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JNMU
Journal of Nanjing Medical University, 2007, 21(4): 272-276
www.elsevier.com/locate/jnmu
Research Paper

A preliminary screening study on the associated proteins in human psoriasis vulgaris by serum proteomics technologies

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Received 26 February 2007

Abstract

Objective: To investigate the optimum screening conditions of associated proteins in human psoriasis vulgaris by serum proteomics technique, and to screen the different expression proteins related with psoriasis vulgaris. **Methods**: Serum samples of peripheral blood were collected from newly diagnosed psoriasis vulgaris patients in the clinic, and 20 matched healthy persons. Serum albumin IgG was removed by filtering with ProteoExtract Albumin/IgG. After comparative proteomics analysis the different protein spots were identified using 2-DE and MS. **Results**: Electrophoresis figures with high resolution and reproducibility were obtained. Three different expression proteins were found only in the serum from psoriasis vulgaris patients, while nine other different proteins expressing from healthy volunteers. **Conclusion**: The protein expression was different in the serum between the psoriasis vulgaris patients and healthy volunteers. It was hoped that we could find the biomarkers related to psoriasis vulgaris by using proteomics.

Keywords: electrophoresis, gel, psoriasis vulgaris, proteomics, biological

INTRODUCTION

Serum, as a major component of blood, plays important physiological functions. Serum proteins take undergo some quality and quantity changes when the human body suffers pathological damage. Detecting these characteristic changes is critical for disease diagnosis and therapeutic monitoring, and serum proteomics technologies provide favorable platforms to understand plasma proteins in healthy and sick states. Proteomics is becoming a hot field in the biological study of functional genomic era, especially in screening serum tumor biomarkers. However, the application of proteomics in dermatologic investigations is rarely reported. Here the technique of serum proteomics was adopted to establish the optimized screening of associated proteins in human psoriasis vulgaris, it is hoped that this can help to find the biomarkers related psoriasis vulgaris and to explore the pathogenesis of psoriasis vulgaris, in order to assist the treatment and prevent its recurrence.

SUBJECTS AND METHODS

Subjects

Twenty cases of psoriasis vulgaris were treated for the first time in the Department of Dermatology, Xi'an Jiaotong University Second Hospital (Xi'an, China), were finally diagnosed by senior experts of pathologic diagnosis. 20 subjects were utilized consisting of 11 males and 9 females (mean age 24 ± 7 years old). All 20 patients were in active stage, and presented with symptoms indicative of psoriasis guttata in 5, seborrheic dermatitis-like psoriasis in 3, psoriasis rupioides in 6, psoriasis hypertrophica chronica in 5, and psoriasis nummularis in 1. All patients denied other case history, had not been treated with immune depressant or corticosteroid for one year. 20 healthy control subjects matched gender

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and age came from clinical health examination.

Reagent

Immobilized pH gradient strip (IPG 4-7,17 cm), ProteoExtract Albumin/IgG Removal Kit were purchased from (Merck Chemicals Ltd,Germany);40% w/v Bio-Lyte Ampholyte (pH 3-10, pH 4-7) were purchased from Bio-Rad (Hercules, California, USA);iodo-acid amide, trifluoroacetic acid(TFA), acetonitrile,α-cyano-4-hydroxycinnamic acid, trypsin (mass spectrogram level) (Sigma-Aldrich,Inc,USA);YM-3 were purchased from Millipore(Millipore Trading Co. Shanghai,China);carbamide,DTT, acrylamide,methylene bisacrylamide,CHAPS,SDS, Tris, mineral oil, glycerine, sulfocarbamide, and aminoacetic were purchased from Amresco (Amresco,Inc,USA); broad-ambit Mol wt protein labeling(TaKaRa Biotechnology Dalian,China),all solutions were prepared by deionized water.

Equipment

PROTEAN IEF Cell and PROTEAN II XI Cell for electrophoresis,Image analysis soft(PDQuest7.1) were purchased from Bio-Rad(Hercules,California, USA).

Sample preparation

5 ml of blood samples were obtained by venipuncture from 20 patients and 20 healthy control subjects at Xi'an Jiaotong University Second Hospital(Xi'an, China), Collected blood was allowed to coagulate at room temperature for one hour and centrifuged at 3000 × g for 15 min. The sera were stored at −80°C.

