ENHANCEMENT OF IMMUNOGENICITY IN GLYCOPROTEIN-BASED DNA VACCINE BY THE ADDITION OF DDX41, A MOLECULAR ADJUVANT

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ABSTRACT

The emergence of the first commercialized DNA vaccine for fish had prompted for studies that are focused on the development of a more efficacious vaccine that can protect cultured fish against virus infection. This DNA vaccine designed to express viral glycoprotein (G) gene was proven to effectively give protection to several species of fish against rhabdoviruses (ex. viral hemorrhagic septicemia virus, VHSV). Thus, the addition of molecular adjuvant to the DNA vaccine has been considered to be one of the best methods in improving the vaccine's capability since its presence triggers a more enhanced immune response. DDx41, a cytosolic sensor, has the ability to activate an antiviral cascade of events in response to stimulation of a DNA virus making it a good molecular adjuvant candidate. In the present study, we designed a DNA vaccine consisting of VHSV glycoprotein (VHSVg) and DDx41 which is regulated by EF-1α and CMV promoters, respectively. The aim is to develop a DNA vaccine that can elicit a stronger immune response that could protect the fish against VHSV infection. Four plasmid constructs were prepared for the experiment: pEF-A (empty vector), pEF-G (with VHSVg), pEF-D (with DDx41) and pEF-GD (both VHSVg and DDx41). Expression of individual genes was checked using Western Blot assay, VHSVg in pEF-G and DDx41 in pEF-D were detected using *V5* antibody while DDx41 in pEF-GD was detected using *myc* antibody. Several immune response genes were also evaluated through qPCR to further elucidate the antiviral effect of the plasmid constructs. ΔΔCt method was utilized to quantify the fold changes of the immune gene transcripts after immunization. qPCR results showed that there are significant increases in the transcript number of IRF-1, ISG-15, and IRF-3 ($p < 0.05$) as well as IFN-1 ($p<0.1$) in cells that were immunized with the vaccine-adjuvant construct, pEF-GD, compared to the control plasmid, pEF-A. The results implicated the strength of DDx41 in inducing IFN-mediated immune responses which demonstrates its ability as a molecular adjuvant. This is essential since majority of the DNA vaccines against fish rhabdovirus that we have now are purely based on viral glycoprotein and though it has been proven to be effective, the development of a more efficient DNA vaccine that can improve fish immunity against rhabdovirus infection is significantly beneficial to the aquaculture industry.

KEYWORDS

DDX41, glycoprotein, VHSV, DNA vaccine, molecular adjuvant

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