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SVCV infection triggers fish IFN response through RLR signaling pathway

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ABSTRACT

In mammals, virus infection of host cells triggers innate immune response, characterized by induction of interferon (IFN) and downstream IFN-stimulated genes (ISGs). The initiation of IFN antiviral response is dependent on host recognition of virus infection. In fish, similar IFN antiviral response is induced in response to RNA or DNA virus infection; however, the detailed mechanisms underlying recognition of a given virus and activation of downstream signaling remain largely unexplored. Using an infection model with Epithelioma papulosum cyprini (EPC) cells and spring viremia of carp virus (SVCV), a negative sense single-stranded RNA virus, we reported that fish RLR signaling pathway was involved in SVCV-triggered fish IFN response. IFN response was significantly initiated in EPC cells when infected with SVCV, as evidenced by activation of fish IFN promoters, upregulation of IFN and ISGs at mRNA and protein levels. However, function blockade of RIG-I and MDA5, two cytosolic receptors of fish RLR family, significantly attenuated the activation of fish IFN promoters and also the induction of fish IFN and ISGs by SVCV infection. Consistently, SVCV infection-triggered IFN response were blocked in EPC cells when transfected with the dominant negative mutants of pivotal RLR signaling factors, including MAVS, MITA, TBK1, IRF3 and IRF7. These results together shed light on the conservation of RLR-mediated IFN signaling that contributes to fish cells responding to RNA virus infection.

1. Introduction

In mammals, virus infection of host cells triggers innate immune response, characterized by induction of interferon (IFN) and downstream IFN-stimulated genes (ISGs) [1,2]. Once virus has overcome the physical and chemical barriers, it will be recognized by pattern recognition receptors (PRRs) of host cells. PRRs are germline-encoded sensors and harbor the ability to detect conserved microbial structures that are usually unique to microbes and often essential for microbial existence, referred to as pathogen-associated molecular patterns (PAMPs) [1]. Viruses possess few unique features suitable for detection as PAMPs; therefore, innate immune detection of viral genomic RNA and DNA, which differ from host nucleic acids, is an essential mechanism to mount protective IFN antiviral immune response [1,2]. Extensive studies have shown that RNA virus infection is generally recognized by host cytosolic receptors, namely retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs), and DNA virus infection recognized by DNA receptors, such as intracellular cGAS (cyclic GMP-AMP synthase), DAI (DNA-dependent activator of IRFs, also known as ZBP-1), IFI16 (IFN-γ-inducible protein 16) and so on [1–3].

RLR family is composed of RIG-I, melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [1,2]. They share common domains, including two tandem N-terminal caspase activation and recruitment domains (CARDs), a DExD/H box helicase domain, and a C-terminal RNA-binding domain (CTD), but LGP2 lacks the N-terminal CARDs [2]. RLRs recognize viral RNA through the central DExD/H box helicase domain and subsequently transduce signals to downstream molecules through the N-terminal CARDs. Consistently, RIG-I is determined to recognize dsRNA and 5′-triphosphate ssRNA (5′-ppp ssRNA) of different RNA virus; MDA5 detects long dsRNA of viral genomes, and both dsRNA and ssRNA can be recognized by LGP2 [1,2]. Such recognition issues recruit and activate downstream adaptor protein mitochondrial antiviral signaling (MAVS), further leading to the activation of cytoplasmic kinases TANK-binding

Abbreviations: IRF, IFN regulatory factor; ISG, IFN-stimulated gene; ISRE, IFN-stimulated regulatory element; LGP2, Laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma-differentiation-associated gene 5; MITA, mediator of IRF3 activation; poly(I:C), polyinosinic:polycytidylic acid; RIG-I, retinoic acid-inducible gene I; RLRs, RIG-I like receptors

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kinase 1 (TBK1). The activated TBK1 in turn phosphorylates and activates IFN regulatory factors 3/7 (IRF3/7) to induce the expression of type I IFNs and IFN-stimulated genes (ISGs) for the establishment of host antiviral state. The recognition of intracellular viral DNA recruits a distinct adaptor, stimulator of IFN genes (STING, also known as MITA), to activate the common TBK1-IRF3/7-IFN axis [3]. Notably, the innate adaptor proteins MAVS and MITA activate IRF3 through a similar TBK1-mediated phosphorylation-dependent mechanism [4].

