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ProteoSep®

AN INTRODUCTION TO A NEXT GENERATION BIOMARKER DISCOVERY AND DRUG DEVELOPMENT TECHNOLOGY

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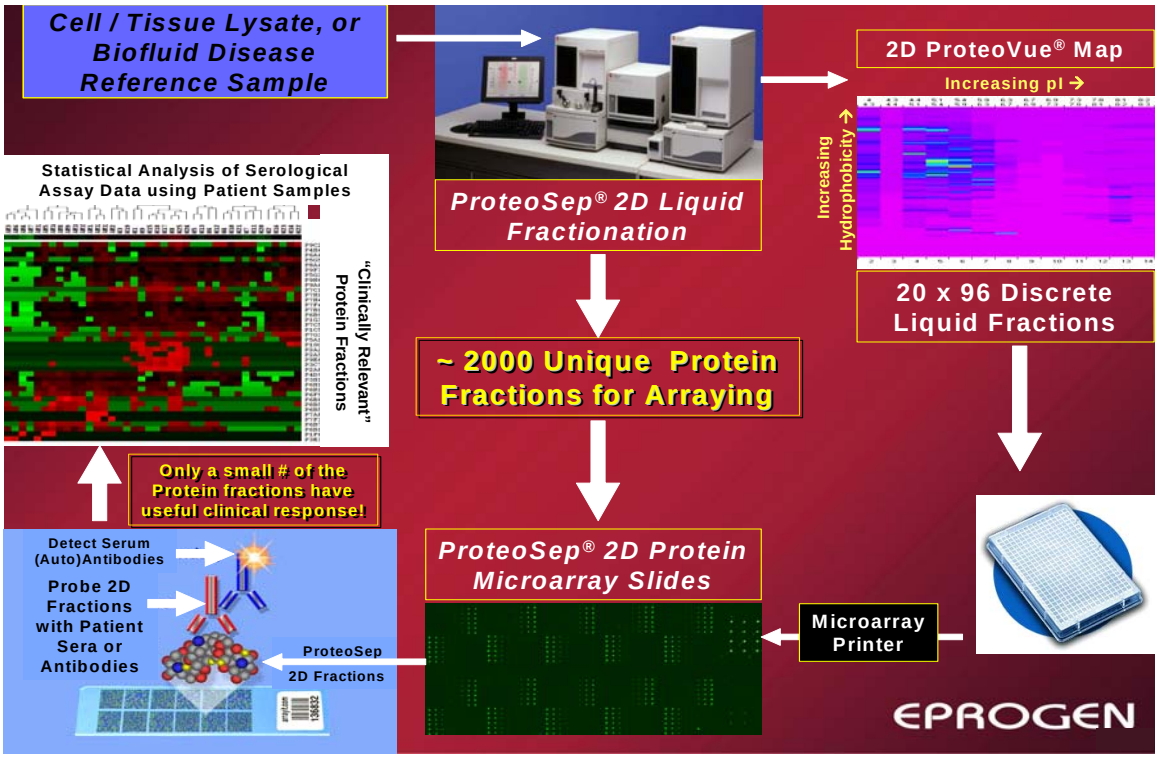
The Critical Need

It is well established that, for a wide range of diseases, a particular drug's clinical benefit during treatment varies widely within patient populations. The discovery and validation of gene and protein biomarkers that classify patients into sub-populations for a more "personalized" approach to therapy have thus emerged as a critical part of drug development, disease diagnosis and management. The enormous heterogeneity of patients, however, presents a very difficult challenge to the general use of comprehensive protein profiling in a clinical setting. New approaches are clearly needed to dig deeper the phenotype of disease onset and progression as well as to better understand how to develop improved, and monitor existing, therapies for patients.

Eprogen's "Bedside-to-Bench" Solution

Eprogen's solution is based on the established notion that surveying a patient's immune response can serve as a sentinel for over- or under-expression of groups of proteins particular to a disease and its expression. Our new approach is to take reference cell lines or protein biofluids specific to a disease of interest and **2D** fractionate them (in the liquid phase) to produce thousands of well-defined subsets of antigens to serve as "bait" for probing the humoral response.

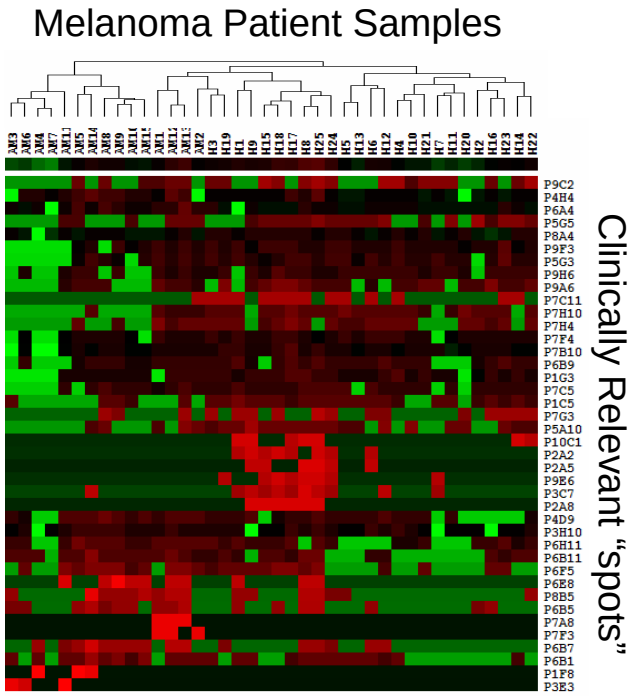
These protein fractions can then be "spotted" onto coated glass slides to produce identical protein microarrays for serological assays. This fractionation process significantly reduces the complexity of the reference sample and allows for simultaneous probing of 1000's of distinct protein fractions in one easy step. Thousands of these disease specific reference microarrays can be produced from one 2D liquid fractionation.



