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Recombinant infectious hematopoietic necrosis virus expressing infectious pancreatic necrosis virus VP2 protein induces immunity against both pathogens

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ABSTRACT

Infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) are typical pathogens of rainbow trout. Their co-infection is also common, which causes great economic loss in juvenile salmon species. Although vaccines against IHNV and IPNV have been commercialized in many countries, the prevalence of IHNV and IPNV is still widespread in modern aquaculture. In the present study, two IHNV recombinant viruses displaying IPNV VP2 protein (rIHNV-IPNV VP2 and rIHNV-IPNV VP2COE) were generated using the RNA polymerase II system to explore the immunogenicity of IHNV and IPNV. The recombinant IHNV viruses were stable, which was confirmed by sequencing, indirect immunofluorescence assay, western blotting, transmission electron microscopy and viral growth curve assay. IHNV and IPNV challenge showed that the recombinant viruses had high protection rates against IHNV and IPNV with approximately 65% relative percent survival rates. Rainbow trout (mean weight 20 g) vaccinated with these two recombinant viruses showed a high level of antibodies against IHNV and IPNV infection. Taken together, our findings demonstrate that rIHNV-IPNV VP2 and rIHNV-IPNV VP2COE might be promising vaccine candidates against IHNV and IPNV.

1. Introduction

Infectious hematopoietic necrosis virus (IHNV) is a member of the *Novirhabdovirus* genus in the *Rhabdoviridae* family [1]. IHNV is regarded as one of the most significant threats to the salmonid farming industry worldwide. The virus causes hemorrhage and necrosis of hematopoietic tissue and other internal organs, with mortality rates as high as 100% [2,3]. IHNV was first described in Sockeye salmon (Oncorhynchusnerka) fry hatcheries in western North America in the early 1950s. It continued to spread to Japan, South Korea, and many European countries causing severe losses in the trout farming industry worldwide [4–10]. IHNV is a non-segmented negative-strand RNA virus with a genome length of approximately 11 kb that encodes six genes in the following order: nucleoprotein (N), polymerase-associated phosphoprotein (P), matrix protein (M), unique glycoprotein (G), and a large RNA-dependent RNA polymerase (L) [11–14]. Additionally, NV protein, located between the G and L genes, is a unique nonstructural protein of the *Novirhabdovirus* genus, which has been shown to be dispensable for IHNV replication in cell culture and is involved in viral pathogenicity [15–18].

Infectious pancreatic necrosis (IPN) is caused by infectious pancreatic necrosis virus (IPNV), which is a non-enveloped, double stranded RNA virus. IPNV affects Atlantic salmon at all stages, especially in young salmonid, causing mortality as high as 70% [19,20]. Among the five IPNV proteins, VP2 and VP3 are the major structural and immunogenic polypeptides of the virus [21]. The VP2 protein contains most of the neutralizing epitopes, which is related to its virulence. These epitopes are capable of eliciting the production of viral neutralizing antibodies against IPNV [22–25]. Vaccines encoding the recombinant VP2 gene had been shown to be sufficient to induce a protective immune response [26–29]. A previous study showed that the central variable domain (positions 243 to 335) of the VP2 coding region encompasses two hypervariable segments [30]. Marie et al. illustrated that the truncated VP2 protein (147–307 amino acids) expressed in bacterial, yeast, piscine, and mammalian cells is capable of inducing an *anti*-IPNV antibody response with antibody titers similar to those for

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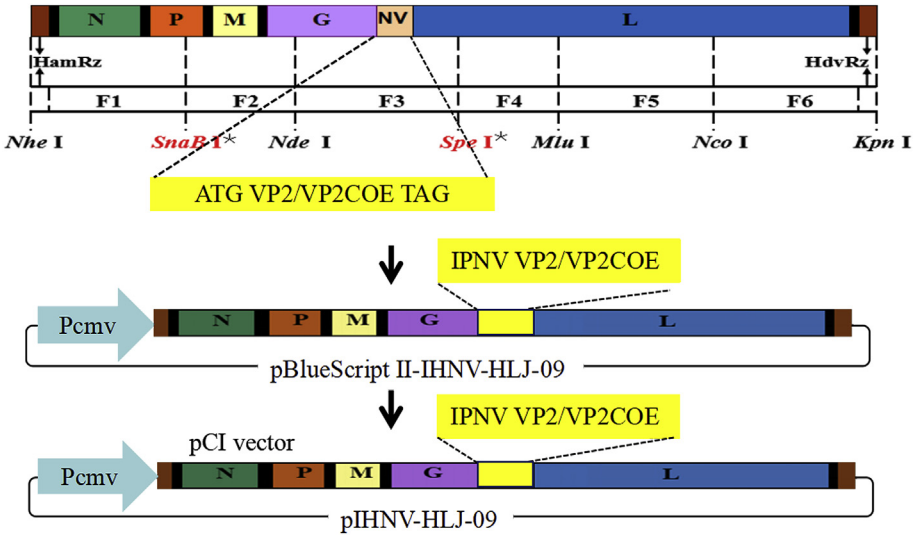

Fig. 1. Construction strategy of the pIHNV-VP2 and pIHNV-VP2COE plasmids. The IHNV NV gene was replaced with the IPNV VP2 or VP2COE gene. The initiator codon (ATG) and stop codon (TAG) were added to the ends of the VP2 or VP2COE genome. Restriction sites (*) presented in the genome were created as the genetic markers.

whole IPNV. Moreover, there is some evidence that a truncated form of the VP2 gene can also be used as an antigen to produce long lasting protection [31]. In this study, the IPNV VP2 and VP2COE genes (146–350 amino acids) containing the major neutralizing epitopes were used to replace the NV gene of IHNV for evaluating their efficacy against IHNV and IPNV.