When responding to virus infection, teleost fish also initiates the innate IFN antiviral response [5,6]. The pivotal genes involved in fish RLR signaling pathway, including RIG-I, MDA5, MAVS, MITA, TBK1 and IRF3/7 have been identified in many fish species [6]. These fish genes are significantly induced by virus infection, and overexpression of them individually results in upregulation of IFN and ISGs and also establishment of host antiviral states [7–12]. Further function characterization of these genes indicates that fish possess the conserved RLR pathway to initiate IFN expression and antiviral response [5,6].

SVCV (spring viraemia of carp virus) is a negative-sense single-stranded RNA virus, causing acute hemorrhagic and contagious disease primarily in cyprinid species, especially in common carp (*Cyprinus carpio*), also in goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), koi (*Cyprinus carpio koi*), bighead carp (*Aristichthys nobilis*), tench (*Tinca tinca*) and orfe (*Leuciscus idus*) [13,14]. Due to its great harm to fish, SVCV-infected zebrafish model has been developed to study fish antiviral immunity [15,16], and using this pathogen model, fish IFN receptors have been identified [17–19]. In addition, SVCV is widely utilized to infect fish cell lines of different origin, showing that the genes involved in fish IFN antiviral response are generally up-regulated in response to SVCV [20–24]. These studies indicate that SVCV infection is potential to trigger fish IFN antiviral response; however, which signaling pathway is exactly activated to initiate IFN response during SVCV infection remains to be investigated. In this study, we found that in SVCV-infected EPC cells, fish RLR signaling pathway was activated, which initiated fish IFN antiviral response.

2. Materials and methods

2.1. Cells and virus

Epithelioma papulosum cyprini cells (EPC) were grown at 28 °C in medium 199 supplemented with 10% fetal bovine serum (FBS). SVCV, a negative sense single-stranded RNA virus in the family *Rhabdoviridae* [13,14], was propagated and titered according to the method of Reed and Muench, by a 50% tissue culture-infective dose (TCID50) assay on EPC cells [15].

2.2. Plasmids

The empty vector (pcDNA3.1) was purchased from Invitrogen. The dominant negative mutant plasmids of zebrafish RIG-I and MDA5, RIG-I-DN and MDA5-DN, were described previously [15]. The dominant negative mutants of other RLR signaling factors, including zebrafish MAVS-ΔTM, crucian carp MITA-CT, crucian carp TBK1-K38M, zebrafish IRF3-DN, zebrafish IRF7-DN were made previously in our lab [9,25,26]. Three fish IFN promoter-driven luciferase constructs (including EPC IFNpro-luc, zebrafish IFNφ1pro-luc, zebrafish IFNφ3pro-luc) were described previously [9,27].

2.3. Transfection and luciferase activity assays

Transfection was performed according to our previous reports [8,9,15]. Typically, EPC cells were seeded overnight in 24-well plates and were transfected with various constructs at a ratio of 10:10:1 (promoter-driven luciferase plasmid/expression plasmid/Renilla luciferase plasmid pRL-TK) using Lipofectamine 3000 (Invitrogen). For the control, empty vector pcDNA3.1 replaced the expression plasmids. If necessary, the cells were treated again with SVCV infection after transfection. At the indicated time points, the cells were harvested and lysed according to the Dual-Luciferase Reporter Assay System (Promega). Luciferase activities were measured by a Junior LB9509 luminometer (Berthold, Pforzheim, Germany) and normalized to the amounts of Renilla luciferase activities. Unless indicated, the results were representative of more than three independent experiments, each performed in triplicate.

2.4. SVCV infection and quantitative real-time PCR (RT-qPCR)

EPC cells were seeded in 3.5 cm dishes overnight, and replaced with 2 ml FCS-free 199 medium containing SVCV at different titers. After incubation at 28 °C for 1 h, the cells were replaced with 2 ml fresh FCS-free 199 medium and further cultured at 28 °C for the indicated time points (0, 12, 24, 48 h post infection). Control cells were mock-infected by incubation with FCS-free 199 medium alone.

Total RNA was extracted by TRIZOL Reagent (Invitrogen), and the RNA was treated with RNase-free DNase I (Promega) according to the manufacturer's protocol. First-strand cDNA was synthesized using random primers and M-MLV reverse transcriptase (Promega), and then kept at −20 °C for RT-qPCR analysis. RT-qPCR was performed in a DNA Engine Chromo 4 real-time system (BioRad) with SYBR green real-time PCR master mix (ToYoBo). Reactions were performed in a 20 μl volume containing SYBR Green I Dye. All samples were analyzed in triplicate and the expression value of target genes was normalized to β-actin according to the $2^{(-\Delta\Delta C(T))}$method. The primers used for RT-qPCR analysis are listed in Table 1.