Sera preparation

35 μl of sera were followed by ProteoExtract Albumin/IgG Removal Kit instruction, and then YM-3 ultrafiltration was used to condense protein, the concentration of serum protein was determined by Bradford and stored respectively.

Two-dimensional electrophoresis

The two-dimensional electrophoresis method was performed as published (refer to reference)[1]. For 2-DE, lyophilised total serum and chromatographic fractions were made soluble by suspension in lysis buffer for 3 h at 30℃ with vigorous shaking. For isoelectric focusing, 150 μg of protein sample was mixed with rehydration buffer up to 350 μl. This mixture was used to rehydrate 17 cm, pH 4-7 linear ReadyStrip™ IPG Strips (4%T; 3%C) for 12 h at 20°. A constant voltage(50 V) applied across the gel strips, which were placed in the Protean IEF cell focusing tray. The re-hydrated gels were electrophoresed at 250 V for 15 min, subjected to a linear voltage ramp from 250 to 10,000 V for 5 h, and then focused until 60,000 Vh. The temperature was maintained at 20°C. The IPG strips were then incubated in SDS-PAGE equilibration buffer with 1% (w/v) DTT with gentle shaking. After 15 min, the procedure was repeated with SDS-PAGE equilibration buffer with 2.5%(w/v) IAA for 15 min. After equilibration, the IPG gel was transferred onto a 9-16% gradient polyacrylamide(30%T; 2.6%C) gel and SDS-PAGE was performed in a Protean II xi Cell at a constant temperature of 15°C (20 mA per gel for 15 min, then 40 mA per gel) until the bromophenol blue marker had reached the bottom of the gel. For computer analysis of 2D-PAGE patterns, ammoniacal silver-stained gels(modified from) were digitised and the protein patterns analyzed with the PDQuest 7.1.1 software package.

Protein identification

Proteins were dried and digested with 10 μg/ml trypsin in 25 mM ammonium bicarbonate at 37℃ overnight. Peptides were eluted with 5%(v/v) trifluoroacetic acid and 75%(v/v) acetonitrile. Finally, 0.7 μl of the samples were mixed with 0.5 μl of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50%(v/v) acetonitrile, then analysed on a MALDI-TOF MS. Data processing was performed with MASSLYNX (Micromass), and database searching to identify the proteins of interest from their peptide fingerprint was done with MASCOT Daemon search engine (Matrix Science Ltd., London, UK) against NCBInr and Swiss-Prot databases.

RESULTS

Two-dimensional electrophoresis maps

To smooth intrinsic individual differences and deplete high abundant proteins in serum, we optimized a serum pretreatment strategy which included mixing the samples of one group, for albumin and immunoglobulin depletion and desalting. After sera pretreatment, 4-6 times more sera were analyzed and proteins were found better separated, more protein spots were detected clearly (***Fig 1***), and the proteins covered by albumin and immunoglobulin were separated completely.

Results of Protein identification

In 2-D maps, 45 disparate points in 12 spot relative volumes between control and psoriasis vulgraris samples were detected (***Fig 2***), and the disparate points in the same place been detected as the same

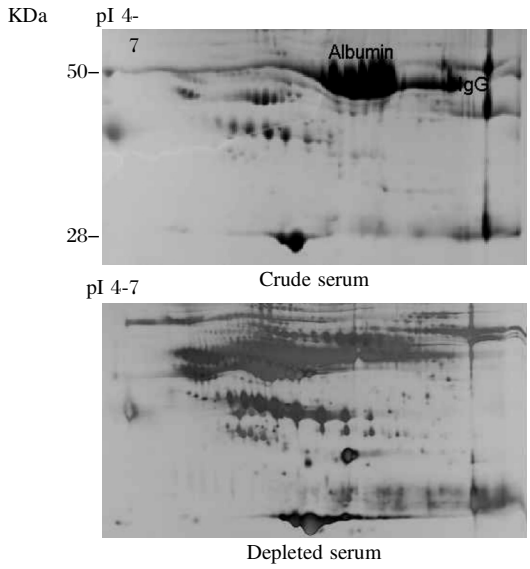
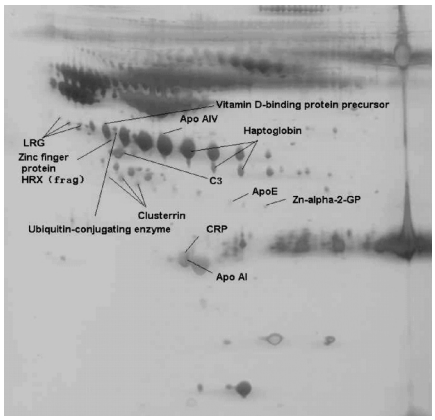


