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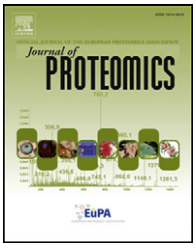

A proteomics approach to the identification of biomarkers for psoriasis utilising keratome biopsy

James C. Williamson[a,*], Peter Scheipers[b], Veit Schwämmle[a], John R. Zibert[b], Hans Christian Beck[c], Ole N. Jensen[a]

[a]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense DK-5230, Denmark
[b]LEO Pharma A/S, Industrieparken 55, Ballerup 2750, Denmark
[c]Center for Clinical Proteomics, Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Sdr. Boulevard 29, Odense 5000, Denmark

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ABSTRACT

The discovery of plasma biomarkers for psoriasis vulgaris may aid clinicians in disease grading and monitoring of treatment response. We have therefore developed a proteomics/mass spectrometry based workflow which enables biomarker discovery from psoriasis patient samples. We have utilised keratome skin biopsy, which results in reduced cellular complexity compared to punch biopsy. Furthermore, we applied short term *ex vivo* culture in order to enrich for a "secretome" sub-proteome reflective of the disease and enriched in potential biomarkers. Using these sample preparation techniques we performed a quantitative proteomics screen of four patients with psoriasis using stable isotope dimethyl labelling and identified over 50 proteins consistently altered in abundance in psoriasis lesional versus non-lesional skin. This includes several canonical psoriasis related proteins (e.g. S100A7 [Psoriasin] and FABP5 [Epidermal Fatty Acid Binding Protein]) and more than 30 novel alterations. From this disease localised dataset we further assessed several proteins as potential biomarkers in the plasma of patients with psoriasis versus healthy controls utilising selected reaction monitoring mass spectrometry (SRM-MS/MS).

Biological significance
The significance of this study for proteome research in psoriasis and dermal disease is threefold. 1) A novel sample preparation method for isolation of dermal proteomes enriched in extracellular proteins is described, which may be of interest to other researchers in the field. 2) Novel psoriasis associated alterations in protein abundance are described at the disease site, bolstering knowledge in an area dominated by transcript level studies and 3) Profilin 1 is described as a candidate plasma biomarker of psoriasis, this is of value in itself and it proves that our workflow can yield results in terms of biomarker discovery.

1. Introduction

Psoriasis is an inflammatory skin disorder affecting 2–3% of the population of the western world. It has a complex disease aetiology involving many cell types, genetic and environmental risk factors [1,2]. This complexity leads to a wide margin of severity from patient to patient and varying responses to treatment. Therapies range from topically applied steroids or

* *Corresponding author at:* Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense 5230, Denmark. Tel.: +45 6550 2412.
E-mail address: James.Williamson@btinternet.com (J.C. Williamson).

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vitamin D analogues for mild to moderate psoriasis, to systemic immune response modifiers for moderate to severe grades of psoriasis [3]. However, even though many effective treatments are available, some patients fail to respond to certain medications which results in a period of trial and error to find an appropriate intervention. Additionally, after some time an effective treatment may lose efficacy due to treatment resistance. Therefore in order to better assess the needs of patients, identify potential treatment resistance, and monitor treatment response, a better understanding of psoriasis as well as the discovery of biomarkers is needed.

Some proteomic techniques such as SELDI, cytokine profiling and glycoprotein enrichment have been applied to the analysis of serum/plasma from psoriasis patients [4–6]. However, plasma remains an extremely challenging material for biomarker discovery as the levels of such proteins are eclipsed in concentration by highly abundant plasma constituents. Clearly, healthy and disease derived plasma must come from different individuals. This necessitates careful patient matching and high inter-individual variability may be prohibitive to the success of such studies. Rather than attempt biomarker discovery in plasma, an alternative is to begin with a discovery phase study at the disease site (in this case at psoriasis lesions) and move into plasma at a later stage when biomarker candidates are defined. The central premise for such an approach is that disease related proteins can drain from the affected tissue into plasma (or other disease proximal fluid), where they may serve as biomarkers. This technique has been adopted for the discovery of biomarkers in the interstitial fluid of resected ovarian tumours [7,8], urine from bladder cancer patients [9] and cerebrospinal fluid from patients with neurodegenerative disease [10]. Furthermore, we have successfully utilised this technique previously to investigate the release of S100A4 protein from epidermal psoriasis skin [11].

