Virology 448 (2014) 322–332

Contents lists available at ScienceDirect

Virology

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

Gig1, a novel antiviral effector involved in fish interferon response

Fan Sun, Yi-Bing Zhang *, Jun Jiang, Bing Wang, Chen Chen, Jun Zhang, Jian-Fang Gui *

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 19 July 2013
Returned to author for revisions
27 August 2013
Accepted 21 October 2013
Available online 9 November 2013

Keywords:
Fish
Gig1
Expression regulation
RLR signaling pathway
Antiviral effects
IFN response
Gene family

ABSTRACT

Vertebrate interferon (IFN) response defenses against viral infection through the induction of hundreds of IFN-stimulated genes (ISGs). Most ISGs are conserved across vertebrates; however, little is known about the species-specific ISGs. In this study, we reported that grass carp reovirus (GCRV)-induced gene 1 (Gig1), previously screened as a virus-induced gene from UV-inactivated GCRV-infected crucian carp (*Carassius auratus*) blastulae embryonic (CAB) cells, was a typical fish ISG, which was significantly induced by intracellular poly(I:C) through retinoic acid-inducible gene I (RIG-I)-like receptors-triggered IFN signaling pathway. Transient or stable overexpression of Gig1 prevented GCRV replication efficiently in cultured fish cells. Strikingly, Gig1 homologs were found exclusively in fish species forming a novel gene family. These results illustrate that there exists a Gig1 gene family unique to fish species and the founding gene mediates a novel fish IFN antiviral pathway.

Introduction

In mammals, the mechanism underlying type I interferon (IFN) response has been well-characterized. Once upon infection, viral proteins and nucleic acids are detected by host pattern recognition receptors (PRRs) including retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and Toll-like receptors (TLRs) (Beutler et al., 2007). Such recognition activates signaling cascades resulting in the induction of type I IFNs, which subsequently establishes the so-called "host antiviral state" in both autocrine and paracrine fashions by inducing the expression of hundreds of interferon stimulated genes (ISGs) (Sadler and Williams, 2008; Schoggins and Rice, 2011). Thus, the cellular effectors that limit the spread of viral infection are the products of ISGs. Whereas a few ISGs have been characterized with respect to antiviral potential, most of them are of unknown or incompletely understood function (Schoggins and Rice, 2011). Recent studies have revealed more targeted antiviral action of function-characterized ISGs in a tissue-, cell- and virus-specific manner (Fensterl et al., 2012, 2008; Schoggins et al., 2011; Szretter et al., 2011).

Since the discovery of IFN more than 50 years ago, the IFN antiviral system has been believed to exist across vertebrates. This notion is strengthened by the recent findings that the lower vertebrate fish possess *IFN* genes and many conserved ISGs (Gui and Zhu, 2012; Zhang and Gui, 2012). Similar to mammals, emerging studies showed that fish RLRs family members RIG-I, MDA5 can trigger *IFN* gene expression by recruitment of MITA (mediator of IRF3 activation), which subsequently activates the TBK1-IRF3-IFN signaling pathway (Chang et al., 2011; Sun et al., 2011). Fish MAVS (mitochondrial antiviral signaling protein) seems to be involved in IFN induction through the RLR signaling pathway (Biacchesi et al., 2009, 2012). The resultant fish IFNs exert antiviral effects through upregulation of the downstream ISGs likely by the Janus kinase (Jak)-signal transducer and activator of the transcription (Stat) pathway (Shi et al., 2012; Skjesol et al., 2010; Yu et al., 2010). In support of these observations, fish ISGs promoters harbor a DNA sequence, termed IFN-stimulated response element (ISRE), which is responsible for IFN induction (Altmann et al., 2004; Jiang et al., 2009; Li et al., 2012b; Liu et al., 2011; Sun et al., 2010); two fish ISGs, *Mx* and *PKR*, similar to their mammalian homologs (Sadler and Williams, 2008), share abilities of restricting virus replication (Larsen et al., 2004; Liu et al., 2011; Zhu et al., 2008). Besides these ISGs conserved in all vertebrates, there exist IFN-inducible antiviral components unique to distinct vertebrate lineages. For example, human schlafen 11, belonging to a gene family that is limited to mammalian organisms, has recently been identified as an IFN-inducible antiviral effector that suppresses HIV protein synthesis by means of codon-bias discrimination (Li et al., 2012a); mouse schlafen 2 and human schlafen 5 induce a growth-suppressive effects of IFNs in malignant cells (Katsoulidis et al., 2009, 2010), highlighting the physiological significance of mammals-specific ISGs in IFN response.

