Fish & Shellfish Immunology 30 (2011) 317–323

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Molecular cloning, characterization and expression analysis of *interferon-β promoter stimulator 1* (*IPS-1*) gene from grass carp *Ctenopharyngodon idella*

Jianguo Su*, Teng Huang, Chunrong Yang, Rongfang Zhang

College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling 712100, China

ARTICLE INFO

Article history:
Received 19 September 2010
Received in revised form
29 October 2010
Accepted 4 November 2010
Available online 13 November 2010

Keywords:
Grass carp (*Ctenopharyngodon idella*)
IPS-1
Gene cloning
mRNA expression
Grass carp reovirus

ABSTRACT

IPS-1 (*interferon-β promoter stimulator 1*), also known as *MAVS/VISA/Cardif*, plays a central role in antiviral immunity. In this manuscript, we cloned and characterized *IPS-1* from grass carp *Ctenopharyngodon idella* (designated as *CiIPS-1*). The *CiIPS-1* cDNA is 2412 bp long and consists of a 5′ untranslated region (UTR) of 124 bp, a 3′ UTR of 497 bp with three cytokine RNA instability motifs (ATTTA) and a polyadenylation signal (AATAAA), and an open reading frame (ORF) of 1791 bp encoding a polypeptide of 596 amino acids with a calculated molecular mass of 64.1 kDa and a theoretical isoelectric point of 4.79. Structural analysis showed that the *CiIPS-1* protein contained an N-terminal CARD (caspase activation and recruitment domain), a central proline-rich domain, a putative TRAF2-binding motif and a C-terminal transmembrane domain. Similarity analysis of the deduced amino acid sequence of the *CiIPS-1* by MatGAT software revealed that the *CiIPS-1* shared 27.8–76.4% identity and 47.4–85.2% similarity with other known piscine *IPS-1* sequences. The *CiIPS-1* mRNA was constitutively expressed in the examined tissues, higher in spleen, and was induced by grass carp reovirus (GCRV) injection by semi-quantitative RT-PCR assay. Quantitative real-time RT-PCR analysis revealed that the *CiIPS-1* mRNA expression was rapidly and significantly up-regulated *in vivo* and *in vitro* after GCRV infection, and the *CiIPS-1* transcripts were also significantly enhanced *in vitro* post the synthetic double stranded RNA polyinosinic–polycytidylic potassium salt (poly (I:C)) stimulation. These results indicated that *CiIPS-1* was an inducible acute-phase protein and involved in the immune reaction to GCRV in grass carp.

1. Introduction

The innate immune system is an evolutionarily conserved system that provides the first line of protection against invading microbial pathogens through germ line-encoded pattern recognition receptors (PRRs) that recognize different but overlapping pathogen-associated molecular patterns (PAMPs) [1]. PRRs discriminate between self and non-self. They interact with products of infectious agents to activate cells of the innate immune system and also stimulate the adaptive immune system [2]. Up to now, at least three families of PRRs, such as the Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), have been identified [3]. TLRs, a well-known class of PRRs, are expressed on cell surfaces or in endosomes, and recognize PAMPs from viruses, bacteria, fungi and protozoa [2]. NLRs are known to main functions in bacterial detection [4]. Melanoma differentiation associated gene 5 (*MDA5*, also known as *IFIH1* or *Helicard*), retinoic acid induced protein I (*RIG-I*, also known as *DDX58*) and laboratory of genetics and physiology 2 (*LGP2*, also known as *DHX58*) compose RLR gene family, which specifically recognize viral RNA in the cytoplasm [5].

The recent findings of *MDA5* and *RIG-I* as cytoplasmic primary sensors of RNA viruses for induction of type I IFNs highlight the RLR pathway in antiviral innate immunity [6]. This signaling pathway is obviously different from that mediated by TLRs and constitutes a major pathway activated by viral infection [7]. Analysis of *MDA5* or *RIG-I* knockout mice demonstrates that this pathway is central for innate immunity against viral infection [8,9]. In zebrafish, the mRNA expression of molecules participating in RLR pathway are much more sensitive and specific to poly(I:C) induction compared with those in TLR antiviral pathway [10].

IPS-1 (*interferon-β promoter stimulator 1*) is the sole adaptor in both RIG-I and MDA5 signalings that mediate effective responses against a variety of RNA viruses [11]. In 2005, first *IPS-1*, also known as MAVS/VISA/Cardif, was identified by four independent research groups as an important adaptor linking RIG-I/MDA5 to the downstream signaling molecules [12–15]. Once intracellular viral dsRNA or ssRNA is sensed by *RIG-I* and *MDA5*, *RIG-I/MDA5* change

* Corresponding author. Tel.: +86 29 87092139; fax: +86 29 87092164.
E-mail address: su.jianguo@gmail.com (J. Su).

1050-4648/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2010.11.010

conformation, enabling interaction with the adaptor *IPS-1* via the CARD (caspase activation and recruitment domain) and subsequently activate inhibitors of κB kinase (IKK)-α, -β, -ε, and TANK-binding kinase 1 (TBK1) to phosphorylate various transcription factors, including interferon regulatory factor-3 (IRF3), nuclear factor-κB (NF-κB), and activating transcription factor-2 (ATF-2)/c-Jun. These transcription factors directly activate type I interferon promoters and downstream inflammatory cytokines [16]. *IPS-1* plays a central role in innate antiviral immunity [17].