2.5. Western blotting and antibodies

Western blotting was performed as describe previously [8,15]. Typically, protein samples were made from SVCV-infected EPC cells by SDS loading buffer (Beyotime). Cell lysates were separated by SDS-PAGE gels, electrophoretically transferred to a PVDF membrane (Millipore), and probed with the indicated antibodies. Three fish polyclone antibodies used in this study were described previously, including crucian carp IRF3-specific antibody [8], crucian carp PKR-specific antibody [10] and zebrafish LGP2-specific antibody [15].

3. Results

3.1. SVCV infection activates fish IFN promoters

In order to determine whether SVCV infection was able to effectively active IFN response, luciferase assays were used to study the effects of SVCV infection on the activation of different fish promoters. Three fish IFN promoter-driven luciferase constructs (EPC IFNpro-luc, zebrafish IFNφ1pro-luc and IFNφ3pro-luc) are made by cloning ISRE-containing IFN promoters in front of luciferase gene and are readily

Table 1
The primers used in this study.

Primer name	Sequence(5′to3′)	Application
EPC-IFN-F	ATGAAAACTCAAATGTGGACGTA	RT-PCR
EPC-IFN-R	GATAGTTTCCACCCATTTCCTTAA	
EPC-Viperin-F	AGCGAGGCTTACGACTTCTG	
EPC-Viperin-R	GCACCAACTCTCCCAGAAAA	
EPC-IRF7-F	AAAGTCTTCGTCAGCACCAGCG	
EPC-IRF7-R	CTCTCCGAAGCACAGGTAGATGGT	
EPC-Mx-F	GGCTGGAGCAGGTGTTGGTATC	
EPC-Mx-R	TCCACCAGGTCCGGCTTTGTTAA	
EPC-Actin-F	CAGATCATGTTTGAGACC	
EPC-Actin-R	ATTGCCAATGGTGATGAC	

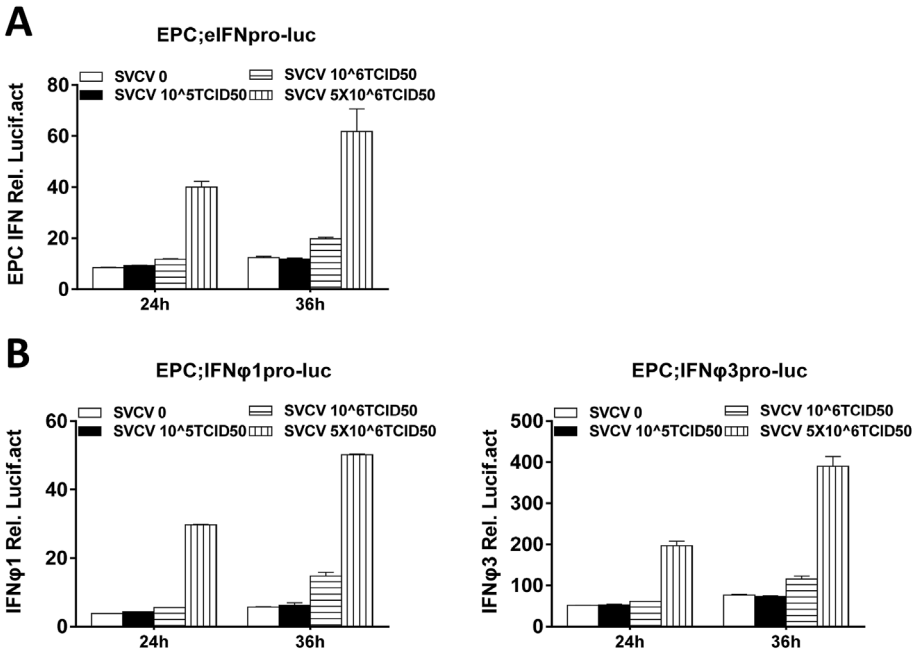

Fig. 1. Activation of fish IFN promoters in EPC cells by SVCV infection.
EPC cells seeded in 24-well plates were cotransfected with IFN promoter-driven luciferase plasmids, EPC IFNpro-luc (A) or zebrafish IFNφ1pro-luc and IFNφ3pro-luc (B) (200 ng each), together with Renilla luciferase plasmid (pRL-TK, 20 ng). 24 h later, the cells were infected with increasing titers of SVCV (10^5TCID$_{50}$/ml, 5×10^6TCID$_{50}$/ml, 10^6TCID$_{50}$/ml). At 24 and 36 h post-SVCV infection, the cells were collected for luciferase assays.

induced by RLR signaling factors [9,26]. Compared to mock infection, SVCV infection induced high levels of luciferase activity in EPC cells when transfected with EPC IFNpro-luc followed by infection of increasing titers of SVCV, and this induction was significantly promoted along with either virus titers or infection duration, indicating that SVCV infection has a potential to stimulate EPC IFN promoter in both dose- and time-dependent fashions (Fig. 1A). Same results were observed when zebrafish IFNφ1pro-luc and IFNφ3pro-luc were transfected instead of EPC IFNpro-luc (Fig. 1B).

