Cancer Antigen Microarrays for Profiling Autoantibodies at the Epitope Level

The additional knowledge gained by studying the response to antigens at the epitope level will help us further understand anti-tumor immunity and may also help us to monitor cancer progress and cancer vaccine efficacy in the future.

Many cancers have a high survival rate if the cancer is detected at the earliest stage. While significant efforts have been made toward this end, the American Cancer Society estimates that close to 600,000 will die of cancer in the US in 2014. It is well-established that antibodies to cancer-associated antigens appear in biological fluids (particularly in blood sera) of cancer patients and the persistence and stability of autoantibodies in the serum of cancer patients is an advantage over other potential markers, which may be released by tumors but rapidly degrade or are cleared after circulating in the serum for a limited time. Detection of these antibody biomarkers in patient sera by immunochemical methods represents a promising approach to minimally invasive cancer diagnostics capable of early detection.

Initially, efforts toward the development of early detection assays for cancers depended on single biomarker molecules; however the lack of specificity of the majority of autoantibodies to cancer-associated antigens makes this a complicated endeavor. These same autoantibodies may be found in patients with inflammatory or autoimmune diseases and so the results have been disappointing.

It has been suggested that the use of multiple serum markers may provide a more sensitive test. The use of antigen arrays which can screen for multiple antibody biomarkers simultaneously allows the identification of so-called “autoantibody signatures”. Draghici & Tainsky were some of the earliest to depart from the reliance on any single marker for detection. They screened large numbers of potential markers on protein microarrays spotted with T7 cDNA clones (SEREX) and ultimately developed an array of 78 antigens capable of identifying stage 1 ovarian cancer. Liu and colleagues developed a reverse capture platform which utilizes a high-density antibody microarray that has been spotted with well-characterized, highly specific monoclonal antibodies. The arrayed monoclonal antibodies serve to capture their corresponding antigens in their native state when incubated with proteins isolated from either cell lines or tissues. These captured antigens then serve as bait for fluorescently labeled IgGs purified from patient sera or plasma samples.

Although response to tumor antigens at the antibody level has now been studied in great detail, detailed epitope mapping of the antibodies has lagged behind. Recently Komatsu et al. have used synthetic peptides with various lengths and NY-ESO-1 full-length protein to assess the specific antibodies in melanoma patient sera. They have demonstrated that shorter overlapping peptide sets provide better resolution for antibody epitope mapping and some antibodies are critically dependent on an individual AA within a short linear peptide. They found that a single AA shift could eliminate the recognition by the antibodies in the serum. By screening the sera of many patients, they have identified the minimal B-cell epitopes located in both the amino and carboxyl terminal regions. Interestingly, the detailed antibody responses to each specific epitope differed significantly in each melanoma patient.

The main points:

• Autoantibodies exist in cancer patients and can be used as biomarkers.
• An antigen signature is more useful (specific) than single biomarker.
• An array or microarray format is the logical choice for multiplex detection.
• Patients sera displays specific epitope signatures.
• Short epitopes are more specific.
• An array of short overlapping cancer antigen epitopes can effectively detect antibody response.

Beeton-Kempen et al. also recently found the ratio of NY-ESO-1 to CTAG2 autoantibody titres to vary considerably across individual patients, suggesting that different patients raised autoantibodies to different epitopes along the length of the NY-ESO-1 protein. Although the cause of the individual epitope specificity is unclear, they point out that this might conceivably impact on clinical outcome of specific patients.

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Thus, miniaturized spatially addressable microarrays of peptides have become powerful tools for high-throughput biomedical and pharmaceutical research and the advancement of proteomics. LC Sciences has developed a **Cancer Antigen Peptide Microarray** capable of measuring cancer related autoantibodies at the epitope level. This peptide microarray contains a curated list of 113 known cancer antigens:

- A group of the top 30 cancer antigens as prioritized by the NCI. This list of cancer antigens is a well-vetted, priority-ranked list of cancer vaccine target antigens based on predefined and pre-weighted objective criteria generated by expert panels.

- Proteins which are currently targeted by anticancer therapies.

- RTK receptors as important cancer antigens, such as EGFR (ERBB1) etc.

These microarrays are built on LC Sciences' proven **PepArray™ technology** which enables in situ (on chip) high density peptide synthesis and multiplex protein assays carried out in a microfluidic picoliter scale microarray (Figure 1). Epitope screening experiments using a cancer antigen p53 antibody (PAb240) and this array technology produced clearly defined binding patterns.

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References