Fig 1 Comparison of the 2-DE map of unpretreated sera(a) and pretreated sera (b)from healthy group with equal protein loading



LRG,Zn-alpha-2-GP,proapo AI,ApoE and Apo AIV were under expressed in psoriasis vulgaris group;Clusterrin precursor,Complement C3 precursor,CRP and Haptoglobin were over expressed in psoriasis vulgaris group;Zinc finger protein HRX (frag),Ubiquitin-conjugating enzyme E2Q2 and Vitamin D-binding protein precursor xpressed significantly and constantly only in psoriasis vulgaris group

Fig 2 Disparation of Two-dimensional electrophoresis maps in group psoriasis vulgaris comparing with group healthy

protein. Overall, Leucine-rich alpha-2-glycoprotein precursor(LRG P02750), Zn-alpha-2-GP(P25311), Apo A-IV(P10263), Apo E(P14682) and Apo A-I (P06727) were under expressed while Complement C3 precursor (P01024),Clusterrin precursor (P10909), C reactive protein (CRP P08276) and Haptoglobinα1 chain (P00738) were over expressed in psoriasis vulgaris group, as compared to the normal group. Vitamin D-binding protein precursor (P02774),zinc finger protein HRX(frag)(Q03164), and Ubiquitin-conjugating enzyme E2Q2 (P20413) expressed significantly and constantly only in psoriasis vulgaris group(***Tab 1***).

Tab 1 **Spots significantly altered in patients with psoriasis vulgaris regarding healthy subjects**

Protein name	Cov %	Mass	PI	Accession No.
LRG	67.1%	38178	6.5	P02750
Zinc finger protein HRX(frag)	62.5 %	431754	9.2	Q03164
Ubiquitin-conjugating enzyme E2Q2	37.5%	42791	4.8	P20413
Clusterrin precursor	48.3%	52495	5.9	P10909
Complement C3 precursor	37.5%	18715	6.0	P01024
Vitamin D-binding protein precursor	43.4%	52929	5.4	P02774
Apo A-IV	36.5%	42961	4.9	P10263
Haptoglobin α1 chain	48.1%	45177	4.1	P00738
C reactive protein	31.8%	39642	3.2	P08276
ApoE	35.8%	36704	6.1	P14682
Zn-alpha-2-GP	45.5%	33872	5.6	P25311
Apo A-I	43.2%	45399	5.3	P06727

DISCUSSION

An important role in finding some biomarkers related to diseases in sera, is to explore the etiopathogenisis in diagnosis, and its relationship to the treatment of disease. However, due to the required proteins being lower in abundance in sera, proteomics approaches have to face many technical problems. For example, 60%-97% of the total protein amount is serum albumin and immune globulin. And remains in sera where less 1% of the total protein amount is potential biomarker. Then there is the problem that how to remove these higher abundance proteins. In our study we optimized a serum pretreatment strategy (as described previously), and obtained better 2-DE maps.

Psoriasis is common, and carries a substantial burden even when not extensive, and is associated with widespread treatment dissatisfaction,Specific disease-related problems in everyday life seem to cause depression in a significant proportion of patients. Especially in psoriasis vulgaris, it's attack rate is 97.98% of all psoriasis patients. It has been

widely accepted that psoriasis is a T cell-mediated autoreactive disorder (psoriasis has now been shown to largely involve the abnormal activity of T cells). It also appears that there is a polygenic inheritance for the predisposition to the development of psoriasis,But up to now, the pathogenesis of the disease has not been definitively understood. In this study we adapted serum proteomics technologies to the pathogenesis of psoriasis vulgaris, to assist the treatment and prevent its recurance.

