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Short communication

A protein microarray prepared with phage-displayed antibody clones

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Abstract

Using a large phage antibody library, a protein microarray spotted directly with phage-displayed antibody clones was created to discriminate between recognition profiles of samples from healthy donors and leukemia patients. The protocol for preparing antibody-displaying phage chips was presented. Some conditions such as substrates and blocking buffers were compared and optimized. The major improvements of this microarray are higher throughput and lower cost compared to previous antibody chips. Due to its convenience and sensitivity, it can be extensively used for rapid and high throughput detection of protein profiles of experimental and clinical samples.

Keywords: Phage display; Microarray; Antibody library; pIII system; pVIII system

1. Introduction

It is well known that proteins play important roles in mediating physiological events and metabolisms in organisms. Characterization of proteins expressed in cells under normal and pathological states may provide important information for diagnostic and prognostic monitoring of diseases. Therefore, development of high throughput approaches to get information on proteome wide scale has become a research focus now (de Wildt et al., 2000; Liu et al., 2002; Wingren et al., 2005). Especially the microarray technology applied to proteomics is being developed rapidly in recent years (Miller et al., 2003; Lopez and Pluskal, 2003).

Generally, the identity of a cellular protein can be identified via specific recognition with a group of monoclonal antibodies. This characteristic makes antibody microarray highly useful for monitoring sensitive biological interaction and a row of publications reported recombinant antibody fragment based microarrays (Angenendt et al., 2004; Wassaf et al., 2006). Traditionally, the antibodies immobilized on chips are monoclonal antibodies produced by hybridoma technology (Stich et al., 2003). It is rather laborious and poorly efficient in generating antibodies by such method and thus unsuitable for high throughput screening. Recent advances in *in vitro* selection techniques such as phage display make the collection of antibodies much faster and cheaper than before (Kramer et al., 2002; Houshmand et al., 1999). Phage display (Smith, 1985) provides a powerful tool for molecular biology to study the recognition and interaction between molecules of protein/protein and protein/other bio-molecules (Willats, 2002). Genetically modified bacteriophages can transport heterogeneous DNA into their bacterial hosts, subsequently replicate and package to express foreign proteins of interest on their surfaces, including variable fragments of immunoglobulin (Jenkins and Pennington, 2001). This technology has particularly made great success in preparing antibodies without immunized animals (McCafferty et al., 1990). The availability of large phage antibody libraries has provided an extensive source for screening against almost any antigen (Holt et al., 2000). Here we describe a new type of protein chip directly prepared with recombinant phage particles based on a large phage antibody library.

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2. Materials and methods

2.1. Construction of large phage antibody library

In order to enlarge the capacity and introduce more sequence diversity, forward directional phagemid DNA concatemers with *cos*λ sites inserted were packaged *in vitro* to construct a filamentous phage display antibody library containing 1.40×10^{10} independent clones. Six antigens were used to check the final library and positive clones corresponding to each of them were found. The details can be seen in our previous publication (Cen et al., 2006).

2.2. Panning

A large phage antibody library constructed by our laboratory (Cen et al., 2006) was used for cell panning (five rounds) with leukocytes from healthy donors and phage antibody clones against common antigens on the surface of leukocytes were obtained. Subsequently, the residual library was used for subtraction panning (three rounds) with leukocytes from leukemia patients and leukemia-specific clones was obtained (Shadidi and Sioud, 2001). The leukocytes used for panning each time was from different individuals. Genes of phage antibodies specific to normal antigens or leukemia-specific antigens on the surface of leukocytes were transferred from pIII display system to pVIII display system, respectively.

2.3. Preparation of chip

As probes, recombinant phage clones were spotted onto the substrate (CapitalBio Corporation) using ArrayIt™ SpotBot® Personal Microarray Robot (TeleChem International Inc.) and microarrays were immobilized at 37 °C over night. SDS with a final concentration of 0.2% was added to the phage suspensions to make spots full.

2.4. Labelling with Cy3

Leukocytes from three healthy donors and three leukemia patients were lysed in 100 μl PBS containing 1% Triton-100. Resulting protein extracts were labelled with fluorescent dye Cy3 (Cy3 Mono-Reactive Dye Pack, Amersham Biosciences) and purified by chromatography (FluoroLink™-Ab Cy3 labelling kit, Amersham Biosciences).

2.5. Detection

The chip was blocked with 2% BSA (Roche) at 37 °C for 30 min and followed by 2% acetylized BSA (Promega) at 37 °C for 30 min. Forty microlitres Cy3-labelled sample was applied to each microarray consisted of 300 spots and kept at 37 °C for 1 h. After unbound proteins were washed away using 0.1% PBST three times and PBS once (5 min/wash), Cy3 fluorescence was scanned with ScanArray Express microarray scanner (Perking Elmer).

2.6. Statistical analysis

For comparison of aldehyde substrate and epoxy substrate, experiments were performed in triplicate, data were compared by *T*-test using Sigmastat software, and considered significant at $P < 0.05$. For fluorescent profiles of proteins extracted from leukocytes, three independent replicates were performed for each probe. Experiments were repeated three times using samples from different individuals. Data were compared using student *T*-test, and considered significant at $P < 0.05$. Data are shown as mean ± standard error (S.E.).

3. Results and discussion

3.1. Preparation of specific antibody-displaying phage sensors

Some tumor cells such as HeLa cells and leukemia leukocytes were used as samples to check the chips developed by our group. In previous study, the entire pool of proteins from lysed lymphocytes and HeLa cells were labelled by fluorescent dye Cy3 and reacted with the analytical chip. A different binding pattern was obtained. Based on these preliminary data, the process was further optimized and used to prepare phage-displayed antibody chip herein for screening leukemia. A large phage antibody library displayed by pIII system was used for cell panning with leukocytes from healthy donors to get clones as normal probes. Subsequently this library was further used for subtraction panning with leukocytes from acute myeloid leukemia patients to get clones as leukemia-specific probes (Supplemental Table 1).

Actually these clones selected through panning can be directly spotted onto microarray as sensors. However, in order to improve the sensitivity of chip, genes of phage antibodies directed to normal or leukemia-specific antigens on the surface of leukocytes were both transferred from pIII display system to pVIII display system, respectively. The resulting pools were designated as pool 1 (normal) and pool 2 (leukemia-specific). pIII system has been routinely used for screening antibody fragments with high affinity because the minor coat protein pIII display system can only display about one copy of the fused protein. It can enable selection of comparatively high specific probes and subsequently improve the signal-to-noise ratio of chip. In contrast, the major coat protein pVIII display system is capable of displaying hundreds of copies of fused peptides over the phage virion, and thus a higher display titer and efficiency can be reached (O'Connor et al., 2005; Chen et al., 2004).

3.2. Comparison of aldehyde substrate and epoxy substrate

Chips prepared by commercially available epoxy substrate (CapitalBio® EpoxySlide™) and aldehyde substrate (CapitalBio® AldehydeSlide™) were compared. Results showed that the former yielded much higher signal-to-noise ratio (S/N ratio) (Supplemental Fig. 1). So the epoxy substrate was chosen for further application.