Journal of Virological Methods xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Selection and characterization of single-chain recombinant antibodies against infectious haematopoietic necrosis virus from mouse phage display library

Q1 Hong Liu[a], Xiaocong Zheng[b], Xiujie Shi[b], Li Yu[b], Peng Jia[b], Jinjin Wang[b], Junqiang He[b], Wensheng Lan[b], Hong Liu[b,*], Zhixin Wu[a,**]

[a] College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China
[b] Shenzhen Entry-Exit Inspection and Quarantine Bureau, Shenzhen 518045, China

Article history:
Received 11 November 2013
Received in revised form 11 March 2014
Accepted 4 April 2014
Available online xxx

Keywords:
Phage display
Single-chain fragment variable antibody
Infectious haematopoietic necrosis virus

A B S T R A C T

Six single-chain fragment variable (scFv) antibodies against infectious haematopoietic necrosis virus (IHNV) were selected from an antibody phage display library by phage display technology. The soluble scFv antibodies showed a molecular weight 32 kDa by Western blot. Dot blot analysis revealed that the six scFv antibodies could recognize IHNV. For enzyme linked immunosorbent assay (ELISA), four scFv antibodies (P1A4, P1A12, P1D5 and P3E2) showed cross-reactivity with spring viraemia of carp virus (SVCV). However, none of the six scFv antibodies had cross-reaction with Pike fry rhabdovirus (PFRV), Soft-shelled turtle iridovirus (STIV), viral haemorrhagic septicemia virus (VHSV), or viral nervous necrosis virus (VNNV). Indirect immunofluorescence results showed that all of these scFv antibodies reacted positively with virus in the IHNV-infected cells. These scFv antibodies will be useful in diagnostic test development and pathogenesis studies for IHNV.

© 2014 Published by Elsevier B.V.

1. Introduction

Infectious haematopoietic necrosis virus (IHNV), a significant pathogen of salmon and trout (Wolf, 1988), is enzootic in river systems throughout western North America and has spread to Asia and Europe by movement of infected fish and eggs (Winton, 1991). It is an economically important fish pathogen, causing severe acute infections in young salmonid fish and resulting in high mortalities which can reach 80%–100% during outbreaks (Alonso et al., 2005). Outbreaks of the diseases have caused great economic losses and became factors restricting aquaculture and the development of fisheries.

The virus is a member of the genus *Novirhabdovirus* in the family *Rhabdoviridae*, and consists of a linear single-strand, negative-sense RNA genome of approximately 11k nucleotides. The IHNV genome contains 6 genes in the order 3′-N-P-M-G-NV-L-5′, encoding the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), nonvirion protein (NV) and polymerase (L), respectively (Kurath et al., 1985; Morzunov et al., 1995; Tordo et al., 2005).

Phage display technology is based on recombinant DNA methods allowing co-selection of recombinant antibodies and their corresponding genes (Wittrup, 1999). If DNA fragments encoding polypeptides are fused to certain bacteriophage coat protein genes, they can be encapsulated within phage particles that also display the encoded polypeptides on their surfaces (Sidhu, 2000; Wong et al., 2001). Recombinant antibody technology allows the display of functional antibody binding sites on the surface of bacteriophage, without the immortalization of B-cell lines used in hybridoma technology (Garet et al., 2010). Generally, phage display libraries consist of a heavy and light chain variable region domains (VH and VL) linked by a flexible Glysine-Serine linker, fused to the phage minor coat protein gene III and displayed as a single-chain fragment variable (scFv) (McCafferty et al., 1990). This technique for the production of recombinant antibodies has several advantages: rapid culture of phage clones; easy handling and detection of secreted antibodies; genetic stability; lower costs compared with monoclonal antibodies (MAbs) (Johns et al., 2000; Foy et al., 2002). The major advantage of this technological advance is that antibodies could be expressed both on the surface of the

* Corresponding author at: Shenzhen Entry-Exit Inspection and Quarantine Bureau, China. Tel.: +86 755 25588410.
** Corresponding author at: College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei 430070, China.
E-mail addresses: liuhong@szciq.gov.cn (H. Liu), wuzhixin@mail.hzau.edu.cn (Z. Wu).

http://dx.doi.org/10.1016/j.jviromet.2014.04.008
0166-0934/© 2014 Published by Elsevier B.V.

