Planar Antibody Arrays for Biomarkers in Nephritis

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Contents

Abstract

Affinity proteomics, represented by planar antibody arrays, is an established methodology for high-throughput disease proteomics. The technology can be used to generate multiplexed protein expression profiles of even crude proteomes. The antibodies are deposited one by one in an ordered pattern, an array, onto a planar, solid support, where they will act as specific catcher molecules. Next, the sample is added, and any specifically bound proteins are detected and quantify using mainly fluorescence as sensing technology. The observed binding pattern is then converted into a high-resolution protein expression map, or protein atlas,

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outlining the composition of the sample at the molecular level. Using state-of-the-art bioinformatics, candidate biomarker signatures are identified. Hence, the technology platforms provide unique opportunities for, e.g., biomarker discovery, disease diagnostics, monitoring, and evidence-based therapy selection, setting the stage for personalized medicine. Nephritis is inflammation of the kidney, a focal or diffuse proliferative or destructive disease, for which new panels of high-performing, blood-based biomarkers could have a clinical impact. In this chapter, we will describe the design and development of planar antibody microarrays for biomarker discovery and illustrate their use for delineating disease-associated biomarkers in nephritis.

Keywords

Recombinant antibodies • Antibody arrays • Protein expression profiling • Disease proteomics • Nephritis • SLE • Biomarkers

Abbreviations

GPS	Global proteome survey
scFv	Single-chain fragment variable
SLE	Systemic lupus erythematosus
TXP	Triple-X Proteomics

Key Facts of Planar Antibody Arrays and Nephritis

- Planar antibody arrays are miniaturized assays for multiplexed profiling of proteins in even crude samples, such as serum.
- Antibody arrays rely on the specific, sensitive, and selective binding properties of the arrayed antibodies for capture of the corresponding proteins (antigens).
- Planar antibody arrays can be used for protein expression profiling, resulting in biomarker discovery.
- Nephritis is a chronic or acute inflammatory condition of the kidneys.
- Nephritis-associated serum and urine biomarkers can provide the clinicians with actionable information (e.g., diagnosis and monitoring).

Definition

Array A miniaturized, ordered pattern of, e.g., dispensed antibodies.

Biomarker A measurable indicator of some biological state, condition, or disease.

Clinical proteomics A branch of proteomics, involving the application of proteomic technologies on clinical samples.

Microarray An array with micro-sized spot features.

Nanoarray An array with nano-sized spot features.

Nephritis An acute or chronic inflammatory condition of the kidneys.

Planar arrays Arrays printed on planar surfaces.

Proteome All proteins in a given sample, cellular system, or organism, at a given time point.

Proteomics Large-scale comprehensive study of all proteins (the proteome) in sample.

scFv The smallest fragment of an intact antibody containing the antigen-binding site.

Introduction

Nephritis is a chronic or acute inflammatory condition of the kidneys, involving the glomerulus, tubule, or interstitial tissue. The disease is due to a variety of causes, including kidney disease, infection, and autoimmune disease, and the treatment depends on the cause. In many cases, the damage is reversible when the cause is identified and removed, but can in severe cases progress to renal failure and fibrosis. Data indicates that this condition could be the ninth highest cause of death in humans across the world. There are several different types of nephritis, such as acute nephritis, chronic nephritis, glomerulonephritis, interstitial nephritis, pyelonephritis, autoimmune nephritis, and lupus nephritis.

Lupus nephritis is caused by systemic lupus erythematosus (SLE) and one of the most serious complications that can result from SLE (D'Cruz et al. 2007; Herbst et al. 2012; Mok 2010; Rovin et al. 2007). Data indicates that 35 % of the patients display signs of nephritis at the time of lupus diagnosis, and about 40–60 % of the patients will show kidney involvement during the course of this chronic autoimmune connective tissue disease. If not diagnosed and treated early enough, kidney nephritis could result in severe condition and even death. The clinical manifestations of SLE vary among the patients, and the signs and symptoms evolve over time and overlap with those of other autoimmune diseases, why SLE is often misdiagnosed and/or overlooked (Liu et al. 2010; Merrill 2005; Manzi 2009). In fact, SLE is often referred to as the "invisible disease." Hence, high-performing blood- and/or urine-based biomarkers would thus have a significant clinical impact, providing the clinicians with actionable information. However, deciphering disease-associated biomarker panels in crude samples, such as serum or plasma, has proven to be technologically very challenging.

