High-density arrays to profile circulating biomarkers

Paul D. Lampe

Background

This presentation focuses on the utility, advantages, and disadvantages of using different protein microarray technologies to examine the proteomics, glycomics, and autoantibody content of plasma, sera, and tumor tissue samples. In general, array technologies use small amounts of biospecimen and offer the advantage of probing large numbers of analytes in a single sample under the same conditions for washing and processing steps. Depending on the format, they can be part of an automated workflow that quickly yields a large amount of data that can be amenable to pathway analysis. Different surfaces can be used to imprint arrays including glass slides (usually with a surface treated to tightly bind the analyte), membranes (e.g., nitrocellulose or PVDF) or patterned microtiter plates. Most of the technology and current basic protocols for producing these arrays have been adapted from early nucleic acid expression arrays (1). Although the more cutting-edge technologies are produced by individual laboratories, several manufacturers now produce a variety of protein arrays. The discussion here will focus on four types of arrays: protein arrays, peptide arrays, reverse phase arrays, and antibody arrays.

Discussion

Protein microarrays

These arrays can be used to probe for protein-protein interactions with specific purified proteins or in cellular lysates and the presence of antibodies specific for the protein in samples such as plasma or sera (2). The proteins to be arrayed are typically expressed in bacteria, yeast or mammalian cells and are purified and spotted onto the array surface. Several commercial companies now produce these arrays with some containing from hundreds to 20,000 expressed proteins, often spotted in triplicate. A clever alternative approach has been devised that generates the protein directly on the array; spotted DNA is transcribed and translated to yield protein within the spot, so called Nucleic Acid-Programmable Protein Arrays (NAPPA) (3). After incubation of a sample with the protein array, a sensitive detection system is required. Typically, for protein-protein interaction studies this can involve fluorescent tagging of the proteins or molecules in the analyte in a broad screen or a primary antibody specific for a particular protein of interest in a targeted study. For antibody (often used for determination of exposure to pathogens (4)) or autoantibody (used for autoimmune diseases and cancer biomarker discovery (5)) detection this can simply involve subsequent incubation of the array with fluorescently labeled anti-immunoglobulin (e.g., anti-human IgG or IgM). Often these can be multiplexed with different fluorophores (e.g., fluorescein-labeled anti-IgG and Alexa-Fluor680 labeled anti-IgM). Fluorescence levels are then detected in a scanner specific for...
Peptide microarrays

Peptide microarrays can be used for the same purposes as protein arrays but given the smaller size of peptides, they can yield more information about the specific region of a protein or epitope that interacts with an antibody or with another protein (6). Often this involves the synthesis of overlapping peptides (7) or specific types of protein domains (8). Given that peptides bind less tightly to surfaces such as nitrocellulose or protein-absorbing plastic surfaces, they are usually covalently linked to the array surface, often via a terminal cysteine or amine that is added to the peptide sequence during synthesis. Some of the advantages of peptide arrays include the fact that the cost of peptide synthesis has decreased dramatically and many companies will sell large libraries of high quality peptides at relatively low prices. Some disadvantages are that peptides may not have the same 3D structure as the matching sequence within a protein. Further, those interactions, as with expressed proteins, may not have key post-translational modifications. In these cases, interactions would potentially not be detected yielding a false negative signal.

Reverse phase protein arrays (RPPA)

RPPAs are produced by spotting a number of samples onto a surface and then detecting the presence of a specific protein or analyte in each of the spots usually via antibody detection or other specific reagent binding (9–11). Samples often include either partially purified cellular lysates or crude plasma, sera, and other bodily fluids and can sometimes be arrayed at different amounts in order to determine an optimal signal and analyte level. In essence, the most common form of RPPA is a “dot-blot” in microarray format. If a large number of different samples are spotted onto an array, one can potentially determine the level of a particular protein or analyte in all of the samples simultaneously and depending on the detection system, they potentially can be multiplexed. However, a potential disadvantage is cross-reaction of primary or secondary antibodies (i.e., non-specific interaction) with partially purified or crude samples and, therefore, the user must have a good antibody or detection reagent. Further, because these arrays are custom made and the proteins selected up front, a priori knowledge of the specific proteins that might vary with disease or condition is important.

