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Induction of antiviral state in fish cells by Japanese flounder, *Paralichthys olivaceus*, interferon regulatory factor-1

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Abstract

Interferon regulatory factor-1 (IRF-1) mediates an antiviral state in cells by regulating the expression of the interferon (IFN-α/β) system. To elucidate the role of IRF-1 in fish during virus infections, we constructed a recombinant plasmid of the Japanese flounder, *Paralichthys olivaceus* IRF-1 (JF IRF-1) under the control of the cytomegalovirus (CMV) immediate/early enhancer promoter. The antiviral mechanism of JF IRF-1 was studied using transfection experiments in a homologous cell line. Here, we show that cell supernatants obtained from transiently transfected cells enhanced cell viability of a heterologous cell line upon incubation, reduced the titers of hirame rhabdovirus (HIRRV) and viral hemorrhagic septicemia virus (VHSV), and possessed cytokine-like activity, as shown by their ability to protect cells against virus infections. The supernatants also inhibited the replication of the rhabdoviruses during the early stages of infection as indicated by the reduction of viral titers in the presence of the supernatants obtained from the transfected cells. Further analysis showed that the cell culture supernatants contain cytokine-like substances that possess acid-labile and temperature-resistant properties. These results indicate that JF IRF-1 induces an antiviral state in cells by mediating the production of cytokine-like substances. Thus, JF IRF-1 might be useful as an adjuvant in the development of DNA vaccines against commercially important viral pathogens in Japanese flounder aquaculture.

Keywords: Japanese flounder; *Paralichthys olivaceus*; IRF-1; Rhabdovirus; Antiviral mechanism

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1. Introduction

The regulation of interferon (IFN) genes and IFN-inducible genes in mammals is mediated by transcription factors, called interferon regulatory factors that bind to sequence elements in the 5′ flanking regions of their promoters [1]. Several such transcription factors have been identified, including IRF-1 through IRF-9 [2–4]. IRF-8 is also known as ICSBP and IRF-9 is also known as p48, ISGF3γ. These transcription factors have been shown to have a role in activating the IFN-α/β system in the host response during viral infection [5].

Human IRF-1, which has been well characterized, activates cellular genes inducible by IFN and other cytokines [6]. Other cellular functions attributed to IRF-1 include: targeting of the *cis*-elements in viral promoters [7]; suppression of tumor activity [8]; acting as an intermediate in some signal pathways leading to cell apoptosis [3]; regulating T-cell selection and differentiation [9]; and playing a key role in some inflammatory responses and autoimmune diseases [3].

In mammals, IRF-1 appears to promote viral resistance against some RNA viruses [10]. GM-637 and L929 cells expressing IRF-1 were shown to have reduced susceptibility to encephalomyocarditis (EMC) virus infection [11,12]. However, a chicken IRF-1 failed to confer viral resistance upon transfection in a homologous fibroblast cell line [9]. In fish, a novel IRF cDNA was cloned in Japanese flounder, *Paralichthys olivaceus* [13]. It has a length of 1746 bp and encodes 297 amino acids. Its amino acid sequence shares approximately 40% identity with the avian and mammalian IRF-1 and IRF-2. The carboxy-terminal region is not particularly rich in basic amino acid residues, which suggests that the cloned IRF cDNA is a member of the IRF-1 family [13].

Fish were found to produce molecules with interferon (IFN)-like activity [14] based on a cell protection assay [15]. Recently, IFN genes have been cloned from zebrafish, *Danio rerio* [16], Atlantic salmon, *Salmo salar* [17], and from channel catfish, *Ictalurus punctatus* [18]. These IFN-like substances were produced either in response to a virus infection or by incubation with dsRNA polyinosinic: polycytidylic acid (poly I:C) [14,15,19–21]. Since IRF-1 regulates IFN activity in fish [22], our present study aimed to determine whether the Japanese flounder IRF-1 (JF IRF-1) has a role in the induction of an antiviral state in cells during virus infection. We obtained supernatants from a homologous cell line transiently transfected with JF IRF-1 and tested their effects on cellular viability as measured by the MTT assay as well as their ability to protect a heterologous cell line, epithelial papilloma of carp (EPC) against hirame rhabdovirus (HIRRV) and viral hemorrhagic septicemia virus (VHSV) infection. We used a heterologous cell line to test the protective effect of the supernatants because we wanted to eliminate the additive effect of the production of cytokine-like substances in homologous cells as a consequence of virus infection. We also tested whether the supernatants have the ability to suppress rhabdoviral replication during the early stages of replication by determining their titers in a susceptible cell line. Both these viruses are negative single-stranded RNA viruses that pose serious threats to both cultured and wild-caught Japanese flounder [23–25]. The results of these in vitro studies raise the possibility of using JF IRF-1 as a vaccine adjuvant for controlling viral infections in Japanese flounder aquaculture.

2. Materials and methods

2.1. Construction of plasmid expressing JF IRF-1

pGEM T-Easy vector (Promega, Madison, WI, USA) ligated with the full length Japanese flounder interferon regulatory factor-1 cDNA (GenBank accession number AB005883) was digested with *Eco*RI, purified with phenol:chloroform:isoamyl alcohol and the resulting *Eco*RI-digested JF IRF-1 fragment was