Proteomics is the large-scale comprehensive study of all proteins in a given sample, cellular system, or organism, defined as the proteome. Clinical proteomics is a branch of proteomics, involving the application of proteomic technologies on clinical samples, such as blood. The aim is to decipher disease-associated biomarkers for, e.g., diagnosis, prognosis, classification, and therapeutic prediction, as well as for screening and/or monitoring how well the patient responds to a given treatment. In addition, and most importantly, the traditional approach of searching for a single, unique biomarker as the solution to an unmet clinical need (e.g., diagnosis) has been replaced by the concept of defining multiplexed biomarker panels. Such biomarker panels have been validated to provide a much more selective, specific, and robust disease classifier (Cordero et al. 2008; Hanash et al. 2008; Mischak et al. 2007; Borrebaeck and Wingren 2007) and will become the golden standard to aim for. In this process, the need for multiplexed, high-performing (e.g., resolution, specificity, sensitivity, and reproducibility) protein bioassays capable of handling also crude, complex samples (e.g., non-fractionated plasma) has become evident (Hanash 2003; Hanash et al. 2008). The challenging analytical nature of a proteome is well illustrated by plasma, containing thousands of individual proteins, ranging in concentration over more than nine orders of magnitude. This has been a major driving force in the development of a new line of proteomic technologies, denoted affinity proteomics, mainly represented by antibody microarrays (Borrebaeck and Wingren 2009a, 2011). The antibody microarray-based technology has rapidly evolved from early proof-of-concept setups to multiplexed, high-performing protein bioassays and today constitutes a key established approach within clinical proteomics at frontline laboratories (Borrebaeck and Wingren 2009a).

In 2000, the first set of papers was published, reporting focused efforts toward developing antibody microarrays (Haab et al. 2001; MacBeath and Schreiber 2000). In these publications, low-density (<10) antibody microarrays were generated by printing polyclonal and/or monoclonal antibodies one by one. The basic concepts of the antibody microarrays were demonstrated, but the work also highlighted some of the technical challenges that would have to be addressed and resolved before the technology would become an established proteomic approach. During the last 15 years, major efforts have therefore been launched to develop the technology further. As a result, a set of high-performing antibody micro- and nanoarray technology platforms are now at hand, providing novel opportunities for large-scale protein expression profiling of high- and low-abundant targets in crude, non-fractionated proteomes, such as serum (Borrebaeck and Wingren 2009a).

Antibody Micro- and Nanoarray: Basic Technological Concepts

An antibody array is a specific form of protein array that relies on the specific binding property of the antibody. More specifically, the antibodies are printed one by one onto a solid support in an ordered pattern, an array, where they are exploited as capture molecules, or probes, for the corresponding antigens, with the aim of detecting and quantifying the levels of the target proteins in the sample at hand

