Journal of Immunological Methods 446 (2017) 37–46

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Proteomic analysis of human plasma and peripheral blood mononuclear cells in Systemic Lupus Erythematosus patients

Sara Pinna [a], Sara Pasella [a], Marta Deiana [b], Angela Baralla [a], Andrea Mannu [a], Antonio Giovanni Emilio Masala [c], Piera Veronica Pileri [c], Nicola Deiana [b], Fabrizio Scognamillo [d], Carlo Pala [d], Angelo Zinellu [a], Ciriaco Carru [a], Luca Deiana [a,b,*]

[a] Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[b] Associazione "L'Isola dei Centenari", Sassari, Italy
[c] Internal Medicine 1, A.O.U. University of Sassari, Sassari, Italy
[d] Surgical Pathology, A.O.U. University of Sassari, Sassari, Italy

ARTICLE INFO

Article history:
Received 26 October 2016
Received in revised form 9 March 2017
Accepted 28 March 2017
Available online 5 April 2017

Keywords:
Autoimmune diseases
SLE
PBMC
Two dimensional SDS PAGE
Proteomic

ABSTRACT

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with a broad spectrum of clinical presentations and incompletely understood pathogenesis. This autoimmune disease is characterized by alterations in both the innate and adaptive immune system that lead to the loss of immunologic tolerance.
In autoimmune diseases particularly in SLE, early diagnosis, flare or remission phases can be difficult to identify. Proteomics can help to find new therapeutic targets and it also could help to better understand the cellular mechanisms. The aim of this study was to observe the variations in plasma and Peripheral Blood Mononuclear Cells (PBMCs) proteome in order to increase our knowledge about pathogenesis and to find possible diagnostic markers and/or therapeutic targets for improving diagnosis and treatment.
The comparative proteomic analyses showed that several proteins were differentially expressed in the PBMCs from SLE patients. Among these, PRDX2 may be used as candidate biomarker or target protein for further investigations. In plasma, we showed that plasma clusterin levels increased in SLE patients compared to healthy controls, but this increase is not statistically significant. These proteomic results provide suggestions for understanding the molecular mechanisms of SLE, as well as the physiological changes correlated with SLE disease.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with a broad spectrum of clinical presentations and incompletely understood pathogenesis. This autoimmune disease is characterized by alterations in both the innate and adaptive immune system that lead to the loss of immunologic tolerance and occurrence of auto-antibodies against nuclear material (Rahman and Isenberg, 2008).

The aetiology of SLE includes both genetic and environmental components with female sex strongly influencing pathogenesis (Matucci Cerinic et al., 2014).

Disease pathogenesis in SLE manifests itself as systemic acute and chronic inflammation that affects many organ systems. Furthermore, its onset can vary from patient to patient.

In SLE, the presence of specific serological markers is limited, and the pathogenesis has not yet been fully elucidated. The diagnosis of SLE during the initial stages may be mistaken, this happens because the symptoms may be similar to those of other rheumatic diseases (such as rheumatoid arthritis (RA) and systemic sclerosis (SSc)); moreover, it requires a complex interpretation of the American College of Rheumatology (ACR) criteria.

The disease being systemic, it could also manifest itself in different sites, and so far there are no specific tests to indicate accurately the picture.

From the present literature (Pavón et al., 2006; Schulz et al., 2007; Kim et al., 2008; Dai et al., 2008; Zhang et al., 2010; Rana et al., 2012; Wang et al., 2012), we note the importance of proteomics, which can help to find new therapeutic targets and it also could help to better understand the cellular mechanisms.

Proteins are indeed important executors of physiological and cellular functions; changes in their expression reflect the different physiological conditions (e.g. healthy and sick). Observing the variations may help to

* Corresponding author at: Department of Biomedical Sciences, University of Sassari, Sassari, Italy.
E-mail address: deiana@uniss.it (L. Deiana).

http://dx.doi.org/10.1016/j.jim.2017.03.019
0022-1759/

better understand the pathogenesis in order to improve diagnosis and treatment.

Plasma and Peripheral Blood Mononuclear Cells (PBMCs) can be obtained easily and non-invasively. Both the matrices are subjected to rapid changes in response to different signals, such as during inflammatory states. Plasma is one of the most widely used blood component, while in recent years we have seen a greater interest in PBMCs.

PBMCs are mainly composed of monocytes and lymphocytes, these cells are the most involved in the immune response. Observing the changes in their proteome may help us to have a deeper knowledge of their function in various diseases (Maes et al., 2013; de Roos, 2008; de Roos et al., 2008).

In autoimmune diseases, particularly in SLE, early diagnosis, flare or remission phases can be difficult to identify, because of complex etiopathogenesis, heterogeneous presentation of symptoms, and unpredictable course. In addition, the initial symptoms may include signs or symptoms common among different ADs.

The definition of specific serological markers is essential for early differential diagnosis. There are no set of biomarkers that can be used reliably to confirm the presence of SLE or to monitor its progression with specificity and sensitivity.

Starting from these assumptions, the aim of this study was to observe the changes in the proteome of SLE patients, in order to increase our knowledge about pathogenesis and to find possible diagnostic markers and/or therapeutic targets for improving diagnosis and treatment.

2. Materials and methods

2.1. Patients

Twenty one SLE patients, (20F:1M, 48,2 ± 6,14 years), twelve RA patients (12F, 64,15 ± 12,17 years, used as pathological control) and twenty one healthy volunteers (20F:1M, 42,14 ± 9.58 years) were enrolled in the study.

