Human Proteome Arrays for Autoantibody Identification in Clinical Cancer Studies

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Abstract

Early detection of cancer is a critical factor for successful treatment of cancer patients. Autoantibody signatures have value in the diagnosis and management of autoimmune disorders and may also be valuable for detection of cancers. Previous groups have applied several autoantigens, such as DEK (Figure 6). In addition, we identified some new cancer-associated autoantigens such as GMPR2 (Figure 7).

Methods

Automated Array Production (continued)

Protein arrays containing ~700 recombinant autoantigens were generated using a high-throughput system. Three hundred and sixty proteins from 3 plates of TnT reactions which were combined into 12 pools of 24 proteins coupled to unique linkers. These 12 protein-linker pools were each added to a column of wells to facilitate dispensing into an array plate. Many of these steps were automated using an epMotion® 5320 (Eppendorf, Hamburg, Germany)

High-throughput, sensitive, and specific assay platform, with large-scale protein arrays. This approach also enables a rapid transition from antigen discovery to clinical validation using small focused arrays on the same diagnostic platform.

Results

Screening

The primary screening of our protein arrays involved a range of tumor and plasma samples. Samples were selected from our patient database, which includes patients with breast, lung, ovarian, prostate, colon, kidney, melanoma, and pancreatic cancers. Each patient sample was tested against a set of 12 protein-linked arrays at a concentration of 10 μg/mL. A total of 188 antigens from our primary screens (96 from sample set 1 and 92 from sample set 2) were selected for secondary screening using individual patient samples.

The antigens selected from the primary screen were recovered from our source library of bacterial clones and cultured, and then new proteins were immobilized on assay plates. The antigens were then screened against a large set of patient samples. The patient samples were initially screened as pools, followed by screening of individual samples.

Screening was done using samples from patients with breast, lung, ovarian, prostate, colon, kidney, melanoma, and pancreatic cancers. The antigens were selected based on signals that exceeded specific thresholds according to any of the following criteria:

• Sample set 1: Ratio between response in a disease pool and median response across all tested normal samples. This criterion was used to identify antigens that were significantly overexpressed in each disease pool. For example, if a sample set 1 antigen had a ratio of 5, it would be considered significantly overexpressed in the disease pool.

• Sample set 2: Ratio between response in a disease pool and the median response across all tested normal samples. This criterion was used to identify antigens that were significantly overexpressed in each disease pool.

• Sample set 1: Correlation between response in a disease pool and the median response across all tested normal samples. This criterion was used to identify antigens that were significantly correlated with the disease.

• Sample set 2: Correlation between response in a disease pool and the median response across all tested normal samples. This criterion was used to identify antigens that were significantly correlated with the disease.

The antigen Yielded the highest percentage of positive autoantibody responses with 21% for TP53, 8% for DEK, and 6% for GMPR2. The classification of patient samples, especially in combination with patient-specific protein arrays determined from tumor whole-genome sequencing or other disease, and benign prostate hyperplasia. Samples from patients with breast, lung, ovarian, and prostate cancers were sub-divided into disease, and benign prostate hyperplasia. Samples from patients with breast, lung, ovarian, prostate, colon, kidney, melanoma, and pancreatic cancers. The classification of patient samples, especially in combination with patient-specific protein arrays determined from tumor whole-genome sequencing or other disease.