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Original Research

Differences in expression of serum protein in patients with psoriasis vulgaris and blood heat syndrome and healthy volunteers

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Abstract: To explore the mechanism of psoriasis vulgaris (PV), serum protein expression profiles between PV patients with blood-heat syndrome and healthy volunteers were detected by isobaric tags for relative and absolute quantitation (iTRAQ). First, sera from 15 PV patients with blood-heat syndrome and 10 healthy volunteers were collected; then, serum proteins were separated and hydrolyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and a specific iTRAQ marker enzyme respectively after further purification and protein abundance treatment. Compared with the control group, differentially expressed proteins in PV patients with blood-heat syndrome were identified and analyzed by tandem mass spectrometry. A total of 787 proteins were identified and 718 proteins had a functional annotation with gene ontology (GO) by iTRAQ in the current study. Significant differences ($P < 0.05$) and great differences ($P < 0.01$) were found in 681 proteins and 536 proteins respectively between the patient group and healthy group.). Different protein expression profiles in serum existed between PV patients with blood-heat syndrome and healthy volunteers; the differences largely involved immune-related proteins and lipoproteins. The proteins specific for PV with blood-heat syndrome deserves further investigation.

Key words: Psoriasis vulgaris; Blood-heat syndrome; Proteomics; iTRA.

Introduction

Psoriasis vulgaris (PV) is a common erythematous scaly skin disease, with a worldwide prevalence of 2%-3% (1). PV is mainly characterized by keratinocyte hyperplasia, inflammatory cell infiltration, neovascularization, and proliferation (2). It is still a hot topic for dermatologic research due to its unknown mechanism, the probability of relapse, and the difficulty in achieving a cure.

Currently, the most popular ways for PV treatments are ladder treatment methods of Western medicine; however, the treatment effects are still far from stastifactory and breakthough is still expected. In addition, some effective drugs, e.g., bimolane, have significant side effects, and PV is likely to relapse without continuous drug treatment. In contrast, PV treatments with natural drugs in traditional Chinese medicine have shown remarkable results. In traditional Chinese medicine, related syndromes of patients are obtained using four diagnostic parameters, i.e., look, smell, ask, and palpation of the pulse; and pateint may have different pathologic states during different periods. Nevertheless, the treatment effects of traditional Chinese medicine highly depend on the experience of the physician. For example, it is quite difficult for an unexperienced physician to distinguish the four syndromes existing in PV patients, i.e., damp heat syndrome, blood heat syndrome, blood stasis syndrome, and blood deficiency syndrome, with traditional Chinese medicine methods (3). Thus, it is still a great challenge for Chinese medicine-mediated therapy to define these symptoms precisely and provide a timely remedy.

With the rapid development of proteomics in therapeutic areas, protein quantitative methods have been used in the treatment of related diseases (4). Isobaric tags for relative and absolute quantitation (iTRAQ) method, which was developed by Applied Biosystems Inc, is a technology for peptide labeling *in vitro* (5). iTRAQ utilizes 4 or 8 kinds of isotope labeling to specifically bind amino acids in polypeptides. Tandem mass spectrometry was then used for further analysis. As a new protein quantitative technique, iTRAQ can detect 4 or 8 proteins with relative and absolute quantification among different samples at the same time. Compared with two-dimensional gel electrophoresis, various proteins, such as cytoplasmic and membrane proteins, low abundance proteins, acidic and basic proteins, and low (< 10 Kda) and high molecular weight (>100 Kda) proteins, can be detected by iTRAQ. Therefore, iTRAQ is an important technology in protein quantification.

In the present study, iTRAQ was used to screen different and specific proteins in human serum from PV patients and healthy volunteers. Specific proteins associated with blood heat syndrome in PV were detected to lay a good foundation in the standardization and therapy of patients with PV and blood heat syndrome.

Materials and Methods

Patients and sera

In this study, forty patients were recruited in the

Affiliated Hospital of Chengdu University of Traditional Chinese Medicine. The PV patients were diagnosed with blood heat syndrome between December 2014 and October 2015. To further screen the optimal individuals, 15 patients were deemed to be eligible research participants by experts according to Western and traditional Chinese medicine diagnostics, as shown below. Among the 15 patients, 9 were males and 6 were females. The average age and duration of disease were approximately 34 years and 44 months, respectively. Ten healthy volunteers (4 males and 6 females) served as the control group, with the average age of 31 years. All procedures were approved by the Ethics Committee of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, and informed consent was signed by all patients.

Western and traditional Chinese medicine diagnostics

Western diagnosis was performed according to the PV-related criteria by Johnson *et al.* (6) (7). Traditional Chinese medicine diagnosis referred to Chinese medicine clinical research guidelines by the State Drug Administration and a recent report (8).

Standard of PV patients

Patients meeting the following standards were selected as research participants: fulfilling the diagnostic criteria of PV; compliance with the standards of heat blood syndrome in traditional Chinese medicine; 18-70 years of age; no co-existing diseases and other syndromes; and no treatment with retinoids, glucocorticoids, and methotrexate within the last month.