Global proteomic analyses of psoriasis skin are rather uncommon compared to microarray and other transcript level approaches [12–14]. This is perhaps due to the difficulty of analysing dermal tissue with proteomics techniques. The variety of infiltrating immune cell types present in dermal tissue is large, especially in psoriasis skin [1]. This leads to an extractable proteome with a challenging high complexity and broad range of protein concentration. Such samples are increasingly within the reach of modern proteomics/mass spectrometry techniques. However it remains desirable to find intelligent methods of sample preparation to alleviate protein complexity and dynamic range issues prior to analysis. There are a number of ways this may be achieved for clinically derived dermal samples. It is possible to study some resident cell types in isolation by primary culture from clinical isolates [15,16]. However, passage and isolation of cells from the dermal microenvironment may have an impact on the proteome. Some studies have shown that immune cell populations distal from the disease site (both blood born and from bone marrow) are of interest and can be analysed without culturing [17,18]. Finally, two sampling methods, namely microdialysis and suction blister have been used to access interstitial fluid-like proteomes at or close to the site of skin disease [19,20]. While such samples are more amenable to proteomics analysis than plasma, high levels of contaminating albumin are still typically present. We therefore identify a need for a sample preparation technique which results in the generation of a sub-proteome more appropriate for modern proteomic analysis of dermal tissue and identification of candidate plasma biomarkers.

We have chosen to use keratome biopsy of epidermal tissue as a starting clinical isolate. Due to its reduced sampling depth compared to conventional punch biopsy, we predict a reduction in cellular and proteome complexity. Additionally, we chose to enrich a "secretome" from these biopsies using a short term culture method, which will effectively enrich proteins more likely to be reflected in plasma. With the combination of these two techniques we hypothesised that the extracted sub-proteome would be more convenient to analyse than blood plasma and with a greater potential to contain plasma biomarkers than a tissue lysate.

Using this sub-proteome we performed a global proteomic analysis of four psoriasis patients comparing peptide abundance in psoriasis involved and uninvolved skin, thus every patient was its own control. We applied stable isotope dimethyl labelling and mass spectrometry to assess the relative abundance of proteins in these samples. This discovery dataset contains a number of known psoriasis associated alterations in protein abundance as well as interesting novel observations. The abundance of selected candidates from this study was assessed in plasma of psoriasis patients and controls by selected reaction monitoring mass spectrometry (SRM-MS/MS).

2. Materials and methods

2.1. *Dermal tissue collection and short term culture*

Keratome biopsies were obtained from 4 patients diagnosed with psoriasis vulgaris including both involved (plaque psoriasis) and uninvolved psoriasis skin to a depth of ~0.5 mm. Prior to sampling, none of the patients had used any systemically immunosuppressive medications for 4 weeks or any local treatment for 2 weeks. The study was carried out in agreement with the Declaration of Helsinki Principles. All subjects gave informed consent. Patient meta-data were destroyed in order to preserve anonymity. Keratome biopsies contained mostly the epidermal layer and papillary dermis (Fig. 1). From the keratomes, 4 mm diameter biopsies were taken from psoriasis involved and uninvolved areas. These tissue biopsies were transferred to 0.5 mL of RPMI media (without serum) with 50 IU mL-1 penicillin, and 50 IU mL-1 streptomycin (Cambrex, Belgium), cut into small pieces, and incubated at 37 °C, 5% CO_2, in 24-well incubation plates for 24 h, followed by centrifugation to remove the cells and debris [11]. The proteins contained in the culture media were used for the discovery proteomics workflow.

2.2. *Clean-up, quantification and digestion*

To remove media components and concentrate protein, samples were applied to 10 kDa centrifugal ultrafiltration devices (Amicon from Millipore, Denmark) and washed 5 times with 20 mM HEPES (pH 7.4) including protease inhibitors (Roche Diagnostics, Germany). Protein content in all samples was quantified by BCA assay (Pierce, Germany). 20 μg of protein from each sample was reduced with 10 mM DTT at 57 °C for