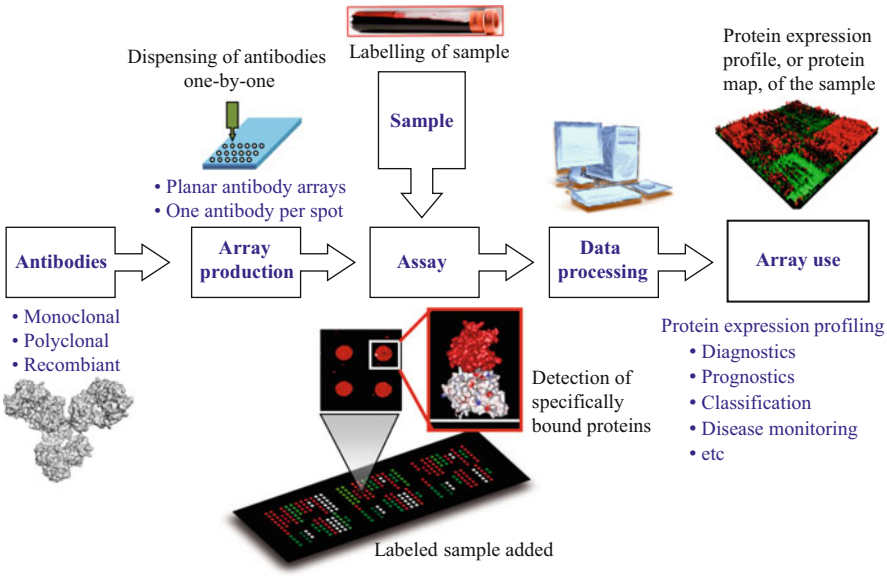

Fig. 1 Schematic illustration of planar antibody microarray setup

(Borrebaeck and Wingren 2009a; Wingren and Borrebaeck 2009) (Fig. 1). Producing such miniaturized, high-density arrays based on antibodies with a broad range of specificities enables the simultaneous screening of many protein targets, while consuming minute (μL range) amount of reagents. When the antibody microarray has been produced, the assay is run like a conventional immunoassay (e.g., ELISA). The observed signal intensities are then transformed into a protein expression map, or detailed protein atlas, revealing the composition of the sample at a molecular level. In other words, the antibody array technology provides unique opportunities for performing protein expression profiling of crude, non-fractionated proteomes that will enhance our fundamental knowledge of biological processes in both disease and health (Borrebaeck and Wingren 2007, 2009b; Haab 2006; Hartmann et al. 2009; Kingsmore 2006; Wingren and Borrebaeck 2006).

The current concept of generating miniaturized antibody arrays, ranging in size from mm^2 to cm^2, is based on either printing (pL scale or less) (Borrebaeck and Wingren 2007; MacBeath 2002; Wingren and Borrebaeck 2006), self-addressing (Wacker and Niemeyer 2004; Wacker et al. 2004), or self-assembling (He et al. 2008a, b; He and Taussig 2001; Ramachandran et al. 2004, 2008) small amounts (fmol range) of individual antibodies with the desired specificity onto a solid support (Fig. 2). Direct printing is by far the most commonly used approach and is based on using various dispensing methodologies, with non-contact ink-jet printers dominating the scene (Borrebaeck and Wingren 2007; Wingren and Borrebaeck 2007). The purified probes are printed one by one in the pL scale, generating ~150 μm sized spot features, depending on the printing buffer and the surface properties of the solid support. Self-addressing is a new method for

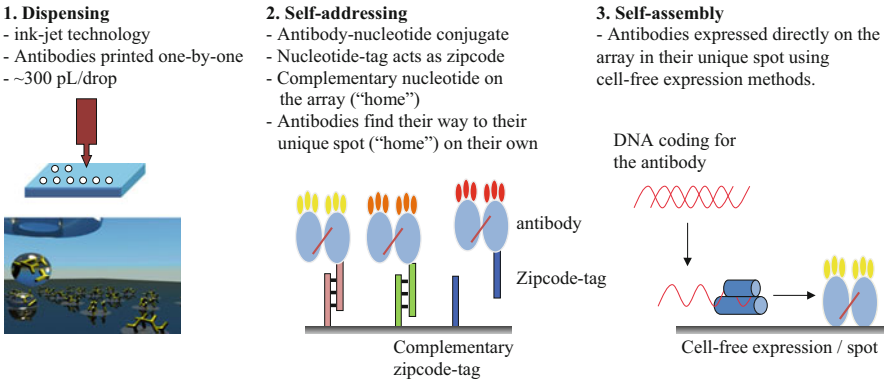

Fig. 2 Three main ways of producing planar recombinant antibody arrays

potentially producing truly high-density arrays, but still in its exploratory phase (Wacker and Niemeyer 2004; Wacker et al. 2004). In this approach, each individual antibody is tagged with a unique zip code tag, a short stretch of DNA. When added to the array in bulk, the antibodies will find their way on their own to their unique home (spot) on the array, composed of complementary DNA. Self-assembling antibody microarrays is also a new, exploratory approach to potentially generate high-density antibody arrays (He et al. 2008b). In this setup, the antibodies are produced directly on the chip in their unique position, using cell-free protein expression (He et al. 2008a, b; He and Taussig 2001; Ramachandran et al. 2004, 2008).

