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# Phage Display Libraries in Sequelization of Autoantibody Signature Analyses in Cancer Immunomics

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## Editorial

In current researches in clinical chemistry, an increasing number of projects are utilizing autoantibody signatures to test their diagnostic and prognostic values for cancer. For various types of cancer, it has been established or suggested that circulating immunoglobulins from the sera of cancer patients contain a repertoire of antibodies elicited against the tumor [1]. Interest of researchers toward this topic has been ignited partly by the findings that production of autoantibodies against tumor-associated antigens may precede clinical confirmation of tumors by several months or years [1]. Beyond their potential usefulness for cancer diagnosis, autoantibody signatures may show usefulness in occasions where a fine discriminatory power is required, such as inference of cancer stages. In the present issue of our journal, Partin et al report the performance of the panel of ten phage-peptides recognized by autoantibodies from prostate cancer patients in discriminating patients with prostate cancer of advanced stages (Gleason score 7 or greater) from those with Gleason score 6 [2].

Phage display strategy can enrich autoantibody-binding peptides from a pool of peptides derived from cDNA as well as from a pool of combinatorial peptides [3]. Of note, the phage cDNA library used in Partin et al was the same as the one used in Wang et al [4] and Schipper et al [5]. Wang et al conducted iterative biopanning followed by phage-peptide microarray analyses and identified autoantibody biomarkers that can be used for screen of prostate cancer [4]. The 22-phage peptide panel performed well to discriminate patients with an intermediate range of PSA (4-10 ng/mL) from healthy controls. Intriguingly, of the 22 selected peptides, 17 were untranslated region or out of frame in the coding sequences of known genes. In Schipper et al [5], biopanning and subsequent analyses led to 62 peptides, but the authors' training analyses led to a logistic regression model based on eight peptides that are totally different from those selected in Wang et al [4]. Three of the eight peptides were from the products of androgen response genes or their regulators. The panel of the eight clones discriminated the PCA patients from healthy controls with area under the curve (AUC) of 0.69 without PSA. Comparison of these analyses highlights the heterogeneity and/or high complexity of the repertoire of autoantibodies; different sets of patient's serum samples used for the training lead to different panels of enriched peptides. Interestingly, both panels worked well.

In Partin et al [2], sera from the subjects with low-grade prostate cancer (Gleason score 6 or lower, which is likely insignificant cancer in men with a prostate cancer negative diagnosis) were used as as the control group sera. The panel led to AUC of 0.69 in receiver operating characteristic analysis whereas it was greater than 0.55 with PSA alone. This demonstrates the usefulness of autoantibodies signatures as the biomarkers not only for diagnosis of prostate cancer but also for inference of the specific stage of the cancer, where the conventional maker PSA is not very useful. Phage display libraries can also be used to identify important tumor-associated antigens (TAA). Notable such studies on prostate cancer using a combinatorial phage-peptide library include Mintz et al [6] that led to demonstration that immunoreactivity to fetuin-A becomes positive specifically in aggressive castrate-resistant type prostate cancer patients.

Besides phage display strategies, there are several strategies to obtain the panel of peptide/proteins recognized by autoantibodies such as serum proteomics analyze (SERPA), serological analysis of recombinant expressed cDNA clone (SEREX), and high-throughput protein microarray [7]. SERPA identifies antigens through standard proteomics analyses typically involving two-dimensional protein electrophoresis. In general, an increasing number of studies are using protein (or peptide) microarray, as discussed in a review article by Ayoglu et al on various antigen arrays useful for autoantibody profiling [8]. Recent improvements of scanner, image analysis software, and protein printing robot's necessary for planar microarrays as well as innovation in user-friendly bead-based platforms have now offered a variety of tools that complement each other at different phases of biomarker discovery studies. An example of current applications of protein array strategies can be found in Anderson et al [9], in which selection was performed based on the NAPPA (nucleic acid programmable protein arrays). In NAPPA, cDNAs are printed, then transcribed and translated and immobilized using epitope tags fused to the proteins. Compared to these protein microarray strategies, phage display is a technique that has been widely used in biotechnology, and therefore it does not require an expensive facility for enrichment of peptide clones. Several rounds of biopanning allows enrichment of autoantibody-binding peptides.

It is of clinical interest to consider overall usefulness of phage display in comparison with protein microarray strategies. For both strategies, typical sensitivities of individual autoantibody biomarkers are not high (~10-25%) although their specificities are high. Both approaches usually lead the authors to focus only on ~10-20 candidates TAAs or peptides. To my knowledge, phage-display and protein microarray performance have not been directly compared in terms of the performance. Once a panel of useful clones is established, the phage display strategy typically translates to beads-based microarray strategies. So, this comparison is not about their running costs in large-

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scale studies, but about the costs for obtaining a good panel of autoantibody-binding peptides.

Of note, while it takes one or two weeks to prepare a phage library from tissues or cells, the same library can be shared by related studies. So, it is understandable that one library can serve many analyses that utilize diverse combinations of sera from patients and controls. For example, Zhang et al recently used the sera from patients of HBV and/or HCV infection as controls in the analysis of hepatocellular carcinoma serum biomarker screening based on a random phage display strategy [10]. Thus, phage display strategies seem to expedite researchers to try to see the performance of the panel of peptides in various related settings at low cost.

It is of interest to compare performance of combinatorial peptide phage libraries as opposed to cDNA phage libraries. In theory, a recombinant peptide library can cover more diverse sequences in an unbiased manner than cDNA library, but an epitope may consist of peptide portions separated in sequence or its recognition may depend on protein folding stabilized by flanking sequences. Future studies may focus on comparison among various phage display strategies.

By way of the random peptides approaches, my personal question is directed to the fact that the peptide clones that are enriched by a phage-display method and show diagnostic usefulness often include protein products of untranslated region of genes and out-of-frame cDNA fragments [4]. Somehow, such apparently meaningless peptides work well as autoantibody-binding antigens, so it would be interesting to envisage that the repertoire of autoantibodies generated in cancer patients could have amazingly high complexity possibly due to anomalous ways of protein degradations that may lead to autoantibody generation against epitopes that are normally hidden.

It is also important to recognize that such high-throughput analyses are enabled by a number of supports including organization and management of bioresources. For future retrospective cohort analyses on earlier stages of cancers, it is crucial to obtain and reserve serum samples not only from cancer patients but also from healthy subjects.

I am often impressed with frequent revisions of textbooks of clinical chemistry compared to those of biochemistry and physiology, and reason that this reflects the multidisciplinary nature of clinical chemistry encompassing practical importance as well as biomedical understanding. Until a decade ago, however, we had little need to talk about autoantibody cancer markers other than anti-p53 in the classes on clinical chemistry [11]. Given the recent findings signifying an extraordinarily rich source of information in autoantibody signatures, high-throughput analyses of autoantibody signatures may become a basic knowledge of future students.

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