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Short communication

Functional characterization of interferon regulatory factor 5 and its role in the innate antiviral immune response

Kete Ai[a,b,1], Kai Luo[a,1], Lihai Xia[a], Weihua Gao[a], Wei Hu[a,c], Zhitao Qi[a,c], Qiaoqing Xu[a,c,*]

[a] Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education, Jingzhou 434020, China
[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province 430072, China
[c] School of Animal Science, Yangtze University, Jingzhou 434020, China

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ABSTRACT

In mammals, type I interferons (IFNs) are primarily regulated by transcription factors of the IFN regulatory (IRF) family. Interferon regulatory factor 5 (IRF-5) plays pivotal roles in antiviral and inflammatory responses. In the present study, we found that zebrafish (*Danio rerio*) IRF5 is a key player in the regulation of the expression of type I IFN and its antiviral immune response. IRF5 was upregulated in zebrafish embryonic fibroblast cells (ZF4) when challenged with grass carp reovirus (GCRV). Moreover, the expression profiles of Mx, IFN, Viperin, and IRF7, but not IRF3, were upregulated by overexpression of IRF5 in Epithelioma papulosum cyprinid cells (EPCs). Luciferase assays revealed that the activation of the IFNϕ1 promoter was stimulated by overexpression of IRF5 and IRF5-△IAD (IRF5 lacking the IRF-associated domain), respectively. However, overexpression of IRF5 or IRF5-△IAD inhibited the activity of the IFNϕ3 promoter. IRF5-△DBD (lacking the DNA-binding domain) had no influence in the activation of the IFNϕ1 and IFNϕ3 promoters. Furthermore, the determination of the cytopathic effect (CPE) numbers and viral titers revealed that the viral concentration was reduced by ectopic expression of IRF5 in EPC cells. Ectopic expression of IRF5 in EPC cells could protect cells from GCRV and significantly inhibited GCRV virus replication. These data indicated that IRF5 could limit viral replication through an IFN-dependent pathway.

1. Introduction

Interferon (IFN) regulatory factors (IRFs) are a family of transcription factors that bind a specific DNA motif known as the IFN-stimulated response element (ISRE), and have critical effects on their target genes. IRFs have diverse biological functions, including the induction of both innate and adaptive immune immunity, antiviral response, proliferation, apoptosis, and tumor proliferation [1,2]. The IRF family has nine members in mammals, ten members in birds, and eleven members in fish [2–6]. All IRFs share a well-conserved DNA-binding domain (DBD), which is characterized by five tryptophan repeats, and is responsible for binding the promoters of target genes [1]. The C-terminus of IRFs possesses an IRF-associated domain (IAD), which is conserved in IRF3–10. The IAD mediates the interactions between IRFs or other transcription factors and other proteins to form complexes [7].

Mammalian IRF5 has multiple functions, including regulating the expression of IFN-α and IFN-β to participate in the antiviral response, activating inflammatory factors, and tumor suppression [8–10]. IRF5-mediated activation is a virus and cell type-specific immune response [11–13]. IRF5 is generally involved downstream of the TLR–MyD88 signaling pathway for gene induction of proinflammatory cytokines, such as interleukin-6 (IL-6), IL-12 and TNFα [9]. IRF5 is also critical for the retinoic acid-inducible gene I (RIG-I) immune pathway. IRF-5 regulates the type I IFN response in myeloid dendritic cells (DCs) downstream of MAVS signaling [14] and mediates the Dectin-Syk signaling pathway controlling IFN-β expression by DCs in response to *Candida albicans* [15]. IRF5 was also reported to be involved in NOD2-mediated IFN induction [16]. However, the biological function and signaling pathways of IRF5 have not been characterized in teleosts.

In teleosts, IRF5 has been characterized recently in *Ctenopharyngodon idella*, *Danio rerio*, *Polyodon spathula*, *Scophthalmus maximus*, *Paralichthys olivaceus*, *Oplegnathus fasciatus*, *Cynoglossus semilaevis*, and *Cyprinus carpio* L, and is upregulated by grass carp reovirus (GCRV), turbot reddish body iridovirus (TRBIV), lymphocystis disease virus (LCDV), poly(I:C), iridovirus, IFN1, and LPS, indicating that fish IRF5 is involved in the antimicrobial process of the innate immune response [17–24]. Thus, in the present study, we report the functional characterization of zebrafish (*Danio rerio*) IRF5 in the regulation of IFN

* Corresponding author. School of Animal Science, Yangtze University, Jingzhou 434020, China.
E-mail address: xuqiaoqing@163.com (Q. Xu).
[1] The two authors contributed equally.

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gene transcription. The expression of IRF5 was studied in ZF4 cells in response to GCRV infection. Overexpression of IRF5 in EPC cells could activate the expression of IFN, Viperin, and IRF7. Luciferase assays revealed that the regulation of the IFNϕ1 and IFNϕ3 promoters was affected by overexpression of IRF5. Furthermore, domain analysis demonstrated that the DBD had the same influence on these promoters as full-length IRF5. Lastly, overexpression of IRF5 in EPC cells could protect cells from GCRV infection. Thus, we demonstrated that IRF5 plays a role as a key cytokine in the antiviral immune response in teleosts.

2. Materials and methods

2.1. Cells and viruses

ZF4 cells were cultured at 28 °C, 5.0% CO_2 in Dulbecco's minimal Eagle's medium (DMEM)/F12 medium supplemented with 10% fetal bovine serum (FBS). Epithelioma papulosum cyprinid cells (EPC) cells were grown at 28 °C, 5.0% CO_2 in M199 medium supplemented with 10% fetal bovine serum (FBS). GCRV (double-stranded (ds) RNA virus) was propagated in EPC cells as described previously [25].