The size of the individually printed spots determines whether the array is denoted a microarray (spot diameter (Ø) in the μm range) or a nanoarray (Ø in the nm range) (Wingren and Borrebaeck 2007). Regarding antibody microarrays, arrays with an overall footprint of < 1 cm^2, based on 18×10^3 μm^2 (diameter (Ø) of ~150 μm) sized spots at a density of $\leq$ 2,000 spots/cm^2, have mainly been produced and applied (Hoheisel et al. 2013; Borrebaeck and Wingren 2009a; Kingsmore 2006; Sanchez-Carbayo 2010). Further, the multiplexity, i.e., the number of antibodies with different specificities per array, has been in the range of <900 different antibodies/array. Adopting ink-jet-based printers to produce the arrays, the antibodies have been sequentially spotted in parallel (1 to 4 antibodies at a time), and the multiplexity has been achieved by washing the nozzles and loading them with new antibodies.

In the case of nanoarrays, conceptual protein (antibody) nanoarrays displaying truly miniaturized (spot size; <0.8 μm^2, Ø < 1 μm) and high-density (spot density; >100,000 spots/cm^2) features have been designed and produced (Nettikadan et al. 2006; Lee et al. 2010; Hoff et al. 2004; Backmann et al. 2005; Arntz et al. 2003; Zheng et al. 2005; Ellmark et al. 2009; Ghatnekar-Nilsson et al. 2007; Bruckbauer et al. 2004; Tran et al. 2010) (for review see Wingren and Borrebaeck 2007). Despite the success, these nanoarray designs have been shown to be associated with three key technical bottlenecks. First, the production methodologies at

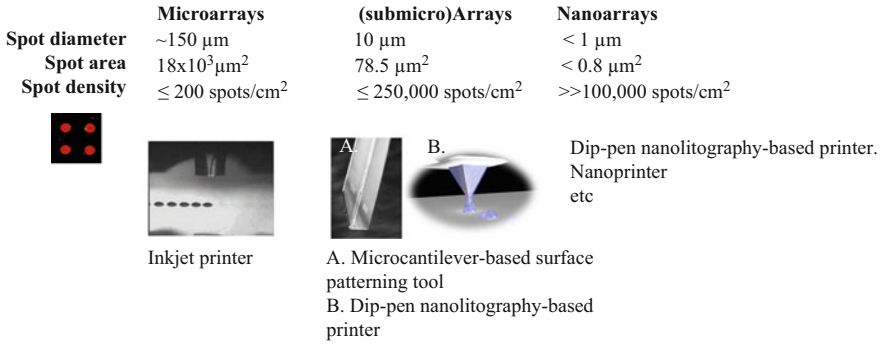

Fig. 3 Three main types of planar antibody arrays (with respect to size of the spots)

hand are currently limited to producing only 1-plex arrays (i.e., arrays composed of multiple spots of a single antibody), or in rare cases <5-plex designs (Wingren and Borrebaeck 2007; Nettikadan et al. 2006; Lee et al. 2002, 2010; Hoff et al. 2004; Backmann et al. 2005; Arntz et al. 2003; Zheng et al. 2005; Ellmark et al. 2009; Ghatnekar-Nilsson et al. 2007; Bruckbauer et al. 2004; Tran et al. 2010; Berthet-Duroure et al. 2008; Meister et al. 2004). Second, reducing the spot size < 1µm will lead to impaired rather than improved assay performance (e.g., sensitivity) (Ekins 1998). Third, hardware for sensitive (such as fluorescence-based) sensing of high-density nanoarrays remains to be established (Wingren and Borrebaeck 2007).

However, the density and multiplexity of antibody arrays are essential for large-scale protein expression profiling endeavors. In order to meet this demand without having to further develop the technologically challenging antibody nanoarray designs, miniaturized arrays based on submicron-sized (Ø 10 µm) rather than nano-sized (Ø < 1 µm) spot features have surfaced (Fig. 3) (Irvine et al. 2011; Jang et al. 2010; Lynch et al. 2004; Nettikadan et al. 2006; Petersson et al. 2014b). Using a nanoarrayer, based on dip-pen technology, the first 12- and 48-plex planar recombinant antibody arrays, based on 78.5 um^2 (Ø 10 µm) sized spots at a density of 38,000 spots/cm^2, interfaced with a fluorescent-based readout were recently produced (Petersson et al. 2014b, c). Importantly, their use for biomarker discovery in serum was also outlined, using systemic lupus erythematosus as showcase (Petersson et al. 2014c). Interestingly, adopting a microcantilever-based surface patterning tool, it was recently demonstrated that 16-plex recombinant antibody arrays, based on miniaturized spot features (78.5 um^2, Ø 10 µm) at a 7- to 125-times increased spot density (250,000 spots/cm^2 vs. 38,000 spots/cm^2 (Petersson et al. 2014b) or 2,000 spots/cm^2 (Borrebaeck and Wingren 2009a)), interfaced with a fluorescent-based readout could be produced (Petersson et al. 2014a). The feasibility of this conceptual array platform for serum protein profiling was also indicated (Petersson et al. 2014a).

