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Phage Display Technique and Hepatitis Viruses Studies

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Phage display is an in vitro selection method in which a peptide or protein is expressed as a fusion with a coat protein of a bacteriophage. This versatile technique has a lot of application in life science. In this literature review the applications of phage display in Hepatitis studies evaluated. This is the first review that considers this issue.

Keywords: Phage display, Hepatitis viruses.

Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis may occur with limited or no symptoms, but often leads to jaundice (a yellow discoloration of the skin, mucous membrane, and conjunctiva), poor appetite, and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persists longer. Acute hepatitis can be self-limiting (healing on its own), can progress to chronic hepatitis, or, rarely, can cause acute liver failure[1].

Viral hepatitis is a major public health problem worldwide. The infection is caused by five taxonomically unrelated human viruses, namely, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV).

HAV or hepatitis A virus cause an acute and commonly self-limiting infection followed by long immune protection against the virus[2].

HBV, responsible for 600000 deaths each year, caused a life threatening liver disease. The infectious virus particle, also called as Dane particle, is responsible for causing infection in 5 percent of world's population with 2 billion people infected with the virus and 350 million as carrier of chronic infection[3-5].

Hepatitis C virus (HCV) is the major causative agent of non-A, non-B hepatitis worldwide. HCV is blood borne pathogen which causes severe liver disorders, including hepatocellular carcinoma, hepatic steatosis, liver cirrhosis, end stage liver disease and various metabolic disorders. HCV was identified by Choo et al. as a positive stranded RNA molecule related to Togaviridae or Flaviviridae[6-7].

HDV is a unique agent characterized by a single-stranded RNA genome encapsidated by the hepatitis B surface antigen (HBsAg) and a peculiar strategy of infection of the target organ[8]. In fact, HDV requires the helper functions provided by hepatitis B virus (HBV) in order to propagate to hepatocytes, it can only infect subjects with co-existing HBV infection due either to the simultaneous transmission of the two viruses or super infection in an established HBV carrier[9-10].

HEV is the sole member of the genus Hepevirus in the family of Hepeviridae, is the major cause of waterborne hepatitis in tropical and subtropical countries and of sporadic cases of viral

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hepatitis in endemic and industrialized countries[11].

There are a lot of techniques used for studying of these viruses, herein we focused on phage display technique used in hepatitis researches though.

Literature search

Articles were searched from Google Scholar and Pubmed with key words of phage display and hepatitis viruses, Phage display and BAV, HBV, HCV. The valued information was subjected for review.

Phage display technique

Phage display, created by G. Smith in 1985, describes an in vitro selection method in which a peptide or protein is expressed as a fusion with a coat protein of a bacteriophage, resulting in display of the fused protein on the surface of the phage particle, while the DNA encoding the fusion resides within the phage virion. Phage display has been used to generate a physical linkage between vast libraries of random peptide sequences to the DNA encoding each sequence, allowing rapid detection of peptide ligands for a variety of target molecules (antibodies, enzymes, cell-surface receptors, etc.) by an *in vitro* selection process called biopanning[12-13]. In its simplest form, biopanning is carried out by incubating a library of phage-displayed peptides with a plate coated with the target, washing away the unbound phage, and eluting the specifically-bound phage. The eluted phages are then amplified and taken through additional cycles of biopanning and amplification to successively enrich the pool of phage in favor of the tightest binding sequences. After 3-4 rounds, individual clones are characterized by DNA sequencing and ELISA.

Random peptide libraries displayed on phage have been used in a number of applications[13], including epitope mapping[14-16], mapping protein-protein contacts[17], and identification of peptide mimics of non-peptide ligands[18-22]. Bioactive peptides have been identified either by panning against immobilized purifed receptors[23] or against intact cells[24-26]. The most common bacteriophages used in phage display are E.coli filamentous bacteriophages (f1, fd, M13)[27-28],thoughT4, T7, and » phage have also been used[29-31].

Phage display technique applications

Applications of phage display technology include determination of interaction partners of a protein (which would be used as the immobilized phage "bait" with a DNA library consisting of all coding sequence of a cell, tissue or organism) so that the function or the mechanism of the function of that protein may be determined[32]. Phage display is also a widely used method for *in vitro* protein evolution, also called protein engineering. As such, phage display is a useful tool in drug discovery. It is used for finding new ligands (enzyme inhibitors, receptor agonists and antagonists) to target proteins[33-35]. The technique is also used to determine tumor antigens (for use in diagnosis and therapeutic targeting)[36] and in searching for protein-DNA interactions using specially-constructed DNA libraries with randomized segments[37]. Beside these, phage display technique has been used in hepatitis studies too. In this review we focused on some applications of phage display in hepatitis researches.

