BIOMARKER DISCOVERY: AN ARRAY OF POTENTIAL

Candidate Screening: Low Abundance with High Sensitivity

Journey of a Biomarker Candidate

A Multiplexed Approach

Overcoming Bottlenecks in Cancer Treatment
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Candidate Screening: Low Abundance with High Sensitivity

When processes regulating life – such as growth, reproduction, and movement – go awry, disease can result. Chemical moieties become imbalanced in these disease states, often resulting in measurable levels of certain signature molecules, termed biomarkers. Biomarker discovery has traditionally been performed through proteomic approaches. However, these techniques, in the past, have been limited in their ability to detect low abundance molecules. Moreover, they are generally unable to scan for chemokines, cytokines, growth factors, and other secreted molecules. Antibody arrays can overcome many of these issues and have become a more utilized proteomics approach in recent years, thanks largely to intensified efforts to test and validate antibody pairs, facilitating the development of broad, high-throughput screens.

Complementing Traditional Biomarker Discovery Techniques

Traditional proteomic methods, such as 2-D gel electrophoresis and mass spectrometry (MS, including liquid chromatography–MS; LC–MS) have become indispensable tools in biomarker discovery. These techniques have particularly advanced cancer biomarker discovery. The unbiased nature of mass spectrometry-based screens makes them ideal techniques for mining protein targets that don’t have antibodies or other affinity reagents available. In recent years, significant efforts have been made to improve MS-based approaches in an effort to overcome associated biomarker-discovery challenges (e.g., the need to perform high-throughput assays and the wide dynamic range of biomarker candidate concentrations in the human body).1

However, due to ion suppression, certain secreted and low abundance biomarkers can be missed using MS– or 2-D gel-techniques, and different approaches and equipment are required to fully mine the proteome for these biomarkers. Furthermore, the cost of equipment, need for highly trained personnel, and high costs per sample tested can be prohibitive to many researchers wanting to use traditional proteomic methods.

High-density array screening, i.e., using antibody panels with more than 200 markers, requires just a fraction of the cost of proteomic analysis and requires little equipment or training to get up and running. Large-panel arrays allow researchers with modest budgets to screen for hundreds of potential biomarkers, including cytokines, chemokines, and other secreted molecules that could be missed through traditional proteomics approaches.

A Streamlined Workflow: From Discovery to Validation

Multiplex immunoassays offer the ability to streamline the biomarker workflow, from discovery to validation, by making use of common antibodies in multiple assays. Once thought of as being too narrow in scope for the biomarker discovery phase, arrays containing thousands of antibodies are now capable of targeting a large selection of candidate markers with known importance in many key cellular pathways and diseases.

MS-based biomarker verification usually involves a targeted approach, such as multiple-reaction-monitoring (MRM). Array-based biomarker verification instead makes use of high-density quantitative arrays, which may simplify the verification stage.

The final step in the biomarker journey, validation, requires assessment of biomarker performance in a large cohort study in the target population. Array-based technology again allows this stage to be simplified, permitting the use of the same antibodies employed in the verification stage.

Sensitive and High-Throughput

Multiplex immunoassays offer high sensitivity (as low as 1 pg/ml). Furthermore, antibody arrays are adaptable to high-throughput analysis, and the choice of array platforms offered is large and always expanding. The development of high-density arrays has placed the technique securely into the spotlight when it comes to antibody discovery and validation, making antibody arrays a perfect complement to traditional proteomic techniques.

For references, please see page 7.
Potential biomarkers for a particular disease or condition must first be discovered: high-density screening antibody arrays are an excellent tool for this stage, allowing researchers to profile hundreds of disease-related proteins simultaneously with minimal material and cost.

For biomarker candidate validation and verification, multiplex antibody arrays or single-target ELISAs are a logical choice: there is no need to develop new antibodies or immunoassays for validation or clinical applications using an antibody array approach.
A Multiplexed Approach

More and more studies are finding that a single cytokine or other chemical moiety is insufficient for use as a true disease biomarker, and that a more global perspective is needed to truly understand the presence, or development, of disease. High-density multiplexed antibody arrays, which can now contain 2,000 antibodies or more in a single panel, combined with an ever-increasing pool of validated antibodies, puts arrays at the forefront of biomarker discovery and development, and makes finding multiple biomarkers more accessible than ever before.

Evolution of the Antibody Array

Microarrays were first conceptualized in the early 1980’s by Dr. Tse Wen Chang, who demonstrated that 20x20 grids of antibody spots could be placed on a small surface. Antibody array models were further developed by Dr. Roger Ekins and colleagues in the late 1980’s when they created a model that permitted simultaneous screening of an analyte panel. Initial concepts tried to miniaturize immunological assays and were normally performed in 96-well plates. However, glass slides and membranes were soon found to be better substrates for placing minute antibody spots, as they could accommodate larger arrays.

In late 2000, the idea of simultaneously detecting multiple cytokines came to fruition in the mind of Dr. Ruo-Pan Huang at Emory University, who developed nitrocellulose membrane-based antibody arrays as a strategy to increase the efficiency of profiling blood proteins. Dr. Huang began developing antibody arrays to replace cumbersome and expensive biochemical techniques such as single-target ELISAs and Western blots, first publishing a paper about the new technique in 2001 titled “Simultaneous detection of multiple cytokines from conditioned media and patient’s sera by an antibody-based protein array system.”

Following this publication, Dr. Huang received numerous requests for his antibody arrays from other researchers, leading him to found RayBiotech, which provided the first commercially available cytokine arrays to the research community.