IHNV and IPNV are widespread in salmonid hatcheries from the Americas to Europe, Asia, and Australia [32,33]. In general, IHNV and IPNV transmission occurs horizontally. Fish that survive an IHNV or IPNV infection become persistently infected and are carriers of the virus for a long period, consequently infecting other susceptible fish [34–36]. Because there are no efficient measures to control these diseases, development of effective vaccines has been one of the best methods to resist the invasion of these diseases in aquaculture farms. Several types of vaccines against IHNV have been designed, such as attenuated vaccines or killed virus and DNA vaccines [37–41]. However, only one DNA vaccine has been commercialized by the Canadian Food Inspection Agency [42]. Although various vaccines against IPNV such as inactivated vaccines [43], attenuated vaccines [44], DNA vaccines [45–47], and subunit vaccines [48–51] have been developed, they demonstrate an inability to induce a sufficient immune responses [24]. The poor immune response may be due to fish being sensitive to several pathogens concurrently in the field. Therefore, it is necessary to develop multivalent vaccines against two or more pathogens in aquaculture [52]. Xu et al. constructed a bivalent DNA vaccine based on the G gene of IHNV and the VP2–VP3 genes of IPNV, which induced protective immune responses against IHNV infection, IPNV infection, and co-infection with IHNV and IPNV in rainbow trout [52]. This demonstrates the feasibility of developing multivalent vaccines with the immunogenic polypeptides of the virus.

Reverse genetics is a powerful tool for modifying the viral genome and producing attenuated live vaccines, which may help to fight this rapidly spreading and emerging virus. A reverse genetics system for IHNV is currently available. Moreover, these recombinant viruses developed using reverse genetics are less pathogenic but maintain their immunogenicity *in vivo*, providing a new approach for vaccine development [53,54]. Therefore, in this study, we constructed two recombinant viruses using rIHNV-HLJ-09 as a vector replacing the NV gene with the VP2 or VP2COE gene of IPNV. We show that IHNV can be used as a gene vector as well as an antigen-presenting platform for vaccination purposes.

2. Materials

2.1. Cells, viruses, plasmids, and antibodies

Endothelial progenitor cells (EPCs) were obtained from the American Type Culture Collection (ATCC) and grown in L-15 (Leibovitz) supplemented with 10% fetal bovine serum (FBS). The Sp strain of IPNV from American Type Culture Collection (ATCC VR-1318) was used in this study [55]. The wild-type (wt) IHNV HLJ-09 strain (Accession number JX649101) was obtained from dead rainbow trout tissue from an acute disease outbreak at Bohai experiment station, Heilongjiang Academy of Agricultural Sciences in 2009 [56]. The rIHNV HLJ-09 recombinant virus was rescued by EPCs as described previously [57]. The G, N, P, L, and NV protein expression plasmids and the full-length genome plasmid pBlueScriptII-IHNV HLJ-09 were described previously [57]. The VP2COE protein expression plasmid (pCI-VP2COE) was constructed in our laboratory. Rabbit *anti*-IHNV and Rabbit *anti*-IPNV VP2 antibodies were prepared previously in our laboratory [29,57]. The rabbit *anti*-IgM was prepared from the healthy New Zealand rabbits as described previously [57]. Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, Tetraethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG, and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibodies were purchased from Thermo Fisher Scientific (Thermo, Waltham, MA, USA).

2.2. Plasmid construction

The recombinant plasmids were generated based on the pBlueScript II-IHNV HLJ-09 [57]. The IHNV NV gene was replaced with the IPNV VP2 or VP2COE gene. The recombinant F3 fragments (shown in Fig. 1) were amplified by two-step overlapping, and the six primer pairs used are shown in Table 1. The purified PCR products were sequenced by Comate Bioscience Company Limited (Jilin, China).

Considering the limitations of enzyme sites, the pBlueScript II-IHNV HLJ-09 plasmid was used as an intermediate vector. The VP2 and VP2COE segments, which replaced the NV gene, were cloned into the pBlueScript II-IHNV HLJ-09 plasmid by *Nde I* and *SpeI* respectively. The correct recombinant plasmids were designated pBlueScript II-IHNV-VP2 and pBlueScript II-IHNV-VP2COE. The pIHNV-VP2 and pIHNV-VP2COE recombinant plasmids were constructed by digesting *NheI* and *KpnI* using the pBlueScript II-IHNV-VP2 and pBlueScriptII-IHNV-VP2COE plasmids. The construct strategy is shown in Fig. 1.