Phage display in developing a TaqMan real-time immuno-PCR method for detection of hepatitis viruses

PD-IPCR has been proven to be a highly sensitive assay for the detection of Hantaan virus nucleocapsid protein, prion protein[38] and the IgG in multiple sclerosis[39].

A TaqMan real-time detection assay based on the concept of phage display mediated immuno-PCR (PD-IPCR) for the detection of HBcAg has been developed by Monjezi et. al (2012). PD-IPCR combines the advantages of immuno-PCR (IPCR) and phage display technology.

Previously, a phage bearing a constrained peptide (C-WSFFSNI-C) which interacts tightly with HBcAg was isolated[40] and specificity study showed that the phage only reacted with HBcAg but did not react with HBsAg and HbeAg[41]. In this strategy this phage was used to establish a PD-IPCR as an alternative choice for diagnosis of HBcAg.

Detection of HBcAg in serum by this method sonsist of 5 steps: a) HBcAg particle separated from the virion and dissociation to HBcAg dimers according to Kimura protocol[42]. b) The obtained dimmers coated on a microtiter plate well. c) M13 phages displaying the sequence C-WSFFSNI-C which interacts tightly with HBcAg[40] were added and allowed to interact with HBcAg.

d) In order to lyse the bound phages and to release their genome, the plates well were heated at 95° C. e) the released genome of phage used as a template in the TaqMan real-time PCR[43].

The established TaqMan based real-time PD-IPCR can detect 10 ng of HBcAg by using 108 pfu/ml of the recombinant phage so it's about 10,000-fold more sensitive than the phage-ELISA. Therefore, the suggested PD-IPCR method may be an alternative option for the detection of HBcAg in serum samples. Collectively, these results indicate that this novel test could be a sensitive and highly reproducible detection method in hepatitis studies.

Phage display and finding antibodies against the hepatitis viruses

The significance of phage display in antibody production is increasing. Phage display of antibody libraries has provided a powerful tool for the isolation of human MAbs to important viral pathogens[44-49]. Various formats of antibodies can be displayed on the surface of filamentous phage particles (e.g., M13), and antibodies with desired specificity can be isolated by panning on the antigens of interest[50-51].

To date, a lot of antibodies have been developed in hepatitis researches. Among the first obtained antibodies against the hepatitis viruses was the successful molecular cloning of the antibody repertoire from an HCV-positive patient and the subsequent isolation of genes coding for anti-HCV human antibody fragments in Plaisant study. Availability this combinatorial library was panned against HCV and sixteen human antibody Fab fragments able to bind to HCV-specific antigens were generated which majority of them appeared to have specificity for the HCV c33 peptide[52].

Beside Fab fragment libraries, human single chain Fv antibody (scFv) phage display library against hepatitis C virus has been screened. Yan et. al panned a human single chain Fv (scFv) phage antibody library against hepatitis C virus E2 antigen. The identified antibody was successfully applied in immunohistochemistry staining[53].

In two similar studies, an antibody antigen-binding fragment (Fab) phage display library generated from a donor chronically infected with HCV screened against HCV E2 glycoprotein and finally within a total of more than 120 clones, ten Fabs from different heavy-chain groups recognizing the five different antigenic regions were converted into full-length IgG1s. All recombinant mAbs bound the genotype 1a HCV E1-E2 complex with approximately similar apparent affinities, but only one of these mAbs reacted with genotype 2a HCV. This finding suggesting that the epitopes related to that antigenic part are highly conserved. So using phage display library it could be possible to find neutralizing antibodies protect against hepatitis viruses. Such results provide evidence that protection against heterologous viral infection is possible, suggesting prophylactic vaccine against hepatitis viruses may be achievable. The mAb panel may also be useful for probing the antigenicity of E1-E2-based HCV vaccine candidates and guide the design of immunogens to elicit cross-Nabs (neutralizing antibodies) to HCV[54-55].

Such studies have been done for HBV and HEV too. In Gwang study, for in vitro selection of antibodies that neutralize HBV, a large nonimmunized human phage antibody library in scFv format panned and two anti-pre-S1 antibodies obtained. These antibodies may be a good candidate for immunoprophylaxis against HBV infection[56].