The Growth of Arrays

Today, antibody arrays containing 2,000 antibodies in a single panel are available. Screening antibody arrays can target proteins (antigens), glycoproteins, phosphoproteins, and many other moieties. With the number of validated antibodies growing every year, the gap in target throughput between arrays and mass spectrometry continues to narrow. Antibody arrays are designed with a pre-selected panel of antibodies that target markers having known or suspected relevance in many disease processes. Thus, this targeted approach can efficiently identify proteins that are more likely to be effective biomarkers. Furthermore, their high sensitivity makes them ideal for identifying secreted molecules that may, in combination with other moieties, provide the global perspective necessary to tailor therapeutic regimes.

Arrays in Precision Medicine

For precision medicine, antibody arrays are proving to be fundamental for diagnosis and treatment of disease. For example, high-density arrays were used to better characterize the expression levels of 200 human cytokines, leading to the identification of a common cytokine signature and guiding the diagnosis of a patient with idiopathic uveitis. Personalized treatment reversed the visual loss, illustrating how arrays may assist in individualizing therapy.

Uncovering New Drug Targets

Antibody arrays, with their high-throughput multiplexed design, are a tool for biomarker discovery and not only offer insight into disease, but also help mine potential new drug targets. From asthma to renal cell carcinoma, neurological dysfunction to end-stage heart failure, antibody arrays have been successful in discovering biomarkers in many disease areas.

Multiplexed immunoassays can be applied in multiple aspects of the drug discovery process, including in target identification, investigating mechanisms of drug resistance, elucidating the molecular mechanisms of drug action, understanding drug side effects, and in clinical trials and managing patient care.

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Overcoming Bottlenecks in Cancer Treatment

Since their introduction into mainstream research labs, antibody arrays have accelerated biomarker research. Array-based biomarker discovery has shown particular promise in the field of cancer research, where unique cytokine “biosignatures” have been linked with certain cancers. Antibody arrays have also proven useful in providing insight into cancer mechanisms, and in overcoming the bottleneck associated with innate resistance.

Accelerating Cancer Biomarker Discovery

Protein and antibody arrays represent new opportunities to profile protein expression levels in patients suffering from various cancers; arrays are used to identify protein panels, which can be used as biosignatures for clinical diagnosis, disease classification, disease prediction, drug development, and for optimizing patient care.

Arrays have already proven their worth in the cancer biomarker field for a myriad of cancers. In a study by Jiang et al., arrays were used to identify five serum protein markers (MSP, TIMP-4, PDGF-Ra, OPG, and CA125), which could effectively detect ovarian cancer with high specificity and high sensitivity.

Vasquez-Martin et al. identified a panel of cytokine biomarkers from the IL-8 and GRO chemokine families, which may be useful for monitoring breast cancer responses to endocrine treatments and/or HER2-targeted therapies.

A stomach cancer biosignature was put forward by Cui et al., where antibody arrays were used to identify 67 out of 136 computationally predicted potential serum biomarkers, of which 24 were found to be abundant in cancer patients versus the control group. Hong et al. also used antibody arrays to validate computationally predicted markers excreted into urine for gastric cancer patients. Other cancers for which antibody arrays have helped to discover biomarker signatures include lung, colon, prostate, glioblastoma, renal cell carcinoma, and HBV-related hepatocellular carcinoma.

Phosphorylated Proteins: Insight into Cancer Mechanisms

Antibody arrays are not only pivotal in helping to discover biomarkers for cancers, but also in elucidating cancer mechanisms. In a study by Al-Aidaroos et al., antibody arrays against 71 unique tyrosine kinases (RTK activation study) and 41 unique growth factors (secreted growth factor analysis) were used to help identify PRL-3-driven EGFR hyperactivation and consequential addiction to EGFR signaling, opening new avenues for inhibiting PRL-3-driven cancer progression.

The authors propose that elevated PRL-3 expression is an important clinical predictive biomarker for favorable anti-EGFR cancer therapy.

Cancer-Drug Resistance: Overcoming Bottlenecks

Drug resistance is a bottleneck in cancer treatment, and is sometimes caused by innate resistance within the tumor microenvironment (TME). Although the growth- and metastasis-promoting effects of the TME have been well documented, the TME’s role in drug resistance has only been partially explored. Antibody arrays have been used to identify secreted factors in stromal cell culture that contributed to cancer drug resistance, revealing targets for combination therapies with increased efficacy.

In a study by Straussman et al., 45 different cancer cell lines treated with 35 different anti-cancer drugs were cultured with 23 different stromal cell lines. Strikingly, the researchers found that 16 of the 35 drugs tested were rendered ineffective by the presence of stromal cells; the effect was particularly pronounced for targeted drugs as opposed to conventional cytotoxic agents. From here, they further studied RAF inhibitor PLX4720 and its resistance in BRAF-mutant melanoma cell lines, discovering that the stromal cell-preconditioned medium was sufficient to confer resistance to melanoma cells, suggesting a secreted factor was driving resistance. Using a custom label-based array that detected 567 proteins, the researchers identified a single factor, hepatocyte growth factor (HGF), that was confirmed to be implicated in melanoma drug resistance. These findings support the clinical relevance of HGF-mediated resistance to BRAF (proto-oncogene B-Raf) inhibitors; the authors suggest that combination therapy with MET and RAF inhibitors could be effective for BRAF-mutant melanomas.

The TME is an important but understudied source of anti-cancer drug resistance, but high-density arrays are incredibly useful tools for further uncovering the TME’s secrets.

For references, please see page 7.
**References**

**Article 1 - Candidate Screening: Low Abundance with High Sensitivity**


**Article 2 - A Multiplexed Approach**


**Article 3 - Overcoming Bottlenecks in Cancer Treatment**

RayBio® Quantitative Proteomics Services

- Extensively published Quantibody® array platform
- 1000 human proteins | 640 mouse proteins
- pg/ml detection
- Any biological fluid
- Full data report