In order to achieve high density, access to numerous renewable antibodies is a must. By using large antibody libraries, with, e.g., 10^{10} members (Söderlind et al. 2000), as a probe source, renewable antibodies displaying "any" specificity

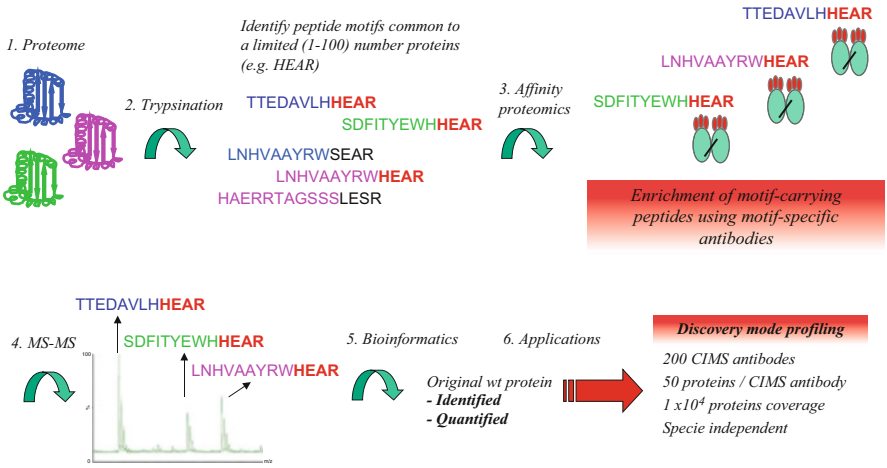

Fig. 4 Schematic illustration of the global proteome survey (*GPS*) setup, designed for global proteome profiling

can readily be selected and included on the arrays. The logistics behind large-scale selections could potentially constitute a logistical bottleneck. If so, two similar concepts were recently presented, demonstrating one solution to how to use a smaller set of antibodies while still targeting numerous proteins. The two concepts denoted Triple-X Proteomics (Poetz et al. 2009) (TXP) and global proteome survey (Wingren et al. 2009) (GPS) (Fig. 4) are based on the same fundamental principle and is based on combining antibody arrays with mass spectrometry. Briefly, antibodies are generated against short peptide motifs, four to six amino acid residues long, each motif being shared among 2 to 100 different proteins. These motif-specific antibodies could then be used to target motif-containing peptides. From a practical point of view, the proteome is first digested (e.g., trypsinated), and the peptide-specific antibodies are then used to specifically capture and enrich motif-containing peptides. Next, the motif-containing peptides are detected, identified, and quantified using tandem mass spectrometry, thereby allowing us to backtrack the original proteins in a quantitative manner. By using 200 such motif-specific antibodies, each targeting a motif shared among 50 unique proteins, would thus enable us to target about half the non-redundant proteome. As an example, a recent study showed that about 1400 tissue proteins could be profiled in a quantitative manner using only nine such motif-specific antibodies (Olsson et al. 2013).

Planar antibody arrays, printed on (microscope) slides ($\leq$16 subarrays/slides; made of plastic, glass, or polymer) or on the bottom of ELISA plates, are the dominating format, although bead-based arrays, or arrays in solution, have also been manufactured (Borrebaeck and Wingren 2009a).

The assay is run like a traditional immunoassay (~4 h assay time), but consuming only μL scale volumes of the samples. It should be noted that crude, non-fractionated

proteomes, such as serum, plasma, urine, cell lysates, and tissue extracts, can, in contrast to many competing proteomic technologies, be directly used without having to pre-fractionate the sample (Belov et al. 2001, 2003; Campbell et al. 2006; Dexlin et al. 2006; Mischak et al. 2007; Ingvarsson et al. 2007; Haab 2003; Wingren et al. 2007; Haab et al. 2001; Wingren and Borrebaeck 2009).

