PHAGE DISPLAY IN DISEASE DIAGNOSIS: FROM ANTIBODY SELECTION TO CLINICAL TRANSLATION

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ABSTRACT

With the advent of molecular biology, a number of display techniques have been developed for the generation of recombinant antibodies. Phage display remains the most effective technique for generating recombinant antibodies. The key factor driving its success is the resilience of the phage particles, facilitating automation and adaptability to modifications. The production of monospecific binders provides an essential diagnostic tool at a more economical and efficient cost. Recombinant antibodies may be altered with significant versatility, rendering them highly appropriate across various platforms.

INTRODUCTION

Phage display is a potent biotechnological method that enables the production of exogenous polypeptides on the surface of phage particles (Pande et al., 2010). The phage display technique has transformed the domain of protein engineering, significantly influencing antibody engineering. The initial advancement in the evolution of phage display approach, which emerged as a highly effective technological platform for the production of antibody-based therapeutics, was reported in 1985 by George Smith. Smith demonstrated that foreign DNA pieces may be integrated into the gene producing the filamentous phage coat protein III (pIII), produced as peptide-phage fusions. Three years later, Parmley and Smith elucidated that peptide-phage fusions, even at a ratio of one peptide-phage fusion per million wildtype virions, could be enriched through the application of biotinylated antibodies specific to the peptides, a technique termed biopanning. Later, it was demonstrated that libraries comprising millions of random short peptides produced specific peptides capable of tightly binding to various antibodies, thereby establishing the groundwork for identifying specific peptides for any antibody without prior knowledge of its specificity.

PRINCIPLE OF PHAGE DISPLAY TECHNOLOGY

Phage display is a multi-step process that begins with the design of a library. A classical phage display experiment involves screening a library of various protein variants to identify high-affinity binders to a specific ligand. This process begins with cloning the protein into a phagemid vector or recombinant phage and confirming its functional expression on the surface of the generated phage particles.

1. **Library construction**

A diversified library of DNA sequences that encode target proteins or, peptides are created. The sequences are inserted into a phage vector, commonly M13 bacteriophage, and fused with a gene that encodes a coat protein, typically pIII or pVIII. So that each phage exhibits a distinctive peptide or protein on its exterior that corresponds to the DNA inside.

2. Phage introduction and Amplification

Recombinant phage vectors are delivered into *E. coli* cells. *E. coli* synthesizes and secretes phage particles that exhibit peptides and proteins.

3.Biopanning

The phage library is subjected to an immobilized target (e.g., receptor, antigen). Phages possessing peptides that will bind to the target, while unbinders will be eliminated during washing. Bound phages are eluted, typically using low pH.

4. Amplification of specific Phages

Eluted phages are employed to infect new E. coli, hence increasing the selected binders. The phages are collected from the elute to create a new pool of phages.

5. Multiple Rounds of Panning

Generally, three to five times are reiterated to enhance high-affinity binders. The stringency (concentration of target, washing strength) is augmented with every repetition.

7. **Evaluation and Analysis**

Individual phage clones are extracted and evaluated (e.g., using ELISA) for their affinity to the target. DNA sequence from high-affinity binders can be sequenced to find the protein or, peptide sequence.

COMMON VEHICLES IN PHAGE DISPLAY

1. Filamentous bacteriophages

Filamentous bacteriophages of *E. coli* (f1, fd, M13) are frequently employed in phage display techniques. Antibodies and peptides are predominantly displayed on phage proteins pIII and pVIII. The main drawback of phage display technique is the loss of coat protein functionality; however, this issue is addressed through hybrid phages and modifications of the coat protein. These virions comprise the whole wild-type genome plus a fusion gene, which may exist as an insertion in the phage genome or as a phagemid vector including the origins of replication for both the phage and its host, gene with suitable cloning sites, and an antibiotic-resistance gene. Consequently, both wild-type protein and fusion protein will be exhibited on the phage surface.

2. T7 phage

The utilization of T7 serves as an alternative to M13 display. The T7 phage display system is extensively utilized because of its remarkable robustness and stability under conditions that inactivate other phages. The advantages of employing T7 instead of M13 display techniques are attributable to the non-involvement of the capsid in phage-host adsorption and the elimination of the necessity for peptide secretion through the periplasm and cell membrane; however, this method constrains the potential for post-translational modification of polypeptides in eukaryotic systems.

3. Lambda Phage

Phage lambda can display complex, high molecular mass proteins as fusions with either the N- or C-terminal of the head protein. Furthermore, translocation across the E. coli membrane is not required in this system. In comparison to the filamentous phage system, lambda display elicits a higher immune response, despite presenting a diverse array of proteins in multiple copies.