In this work, the spots identified as C reactive proteins (Swiss-Prot accession number P08276), were widely known to alter during acute phase and inflammation [2,3]. Also complement C3 precursor (Swiss-Prot accession number P01024) increases in plasma and over-expressed in psoriasis vulgaris skin. These have been considered the two proteins increasing resulting from inflammation. The other 10 proteins (Leucine-rich alpha-2-glycoprotein precursor, Zn-alpha-2-GP, Apo A-IV, Apo E, Apo A-I, Clusterrin precursor, Haptoglobinα1 chain, Vitamin D-binding protein precursor, Zinc finger protein HRX (frag),Ubiquitin-conjugating enzyme E2Q2) have seldom been reported in dermatological research. Zn-alpha-2-GP can be widely found in plasma, body fluid, and normal epidermatic cormeous layer. It closely correlated with apoptosis, and The largest effect on Zn-alpha-2-GP expression in the proinflammatory cytokine TNF-α and TNF-γ and dexamethasone, PPARγ nuclear receptor[4-6]. IFN-γ can significantly increase the Zn-alpha-2-GP expression in the normal Malpighian cell, but in psoriasis skin, IFN-γ decrease the Zn-alpha-2-GP expression [7-10]. Apo A-IV,Apo E,Apo A-I is the dominant structural apolipoprotein of HDL particles, research has identified that high-density lipoprotein (HDL)-associated apolipoprotein A-I inhibits monocyte activation, and thus both TNF-α,IL-6,and IL-8 production [11]. Apo E could adjust immunity by inhibiting the generation and differentiation of IL-2 dependent peripheral mononuclear cells(PMC) and lymphocytes[12-16]. This study showed that Zn-alpha-2-GP, Apo A-IV, Apo E,Apo A-I was expressed lower in psoriasis vulgaris sera in comparison with control group, however the reason was still undefined.

Upon further investigation, we found that Clusterrin precursor (P10909) was overexpressed in comparison with the control group and psoriasis vulgaris group, and we believe Clusterrin precursor could be closely related to the genesis, development, prognosis, and recidivation of psoriasis data arranging. Clusterin has been reportedly implicated in several diverse physiological processes such as sperm maturation, lipid transportation, complement inhibition, tissue remodeling, membrane recycling, cell-cell and cell-substratum interactions and stabilization of stressed proteins in a folding-competent state and the promotion or inhibition of apoptosis. The question of whether clusterin is a multifunctional protein, or if it deploys a single primary function(influenced by cellular context) remains a central issue continuing to stimulate interest in this unusual molecule. Earlier reports about clusterin focused on the promotion of apoptosis, and been described as apoptosis biomarker[17-19], then contradictory data indicated clusterin as high expressed in testicular sustentacular, but having no relation with apoptosis In addition not all apoptosis cell expressed clusterin. Another view considered is that clusterin promotes an inhibition of apoptosis, and it is widely accepted for its inhibiting function of apoptosis. Sintich[20] speculated its possible sites of action in the protection from cellular apoptosis as follows. First, clstertin has been characterized as a "sticky" protein:its protective effect may be mediated through direct interaction with the TNF molecules, rendering the cytokine unavailable to target cells. Another possibility is that clusterin may act at the level of TNF receptors. If clusterin competes with TNF for its 55-KDa receptor, it could protect target cells from the cytotoxic effect of TNF. A third possibility is that clusterin may interact with cell surface molecules such as sphingomyelin and interfere with TNF signal transduction. For immune reaction, Clusterin is a soluble complement regulatory protein that binds to C5b-7, C5b-8 and inhibits generation of membrane attack complex, C5b-9. Glomerular deposition of clusterin has been observed in human and experimental membranous nephropathy in association with C5b-9 and immune deposits. This up-regulation of clusterin may play a critical role in protecting mesangial cells from complement attack. Moreover, clusterin has been found to have a synergistic action with vitronectin to make MAC dissolvable(losing the function of dissolving cell)[21]. It has been reported that serum vitronectin increased significantly in severe psoriasis patients, due to it being an acute phase reactive protein, speculating this increase was associated with C5b~9.

In the present study, we identified that there were 12 proteins differentially expressed in the psoriasis vulgaris group, as compared to normal group for these proteins. Although there is a large amount still unclear in exploring dermatology research using proteomics technology, we believe firmly our investiga-

tion is a well-disposed experiment.

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