Please cite this article in press as: Liu, H., et al., Selection and characterization of single-chain recombinant antibodies against infectious haematopoietic necrosis virus from mouse phage display library. J. Virol. Methods (2014), http://dx.doi.org/10.1016/j.jviromet.2014.04.008

UNCORRECTED PROOF

phage (antibodies linked to the phage) and in the medium (soluble antibodies) (Gourdine et al., 2005). Therefore phage display is a powerful technology for selecting and engineering polypeptides with novel functions and has been used widely to produce antibodies.

Due to the extensive losses caused by IHNV in salmon and trout aquaculture, rapid detection and identification of infectious viruses are important for disease control. Many methods have been developed for the diagnosis of fish viruses. These include isolating virus from candidate fish by infection of established cell lines then confirming the identity by serum neutralization assay (OIE, 2000), immunofluorescent antibody techniques (IFAT), enzyme-linked immunosorbent assay (ELISA), immunohistochemical and immunogold labelling (Drolet et al., 1995), reverse transcriptase-PCR (RT-PCR) (Barlic-Maganja et al., 2002; Jakob et al., 2011), and real-time RT-PCR (Purcell et al., 2006, 2013). PCR technology has high sensitivity, but it suffers from false positive rate. MAbs as useful tools, have been used widely for development of effective diagnosis of fish viral pathogens (Monini and Ruggeri, 2002; Chen et al., 2008), but the cost and time for production are main limitations.

In this study, selection and characterization of single-chain fragment variable (scFv) antibody against IHNV from phage antibody library were performed. This work will serve as the basis for further development of a rapider and simpler detection kit for IHNV.

2. Materials and methods

2.1. Virus and phage display library

The viral pathogen infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicemia virus (VHSV) strain J167 were gifted by Centre for Environment, Fisheries and Aquaculture Science (CEFAS) of UK. Spring viraemia of carp virus (SVCV) strain A-1 was originally isolated from virus-infected common carp in China and preserved in our laboratory (Teng et al., 2007). Q2 Pike fry rhabdovirus (PFRV), Soft-shelled turtle iridovirus (STIV) and viral nervous necrosis virus (VNNV) strain SSS0607 were isolated and preserved in our laboratory. The cell lines of epithelioma papulosum cyprini (EPC) were preserved in our laboratory. Immunotube were purchased from Becton, Dickinson and Company (USA). The phage-displayed native mouse antibody library was provided by Professor Dai Heping of Institute of Hydrobiology, Chinese Academy of Sciences.

2.2. Panning

The antibody library was screened by three rounds of panning against IHNV, as described by Zhao et al. (2010). The transformed *Escherichia coli TG1* cells were infected with M13KO7 helper phage to rescue the phagemid with its scFv insert. The phage library was precipitated by addition of PEG/NaCl (PEG8000 in 2.5 M NaCl (20%, w/v)) for more than 1 h on ice bath and collected by centrifugation. Then the phages were resuspended in 5 ml 2YT medium. Panning of the phage scFv library was performed against IHNV which was coated on immunotube at a concentration of 50 μg/5 ml PBS overnight at 4 °C. After 3 rinses with PBS, the unoccupied sites in the immunotube were blocked with 5% bovine serum albumin (BSA) in PBS at 37 °C for 1 h. Then the prepared phages were added and incubated at 37 °C for 2 h. Unbound phage was removed by 20 rinses with PBST (PBS containing 0.1% Tween-20) followed by 20 rinses with PBS. Bound phages were eluted by adding 1 ml 100 mM triethylamine to the tube and rotating for 10 min. Eluted phages were neutralized with 0.5 ml of 1 M Tris–HCl (pH 7.5) and then added to 5 ml *E. coli TG1* mid-log cells. The mixture was incubated at 37 °C for 1 h and then plated onto a SOBAG (SOB containing 100 μg/ml ampicillin and 2% glucose) plate and incubated at 30 °C overnight. Then the first round of panning was completed. The titres of input and output phage were calculated for each panning round according to the numbers of colonies on petri dishes of series dilution. The other two rounds of panning were performed according to the above.