PHAGE DISPLAY DRIVEN ANTIBODY DISCOVERY

1. Employing Phage and Yeast display: The case of tuberculosis

The current methods to screen for tuberculosis (TB) are laborious and inadequate. Antigen 85 (Ag85) is the most abundant protein in tuberculosis a target for early diagnosis. The availability of strong, sensitive, and specific antibodies presents a significant bottleneck. A novel method utilizing Ag85 as a model is established to identify antibodies against several possible targets through a combination of phage and yeast display techniques. The potency of this approach is evidenced by the identification of 111 unique antibodies in

preliminary screenings. The innovation consists of screening the complete output of phage antibody selection in a singular experiment using yeast display, providing a benefit over conventional monoclonal antibodies generated using costly and labor-intensive methods.

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2. **Identifying immunogenic proteins and the development of antibodies against *Salmonella* Typhimurium**

Salmonella Typhimurium, a foodborne pathogen responsible for approximately 100,000 infections each year in Europe, necessitates improved detection methods to avert foodborne illnesses. Presently commercial ELISA techniques have been limited by cross-reactivity and necessitate the discovery of novel immunogenic proteins for enhanced specificity in diagnostic assays. A phage display library encompassing the complete *S.* Typhimurium genome is established, resulting in the identification of 47 immunogenic oligopeptides utilizing convalescent serum from pigs afflicted with the disease. Complete genes matching to seven discovered oligopeptides were cloned, and five were expressed in *E. coli*. The immunogenic properties of these antigens are confirmed using sera from pigs infected with *S.* Typhimurium and control samples from uninfected animals. Human antibody fragments are isolated and analyzed by antibody phage display techniques. This study discovered novel immunogenic proteins of *S.* typhimurium and produced antibody fragments targeting them by phage display technology. Five proteins are validated using positive and negative sera for possible diagnostics of *S.* Typhimurium.

3. **Immuno-PCR (IPCR)**

Immuno-PCR (IPCR) is an advanced detection tool for immunological research and clinical diagnostics, because of its high sensitivity. Instead of monoclonal antibodies a recombinant phage particle is used in conventional IPCR. The surface displayed single chain variable fragment (scFv) and phage DNA, which can function as detecting antibodies and PCR templates. This aims to enhance the detection sensitivity of scFv and promote its utilization in immunoassays. The concept is demonstrated by utilizing prion protein (PrP) and Hanta virus nucleocapsid protein (NP) as detection targets in S-ELISA, iELISA, and real-time PD-IPCR, achieving a 1000- to 10,000-fold enhancement in detection sensitivity relative to traditional enzyme-linked immunosorbent tests. The results of this study may establish a novel framework for creating an easy-to-use, cost-effective, and ultrasensitive immunoassay approach.

4. **Single-Domain Antibody (sdAbs) based ELISA against Swine Influenza virus**

ELISA is a common diagnostic technique for swine influenza virus (SIV); However, production of classical antibodies are costly and labor-intensive. Single-domain antibodies (sdAbs) provide faster production, enhanced stability, and elevated affinity. The Phage display technique is used to isolate sdAbs targeting the SIV-NP(Nucleoprotein) protein. The sdAb5 is conjugated with the biotin acceptor peptide (BAP) and a His-Tag for its expression as a monomeric and site-specific biotinylation in E. coli. Anti-SIV antibodies derived from swine samples are employed to inhibit the interaction between biotinylated sdAb5 and SIV-NP protein immobilized on the ELISA plate. The sensitivity, specificity, and reproducibility of sdAb-ELISA are assessed, and the concordance among sdAb-ELISA, a commercial ELISA kit, and Western blot is analyzed. Six SIV-NP-specific sdAbs are isolated, with sdAb5 recognized as the predominant sdAb exhibiting enhanced reactivity. The sdAb-ELISA demonstrated 100% specificity for five distinct swine viruses, with a

detection threshold of 1:160 in an anti-SIV positive reference serum. The commercial ELISA kit and sdAb-ELISA showed a consistency of 94.17% in the serum samples of swine.

CONCLUSION

Phage display has evolved into a potent platform for selecting high-affinity antibodies, transforming the domain of disease diagnostics. Its versatility facilitates quick identification of disease-specific binders, hence enabling the development of specific and sensitive detection tools. Advancements in bioengineering and screening technologies are facilitating the transition of phage display from laboratory research to clinical application. It offers significant potential for the early, precise, and cost-effective disease diagnosis.

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