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An overview on application of phage display technique in immunological studies

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ABSTRACT

Phage display is very strong technique in drug discovery and development. Phage display has many applications in improving the immunological studies. Development of monoclonal antibody, peptides, peptidomimetics and epitope mapping are main application of phage display. Selection of monoclonal antibody or peptides that are displayed on the surface of the phages can be occurred through biopanning process. In biopanning process phage library is incubated with antigen and particular phages can be identified and isolated. Increasing the stringency in the biopanning rounds can be help to select phages with high affinity and specificity. Here, we describe an overview of phage display application with focusing on monoclonal antibody production and epitope mapping.

1. Introduction

Phage display is generally new strategy to exhibit peptide (polypeptides) on the surface of bacteriophage. For the first time, phage display introduced by George Smith in 1985. As per this system, phages can be expressed the desired (poly) peptides as a component of their surface proteins. In this technique, the (poly) peptide express as fusion with one of the surface proteins of the bacteriophage and in this way the desired (poly) peptide is chosen by binding to different target [1]. In fact, this method is based on the reaction between the ligand and receptor. Displaying of proteins on the surface of the phages is dependable strategy for choice of uncommon qualities that cod proteins with binding activity. Phage display can be set to show different quantities of peptides with substantial differences in polyvalent configuration. The level of display relies on upon the sequence and also length of poly (peptides) [2,3]. In case of small peptides (smaller than 8 residues), hundreds or thousands copies of them can be displayed [4,5]. This technology is fit for delivering small peptides (1–20 residues) with high binding affinity to any protein [6]. Phage display ygetarts is based on genetic manipulation of genes of surface proteins of filamentous phages like M13 and Fd. Foreign DNA inter into the genome of filamentous phage (for example gene III) and foreign peptide encoded as a fusion protein with a surface coating protein [1,7,8].

Rapid identification and isolation of high specific phage to its own target (through biopanning process) is advantage of phage display technique. Biopanning cause recognizable proof of individual peptides in the scope of micromolar to nanomolar range [9,10]. In this review we outlined distinctive application of phage display from the past to present.

2. General view to biology of filamentous phages

Filamentous phages are group of non-lytic phages that incorporate round single stranded DNA. The family of Ff, M13, fd, and f1 are vital phages which have utility in phage display which among them M13 phage is the most generally used [11].

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M13 phage, is a bacteriophage that is (6–7) nm in diameter and 900 nm long and is a member of F positive family [12]. The most important feature is that in contrast to other phages, M13 can be effortlessly purified and used. Replication of M13 phage initiate through binding of M13 to its receptor on the bacterial cell (bacterial F-pilus). Therefore bacterial cells that contain pili have infected with M13 phages [12]. In fact, bacterial cell acts as a production factory of M13 phages. M13 phages not kill host cells but slow the cell growth due to stress in the production of phage particles. The genome of M13 phage is 6407 bp long that encodes three groups of phage proteins: 1 – proteins involving in replication (pII, pX and pV), 2 – morphogenetic (pI and pIV), and 3 – structural (pVIII, pIII, pVI, pVII and pIX). The coating proteins of phage categorized into major (pVIII) and minor (PIII and pVI) proteins. Each phage has about 2700 copies of pVIII. The N-terminal region of pIII plays important role in binding of phage to the F-pilus of the bacterium [13–15]. Infection process of bacterial cell initiate with binding of pIII of phage particles to F-pilus. Each phage contains three to five pIII proteins at one end [2,16]. Then single-stranded DNA with the help of cytoplasmic enzymes of bacterium converts to double-stranded DNA which is known as replicative form (RF). The protein pV of phage binds to single strand DNA and inhibits its conversion to replicative form. In fact the amount of pV play critical role for conversion of single strand DNA to RF [17]. Assembly of phage particles start from inner membrane of bacterium with help of pI, pIV and pXI. pVII and pIX play role in secretion of phage. pV is replaced by pVIII during deportation and then pVI and pIII add to the end of the phage [18]. At the first generation of bacterial infection about 1000 phage particles are produced that decrease to 100 to 200 particles per generation at next steps [19,20].