In a majority of cases, the samples are labeled with a fluorescent dye, either directly or indirectly, and interfaced with a fluorescent-based readout (Kusnezow et al. 2007; Wingren and Borrebaeck 2008; Wingren et al. 2007). A dynamic range of at least four orders of magnitude and an assay sensitivity in the pM range can be obtained, thus allowing low-abundant (pg/ml) analytes to be targeted in crude proteomes. By quantifying the signal intensity of each spot in the array, the array images are transformed into protein expression profiles, outlining the protein composition of the sample. State-of-the-art bioinformatics is then applied in order to identify any disease-associated biomarker panels that can be explored and exploited for, e.g., diagnosis, prognosis, and classification (Borrebaeck and Wingren 2009a, b; Wingren and Borrebaeck 2009).

Potential Applications to Prognosis, Other Diseases, or Conditions

To date, planar antibody microarrays have been used for protein expression profiling of almost any kind of crude sample format, such as plasma and serum, with the aim of deciphering disease-associated biomarker signatures (for review see, e.g., Borrebaeck and Wingren 2007; 2009b; 2009a; Haab 2005; Haab 2006; Hartmann et al. 2009; Kingsmore 2006; Griffiths et al. 2005; Wingren and Borrebaeck 2009). The design of the applications ranges from small proof-of-concept studies to large semi-global protein expression profiling efforts. Reviewing the antibody array field, from early to recent applications, shows that the technology can be used in, but not limited to, the following areas: (1) autoimmunity, (2) allergy, (3) bladder proteomics, (4) cell proteomics, (5) drug abuse, (6) glycomics, (7) heart proteomics, (8) hereditary disease, (9) inflammatory conditions/infections, (10) liver proteomics, (11) lung proteomics, (12) medical microbiology, (13) neurology/psychiatry, (14) obstetrics/gynecology, (15) oncoproteomics, (16) periodontology, (17) phosphoproteomics, (18) protein expression, and (19) protein signaling (Table 1).

Cancer is by far the most targeted disease using this technology, and several publications have demonstrated the potential of the antibody microarray methodology for pin-pointing cancer-associated biomarkers for, e.g., diagnosis, prognosis, classification, predicting the risk for relapse, and evidence-based therapy selection, as illustrated by a few selected representative references (Sanchez-Carbayo 2010; Alhamdani et al. 2012; Wingren et al. 2012; Hoheisel et al. 2013). While planar antibody arrays have been frequently applied within the field of cancer, nephritis has so far only been addressed in a limited set of studies. Below, we have outlined the

Table 1 General overview of planar antibody array-based applications and area of use

A. Area of use (example disease, biological process)		B. Applications
A1. Autoimmunity	Systemic lupus erythematosus	B1. Protein expression profiling
A2. Allergy	Cytokine profiling	B2. (Early) Diagnosis
A3. Bladder proteomics	Smooth muscle hypertrophy	B3. Differential diagnosis
A4. Cell proteomics	Blood phenotyping	B4. Classification
A5. Drug abuse	Screening	B5. Phenotyping
A6. Glycomics	Pancreatic cancer	B6. Evidence-based therapy selection
A7. Heart proteomics	Myocardial infarction	B7. Predicting the risk for relapse
A8. Hereditary disease	Cystic fibrosis	B8. Drug abuse screening
A9. Inflammatory conditions/infections	Atherosclerosis, obesity	B9. Bacterial detection/ profiling
A10. Liver proteomics	APAP-induced liver disease	B10. Bacterial toxin detection
A11. Lung proteomics	Chromium(VI) treatment	
A12. Medical microbiology	Detection of bacteria/toxin	
A13. Neurology/psychiatry	Cerebral palsy	
A14. Obstetrics/gynecology	Preeclampsia	
A15. Oncoproteomics	Pancreatic cancer, breast cancer, lymphomas	
A16. Periodontology	Model system	
A17. Phosphoproteomics	Lung cancer	
A18. Protein expression	Posttranslational profiling	
A19. Protein signaling	Various model systems	

findings of some of those applications by selecting a set of representative publications (Table 2).

