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Proteomic plasma profile of psoriatic patients

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ABSTRACT

Background: Psoriasis is a chronic, immune-mediated inflammatory skin disease with severe consequences for the whole organism. The lack of complete knowledge of the main factors predisposing an individual to the appearance of psoriatic lesions, has recently led to the search for modifications in biochemical pathways participating in the development of this disease. We therefore aimed to investigate changes in the plasma proteomic profile of patients with psoriasis.
Material and methods: A proteomics approach was used to analyze the expression of proteins in plasma from psoriatic patients and healthy controls (sex- and age-matched individuals). The analysis was performed using gel electrophoresis, followed by nanoflow LC–MS/MS using a Q-Exactive OrbiTrap mass spectrometer.
Results: Proteomic data indicated a significant decrease in the level of proteins involved in lipid metabolism, such as apolipoprotein M, and proteins involved in the management of vitamin D levels in psoriatic patients' plasma. These changes were accompanied by the expression of proteins involved in immune response and signal transduction. This was particularly evident by the level of transcriptional factors, including AT motif binding factor 1, which regulates excessive cellular proliferation and differentiation. It was also suggested that psoriasis development was associated with increased expression of proteins directly involved in signaling molecule secretion [biotinidase and BAI1-associated protein 3]. In addition, the lipid peroxidation product – 4-hydroxynonenal (4-HNE) generates higher level of adducts with proteins in the plasma of psoriatic patients. Moreover, plasma proteins from healthy subjects creating with 4-HNE adducts were mainly characterized as structural, while in the plasma of psoriatic patients, increased levels of 4-HNE-protein adducts with catalytic activity were observed.
Conclusion: The results presented herein confirm the current knowledge about the profile of proteins responsible for the immune response and management of vitamin D in the plasma of psoriatic patients. However, several new proteins were also identified, which are involved in signal transduction and lipid metabolism as well as catalytic activity. The expression or structure of these proteins was shown to change through the course of the development of psoriasis. This knowledge may help contribute to the design of more specific pharmacotherapy.

1. Introduction

Psoriasis, a chronic, immune-mediated inflammatory skin disease, is estimated to affect approximately 2–4% of global the population [1]. Symptoms of psoriasis may affect the body's entire surface, causing considerable psychosocial disability and majorly affecting a patients' quality of life [2]. Psoriasis prevalence may be associated with depressive illness, psoriatic arthritis, and even cardiovascular disease [3].

It is well recognized that psoriasis prevalence is associated with chronic inflammatory reactions *in vivo*, resulting in the increased expression of proinflammatory factors in both human and rat skin cells, as well as human plasma [4–6]. Changes in the signal transduction pathways in psoriatic skin tissue, including the activation of mitogen-activated kinases (such as p38, ERK1/2, JNK) [7,8]. and transcription factors involved in the inflammatory response (e.g. NFκB, Foxp3, HIF-1α), cause cells to proliferate (STAT3) and an antioxidant response (Nrf2) occurs [9–12]. In addition, the level of

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proteins involved in the antioxidant defense (including superoxide dismutase) are significantly increased in psoriatic skin tissue [13]. In psoriatic skin biopsies, there exists a strong imbalance between pro- and anti-apoptotic proteins, with a significant shift toward the pro-apoptotic direction [14].

As a consequence of the local changes described above, psoriasis development is associated with increased inflammatory and oxidative conditions, resulting in oxidative modifications to the structure and function of certain plasma antioxidant proteins and lipids [15]. Moreover, low molecular weight electrophilic lipid peroxidation products may also interact with proteins and, via adduct formation, alter protein structure and function further [16]. To date, increased levels of 4-hydroxynonenal [4-HNE] and 4-HNE-protein adducts were observed in the blood of patients with another skin disease – vitiligo [17]. The influence of lipid peroxidation products on the proteomic profile of psoriatic patients' plasma remains to be elucidated. It is therefore assumed that psoriatic patients' cellular metabolic disorders may affect the proteomic profile, including structural modifications and 4-HNE-protein adduct formation. Evaluating protein profile changes will help identify metabolic pathways that could be useful for early disease diagnosis and improving or selecting appropriate pharmacotherapy.

Therefore, the aim of this study was to analyze the proteomic profile of plasma from psoriatic patients, with special attention paid to protein modifications caused by lipid peroxidation products.

2. Material and methods

2.1. Plasma samples

Blood samples were collected from 6 untreated psoriatic patients (3 men and 3 women; age range 27–54 years, mean 40) and 6 healthy people (sex- and age-matched individuals forming a control group; age range 28–55 years, mean 40). Eligible patients were those who were given a diagnosis of plaque psoriasis for at least 6 months with at least 10% of the total body surface area affected. The severity of psoriasis was assessed using the Psoriasis Area and Severity Index (PASI) score (median 17; range 10–25). None of the patients or healthy subjects had received topical or oral medications during the 4 weeks before the study. Individuals whose history indicated any other disorders were excluded from the study. None of the participants were smokers. The study was approved by the Local Bioethics Committee Medical University of Bialystok (Poland), No. R-I-002/502/2015. Written informed consent was obtained from all the patients.

