Development of novel DNA vaccines by using genetically modified antigens of fish pathogens

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Development of novel DNA vaccines by using genetically modified antigens of fish pathogens (魚類感染症に対する組み換え抗原を用いた新規 DNA vaccine の開発)		

Vaccination is one of the prophylactic methods to protect the animals against disease. DNA vaccination have shown to induce immunity against viral and bacterial pathogens in fish, however, the induced protection showed variable results, which demands the search for new approaches to improve the vaccination efficacy. The use of sorting signals associated with an antigen in a DNA vaccine have shown promising results in animal models, taking advantage of the different sorting motifs of molecules to drive the movement of the antigens inside the cell. Among them, sorting signals from lysosomal membrane proteins can be candidates to improve the efficacy of a DNA vaccine. In the present study, lysosome-associated membrane protein-1 from Japanese flounder, *Paralichthys olivaceus*, (JfLAMP-1) was used as a carrier for the major capsid protein (MCP) from red sea bream iridovirus (RSIV) in order to evaluate its potential as DNA chimeric vaccine. First, JfLAMP-1 gene ORF was obtained by analyzing EST data from previous study in our lab and amplified by using specific primers. JfLAMP-1 amplicon was cloned in T vector, sequence was confirmed and bioinformatics analysis was done. Tissue expression analysis by RT-PCR and qPCR from gill, brain, muscle, liver, spleen, intestine, kidney, blood and Hirame natural embryo cell line was assessed in healthy animals. JfLAMP-1 gene expression in spleen was assessed under poly I:C (polyinosinic:polycytidylic acid) stimulation at 22℃ and Edwardsiella tarda FKC (Formalin-killed cells) injection at 15 ℃ and 22 ℃. JfLAMP-1 expression was assessed in HINAE cells by western blot and localization of the protein was evaluated by immunofluorescence assay. JfLAMP1 gene has a length of 1248 bp that encodes for 415 aa (43,8 kDa) and exhibit a signal peptide, a luminal domain, transmembrane domain and cytoplasmic domain similar with reported in higher vertebrates. JfLAMP-1 gene expresses constitutively in all the tissues with a higher expression in brain. In E. tarda FKC injection experiment, JfLAMP-1 mRNA level showed higher at 3 h, 12 h and 7 days post-injection at 22 ℃ and 1 day and 7 days post-injection at 15 ℃. In poly I:C stimulation, JfLAMP-1 showed no changes in the expression at mRNA level. In the protein analysis, JfLAMP-1 was detected in HINAE cells as a 56 kDa band and the immunofluorescence analysis showed it distributed in small and large granules in the cytoplasm and grouped close to the nucleus. After its characterization, JfLAMP-1 was fused with the MCP from RSIV to produce a chimeric DNA vaccine. For this, the DNA encoding the luminal domain of JfLAMP-1 was replaced with the gene for the RSIV MCP, and the construct was cloned in an expression vector (pCIneo). Japanese flounder juveniles (n=30) were distributed in the experimental groups (pCIneo, pCMCP and pCLAMP-MCP), vaccinated and the antibody titers measured 30 days post-vaccination. Fish vaccinated with the chimeric vaccine pCLAMP-MCP showed significantly higher antibody levels than fish vaccinated with pCIneo vector harboring the MCP gene ($p<0.05$). Then a new chimeric vaccine was designed, inserting the MCP gene and keeping the luminal domain (LumD) of JfLAMP-1 gene. In this experiment, Japanese flounder juveniles (n=60) were distributed in six groups (PBS, pCIneo, pCLAMP, pCMCP, pCLAMP-MCP and pCLAMP-MCP-LumD). After 30 days of vaccination, fish vaccinated with the chimeric vaccines showed significantly higher antibody levels than those vaccinated with pCIneo vector harboring the MCP gene ($p<0.05$). The inclusion of the LumD did not induce statistically higher antibody titer than the pCLAMP-MCP. Then, a vaccination and challenge test were performed using JfLAMP-1 chimeric vaccine in a highly susceptible fish species to RSIV. For this, striped

beakfish, Oplegnathus fasciatus, individuals were distributed in four experimental groups (PBS, pCIneo, pCMCP and pCLAMP-MCP; n=30 per group) and after 30 days of vaccination, a challenge test was done by using RSIV in low and high dose. In the vaccinated group challenged with low dose of RSIV, pCMCP and pCLAMP-MCP showed similar relative percentage of survival of 13%, however in the high dose challenge, pCLAMP-MCP vaccinated group showed relative percentage of survival of 19%, compared with 0% of the pCMCP. JfLAMP-1 chimeric vaccine induced higher protection than conventional DNA vaccine. In conclusion, chimeric DNA vaccines using sorting signals from specific molecules can be candidates to enhance the immune response against specific pathogens, for example, by modulation of the traffic of antigen.