Resistin as a Potential Marker in Lupus Nephritis

In this study, the authors used commercially available planar antibody arrays to discover candidate biomarkers in serum and urine of patients suffering from SLE and lupus nephritis (Hutcheson et al. 2015). The hypothesis was to explore whether serum and urine levels of adipokines could act as biomarkers for lupus nephritis. In previous work, adipokines have been associated with SLE and cardiovascular disease. Based on the antibody array work, 15 adipokines, adiponectin, leptin, and resistin were selected. Next, ELISA was applied in an attempt to validate the biomarkers. Compared to matched controls, the results showed that the expression

Table 2 Overview of the selected applications, using (planar) antibody arrays for protein expression profiling and biomarker discovery in nephritis, used as representative examples

Study (aim/target disease/ reference)	Antibody array (design/ antibodies)	Key finding(s)
1. Serum and urine protein profiling for biomarker discovery 2. SLE and lupus nephritis 3. Hutcheson et al. 2015	Commercially available planar antibody arrays Mono-/ polyclonal antibodies	Resistin was indicated as a potential biomarker in lupus nephritis
1. Plasma protein profiling for biomarker discovery 2. Glomerulonephritis, diabetic nephropathy, obstructive uropathy, and analgesic abuse 3. Neiman et al. 2011	In-house designed bead-based arrays Polyclonal antibodies	Fibulin was outlined as a candidate biomarker for renal impairment, in particular for glomerulonephritis
1. Urine profiling for biomarker discovery 2. Lupus nephritis 3. Wu et al. 2013	Commercially available planar antibody arrays Mono-/ polyclonal antibodies	Angiostatin was outlined as a candidate urinary biomarker of renal disease in SLE
1. Design of miniaturized planar antibody arrays and serum protein profiling for biomarker discovery 2. SLE 3. Petersson et al. 2014c	In-house designed miniaturized, planar arrays Recombinant single-chain Fv antibodies	First generation of miniaturized planar antibody arrays. Three serum biomarkers associated with SLE were detected
1. Serum protein profiling for biomarker discovery 2. SLE (including lupus nephritis) and systemic sclerosis 3. Carlsson et al. 2011	In-house designed miniaturized, planar arrays Recombinant single-chain Fv antibodies	Multiple serum biomarker signatures for diagnosis, classification, and prognosis of SLE. SLE and systemic sclerosis could be differentiated

levels of adiponectin and resistin were increased in both serum and urine, while leptin was increased in lupus nephritis. Further, the levels of resistin in serum, but not in urine, were found to correlate with renal dysfunction in lupus nephritis. Taken together, resistin might thus prove useful as a biomarker of renal dysfunction in patients with lupus nephritis. Additional work targeting additional, independent patient cohorts will, however, be required to validate the data and to preferentially extend this single biomarker into a multiplex marker panel to increase the anticipated assay performance (specificity, sensitivity, and selectivity).

Plasma Profiling Reveals Candidate Biomarker for Renal Impairment

The ability to detect early signs of kidney toxicity and to monitor progression of disease represents essential unmet clinical needs. Spurred by this, the authors applied an in-house designed antibody suspension bead array to perform plasma protein expression profiling targeting four types of kidney disorders, including glomerulonephritis, diabetic nephropathy, obstructive uropathy, and analgesic abuse (Neiman et al. 2011). To this end, 129 polyclonal antibodies, targeting 94 unique proteins, were used to produce the bead-based array. In total, 200 clinical plasma samples, including renal-associated cases and controls, were profiled. Significantly higher expression levels were observed for 1 of 94 proteins, fibulin-1, in glomerulonephritis patients compared to all of the other patient cohorts, indicating a potential for differential diagnosis. Most importantly, using three different antibodies directed toward three separate, non-overlapping epitopes on fibulin-1 showed similar expression levels, further supporting the data. In addition, Western blot analysis of selected plasma samples confirmed the observations. Next, a novel, independent patient cohort, including glomerulonephritis and controls, was applied in an attempt to validate the findings in the discovery cohort. The data confirmed the indications, outlining fibulin-1 as a potential indicator to monitor kidney damage or kidney malfunction. The performance of the biomarker might be even further improved by finding additional markers, in the end resulting in a multiplexed panel.

