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Recombinant scFv antibodies against infectious pancreatic necrosis virus isolated by flow cytometry

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Infectious pancreatic necrosis is a significant disease of farmed salmonids in China. In this study, a single chain variable fragment (scFv) antibody library derived from rainbow trout (*Oncorhynchus mykiss*) and viral protein VP2 of a Chinese infectious pancreatic necrosis virus (IPNV) isolate ChRtm213 were co-expressed by a bacterial display technology. The library was subjected to three rounds of screening by flow cytometry (FCM) to select IPNV specific antibodies. Six antibody clones with different mean fluorescence intensities (MFI) were obtained by picking colonies at random. The antibody clones were expressed and purified. The purified IPNV-specific scFv antibodies were used successfully in Western blotting, enzyme linked immunosorbent assay (ELISA) and an immunofluorescence antibody test (IFAT). This method provides a high throughput means to screen an antibody library by flow cytometry, and isolate a panel of antibody that can be used as potential reagents for the detection and study of IPNV that are prevalent in China.

1. Introduction

Infectious pancreatic necrosis (IPN), a significant disease of farmed fish worldwide, is caused by infectious pancreatic necrosis virus (IPNV), a member of the family *Birnaviridae*, a family of non-enveloped, double-stranded RNA viruses with a bisegmented (A and B) genome encoding five or six proteins (Cohen et al., 1973; Macdonald and Yamamoto, 1977; Ruane et al., 2015). IPNV is the causal agent of a highly contagious disease that affects many species of fish and shellfish (Dadar et al., 2013). IPN disease can induce high mortality, which can result in huge economic losses in both fry and juveniles of rainbow trout, brook trout and Atlantic salmon (Wolf et al., 1968). Fish that survive an IPNV infection may become carriers of the virus for long period and serve to sequentially transmit the virus to other susceptible species of fish and shellfish (Bang Jensen and Kristoffersen, 2015). IPN was first described in a freshwater brook trout (*Salvelinus fontinalis*) facility in North America in the 1950s (Wood et al., 1955) with the subsequent isolation of the virus reported by Wolf et al. (Wolf et al., 1960). In Europe, the disease was initially reported in freshwater rainbow trout (*Oncorhynchus mykiss*) farms in France (Besse and De Kinkelin, 1965), Denmark (Vestergård Jørgensen and Bregnballe, 1969), Scotland and Norway (Ball et al., 1971; Hastein and Krogsrud, 1976; Ruane et al., 2015). By the mid-1970s, IPN had been reported in North America, Europe and Japan (Hill and Way, 1995). The mid-1980s, the first outbreak of IPN was recorded in hatcheries for juvenile rainbow trout in China (Jiang et al., 1989). This was followed by a series of IPN outbreaks in cultured juvenile rainbow trout in various districts of China (Hu et al., 2012; Niu and Zhao, 1988; Sun et al., 1995). Despite wide spread vaccination, IPN has remained be a significant disease of farmed salmonids in the majority of countries, resulting in direct economic losses due to high mortality and disease-management costs (Bang Jensen and Kristoffersen, 2015; Ruane et al., 2015).

In previous studies, a bacterial display technology was established and successfully applied to antibody library screening (Xu et al., 2014a). However, by using the bacterial display technology, purified recombinant antigen was required in the screening process. The preparation of antigen is time consuming and labor intensive. In this study, an antigen-antibody co-expression display technology was established. In the co-expression vector, the new lipoprotein A (NlpA) leader peptide (Harvey et al., 2004) with the first six amino acids of the mature NlpA was utilized to anchor antibody on the periplasmic side of the inner membrane,

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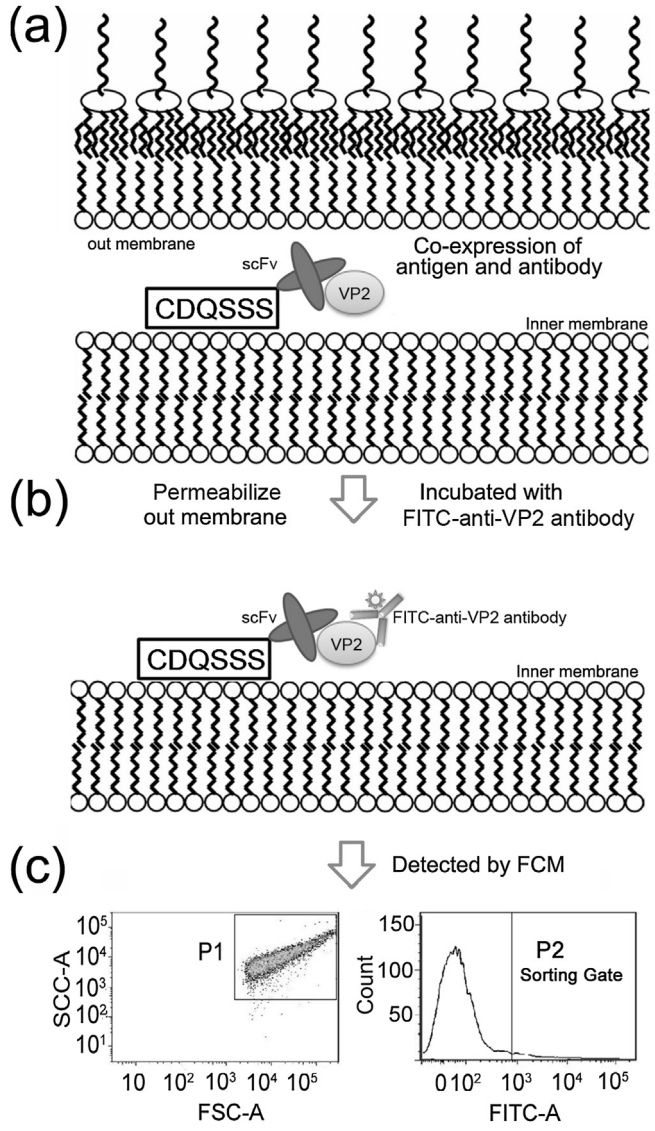

