Screening for protective antigens of Cyprinid herpesvirus 2 and construction of DNA



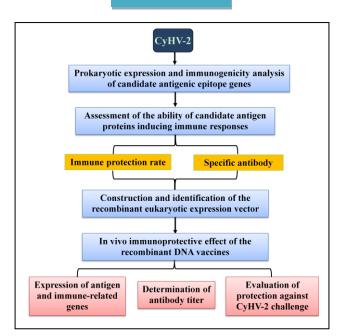
vaccines

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Abstract

In recent years, crucian carp hematopoietic necrosis caused by *Cyprinid herpesvirus* 2 (CyHV-2) infection has caused an enormous economic loss to the aquaculture industry. In this study antigenic epitope analysis was performed on the membrane proteins of CyHV-2, and 8 antigen-rich peptide fragments were selected for prokaryotic expression. Then, the immunogenicity of the recombinant proteins was analyzed. On this basis, DNA vaccines were constructed for immunization of hybridized Prussian carps. The protective effect of DNA vaccines against challenge in hybridized Prussian carps was evaluated.

Methods



Results

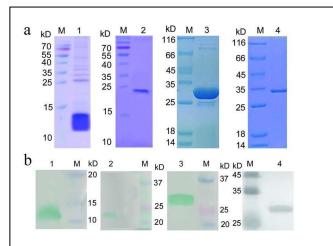


Fig. 1. SDS-PAGE and Western blot identification of the purified recombinant proteins. a is the SDS-PAGE electrophoretogram and b is the Western blot diagram.M is the protein marker. 1-4 are the purified recombinant ORF16, tORF25, tORF64, and ORF146 proteins, respectively.

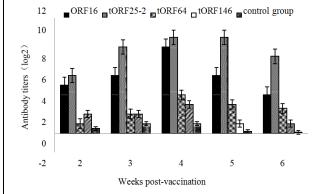


Fig. 3. Antibody titers determined by indirect ELISA in the immunized carps.

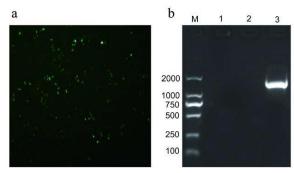


Fig. 4. Transfection with the recombinant plasmid, pEGFP-N1-ORF25.

a. Fluorescence pattern after transfection with the recombinant plasmid, pEGFP-N1-ORF25; b. RT-PCR result of ORF25 expression in the transfected cells. M. 2000 bp DNA marker; 1. Amplification products of the 293T cells transfected with the plasmid, pEGFP-N; 2. Amplification products of 293T cells; 3. Amplification products of 293T cells transfected with the plasmid, pEGFP-N1-ORF25.

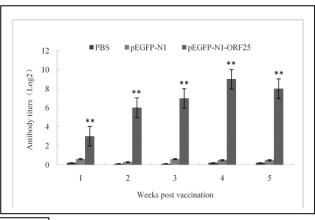


Fig. 5. Relative expression of the four cytokines in the kidneys of carps. "**" indicates an extremely significant difference (P<0.01). The level of cytokine expression in the control group was defined as 1.

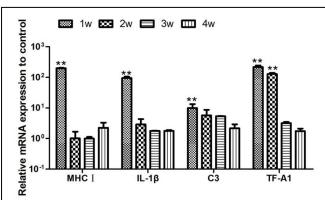


Fig. 6. Antibody titers detected by indirect ELISA at different time points post-immunization."**" indicates an extremely significant difference (P<0.01).

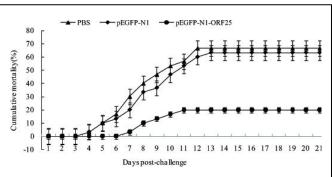


Fig. 7. Cumulative mortality in each experimental group post-challenge.

Conclusions

Our study showed that all 8 recombinant proteins were successfully expressed. Among the recombinant proteins, ORF16, tORF25, tORF64, and ORF146, gave a positive serum reaction with CyHV-2. Of the four proteins used for the immunization of silver crucian carps, the antibody titer induced by tORF25 was the highest. The DNA vaccine, pEGFP-N1-ORF25, was constructed based on ORF25 and able to induce production of specific antibodies in carps, while up-regulating the expression of MHC I, IL-1β, C3, and TF-A in the kidneys of carps. Moreover, the immunoprotective rate was increased to 70% in hybridized Prussian carps. The results showed that the DNA vaccine constructed based on the ORF25 gene had a greater immune protective effect and can be used as a candidate vaccine for immunoprotection against CyHV-2.

Significance statement

Hematopoietic necrosis caused by CyHV-2 infection has always been a difficult problem. In this study, we constructed a novel DNA vaccine based on the ORF25 gene, which has a strong immune protection effect. This vaccine can be used as a candidate vaccine against CyHV-2 infection.

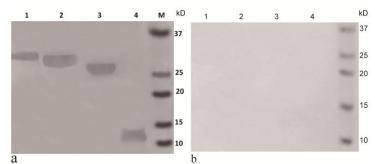


Fig. 2. reaction results of candidate antigen protein with crucian carp serum. a: The result of interaction between candidate antigen and CyHV-2 positive crucian serum; b: The result of interaction between candidate antigen and normal crucian carp. M is the protein marker, and 1, 2, 3 and 4 are the purified prokaryotic expression products of ORF16、tORF25-2、tORF64 and tORF146, respectively.