Biomarker of Renal Pathology Chronicity in Lupus Nephritis

In this study, the authors used a commercially available, multiplexed antibody microarray to perform protein expression profiling of about 280 proteins in urine targeting lupus nephritis (Wu et al. 2013). The data indicated elevated levels of urine angiostatin. Angiostatin has been shown to have modulatory function in inflammation and angiogenesis. Using ELISA, the increased levels of urinary angiostatin were then validated in an independent cohort of SLE patients. Next, the authors investigated whether the levels of angiostatin also reflected the SLE disease activity. Indeed, the results showed that higher levels were observed in active SLE versus inactive SLE. In fact, the patients with the most severe form of SLE were found to have the highest levels of urinary angiostatin. The biomarker might also be used to differentiate SLE patients with active SLE versus inactive SLE, as illustrated by receiver operating curve analysis resulting in an area under the curve of 0.90. Finally, when analyzing lupus nephritis patients, urine-angiostatin levels were found to correlate with renal pathology chronicity index, but not with the activity index. Hence, angiostatin surfaced as a novel, candidate noninvasive biomarker of renal disease in SLE. Further studies will be required in order to validate these promising findings, targeting novel, independent patient cohorts.

Planar Antibody Arrays for Biomarker Discovery in Lupus Nephritis

In this exploratory work, the authors first developed and designed a 48-plex miniaturized recombinant single-chain Fv antibody array platform (Petersson et al. 2014c). In more detail, individual spot features with a diameter of 10 μm and an area of 78.5 μm^2 were printed at a density of 38,000 spots per cm^2 using dip-pen nanolithography. The setup was interfaced with a high-resolution scanner for fluorescence-based sensing. The performance and applicability of the in-house designed planar antibody arrays were demonstrated by performing protein expression profiling of lupus nephritis. To this end, the observed serum profiles of lupus nephritis ($n = 45$) versus healthy controls ($n = 30$) were compared, and differentially expressed proteins were defined. The results showed that differentially expressed serum levels of three proteins in lupus nephritis versus healthy controls were detected, including complement protein C1q (downregulated), interleukin 6 (upregulated), and low-density lipoprotein (upregulated). Of note, these data supported previous findings, based on using conventional recombinant antibody microarrays (Carlsson et al. 2011). Taken together, the data outlined that planar recombinant antibody arrays could be used to define lupus nephritis-associated serum biomarkers, while consuming minute amount of sample (<1 single drop of serum).

Planar Antibody Microarrays: Biomarker Discovery in Systemic Lupus Nephritis

In this discovery study, the authors used in-house designed 135-plex recombinant single-chain Fv antibody microarrays to perform protein expression profiling of systemic sclerosis, systemic lupus nephritis, and healthy controls (Carlsson et al. 2011). The 135 antibodies were directed against 60 different proteins, including mainly immunoregulatory proteins. The hypothesis was to explore (parts of) the immune system as an early, specific, and sensitive sensor for disease. The results showed that several candidate SLE-associated multiplexed serum biomarker panels were successfully deciphered, reflecting disease (with impact on diagnosis), disease severity (enabling phenotyping), and disease activity (indicating ability to detect, monitor, and potentially even predict flares). In addition, biomarker panels differentiating SLE and systemic sclerosis were detected, and the observed differences increased with severity of SLE. Hence, the study demonstrated that molecular portraits of systemic lupus nephritis (and systemic sclerosis) could be extracted from a crude serum sample. Of note, the assay was performed while consuming less than a single drop of serum, and low-abundant biomarkers (pg/ml) could readily be detected. In the end, the disease-associated marker panels might also enhance our fundamental understanding of these complex autoimmune diseases. Of note, the authors have a set of additional manuscripts in the pipeline, further validating the candidate serum biomarker signature for diagnosis and

outlining additional marker panels for classification and prognosis (Wingren et al., unpublished observations).

Summary Points

- This chapter focuses on the design of planar antibody arrays for protein expression profiling and biomarker discovery in nephritis.
- Planar antibody arrays have been developed for biomarker discovery in clinical proteomics.
- Miniaturized planar antibody arrays can be used to perform multiplexed protein expression profiling, targeting crude proteomes.
- Planar antibody arrays have been successfully used for biomarker discovery in nephritis.
- Nephritis-associated urine, serum, or plasma biomarkers have been deciphered using planar antibody arrays.
- Multiplexed biomarker panels can be deciphered in a single drop of blood, or less, using planar antibody arrays.

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