Proteomic determination of blood heat syndrome in PV

Main reagents and equipment

SDS, urea, Trisbase, DTT, and IAA were purchased from Bio-rad. The iTRAQ Reagent-8plex Multiplex Kit and dissolution buffer were purchased from AB SCIEX. Acetonitrile and CAN were purchased from Merck. All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

The equipment used in the present study included a Q-Exactive mass spectrometer (Thermo Finnigan), an AKTA Purifier 10 purification equipment (GE Healthcare), a low-temperature speed centrifuge (Eppendorf, a spectrophotometer (Unico), a centrifugal vacuum concentrator (Eppendorf), and a 600V electrophoresis instrument (GE Healthcare).

Methods

Serum samples from five PV patients and healthy volunteers were mixed equally to form a group. Hence, serum samples from PV patients with blood heat syndrome were divided into groups A1, A2, and A3, and healthy volunteer serum samples were divided into groups C1 and C2. High abundance proteins in each group were removed by Agilent H-14. Following the protein concentration process, the low abundance protein content was measured by the Bradford method. SDS-PAGE, filter-aided proteome preparation (FASP), and peptide analysis were used to further detect protein characteristics. Each group of samples with approximately 81 μg of protein was labeled by the iTRAQ Reagent-8 plex Multiplex Kit according to the user manual. Then, all labeled proteins were mixed for SCX pre-fractionation. In addition, each sample was separated by a high-performance liquid chromatography system (Easy nLC) with a nanoliter flow rate. The iTRAQ experiments were performed by Shanghai Omicsspace Biotech Co. LTD. (Shanghai, China). Finally, Q-exactive was used for mass spectrographic analysis.

Bioinformatics analysis

The results with RAW files from mass spectrometry were screened by filtering parameters (Peptide FDR ≤ 0.01). Then, the proteins were identified and quantitatively analyzed in the database of uniprot human fasta by Mascot2.2 and Proteome Discoverer1.4 software. Finally, the identified protein groups in the ontology of the cellular component, biological process, and molecular function were analyzed by Proteome Discoverer software.

Statistical analysis

The values and differences between groups were measured using single-factor analysis of variance (ANOVA) and Student's t-test analysis with SPSS (version 18.0; IBM, USA). Statistical significance was set at a $P<0.05$.

Results

Removing high abundant proteins in serum and SDS-PAGE

SDS-PAGE and Bradford methods were used to detect protein-related factors. Proteins concentrations on patients and healthy volunteers are shown in Table 1. The results of SDS-PAGE also demonstrated that high abundance proteins in serum had been removed successfully by Agilent H-14 (Figure 1).

Table 1. Protein concentration determination by Bradford.

Sample	A1	A2	A3	C1	C2
Concentrate (μg/μL)	8.6	8.3	7.4	8.8	11.6

Note: A1, A2, and A3 represent patients with blood heat syndrome in PV; C1 and C2 represent control groups with healthy volunteers.

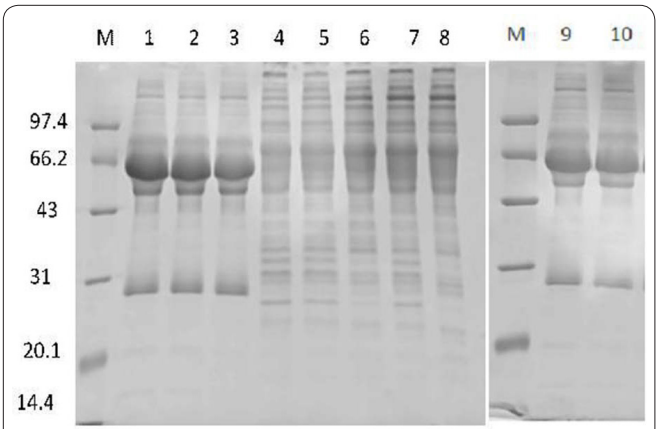

Figure 1. SDS-PAGE of various samples. M represents protein marker; 1, 2, and 3 represents original samples from serum; 4, 5, and 6 represent original samples with removal of high abundance proteins in serum; 7 and 8 represent samples with removal of high abundance proteins in healthy serum; 9 and 10 represent original samples from serum of healthy volunteers.

Table 2. Quantitative analysis of protein identification results (P<0.01).

Accession number	Petides coverage	P-value	Protein quantification (A/C)
Q59H94	0.89	0.00475	0.3411
Q5T0R7	13.22	0.00057	0.5070
P67936	43.95	0.00819	0.4196
Q5T123	32.95	0.00742	0.4800
G3V5M6	45	0.00560	0.5883
Q9Y490	5.71	0.00052	0.5542
B0UXC6	3.69	0.00179	0.5259
B4DTR5	1.11	0.00238	1.2286
P37802	40.2	0.00261	0.5950
P69905	61.97	0.00953	0.6165

Note: Accession number represents protein accession number in Uniport database. Peptides coverage represents detected peptide coverage. Protein quantification represents quantitative analysis results of protein identification; A/C > 1 represents the average value of protein expression in groups A1, A2, and A3 (patients with blood blood syndrome) and was higher in groups C1 and C2 (healthy volunteers). Similarly, A/C < 1 represents the average value of protein expression in groups A1, A2, and A3 and was lower in groups C1 and C2.