At the beginning of discovering *IPS-1*, it was reported that *IPS-1* was sited on mitochondria [14]. Recent studies found that *IPS-1* was located on peroxisomes and mitochondria. Upon viral infection, peroxisomal *IPS-1* induced the rapid interferon-independent expression of defense factors that provided short-term protection, whereas mitochondrial *IPS-1* activated an interferon-dependent signaling pathway with delayed kinetics, which amplified and stabilized the antiviral response [18,19].

Some *IPS-1* genes have been identified in mammals and fishes. Lots of studies have been done in human *IPS-1*, such as location, structure, functions, enhancer, inhibitor and other mechanisms [18,20–24]. In fishes, there are five *IPS-1* sequences deposited in GenBank till now, including *Salmo salar*'s (accession No., NM_001168352), *Danio rerio*'s (accession No., FN178460), *Pimephales promelas*' (accession No., FN178455), *Paralichthys olivaceus*' (accession No., HM113533) and *Oncorhynchus mykiss*' (accession No., FN396360). However, the strongly inducible activation of IFN promoter was only verified by overexpression of *S. salar IPS-1* [25]. The antiviral role of *IPS-1 in vitro* was just confirmed in *D. rerio* and *P. promelas* [25,26]. *S. salar* and *D. rerio IPS-1* transcripts were induced by the synthetic double stranded RNA poly-inosinic–polycytidylic potassium salt (poly(I:C)) in TO cells and adult fish, respectively [25,10].

We employ grass carp (*Ctenopharyngodon idella*) as a model for antiviral immune studies because it is a crucial aquaculture species in China and is susceptible to grass carp reovirus (GCRV), a lethal virus. Better understanding of the immune defense mechanisms may contribute to the development of management strategies for disease control and long-term sustainability of grass carp farming.

In the present study, we cloned and characterized *C. idella IPS-1* (*CiIPS-1*) gene and examined the mRNA expressions in different tissues, *in vivo* and *in vitro* after GCRV infection and *in vitro* post poly(I:C) stimulation, which lay a foundation for further functional studies. After submission of the present work, an article was published, describing cloning and antiviral activity of *IPS-1* in Japanese flounder, *P. olivaceus* [27].

2. Materials and methods

2.1. Degenerate primer design and PCR amplification

When we started this work, there was just predicted *IPS-1* sequence of *D. rerio* in fish in GenBank. To identify *IPS-1* cDNA sequence from grass carp, degenerate primers were designed, based on the multiple alignments of the *IPS-1* sequences in *D. rerio* (accession No., FN178460), *Bos taurus* (accession No., NM_001046620), *Canis familiaris* (accession No., NM_001122609), *Sus scrofa* (accession No., NM_001097429), *Mus musculus* (accession No., NM_144888) and *Homo sapiens* (accession No., BC044952). PCR was set up with degenerate primers IF103a and IR104a (Table 1) using the cDNA generated from grass carp spleen. The PCR product was ligated into pMD18-T easy vector, transformed into the competent *E. coli* TOP10 cells, and plated on the LB-agar petri-dish. Positive colonies containing expected size insert were screened by colony PCR. Three of them were picked up and sent to a commercial company (Nanjing Jinsite Biotechnology Co., Ltd, China) for sequencing.

2.2. Cloning the full-length CiIPS-1 cDNA

Rapid amplification of cDNA ends (RACE) was carried out using the 5′ RACE system (Invitrogen) and BD SMART™ RACE cDNA

Table 1
Primers used in the study.

Primer name	Sequence (5′→3′)	Amplicon length (nt) and primer information
CiIPS-1		
IF103a (forward)	TTAYCTGCMATGCCTCAC	147
IR104a (reverse)	TTCYCCAGRGCWGWGATGAA	Gene cloning
IF162a (forward)	CACAATCACTGATAGGGAAGAGGTC	3′RACE
IF163a (forward)	GGACAATCTACGCAGACGAGAGAAC	
IR180a (reverse)	GTTCTCTCGTCTGCGTAGATTGTC	5′RACE
IR181a (reverse)	GCATAGCAGTGAAGTTTCCAGAAG	
IF183a (forward)	CAGGGACACAAAGAAGAGTT	2142
IR184a (reverse)	GGTTATGTAGGATAGAAGGC	Confirming sequence
IF217 (forward)	GACCGTAAGAAGTCAGCCTCC	111
IR218 (reverse)	CCTGAATAACTCTTGATAGCCCTC	qRT-PCR
18S rRNA		
18F99 (forward)	ATTTCCGACACGGAGAGG	90
18R100 (reverse)	CATGGGTTTAGGATACGCTC	qRT-PCR
EF1α		
EF125	CGCCAGTGTTGCCTTCGT	99
ER126	CGCTCAATCTTCCATCCCTT	qRT-PCR
Universal adaptor primer		
UPM	Long: CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT Short: CTAATACGACTCACTATAGGGC	3′RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	
3′-RACE primer		
3′-CDS	AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}$VN	
5′-RACE adaptor primer		
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	5′RACE
AUAP	GGCCACGCGTCGACTAGTAC	

Note: Y = C/T; M = A/C; R = A/G; W = A/T; V = A/G/C; N = A/G/C/T.