JOURNAL OF PROTEOMICS 73 (2009) 252–266

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Validation of serum protein profiles by a dual antibody array approach

Rebecca Rimini, Jochen M. Schwenk, Mårten Sundberg, Ronald Sjöberg, Daniel Klevebring, Marcus Gry, Mathias Uhlén, Peter Nilsson*

Department of Proteomics, School of Biotechnology, KTH-Royal Institute of Technology, Albanova University Center, SE-10691 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 16 April 2009
Accepted 10 September 2009

Keywords:
Antibody microarray
Planar microarray
Suspension bead array
Serum profiling
Antibody proteomics

ABSTRACT

In recent years, affinity-based technologies have become important tools for serum profiling to uncover protein expression patterns linked to disease state or therapeutic effects. In this study, we describe a path towards the production of an antibody microarray to allow protein profiling of biotinylated human serum samples with reproducible sensitivity in the picomolar range. With the availability of growing numbers of affinity reagents, protein profiles are to be validated in efficient manners and we describe a cross-platform strategy based on data concordance with a suspension bead array to interrogate the identical set of antibodies with the same cohort of serum samples. Comparative analysis enabled to screen for high-performing antibodies, which were displaying consistent results across the two platforms and targeting known serum components. Moreover, data processing methods such as sample referencing and normalization were evaluated for their effects on inter-platform agreement. Our work suggests that mutual validation of protein expression profiles using alternative microarray platforms holds great potential in becoming an important and valuable component in affinity-based high-throughput proteomic screenings as it allows to narrow down the number of discovered targets prior to orthogonal, uniplexed validation approaches.

1. Introduction

During the past decade, proteomic profiling of serum and plasma has become a topic of growing interest and the characterization of their protein and peptide content will enable the discovery of an increasing number of reliable markers indicative of disease states or drug therapy effects. However, analytical challenges for such discovery approaches are introduced by the complexity of these biofluids since they consist of thousands of different proteins spanning a concentration range over 10 orders of magnitude [1], with many low-abundance components often masked by the overwhelming abundance of relatively few proteins, particularly albumin [2].

Despite these difficulties, substantial progress has been achieved in serum analysis with the application of affinity-based methods. By implementing miniaturized and parallelized immunoassays through bead-based or planar microarray formats [3], a significant increase in experimental throughput and output has been achieved towards reliable profiling of complex specimen proteomes in a large number of samples. In planar microarrays, capture reagents for individual tests are immobilized as an ordered two-dimensional grid on functionalized

Abbreviations: 3-D, three dimensional; EC, effective concentration; NHS, N-hydroxysuccinimide ester; PBS-T, phosphate-buffered saline with Tween 20; HPA, human protein atlas.

* *Corresponding author*. Tel.: +46 8 5537 8336; fax: +46 8 5537 8481.
E-mail address: rimini@kth.se (R. Rimini).

1874-3919/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2009.09.009

surfaces commonly in the format of microscope slides and exposed to the analyte solutions [4–6]. To date, several combinations of solid supports and immobilization methods have been employed for the fabrication of protein microarrays based on the use of derivatized glass or nitrocellulose-coated slides [7–13]. Despite the high binding capacity of the latter support, they are not always suitable for the analysis of complex protein specimen because of the unspecific binding to the nitrocellulose [14]. As an alternative to planar microarray strategies, bead-based systems have been developed employing color-encoded, polystyrene microspheres as the solid support for the capture molecules, which are mixed to create an array in suspension [5]. The beads are applied to wells of microtiter plates and a later identification occurs in a flow cytometer where the amount of captured target protein is determined on each individual bead. Currently, planar antibody microarrays are generated with a density ranging from few to thousands capture features [5,6] while bead-based arrays are presently limited to 100 parameters per assay [15], although larger numbers of samples can be screened with this technique in a single setting. During the last few years, several applications of antibody microarray methods for serum proteome profiling have been described [16,17] with main focus towards cancer biomarker discovery [18–22]. Despite the success of these efforts, further improvements are still necessary in order to achieve assay sensitivity, dynamic range and degree of multiplexing capacity appropriate for a comprehensive analysis of the serum proteome.