Mapping New Directions in Proteomics®

In addition, the individual liquid fractions used to prepare the microarray spots are stored for later access and in-depth analysis of the proteins they contain. This comprehensive interrogation of a patient's immune response will produce a unique and much more detailed protein "profile" or signature for a particular disease.

Using well defined patient cohorts, serological assays are performed on each patient sera (or pooled sera) using these reference natural intact protein microarrays. Through statistical analysis of the protein "spots" reactive to autoantibodies present in the patient sera [typically measured using fluorescence], the "antigen" signatures are established for the particular reference sample. This then identifies the "biologically relevant" protein fractions that classify patients into their respective populations or subpopulations. Using normal control patients along with benign or non-related disease patients, the same serum samples can be screened across many different reference cell line microarrays to generate a global patient profile with relative ease.



ProteoSep 2D Microarray Analysis Protocol

1. **Reference Cell Line: Primary Melanoma cells from patient lymph node.**
2. **Patient Cohort:**
   - 25 Healthy Volunteers (H)
   - 50 early stage
   - 15 advanced stage (AM)
   - 25 non-melanoma skin Ca
3. **Prepared 864 spot 2D Microarrays and assayed Humoral Response (HR) profiles using fluorescence.**
4. **Statistical Methods Cluster Advanced patients from Healthy using 42/864 "spots" on the microarray (<5% of the total) [> 95% confidence limit].**
5. **"Prioritized" protein fractions sent to MS to ID HR candidates for AM Patients.**

Once the protein fractions (spots) that differentiate the patient populations of interest are identified (typically < 5% of the total number of spots on the microarray), the liquid fraction used to print the spot on the microarray can be readily accessed for more detailed analysis of the proteins present for validation and further research. This approach in reality "prioritizes" the spots containing the proteins of interest. Now, biologically important candidate biomarkers are pinpointed before resorting to more sophisticated techniques (like MS) to characterize the exact nature of the proteins expressed. Having a sound clinical basis at the outset to pursue a protein or group of proteins as biologically significant is of great value.

These characteristic reference antigen (autoantibody) profiles serve not only to highlight active biological pathways related to the disease onset or progression, but also help to "discover what we do not know" about these active pathways as they are expressed in the patient. These immune response profiles of patient samples (Bedside) are now used to generate new, or improve on existing strategies of drug and biomarker development (Bench) by first discerning the relevant proteins that are truly associated with the disease as measured by real patient response.

The unique aspects this "Bedside-to-Bench" approach offers and the efficiencies this will introduce in both time and cost will have a significant impact on how protein biomarker and drug discovery research as well as clinical trials are performed in the future. If the same process can also be used in both drug development efforts and to monitor patients before, during and after the drug treatment, it would save enormous amounts of time, effort and money in drug and biomarker development and validation costs. It would also serve as a significant aid to clinicians dealing with patients throughout the course of diagnosis and treatment.

ProteoSep Microarray Strategies: Make More Effective Use of Clinical Samples!

Cell Line or Tissue Screening/Profiling

Western Blot alternative:

- 1D & 2D liquid multi-well fractions contain intact proteins for direct probing with antibodies.
- Screening multiple arrays produced from the same sample with many different antibodies!
- Antibody qualification and coverage assays.

PTM analyses – Phospho-, glyco-protein detection and coverage.

Serological (biofluid) assays:

- Drug treated vs. Control studies with patients – clinical trial monitoring
- Autoantibody signatures or profiles – Patient (sub)classification
- Disease progression analysis
- New Biomarker discovery studies

References:

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Differential phosphoprotein mapping in cancer cells using protein microarrays produced from 2-D liquid fractionation. Anal Chem. 2006 Feb 1;78(3):702-10.

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Analysis of protein glycosylation and phosphorylation using liquid phase separation, protein microarray technology, and mass spectrometry. Methods Mol Biol. 2009;492:321-51.

- **2D Humoral Response using Cancer Lysate Arrays**

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The identification of phosphoglycerate kinase-1 and histone H4 autoantibodies in pancreatic cancer patients using a natural protein microarray. Electrophoresis, 2009 June; 30(12) 2215-2226

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Humoral response profiling reveals pathways to prostate cancer progression. Mol Cell Proteomics. 2008 Mar;7(3):600-11.

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