3. Phage display libraries

3.1. Antibody phage libraries

Generally there are four kinds of antibody phage libraries: immune-library, naïve library, semi-synthetic and synthetic library [21–27]. In immune-library, the B-cells are isolated from the spleen of immunized animals and then the repertoires of manipulated V-genes are cloned into phage library vectors. The advantage of such library is that the affinity maturation has already occurred in the body of immunized animals and isolated antibodies have high affinity as well as specificity to their own antigen. However, there are some limitation that drawback the use of immune-libraries in phage display: need to construct new library for each antigen, high cost and laborious process, ethical issues due to use of animal for immunization and the target antigen must be immunogenic in in vivo. The mentioned limitations lead to introduction of different type of non-immune phage library known as single-pot library. Contrary to immune library that V-genes are from IgG mRNA, in naïve library, the V-gene sequences are from IgM mRNA of non-immunized animal. The synthetic libraries are created by combination of gene segment from un-rearranged antibody [28,29]. Synthetic libraries are based on diversity introduced into CDR (complementarity determining region) of antibody. The semi-synthetic libraries are built based on substitution of amino acids in CDR regions by PCR and constructing a library with high diversity that no exist in in vivo models [30].

4. Peptide phage library

There are three types of peptide phage libraries: linear heptapeptide library (Ph.D.-7), loop-constrained heptapeptide library (Ph.D.-C7C) and dodecapeptide library (Ph.D.-12). The displayed peptides on the surface of phage are small and therefore the infectivity of phage through pIII is not affected. Large diversity of combinatorial peptides displayed on the surface of the phages leads to isolation of different peptide to almost any type of targets [31]. Peptide libraries displayed on the surface of phages have many application: drug discovery, identification of ligand-binding activity, identification of biomarkers and epitope mapping [32–36].

5. Application of phage display

5.1. Production of monoclonal antibody by phage display

Hybridoma and phage display technology are common techniques to obtain monoclonal antibodies. Hybridoma technology has been presented by Georges Kohler and Cesar Milstein in 1975. Combination of B cell producing monoclonal antibody with mouse myeloma cells is the basis of hybridoma technology. Despite of advantage of hybridoma technology there are some limitation: poor immunogenicity of some targets, high production cost, unexpected immune response due to murine origin, time-consuming and need to myeloma cells [37–40]. Phage display is a powerful technique in isolation of monoclonal antibodies with high affinity to their target [41–44]. Summary of steps of isolation of antibody using phage display are described as follow [39,40,45].

Production of gene fragment: This phase involves animal immunization with the desired antigen and then isolation of B lymphocytes, mRNA extraction and cDNA synthesis. The synthesized cDNA contain genetic information of all antibodies targeting various antigens and consist of approximately 10^9 to 10^{11} lymphocyte clones.

Cloning of gene fragments in the phagemid vectors: Genes related to the different clones of antibodies are digested with restriction enzymes, clone into phagemid vectors and then display on the surface of phages. Sequence diversity of the fragments at this step leads to optimum isolation of antibodies in the later steps. So far, a variety of phages like phages λ, T4, T7 and M13 have been described for displaying of antibody fragments. These vectors help to the displayed antibodies to maintain their function at the surface of phage. But using of phages like M13 that not destroy the bacterial cells is most applicable. The phagemid vectors need helper phage to package and exit from the bacterial cells and enter to the medium [12,16,46].

Selection of specific phages: After cloning of the fragments into phagemid vectors, because of diversity in antibody gene, variety of clones of antibodies display on the surface of the phage. Selections of specific clone that recognize the antigen (target of interest) perform by biopanning. Since the antibody fragments on the surface of the phage are functional so the phage carrying specific antibody can be isolated from non-specific phages (due to antigen–antibody binding properties). At this step expressed antibodies on the surface of phage based on their ability to bind to the target antigen will be enriched through biopanning. The panning process include: immobilization of antigen, binding of the phages, washing and removing non-

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