, and inflammatory responses (both that of innate and adaptive immunity) are major mechanisms known to be involved in psoriasis pathogenesis, among others. In an additional publication, we summarized the effects of a potentially new therapeutic agent Oxymatrine on these major pathomechanisms of the disease, with the title “Oxymatrine may represent an additional therapeutic tool

for severe plaque psoriasis management", (D1; GG: senior (only), corresponding and executive author; BRITISH JOURNAL OF DERMATOLOGY, https://doi.org/10.1111/bjd.18299).

One of the main aims of our research was to characterize the alterations of the extracellular matrix (ECM) in psoriasis and the effects of these abnormalities. The ECM protein Fibronectin (FN) and one of its isoforms, extra domain A-containing fibronectin (EDA+FN) in the microenvironment of basal keratinocytes may influence proliferation via its receptor α5β1 integrin. Therefore, the regulation of FN and EDA+FN production in psoriasis was investigated. We found that the expression of KGF (keratinocyte growth factor), its receptor FGFR2 (fibroblast growth factor receptor 2) a5 integrin and EDA+FN is elevated in psoriatic NL compared with H skin. KGF mildly induced EDA+FN, but not FN expression through MAPK signaling in healthy fibroblasts. STAT1 negatively regulates both FN and EDA+FN expression in healthy fibroblasts, and this regulation is compromised in fibroblasts derived from NL psoriatic skin. The schematic model of the interactions of investigated molecules is shown in fig. 2.

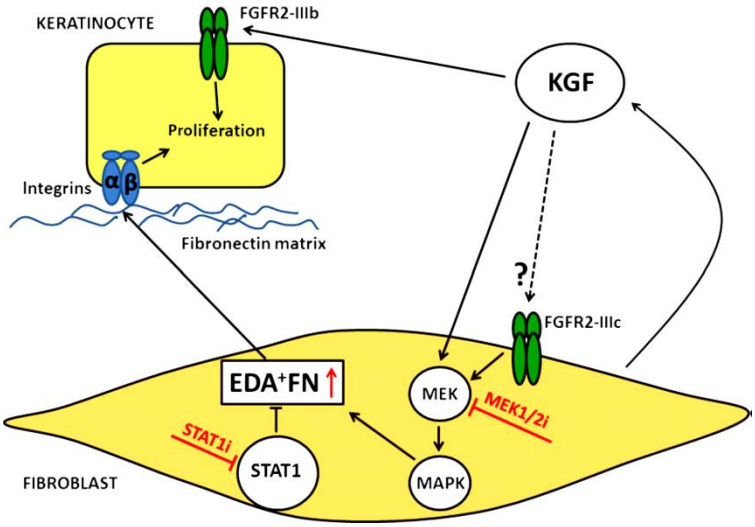

Figure 2. The schematic model of the regulation of EDA+FN production in fibroblasts and its effect on keratinocytes.

The results of our research concluding that the production of FN and EDA+FN by fibroblasts and the signaling of STAT1 are abnormally regulated in psoriatic NL skin are described in the publication "Abnormal regulation of fibronectin production by fibroblasts in psoriasis" (D1; GG: co-author; BRITISH JOURNAL OF DERMATOLOGY, https://doi.org/10.1111/bjd.14219).

In our proteomic study several ECM proteins were identified with altered expression profile. Out of these proteins, the ECM molecule COMP (Cartilage oligomeric matrix protein) – has not been associated with psoriasis and was found to be elevated in the NL skin based on our proteomic results – was characterized in dept in context with the disease. We found that COMP extended deeper into the dermis and

formed a more continuous layer in psoriatic non-lesional skin compared to healthy skin, while in psoriatic lesions COMP showed a partially discontinuous deposition at the dermal-epidermal-junction and confirmed the elevated level of COMP in NL skin (fig. 3).