2.3. Selection of antibodies

After three rounds of panning, single colonies were picked up and cultured in 2YT-AG medium (2YT medium containing 2% glucose and 100 μg/ml ampicillin) overnight at 30 °C and 250 rpm. After that, the bacteria were cultured in 2YT-AK medium (2YT medium containing 100 μg/ml ampicillin and 50 μg/ml kanamycin) supplemented with 1×10^{10} PFU/ml M13KO7 overnight at 30 °C and 250 rpm. The supernatants of the cultures were collected after centrifugation and checked for ability of binding by ELISA utilizing HRP-anti M13IgG as second antibody. Absorbance values twice higher than the background level were considered to have affinity.

2.4. DNA sequencing

The nucleotide sequences of scFv antibody with binding activity to IHNV were determined by Shanghai Sangon Biological Engineering Technology And Service Co., Ltd. Shanghai, China.

2.5. Production of soluble antibody fragments

After DNA sequencing, six positive clones with the highest ELISA signal and different sequences were cultured to produce soluble scFv antibody for further characterization, as described by Dai et al. (2003). Single colony was transferred from a SOBAG plate to 5 ml of freshly prepared 2YT-AG medium and incubated overnight at 30 °C and 250 rpm. A 500 ml volume of 2YT-AG was then inoculated with 5 ml of overnight preculture, and grown at 30 °C with shaking until OD600 nm reached 0.5–0.8. After centrifugation at 4000 × g for 20 min, the bacteria were collected and the supernatant was removed. The bacteria were then resuspended in 2YT-AI (2YT medium containing 100 μg/ml ampicillin and 1 mM IPTG) and incubated for 20 h at 30 °C and 250 rpm. Production of soluble scFv antibody from extracellular or periplasmic extract was based on the manual for the Pharmacia Expression Module (Amersham Biosciences). The scFv antibodies were stored at −20 °C until use.

2.6. Characterization analysis of antibody fragments

2.6.1. Dot blot and Western blot analysis

For dot blot assays, the indicated quantities of IHNV, cell debris and PBS were spotted on nitrocellulose membrane. The membrane was then blocked with 3% BSA in PBS at 37 °C for 1 h. Diluted scFv antibodies were incubated with coated IHNV on the nitrocellulose membrane at room temperature for 1 h. After washing, the binding of the first antibody was assayed with rabbit polyclonal to E-Tag (HRP), and then its substrate, 3,3′-diaminobenzidine (DAB) was used for colour development.

For Western blot assays, the soluble scFv antibodies were subjected to SDS-PAGE (12%) and then transferred to a polyvinylidene fluoride (PVDF) membrane as described by Bhaskaran et al. (2005). The membrane was then blocked in 3% (w/v) BSA in PBS for 1 h at room temperature on a rocker. The membrane was washed for three times, 5 min for each with PBS and PBST. Then the membrane was incubated with rabbit polyclonal to E-Tag (HRP) diluted 1:5000 in PBS containing BSA for 1 h at room temperature. Finally, DAB was used for colour development.

UNCORRECTED PROOF

Please cite this article in press as: Liu, H., et al., Selection and characterization of single-chain recombinant antibodies against infectious haematopoietic necrosis virus from mouse phage display library. J. Virol. Methods (2014), http://dx.doi.org/10.1016/j.jviromet.2014.04.008

Download English Version:

https://daneshyari.com/en/article/6133686

Download Persian Version:

https://daneshyari.com/article/6133686

Daneshyari.com