Antibody microarrays

High-density antibody microarrays allow for the simultaneous determination of the levels of thousands of proteins in complex samples such as cell lysates, sera, plasma, urine, and saliva (12).
As described above for detection of protein interaction, the sample has to be labeled with a tag or the protein can be detected with a second antibody specific for a different epitope on the protein (i.e., like a standard ELISA with a capture and detection antibody) although for the latter, the array size typically is smaller since antibody pairs are required for each spot. Given the number of companies producing antibodies and new efforts to validate their performance such as the Human Protein Atlas (13), obtaining specific and quality antibodies for most of the proteins in the proteome is becoming more and more feasible. This has allowed high-content arrays containing up to several thousand different antibodies, including antibodies specific for post-translational modifications of specific proteins (e.g., phosphorylation-specific antibodies). One of the main advantages of antibody arrays is that they can be used with a variety of probing strategies not only to detect protein levels but also by using lectins (14) or glycosylation-specific antibodies (15), to detect the levels of protein glycosylation. For example, use of anti-sialyl Lewis A antibodies can be used to detect CA19.9 modification in thousands of proteins in a single sample. Alternatively, probing arrayed samples with anti-immunoglobulin antibodies (e.g., anti-IgG or anti-IgM) can be used to detect complexes of antibodies and proteins which can be useful for detection of cancer specific autoantibodies (16). Since spots in high density antibody arrays can be quite small (i.e., less than 300 μm diameter), the amount of antibody needed is quite low. Similarly, depending on the sensitivity of the detection system utilized, sample and reagent usage is low. The main disadvantages of this approach are the cost to accumulate a large library of antibodies and the time and effort necessary to validate the specificity of these antibodies in an array format.

**Future Directions**

Future use of array technology for cancer early detection and pathway analysis will presumably benefit from several advances in technology. Specifically, advanced methods of protein production, peptide library production, and antibodies of increasing quality will provide for arrays of increasing density at presumably lower cost. New methods for label-free quantification will allow for more sensitive protein or analyte detection. All of these will likely lead to more widespread use by investigators and more production of commercial arrays by a variety of companies.

**References**

1. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nature genetics 1999;21:33–7.
2. Robinson WH, Steinman L, Utz PJ. Protein arrays for autoantibody profiling and fine-specificity mapping. Proteomics 2003;3:2077–84.
3. Ramachandran N, Raphael JV, Hainsworth E, Demirkan G, Fuentes MG, Rolfs A, et al. Next-generation high-density self-assembling functional protein arrays. Nat Methods 2008;5:535–8.
4. Cekaite L, Hovig E, Sioud M. Protein arrays: A versatile toolbox for target identification and monitoring of patient immune responses. Methods Mol Biol 2007;360:335–48.
5. Kijanka G, Murphy D. Protein arrays as tools for serum autoantibody marker discovery in cancer. Journal of Proteomics 2009;72:936–44.
6. Amartely H, Iosub-Amir A, Friedler A. Identifying protein-protein interaction sites using peptide arrays. Journal of Visualized Experiments: JoVE 2014:e52097.
7. Katz C, Levy-Beladev L, Rotem-Bamberger S, Rito T, Rudiger SG, Friedler A. Studying protein-protein interactions using peptide arrays. Chemical Society Reviews 2011;40:2131–45.
Novel Platforms for Early Detection of Malignancies

8. Volkmer R, Tapia V, Landgraf C. Synthetic peptide arrays for investigating protein interaction domains. FEBS Letters 2012;586:2780–6.

9. Wilson B, Liotta LA, Petricoin E3rd. Monitoring proteins and protein networks using reverse phase protein arrays. Disease Markers 2010;28: 225–32.

10. Spurrier B, Ramalingam S, Nishizuka S. Reverse-phase protein lysate microarrays for cell signaling analysis. Nature Protocols 2008;3:1796–808.

11. Kornblau SM, Coombes KR. Use of reverse phase protein microarrays to study protein expression in leukemia: Technical and methodological lessons learned. Methods Mol Biol 2011;785:141–55.

12. Haab BB. Antibody arrays in cancer research. Mol Cell Proteomics 2005;4:377–83.

13. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonsson K, Forsberg M, et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol 2010;28:1248–50.

14. Haab BB, Yue T. High-throughput studies of protein glycoforms using antibody-lectin sandwich arrays. Methods Mol Biol 2011;785:223–36.

15. Rho JH, Mead JR, Wright WS, Brenner DE, Stave JW, Gildersleeve JC, et al. Discovery of sialyl Lewis A and Lewis X modified protein cancer biomarkers using high density antibody arrays. Journal of Proteomics 2014;96:291–9.

16. Rho JH, Lamp P. High-throughput screening for native autoantigen-autoantibody complexes using antibody microarrays. Journal of Proteome Research 2013;12:2311–20.