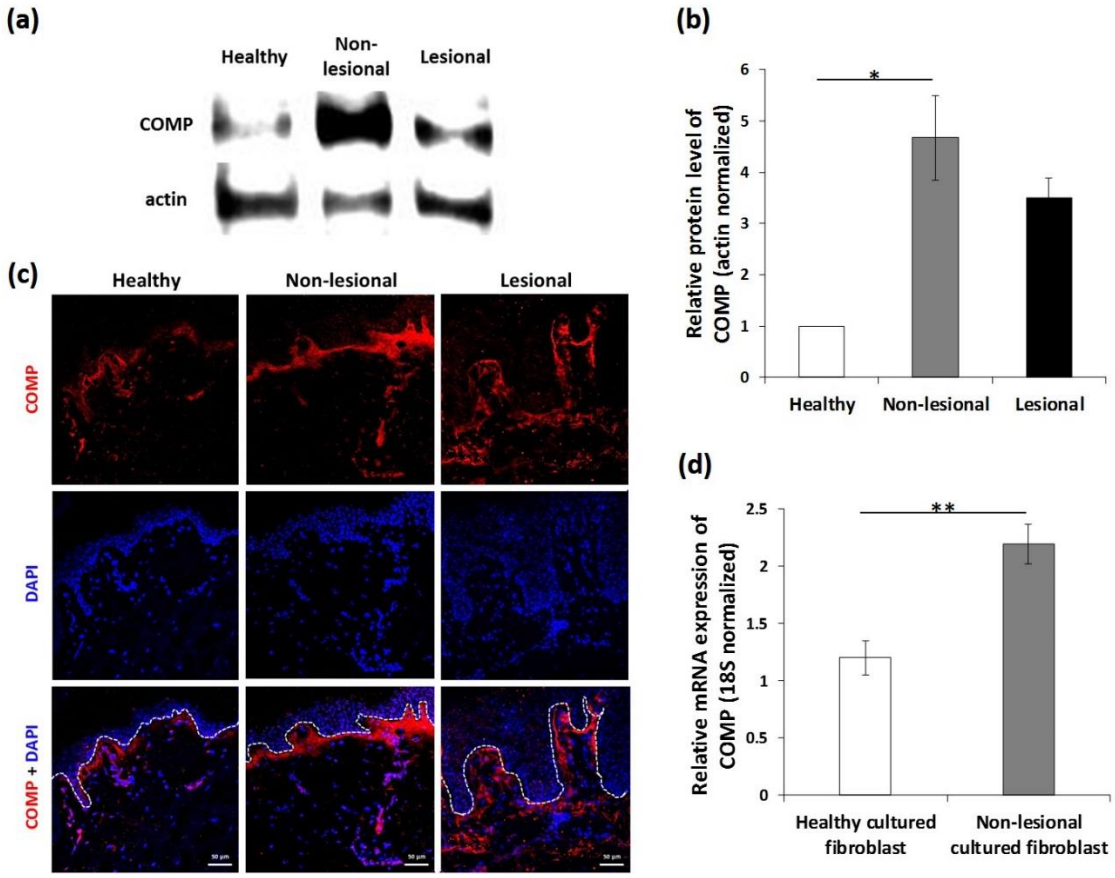

Figure 3. Increased COMP expression in non-lesional skin and altered deposition in lesions. COMP proteins extracted from healthy skin and psoriatic non-lesional and lesional skin were subjected to western blot detection (n=6) (a). Western-blot-based semiquantitative comparison of actin-normalized COMP protein. Data are presented as mean+/-SEM: fold increase is relative to the signal from healthy skin (n=6) (b). Immunofluorescence staining for COMP in healthy (left column), psoriatic non-lesional (middle column) and psoriatic lesional (right column) skin. Dotted lines indicate the border of the dermal–epidermal-junction (n=10) (c). Real-time RT-PCR analysis of COMP cDNA from cultured human dermal fibroblasts of healthy and psoriatic non-lesional origin (d). Data are presented as mean+/-SEM: fold increase is relative to the healthy group.

Furthermore, COMP and β1-integrin showed strong co-localization in non-lesional skin, where the laminin-layer within the basement membrane (BM) is discontinuous. In *in vitro* models, the presence of exogenous COMP decreased the proliferation-rate of keratinocytes and this proliferation suppressing effect was diminished by the blocking

of α5β1-integrin. Our results suggest that COMP can interact with α5β1-integrin of basal keratinocytes through the disrupted BM, and it may stabilize the epidermis in the non-lesional state by contributing to the suppression of keratinocyte proliferation. The antiproliferative effect of COMP is likely to be relevant to other skin diseases in which chronic non-healing wounds are coupled with massive COMP accumulation. These results are summerized in the manuscript entitled "COMP negatively influenced keratinocyte proliferation via α5β1-integrin: Potential relevance of altered COMP expression in psoriasis" that is currently under the third round of revision in JOURNAL OF INVESTIGATIVE DERMATOLOGY (D1).

All data mentioned above were presented at several national and international scientific meetings in the form of abstracts, posters and/or oral presentations (listed at MTMT: https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10040137). (Four other publications were also conceived during the period of the grant, that do not strictly relate to the project are also listed at above MTMT: https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10040137).

Szeged, 19.09.2019 Gergely Groma