Fig. 1. The antigen-antibody co-expression display system. (a) Libraries of scFv antibodies and antigen protein co-expressed in the periplasm of *E. coli* are tethered to the inner membrane by CDQSSS. (b) After outer membrane permeabilization, the positive scFv antibodies-antigen complex anchored on the inner membrane bind with fluorescently labeled antigen specific antibodies. (c) Enrichment of spheroplasts expressing antigen specific antibody by gating the region defined by the distinct scatter of the spheroplasts (FSC and SSC) and the high FITC-A signal. FSC, forward scatter; SSC, side scatter; CDQSSS, NlpA amino acids 1–6.

and the pectate lyase B (pelB) leader peptide was used to transport attached protein to bacterial periplasm. A antibody library (described below) was fused with the NlpA leader peptide, antigen VP2 gene was fused with the pelB leader peptide, and both of these were co-expressed and transported to the periplasm of *E. coli*. If the anchored antibodies could bind to the antigen VP2, an antibody-antigen complex will be formed. Upon removal of the outer membrane by spheroplasting, fluorescent antibodies against the VP2 could enter the periplasmic space where they would be recognized by the membrane-tethered antibody- antigen complex. As a result, antibody-expressing spheroplasts became fluorescently labeled and could be readily enriched and screened by fluorescence activated cell sorter (FACS) (Fig. 1).

Most aquatic birnaviruses belong to serogroup A which includes nine serotypes (A1–A9) (Csermelyi et al., 1988; Dadar et al., 2013), and cluster into seven genogroups based on the nucleotide sequence of segement A (Blake et al., 2001; Zhang and Suzuki, 2004). Since introduced to China along with eyed eggs of rainbow trout from Japan by the 1980s (Jiang et al., 1989), IPNV has spread many districts of China, and outbreaks of IPN caused serious losses due to the high mortality (Hu et al., 2012; Niu and Zhao, 1988; Sun et al., 1995; Tong and Hetrick, 1989). The present study was designed to isolate single chain variable fragment (scFv) antibodies against a Chinese IPNV isolate ChRtm213 from an antibody library assembled from rainbow trout using the antibody-antigen co-expression system combined with flow cytometry (FCM). The IPNV was isolated from rainbow trout under going outbreak of IPN in Yunnan province, southwest China in 2013.

2. Materials and methods

2.1. Materials

E. coli DH5α was used for displaying the scFv library, while *E. coli* Rosetta and pET27b(+) were used for expression of scFvs that were isolated. The pCoex vector (described in 2.2) was used for scFv library display and was generated in our laboratory. IPNV isolates ChRtm213, infectious hematopoietic necrosis virus Sn1203 (IHNV-Sn1203) (described below), rabbit anti-IHNV polyclonal antibody (described below), rabbit anti-VP2 antibody and Chinook salmon embryo (CHSE-214) cell line were from lab stocks. The full-length genomic nucleotide sequence of strain ChRtm213 was deposited in the GenBank database under accession number KX234591 (segment A) and KX234590 (segment B). An FITC antibody labeling kit was purchased from Thermo (California, USA). An FITC-mouse anti-His tag antibody were purchased from eBioscience (Shanghai, China). Protein marker was purchased from Fermentas (California, USA). Prestained protein marker was purchased from Bogoo (Shanghai, China).

2.2. Construction of antigen-antibody co-expression vector pCoex

A previously constructed vector pBFD (Xu et al., 2015) was used as a basic frame to construct the antigen-antibody co-expression vector. The ribosome binding site (RBS) and pelB leader peptide were amplified from plasmid pET-27b and inserted into the pBFD by *Eco*R I and *Xho* I to construct antigen-antibody co-expression vector pCoex. VP2 gene of IPNV ChRtm213 was fused with the pelB leader peptide, and the antibody library (described below in Section 2.3) was fused with the NlpA leader peptide.

2.3. Construction of scFv library

Head kidney and spleen were isolated from rainbow trout (mean weight, 20 g + 2.5) that survived an outbreak of IPN, that caused approximately 70% cumulative mortality. Tissues from 6 rainbow trout were harvested and pooled two month after the outbreak of IPN, and total RNA was extracted using Trizol. cDNA was synthesized from the total RNA sample using Superscript II (Invitrogen) and random hexamer oligonucleotide primers. Primers used to amplify the VH and VL gene fragment for construction of the antibody library were designed according to a previous study (Zhou and Xie, 2015). PCR products of the VH and VL genes were linked by overlapping PCR to construct the scFv antibody library. The linker peptide used in the study were typical $(Gly_4Ser)_3$ sequences. The VP2 gene was fused with the pelB leader peptide, and the antibody library was fused with the NlpA leader peptide. The recombinant plasmids were electroporated into *E. coli* DH5α according to standard procedures, and the library was labeled pCoex-VP2-scFv. The colonies were counted to calculate the transformation efficiency of electroporation. Library diversity was determined by sequencing five random clones obtained from the scFv library.