マダイイリドウイルス病に対する有効なDNAワクチン開発のための研究

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract of Dissertation

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論文題目 Title	Studies for the development of an effective DNA vaccine against red sea bream iridovirus disease		

The World Organization for Animal Health (WOAH) has classified the red sea bream iridovirus (RSIV) as an infectious agent that affects Japanese aquaculture. The infectious disease caused by RSIV results in significant financial losses. The red sea bream iridovirus is a double-stranded DNA virus with an icosahedral shape and a diameter of 200–240 nm. It is a member of the genus Megalocytivirus, which also contains the infectious spleen and kidney necrosis virus. About 120 possible open reading frames (ORFs) can be found in the roughly 110 kbp long DNA genome (Kawato et al., 2017). Comprehensive analyses of the expression kinetics of viral genes in cultured cells (Lua et al., 2005) and juvenile red sea breams (Dang et al., 2007) have resulted in the classification of these genes as immediate-early, early, and late genes. This data can be utilized to find antigens for the creation of new vaccines as well as to comprehend the mechanism of RSIV infection.

WOAH recommended using vaccines for illness prevention, and these days, commercial vaccines are accessible in Japan. The formalin-killed vaccine against RSIVD have been approved and used in red sea bream, striped jack, and Seriola species in Japan. Nevertheless, it is challenging to monitor immunization effectiveness in the field. Additionally, a different kind of vaccination was being researched. These include a recombinant protein vaccine, which protected against RSIV infection (Hajime et al., 2010), an attenuated lived vaccine, which elicited a protective immune response against RSIV (So-Young et al., 2014), and a DNA vaccine, which produced a foreign protein through immunization with antigen-encoding plasmid DNA and elicited a humoral and cellular immune response (Park et al., 2005).

Since DNA vaccines mimic viral infection in the host organism, they will enable the development of more effective vaccines against viral infections in the future. To develop more effective vaccines, it is crucial to simultaneously analyze the mechanisms of viral infection and host immunity. Therefore, the objective of this study is to develop DNA vaccines and investigate virus infection mechanisms.

First, DNA and recombinant vaccines encoding two antigen-candidate genes, which have been reported to be antigens in Japanese yellowtail by Matsuyama et al. (2018), were evaluated in Red Sea Bream (*Pagus major*). DNA vaccines were prepared for two candidate antigen genes, ORF 111 and 450. These vaccines were administered intramuscularly to juvenile red sea bream. Four weeks after inoculation, 10^3 and 10^4 copies of the virus were intraperitoneally injected. In the group injected with 10^4 copies, the survival rate of the DNA vaccine group was 89%, compared to 80% in the control group, whereas the survival rates of the DNA vaccine and PBS group were 72% and 80% in the fish injected with 10^3 copies. No increase in antibody titers against each antigen was observed. A recombinant protein for the candidate antigen gene ORF111 was prepared, and the effects of the DNA vaccine and recombinant protein vaccine were compared. Challenge tests were conducted using 10^4 copies of the virus at 4 weeks post-vaccination. Survival rates of 98% and 88% were observed in the DNA vaccine and recombinant protein groups, respectively, with no deaths in the control group. The recombinant protein vaccine increased specific antibody titers against the antigen, but the results of the challenge test did not allow for a conclusive evaluation of the effectiveness of each vaccine.

Second, to investigate how RSIV replicates in the host and how the host responds to the virus, comprehensive gene expression analysis was performed during RSIV infection in rock bream (*Oplegnathus*

fasciatus), a species particularly susceptible to the virus. After intraperitoneal injection of the viral solution at 10^4 TCID50/fish, the viral genome copy number in the spleen was $10^{4.7 \pm 0.2}$ and $10^{5.9 \pm 0.4}$ copies/μg DNA at 3 and 5 days post-injection (dpi), respectively. By transcriptomic analyses using MiSeq, 6 viral genes, including RING finger domain-containing protein and laminin-type epidermal growth factor-like domain genes, were strongly detected at 3 and 5 dpi. The other virus genes were significantly expressed at 5 dpi. By differentially expressed gene analysis, 334 host genes were identified in comparison to those before infection. They were clustered into four groups based on their expression profiles. Among the immune-related genes, interferon-stimulated genes were strongly upregulated. On the other hand, inflammation-related genes, such as granzyme and eosinophil peroxidase genes were downregulated at 3 dpi. Downregulation of certain genes may contribute to the susceptibility of this fish to the virus.

Although DNA vaccines are important for the future development of viral vaccines, the results suggest that their effectiveness may be weak depending on the choice of antigen. In addition, the expression dynamics of viral genes *in vivo* differ from gene to gene, and the results obtained in this study will contribute to future vaccine development. Furthermore, by analyzing the details of the genes whose expression changes with viral infection, it will be possible to develop more effective DNA vaccines through the development of new adjuvants and other means.