Results of identified proteins

A total of 5814 unique peptides and 787 protein groups were detected by mass spectrometry and checked in Mascot. These protein groups included extracellular matrix proteins (ECMs), immune-related proteins, lipoproteins, metalloproteinases, tumor-associated proteins, keratin, and antimicrobial peptides. ECM contains fibronectin, laminin, and extracellular matrix protein-1 (ECM1). Furthermore, immune-related proteins and lipoproteins were the major components of proteins showing significantly differences between PV patients and healthy volunteers.

Immune-related proteins mainly included peptides of immune-related proteins and complement-associated proteins. The peptides in the present study were from IgG protein. Complement-associated proteins included complement C1 (accession No.: C9IZP8), C2 (accession No.: P06681), C3 (accession No.: P01024), C4 (accession No.: Q6U2E8), C5 (accession No.: P01031), C6 (accession No.: P13671), C7 (accession No.: P10643), C8 (accession No.: P07360), C9 (accession No.: P02748); complement factor 1 (accession No.: G3XAM2), H (accession No.: P08603), P (accession No.: P27918), and C1 inhibitor.

Apo-lipoproteins were the main form of lipoproteins. Apo A1 (P02647), A2 (P02652), A4 (P06727), Apo-B (Q7Z7Q0), B100 (C0JYY2), Apo C3 (P02656), C4 (A5YAK2), Apo E (Q8TCZ8), Apo F (5GXS5), and Apo M (Q5SRP4) were the proteins showing significant differences(*P<0.05*).

Matrix metalloproteinase 2 (MMP-2), metalloproteinase inhibitor 1 (TIMP1), insulin-like growth factor binding protein (IGFBP) 2, IGFBP 4, IGFBP 5, IGFBP 7, pyruvate kinase M2, galectin-related proteome, keratin 2, keratin 9, S-100A8, and other specific proteins were differentially expressed between PV patients and healthy volunteers.

Protein quantitatively analysis between patients and healthy volunteers

Among 787 protein groups, 681 serum proteins showed significant differences between PV patients and healthy volunteers (*P<0.05*), and 536 proteins showed extremely differences (*P<0.01*). These proteins were mainly associated with immunity and lipoprotein0related proteomics. Some proteins presenting extremely differences are shown in Table 2.

Discussion

PV, a common erythematous scaly skin disease, is caused by many factors in susceptible individuals (9, 10).

Tamari M, Saeki H, Hayashi M, et al. An association study of 36 psoriasis susceptibility loci for psoriasis vulgaris and atopic dermatitis in a Japanese population.(J). Journal of Dermatological Science, 2014, 76(2):156-157.

It is difficult to be cured due to a lack of efficient drugs and high probability of relapse, seriously affecting the health and quality of life. The traditional Chinese medicine syndromes in PV mainly include the blood heat syndrome (53.8%), blood dryness syndrome (27.4%), blood stasis syndrome (18.1%), and other syndromes (0.6%) (8). Therefore, we focused on specific protein expression of blood heat syndrome in PV. Expanding results in previous studies (11) (12), we have identified several protein expressions, including lipoproteins, immune-related proteins, antimicrobial peptides, and other specific proteins showing significant difference between healthy subjects and PV with blood heat syndrome. For example, as revealed by Starodubtseva (13) MMP-1 and MMP-12 genes were highly expressed among psoriasis patients, while we showed that only MMP-1 and TIMP1 protein expression displayed significant difference in psoriasis patients.

It should be noted that syndromes in traditional Chinese medicine are real-time assessments and individuals according to the so-called herbalist experience, which has seriously hampered the development and growth of Chinese medicine. Therefore, quantitative methods of symptom characterization are urgently demanded to identify specific indicators of various syndromes. Proteomics, as the large-scale study of proteins, and in particular, structures and functions (14, 15), Chiu C, Chen H, Wu T, et al. Differential proteomics of mo-

nosodium urate crystals-induced inflammatory response in dissected murine air pouch membranes by iTRAQ technology(J). Proteomics, 2015, 15(19):3338-3348.

has indeed been widely used in many fields after the first description of proteomics by Wilkins and Williams (16). The study of (15)proteomics mainly focuses on the entire set of proteins expressed by a genome, cell, tissue, or organism at a specific time., which has laid a good foundation for the mechanism of complicated syndrome doctrine and prescription system in traditional Chinese medicine. Because of the advantages of iTRAQ technology, we adopted this technology to search protein expression profiling. Also note that reagents in iTRAQ could combine with nearly all proteins in samples, and pollutions also may exist, which would lead to incorrect conclusions. Pre-treatment with all samples, such as removal of high abundance proteins, is necessary. Some other deficiencies, such as expensive reagents and high costs, should also be taken into account (17, 18).

In conclusion, using iTRAQ technology, we have identified significant different protein expressions (e.g., lipoproteins, immune-related proteins, antimicrobial peptides) between healthy control and PV with blood heat syndrome. The different protenin expressions may contribute to the the development and occurance of PV, and would be used in the establishement of standardization and treatment of PV with different syndromes.

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