* Corresponding authors. Tel.: +86 27 68780663; fax: +86 27 68780123.
E-mail addresses: ybzhang@ihb.ac.cn (Y.-B. Zhang), jfgui@ihb.ac.cn (J.-F. Gui).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.10.029

In the past decades, great endeavors to identify virus-induced genes in fish found that some are not homologous to any mammalian genes. These genes include *PKR-like/PKZ* (protein kinase containing Z-DNA binding domains) (Hu et al., 2004), *Gig1* and *Gig2* (grass carp reovirus (GCRV)-induced gene 1 and 2) (Zhang and Gui, 2004), which are firstly retrieved from UV-inactivated GCRV-infected crucian carp (*Carassius auratus*) blastulae embryonic (CAB) cells (Zhang et al., 2003), and later found in other fish species (Bergan et al., 2008; Krasnov et al., 2011; Martin et al., 2007; Rothenburg et al., 2008, 2005). Further studies have revealed that *PKZ* encodes a novel fish IFN-induced eIF2α kinase working in concert with *PKR* to block virus infection through phosphorylation of cellular eIF2α (Liu et al., 2011), and that *Gig2* gene is specific to both fish and amphibians (Zhang et al., 2013) and displays an ability to inhibit viral replication in cultured fish cells (Li et al., 2012b). However, the expression regulation and biological function of fish *Gig1* remains to be investigated.

In the present study, we further identified crucian carp *Gig1* as a typical ISG with antiviral function. *Gig1* harbored an ISRE-containing promoter contributing to the induction by IFN and poly (I:C). Poly(I:C) transfection of fish cells activated the RLR signaling pathway to induce the production of IFN protein, which in turn upregulated the expression of Gig1 by the Jak-Stat pathway. We found that fish Gig1 protein suppressed GCRV replication in cultured fish cells, as further verified by real-time PCR analyses showing dramatically decreased amplification of GCRV genome. Finally, *Gig1* homologs existed exclusively in fish lineages forming a gene family with divergence in protein sequences and expression properties. This study identified a novel gene family unique to fish species, and provided evidence for antiviral roles of the founding gene *Gig1* in fish IFN response.

Results

Fish Gig1 is upregulated in cultured cells by poly(I:C) and IFN

Gig1 was first identified as a virus-induced gene in UV-inactivated GCRV-infected CAB cells (Zhang and Gui, 2004; Zhang et al., 2003). Similarly, *Gig1* was transcriptionally induced in a time-dependent manner by UV-inactivated GCRV and extracellular poly(I:C) (Fig. 1A and B). To further investigate the expression of Gig1 at the protein level, we expressed a recombinant Gig1 protein (rGig1) by the prokaryotical expression system, and subsequently obtained a polyclonal anti-Gig1 serum