All subjects were recruited from the AKeA Project (Deiana et al., 1999) in collaboration with the unit of Internal Medicine 1, A.O.U. University of Sassari. All patients and volunteers signed a written informed consent prior to blood sampling. This study was approved by the Institutional Local Ethics Committee (Azienda Sanitaria Locale n°1 di Sassari (Italy) (N° 398/L,,) and was in accordance with the principles of Declaration of Helsinki.

SLE diagnosis is based on the 1987 American College of Rheumatology revised criteria (Arnett et al., 1988). Each SLE patient had the same disease activity, calculated with SLEDAI (Score ≤ 6, stable phase of the disease); the active form of the disease was renal for 33,3% and 66,7% cutaneous. All participants were Caucasians people. Blood samples were collected into vacutainers containing potassium EDTA as anticoagulant (Vacutest Kima). For 2D analyses we have used pooled samples for each group; while for ELISA and Western Blot validations we used single samples.

All SLE patients were on treatment using standard protocol incorporating glucocorticoids (prednisone 5–25 mg daily) and immunosuppressive agents (azathioprine 100 m daily or hydroxychloroquine 400 mg daily). AR patients received low doses of prednisone (2.5–5 mg daily) and methotrexate (10–15 mg per week or hydroxychloroquine 400 mg daily) while healthy subjects haven't any other inflammatory, infectious or other diseases and they were without a family history of autoimmune diseases.

2.2. Plasma samples

The red cell fraction was separated from plasma by centrifugation at 2500*g* at 4 °C for 15 min and the clear plasma supernatant was stored in aliquots frozen at −80 °C until use.

For each group (SLE, RA and Healthy), were created plasma pools.

For protein extraction we used the ProteoMiner™ ProteinEnrichment Kits (Biorad). The Proteominer is a tool used to reduce the concentration of proteins in complex biological samples.

2.3. PBMCs samples

PBMCs were isolated using a Ficoll-Paque Plus density gradient (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

The cells were resuspended in phosphate-buffered saline (PBS). Soluble proteins were extracted from PBMCs by incubation on ice in 200 μl of lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, DNasi (3 μl/ml), RNasi (3 μl/ml) and Complete Protease Inhibitor Cocktail(5 μl/ml)) for 10 min. Were carried 2 freeze-thaw cycles at −80 °C for 5 min. Samples were centrifuged at 14000*g* for 30 min at 4 °C and supernatant containing proteins recovered. For proteomics analyses the protein extract of PBMCs was pooled for each group (SLE,RA and Healthy). The pool is prepared by mixing defined units of protein extract (50 μg of protein) belonging to the same group.

2.4. Protein quantification

The concentrations of plasma and PBMCs proteins were determined using the RC/DC Protein Assay (BioRad) according to the manufacturer's instructions. The RC/DC Protein Assay uses a Lowry method modified to reduce agent compatible (RC) as well as detergent compatible (DC). A standard curve was constructed using albumin as standard sample in a range between 0.2 and 2 mg/ml.

2.5. Two dimensional SDS PAGE

Total protein extracts of plasma and PBMCs samples were separated by 2D-PAGE gels. Analytical gels contained 150 μg of total protein extracts; for preparative gels 1000 μg of protein were applied. Three experimental replicates were performed for each sample pool belonging to the three different groups.

Isoelectrofocusing (IEF).

Plasma: The first dimension (Isoelectric Focusing, IEF) was performed on IPG (Immobilized pH gradient) strips linear pH 3–10, 17 cm from BioRad. At first, the strips were rehydrated for 20 h at 20 °C without voltage in rehydration buffer (Urea 8 M, Tiourea 2 M, 4% CHAPS, Destreak reagent, 10 mM DTT, 1% carrier ampholyte pH 3–10 and 0.05% bromophenol blue) and covered with mineral oil. The proteins were separated using the Protean IEF Cell system (Biorad) according to the following protocol: 2 h at 250 V, 2 h at 500 V, 2 h at 750 V, 2 h at 1000 V, 2 h at 5000 V, 2 h at 8000 V, at 8000 V for 50.000 Volthours, for a total of 80.000 V. Focused IPG strips were equilibrated in two steps (15 min each) in 1 ml freshly prepared sample buffer (50 mM Tris–HCl pH 8.8, 6 M urea, 20% (*v*/v) glycerol and 2% (*w*/*v*) SDS, 1% (*w*/*v*) bromophenol blue) supplemented with 2% dithiothreitol and 2.5% iodoacetamide respectively (Pasella et al., 2013).

PBMCs: IEF was performed on IPG strips linear pH 4–7, 17 cm from BioRad. The strips rehydratetion, the first dimesion and IPG equilibration are the same conditions of plasma.

2.6. SDS-PAGE

To separate the protein in the second dimension we performed a size denaturing sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE). Equilibrated IPG strips were embedded into 0.5% agarose on top of SDS polyacrylamide gels. For the second dimension 13,5% (for plasma proteins) and 12,5% (for PBMCs proteins) SDS-polyacrylamide gels were used. The gels were made using the Multicasting Chamber from BioRad. The gels were run on a Protean Multicell (BioRad) were six gels at a time can be run. The instrument is connected to a cooling bath to avoid excessive heating of the buffer. The lower