Microarray-based immunoassays, whenever applied to analysis of complex specimen, are greatly dependent on the availability of validated high-affinity reagents for capturing the targeted antigens. Among various strategies [23], the Swedish Human Proteome Resource (HPR) initiative combines high-throughput generation of antibodies targeting the non-redundant human proteome with immunohistochemical profiling in a variety of normal and cancer tissues [24,25]. Within the program, in-silico defined epitopes, so-called protein epitope signature tags (PrESTs), are systematically produced for each human protein and used as antigens for immunization and affinity purification of the polyclonal antisera and for the generation of protein microarrays used for antibody quality assessment [26]. The production of such large comprehensive collections of well-characterized specific antibodies represents a fundamental opportunity and challenge for a new generation of antibody microarrays.

A critical issue in microarray analysis is the establishment of efficient methods for the verification of large generated dataset. Currently, Western blot and immunohistochemical analysis are the most widely used techniques to confirm protein microarray data [22,27,28]. Alternatively, antibody microarray measurements are often matched against quantitative data from ELISA assays [21,29]. Since these available techniques rely upon the analysis of one protein at a time, there is a growing need for throughput validation methods. Confirmatory studies based on data concordance between independent microarray platforms have been successfully applied in the analysis of DNA microarray results [30]. Recently, a small-scale example of such a comparative approach was presented by Kusnezow et al [31] for protein microarray analysis, based on concordance in measured serum levels for a limited number of cytokines between the antibody microarray platform they established and a bead-based system. Hence, larger studies are needed to explore mutual validation approaches of protein expression between independent antibody microarray platforms in order to assess whether this approach might become an important component of array-based affinity proteomics.

Here, we describe the implementation of two antibody-based microarray platforms to be used for dual protein profiling of serum specimen. For planar arrays, epoxide and three-dimensional (3-D) solid supports were selected from a comprehensive evaluation study of slides with different surface chemistry and printing buffers with respect to assay reproducibility and sensitivity. For validation, a strategy was employed relying upon data concordance between the established system and a suspension bead-based array platform. Replicate sets of up to 50 clinical human serum samples were profiled simultaneously on each microarray format, and several critical analytical issues were addressed to perform accurate comparative analysis.

2. Materials and methods

2.1. *Antibodies, antigens and serum samples*

Blood serum obtained from 12 healthy donors (Uppsala University Hospital, Sweden) was used for the implementation of planar antibody microarrays. The cross-platform study was carried out on 46 serum samples from a cohort obtained through the EU project MolPAGE. For fourteen individuals, blood had been collected at two independent hospital visits. Two samples generated by pooling the Uppsala and the MolPage sera respectively were used as standards. All samples were stored at −80 °C until use.

A total of 68 mono-specific antibodies were generated within the Human Protein Atlas project as described previously [26,32]. For immunization and subsequent antibody purification, 100–150 amino acid fragments, called Protein Epitope Signature Tags (PrESTs), were recombinantly produced as fusions with a HisABP-tag. The molecular weight of the PrEST proteins was in general 30 kDa as determined by electrospray mass spectrometry. Polyclonal anti-human IgA and C3 antibodies were purchased from DakoCytomation. As controls, unspecific rabbit IgG and anti-human albumin antibody (Jackson ImmunoResearch) were included.

2.2. *Antibody microarray fabrication*

Six slide surfaces were selected and evaluated: poly-L-lysine coated slides (Sigma-Aldrich), SuperAldehyde and SuperEpoxy slides (Telechem), slides AL and P (Schott-Nexterion) and Epoxide coated slides (Corning).

As printing buffers, 1× phosphate-buffered saline (PBS) (Gibco/Invitrogen) was used supplemented with 0.5% (w/v) trehalose (AppliChem), 0.01% (w/v) BSA (Merck), 0.005% (v/v) Tween 20 (Sigma-Aldrich), 5% (v/v) glycerol (Merck), 30% (w/v) polyethylene glycol (PEG, MW 400, KEBOLab) or 0.2% (w/v) polyvinyl alcohol (PVA, MW 30000–70000, Sigma-Aldrich). In addition, the ArrayIt buffer (Telechem) was included. For the