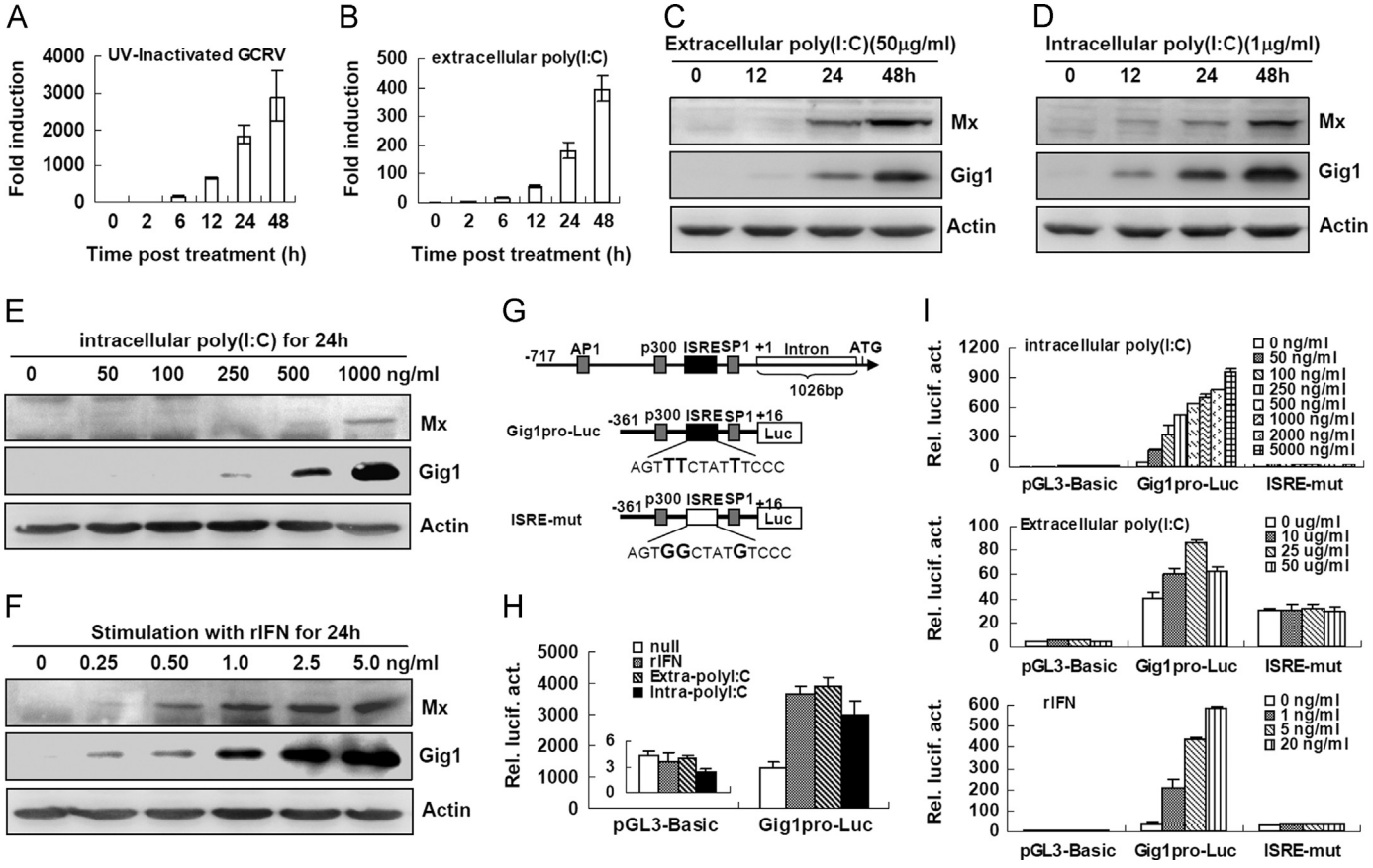

Fig. 1. Induction of crucian carp Gig1 in CAB cells by poly(I:C) and IFN. (A–F) CAB cells in each well of 24-well plates were treated for different times with UV-inactivated GCRV (1×10^9 $TCID_{50}$/ml exposed to UV) (A), extracellular poly(I:C) (50 μg/ml) (B and C), or transfected with 1 μg/ml of poly(I:C)(intracellular poly(I:C)) (D), or treated for 24 h with different doses of intracellular poly(I:C) (E) and recombinant crucian carp IFN (rIFN) (F). At the indicated time points, cells were collected for detection of *Gig1* mRNA by real-time PCR (A and B), or of Gig1 protein by western blot analyses (C–H). (G) Schematic of *Gig1* promoter and Gig1 promoter-driven luciferase constructs. (H) Induction of *Gig1* promoter by IFN and poly(I:C). CAB cells seeded in 24-well plates overnight were cotransfected with 0.25 μg Gig1pro-Luc or control vector pGL3-basic in 500 μl medium. 0.025 μg pRL-TK was included to normalize the expression level. 24 h later, the transfected cells were washed three times to remove transfection mixture, and then treated again with 10 ng/ml rIFN, 50 μg/ml extracellular poly(I:C), or transfected again with 1 μg/ml poly(I:C) as intracellular poly(I:C), or left untreated as a control (null) for an additional 24 h, followed by detection of luciferase activity. (I) CAB cells seeded in 24-well plates were transfected as the same in H with pGL3-basic, Gig1pro-Luc and ISRE-mut, respectively. 24 h later, the transfected cells were washed three times, and then transfected again with different doses of poly(I:C) as intracellular poly(I:C), or treated directly with different doses of poly(I:C) as extracellular poly(I:C) or rIFN for an additional 24 h. Luciferase assays were performed in triplicates and repeated at least three times. Western blot results were representative of at least two different experiments.