2.2. Gene cloning and plasmids

The DNA sequences were retrieved from the NCBI database and the specific primers were designed using Primer premier (version 5.0). The accession numbers and primers were shown in Table 1. The open reading frame (ORF) of IRF5, IRF5-△DBD (aa 115–516), and IRF5-△IAD (aa 1–286) deletion mutants were generated by PCR and cloned into the Hind III/Kpn I restriction endonuclease sites of vector p3xFLAG-CMV-14. The IFNϕ1 and IFNϕ3 promoters were amplified from the genome of zebrafish tail fin and cloned into the Xho I/Hind III and Kpn I/Xho I restriction endonuclease sites of the pGL3-Basic luciferase reporter vector. The zebrafish IFN promoters were described previously [26]. All plasmid constructs were verified by sequencing analyses.

2.3. Transient transfection and virus infection

Using Lipofectamine® 2000 (Invitrogen, USA), transient transfection was performed according to the manufacturer's instructions. EPC cells were seeded in 24-well plates overnight and transfected with 0.5 μg of plasmid DNA in 100 μL of Opti-MEM (Invitrogen). The medium was replaced by free M199 medium with 10% FBS at 6 h. The total RNA was extracted for quantitative real-time PCR (qPCR) analysis at 48 h post transfection.

ZF4 cells were seeded in 12-well plates overnight before stimulation at a concentration of 6×10^5 cells per well. The cells were washed and infected with GCRV at a multiplicity of infection (MOI) of 5 and incubated at 25 °C for 3, 6, 12, 24, and 48 h. Total RNA was extracted for qPCR analysis.

2.4. RNA extraction and qPCR

Total RNAs of ZF4 cells and EPC cells were extracted using the Trizol reagent (Invitrogen). The cDNA was synthesized using Oligo $(dT)_{18}$ primers according to the manufacturer's instructions of RevertAid First Strand cDNA Syntheisi Kit (Thermo Scientific, made in (EU) Lithuania). The qPCR was performed using a BIO-RAD real-time PCR system with the iQTM SYBR® Green Supermix, according to the manufacturer's instructions. The qPCR reactions were performed in a 20 μL volume containing 10 μL of iQTM SYBR® Green Supermix, 1 μL of 10 μM primers, 8 μL of nuclease-free water, and 1 μL of cDNA template. The following thermocycling conditions were used to determine the expression profiles for each gene: 95 °C for 3 min; followed by 40 cycles of 95 °C for 10 s, 60 °C for 25 s, 72 °C for 20 s, and 75.5 °C for 5 s; with subsequent incubations 65–95 °C, with increments 0.5 °C per 5s. The expression of each gene was calculated with reference to the expression of β-actin using the $2^{(-\triangle\triangle Ct)}$ method. Samples were analyzed in triplicate.

Table 1
Primers used in the present study.

Primer	Sequence (5′–3′)	Application	Accession NO
DrIRF5 F	CCAAGCTTGCCACCATGAGTGGTCAACCACGGAG	Eukaryotic expression	NM_001327817.1
DrIRF5 R	GGGGTACCGTTAGTCGGCCAGTTGGGAT		
DrIRF5-△IAD R	GGGGTACCGGGCAGCATGTGTGGGCTG		NM_001327817.1
DrIRF5-△DBD F	CCAAGCTTGCCACCGATCAGTCAGTCAATGGAG		
IFNϕ1-Pro F	TCTCGAGAAGTGAAGTGAAAGTGCA	Promoter activity	NC_007114.7
IFNϕ1-Pro R	CAAGCTTGTTCTCATCTTTGCGTGT		
IFNϕ3-Pro F	GGGTACCGTTCACTGAGCCTGCATG		NC_007114.7
IFNϕ3-Pro R	TCTCGAGGATGCCTATACGGAATCA		
EPC-Actin F	TGTTCCAGCCATCCTTCTTG	Real-time PCR	M24113.1
EPC-Actin R	TGATTTTCATTGTGCTGGGG		
EPC-Mx-RT F	GGCTGGGGCAGGTGTTGGTATT		AY751300.1
EPC-Mx-RT R	TTAACAAAGCCGGATCTGGTGGA		
EPC-IFN-RT F	ATGAAAACTCAAATGTGGACGTA		FN178457.1
EPC-IFN-RT R	GATAGTTTCCACCCATTTCCTTAA		
EPC-Viperin F	GCAAAGCGAGGGTTACGAC		KM099177.1
EPC-Viperin R	CTGCCATTACTAACGATGCTGAC		
EPC-IRF7-RT F	AAAGTCTTCGTCAGCACCAGCG		HQ850444.1
EPC-IRF7-RT R	CTCTCCGAAGCACAGGTAGATGGT		
EPC-IRF3-RT F	AACAAGAATGACACTGCGGA		KJ027520.1
EPC-IRF3-RT R	AACTCGGGAGGGACTTTCAT		
DrActin F	GCCCATCCATCGTTCACAGG		AF057040.1
DrActin R	ACCTCCCTTTGCCAGTTTCC		
DrIRF5-RT F	TAATCCTGCCCACCACAACC		EU274624.1
DrIRF5-RT R	CTCCAGTCCCCATTCCCATA		
DrMx-RT F	CCTGGCAGAATCTATGCTGAAAAC		AF533769.1
DrMx-RT R	AAGGCAGTTTTATTTTCGGTTGTG		

Dr, *Danio rerio*.