Infected chimpanzee, the primate most closely related to humans, derived library has been used for antibody production too. In Schofield study 2 mAbs were obtained from a cDNA phage display library of chimpanzee against ORF2 protein of HEV. These antibodies could be used in western blot and ELISA methods, both of them neutralized HEV and injection of virus-antibody mixtures to rhesus monkeys prevented infection[57].

These researches described in the above showed that it is possible to state that the development of combinatorial antibody libraries displayed on the surface of phage offers the possibility of accessing monoclonal antibodies specificities against hepatitis viruses. Moreover detailed information about different epitopes that appear to be protective and definition of conserved elements in the viral envelope can be of capital importance for rational vaccine and drug design[58].

Ligand identification against hepatitis viruses by phage display method

Identification of ligand binding is very

usual in phage display studies[59-62]. Some researchers have been done for developing of ligand binding peptide against the hepatitis viruses. The first ligand isolation against core antigen of hepatitis B virus (HBcAg) was done by Murray e. al. They screened a random hexapeptide library displayed on filamentous phage and introduced the "ALLGRMK" sequence as a binding ligand against truncated HBcAg. This peptide may represent a lead antiviral agent in chronic infections[63].

Ho et. al isolated 2 other peptides (WSFFSNI and WPFWGPW) form a cyclic (disulfide constrained) phage peptide library reacted with full length HBc Ag[40].

In an existing study, a peptide ligand (LPVRPWT or CD1) against β-cyclodextrin (β-CD) beads was found. A fusion peptide composed of CD1 peptide and residues 9–21 of the HCV core protein C1 (CD1HCV peptide) was designed. This fusion peptide was immobilized on β-CD beads by CD1 peptide. Using enzyme immunoassay, detection of anti-HCV Ab performed. Their findings showed anti-HCV Ab could react strongly, with a detection limit of 1 ng, to HCV core protein C1 conjugated to β-CD via LPVRPWT ligand peptide[64].

These summarized results shows that high affinity ligands related to hepatitis viruses could be obtained from phage display libraries. Such ligands may provide useful information for detection and designing smaller and more potent peptides or small molecules that may be used in hepatitis researches.

Phage display function in finding mimotopes against hepatitis viruses

Phage-displayed peptide approach can be used to identify mimotope of hepatitis viruses. Mimotopes are macromolecules which mimic the structure of an epitope and are frequently obtained from phage display random peptide libraries through screening[65-66].

Puntoriero et.al, in order to identify synthetic surrogates of the HVR1 able to induce antibodies that reacted with virtually all HCV HVR1 variants, derived a consensus profile from more than 200 hyper variable region 1 (HVR1) sequences of different viral isolates and constructed a vast repertoire of synthetic HVR1 surrogates displayed on M13 bacteriophage as fusion to the major coat protein (pVIII). This library was panned against sera from clinically characterized HCV-infected individuals and some effective antigenic and immunogenic mimotopes of a large number of naturally occurring HCV variants isolated, most of which reacted with antibodies present in the majority of the sera from HCV-infected viremic patients (up to 80% of the tested samples). These findings are in good agreement with the hypothesis that HVR1 mimotopes have a higher capability of interacting with different anti-HVR1 antibodies than natural HVR1 variants[67].

In another study a phage display library panned against anti human CD81 (hCD81) molecule (a putative receptor for HCV) and the peptide sequence ATWVCGPCT introduced as hCD81-like small peptide, which can block the binding site of HCV E2 for hCD81[68].

Using the same procedure some mimtopes was isolated against HAV. These mimotopes mimics a structure of VP1 and VP3 capsid proteins. These peptides could bind specifically to serum antibodies from convalescent patients and immunization of mice by them resulted in neutralizing antibodies against HAV[69].

Such finding suggest that such mimotpes could be have great potential for the development of synthetic peptide vaccines, DNA vaccines or could be used to develop a diagnostic assay for hepatitis viruses.

Phage as vaccine against hepatitis viruses

Potential application of phages as a modern platform for vaccines is another advantages of phages. It has been shown that phage could be use as antigen carriers in vaccine design strategies[70-73].

Wan et. al constructed a filamentous phage particles that displayed the Hepatitis B virus epitope S_{28-39} . Injection of such phages to mice resulted in an MHC class I restricted HBs specific CTL response. These results suggest that the phage displaying desired peptide could be potentially suitable for developing the anti-hepatitis viruses vaccine studies[74].

CONCLUSION

The evaluated studies showed that phage display technique could play several roles in hepatitis studies. The function of this octopus like

method, which its arms penetrated into different parts of biology, in hepatitis studies could be very variable. However, it looks that antibody and ligand studies share more concerns in this field of research.

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