Blood samples were taken into ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3000 g (4 °C) to obtain the plasma for analysis.

2.2. SDS-PAGE and in-gel digestion

To partially reduce albumin level in plasma samples, the ProteoExtract Albumin Removal Kit (Calbiochem, San Diego, CA) was used. Total protein content in samples was measured using the Bradford assay [18]. The volume of the sample containing 30 μg of protein was mixed in a ratio 1:2 with sample loading buffer (Laemmle buffer containing 5% 2-mercaptoethanol), heated at 95 °C for 7 min and separated on 12% Tris-Glycine SDS-PAGE gels. Following electrophoretic separation, gels were fixed in methanol: acetic acid: water (4:1:5; for 1 h) and stained with Coomassie Brilliant Blue R-250 (for 4 h). All lanes were cut out of the gel and sliced into 10 sections. Proteins in each section were reduced with 10 mM DTT, alkylated by incubation with 50 mM iodoacetamide, and in-gel digested with sequencing grade trypsin (Promega, Madison, WI, USA). The resulting peptide mixture was extracted from the gel fractions, pulled into 5 sections for each line (Fig. 1), and dried using vacuum centrifugation [19].

2.3. LC–MS/MS analysis

The dried peptides were dissolved in 50 μL 5% ACN + 0,1% formic acid (FA) and 2 μL of this mixture was separated using an Ultimate 3000 (Dionex, Idstein, Germany). Peptide mixture was trapped on a 300 μm id x 5 mm long C18 μ-precolumn (Dionex, LC Packings) and then loaded onto a 150 mm × 75 μm PepMap RSLC capillary analytical C18 column with 2 μm particle size (Dionex, LC Packings) at a flow rate of 0.300 μL/min. The gradient started at 3 min and ramped to 60% Buffer B (90% acetonitrile + 0.03% FA) over a period of 60 min [20]. The eluted from the column peptides were analyzed using a Q Exactive HF mass spectrometer with an nanoelectrospray ionization source (ESI) (Thermo Fisher Scientific, Bremen, Germany). Obtained data were acquired with the Xcalibur software (Thermo Fisher Scientific, Bremen, Germany). The mass spectrometer was externally calibrated and operated in positive and data-dependent mode. Survey MS scans were conducted in the 200–2000 *m/z* range with a resolution of 120,000. In subsequent scans, the top ten most intense ions were isolated and fragmented on an HCD collision cell (collision energy was 30 eV), and the fragments were analyzed at 30,000 resolution. A 10 s dynamic exclusion window was applied, and an isolation window of 4 *m/z* and one microscan was used to collect suitable tandem mass spectra.

2.4. Protein identification, grouping, and label-free quantification

Processing of the raw data generated from LC–MS/MS analysis and protein identification and quantification were carried out using Proteome Discoverer 2.0 (Thermo Fisher Scientific, Bremen, Germany) and Sequest HT (SEQUEST HT algorithm, license Thermo Scientific, registered trademark University of Washington, USA). Protein label-free quantification was prepared according to the intensities of the precursor ions signal. For protein identification the following search parameters were used: peptide mass tolerance set to 10 ppm, MS/MS mass tolerance set to 0.02 Da, mass precision was set to 2 ppm, up to two missed cleavages allowed, minimal peptide length was set to six amino acids, cysteine carbamidomethylation, and carboxymethylation, methionine oxidation and 4-HNE – cysteine/lysine/histidine adducts formation set as a dynamic modification [21]. For each protein, minimal number of identified unique peptides was set to two peptides. Input data were searched against the UniProtKB-SwissProt database (taxonomy: *Homo sapiens*, release 2017-08). Protein grouping was performed according to molecular function using the Gene Ontology (GO) database available in the Proteome Discoverer 2.0 (Thermo Fisher Scientific, Bremen, Germany).

2.5. Statistical analysis

Analysis of each plasma sample was performed in three independent experiments. Data were analyzed in Stata® 13.0 (Stata Corp LP, College Station, TX, US) using principal component analysis (PCA). For determine significant differences between group the Tukey's honestly significant difference (HSD) post hoc test was used. The Shapiro-Wilk test was used for testing the normality of data distribution and the one-way Student's *t*-test for multiple comparisons. Data visualization (clustering and heatmap for top 50) was performed using free available Perseus® 1.6.0.7 software (http://www.coxdocs.org). Results from individual protein label-free quantification are expressed as the mean ± standard deviation (SD) for n = 6. P-values less than 0.05 were considered statistically significant.