3.2. SVCV infection upregulates the expression of IFN and ISGs

The potential of SVCV infection to activate fish IFN promoters indicated a successful initiation of host cellular IFN response. To this end, EPC cells were infected with SVCV, and the transcriptional expression of IFN and selected ISGs was determined by RT-qPCR. As shown in Fig. 2A, the cellular IFN was transcriptionally induced in response to SVCV infection, and its expression level was continuously increased as infection time extended. The transcription of ISGs, including IRF3, IRF7, Mx and Viperin, was also significantly upregulated by SVCV infection, although they peaked at different time points (Fig. 2A). Same results were observed when EPC cells were infected with different titers of SVCV (Fig. 2B).

In subsequent experiments, western blots were used to detect the change in protein levels of three ISGs (LGP2, PKR, IRF3). Fish LGP2 and PKR are two typical IFN-inducible protein [10,15], and unlike mammalian IRF3 that is constitutively expressed and is not induced by viral infection, fish IRF3 is induced by IFN and IFN stimuli [8]. Western blot assays showed that SVCV infection induced a robust increase in LGP2 proteins in both time- and dose-dependent fashions (Fig. 2C). While a relatively high basal level of IRF3 and PKR proteins was observed in resting EPC cells, SVCV infection also resulted in obvious upregulation of both fish proteins. Particularly, a slower-migrating IRF3 protein was observed in SVCV-infected cells, which has been confirmed as a phosphorylated form of fish IRF3 [8,26], indicating the activation of IRF3 protein in response to SVCV infection. These results collectively indicated that SVCV infection has the potential to activate cellular IFN response by induction of IFN and downstream ISGs at mRNA and protein levels.

3.3. SVCV-triggered fish IFN promoter activation is blocked by function knockdown of pivotal components of RLR signaling

In mammals, RNA virus replication in cells produces viral-derived RNAs that are believed to be sensed by RLR receptors [2]. SVCV is a negative sense ssRNA virus in the family *Rhabdoviridae* [13]. We hypothesized that fish RLR could recognize SVCV infection to mediate IFN signaling. To this end, EPC cells were transfected with the dominant negative mutants of RIG-I and MDA5 (RIG-I-DN, MDA5-DN) together with EPC IFNpro-luc followed by SVCV infection, followed by luciferase assays to determine whether SVCV-mediated fish IFN promoter activation was inhibited. As shown in Fig. 3A, SVCV infection resulted in over 3-fold increase of EPC IFN promoter-driven luciferase activity compared to mock infection; however, this activation was severely attenuated by overexpression of either RIG-I-DN or MAVS-DN. Consistently, overexpression of dominant negative mutants of downstream signaling molecules (MAVS-ΔTM, MITA-CT, TBK1-K38M, IRF3-DN, IRF7-DN) also resulted in significantly diminished activation of fish IFN promoter (Fig. 3A).

Previous results showed that both fish MAVS and MITA are involved in poly(I:C)- or RLR-triggered IFN response [9,12]. To determine whether both fish MAVS and MITA cooperated to mediate IFN response by SVCV infection, EPC cells were transfected with EPC IFNpro-luc together with either or both of MAVS-ΔTM and MITA-CT followed by SVCV infection. It showed that overexpression of either MAVS-ΔTM or MITA-CT resulted in obviously diminished activation of fish IFN promoter compared to the control and generally, a much more severe inhibition was observed by overexpression of MAVS-ΔTM than by overexpression of MITA-CT (Fig. 3B). However, the inhibition was not enhanced by combined transfection of MAVS-ΔTM and MITA-CT (Fig. 3B). These results indicated that SVCV infection triggered IFN response through RLR signaling pathway.

3.4. SVCV-induced expression of ISG proteins is blocked by function knockdown of pivotal components of RLR signaling

To corroborate the finding that SVCV infection initiated RLR-directed IFN signaling, western blot assays were used to investigate the protein levels of LGP2, IRF3 and PKR in SVCV-infected EPC cells, which had been pre-transfected with or without the dominant negative mutants of fish RLR signaling molecules (RIG-I-DN, MDA5-DN, MAVS-

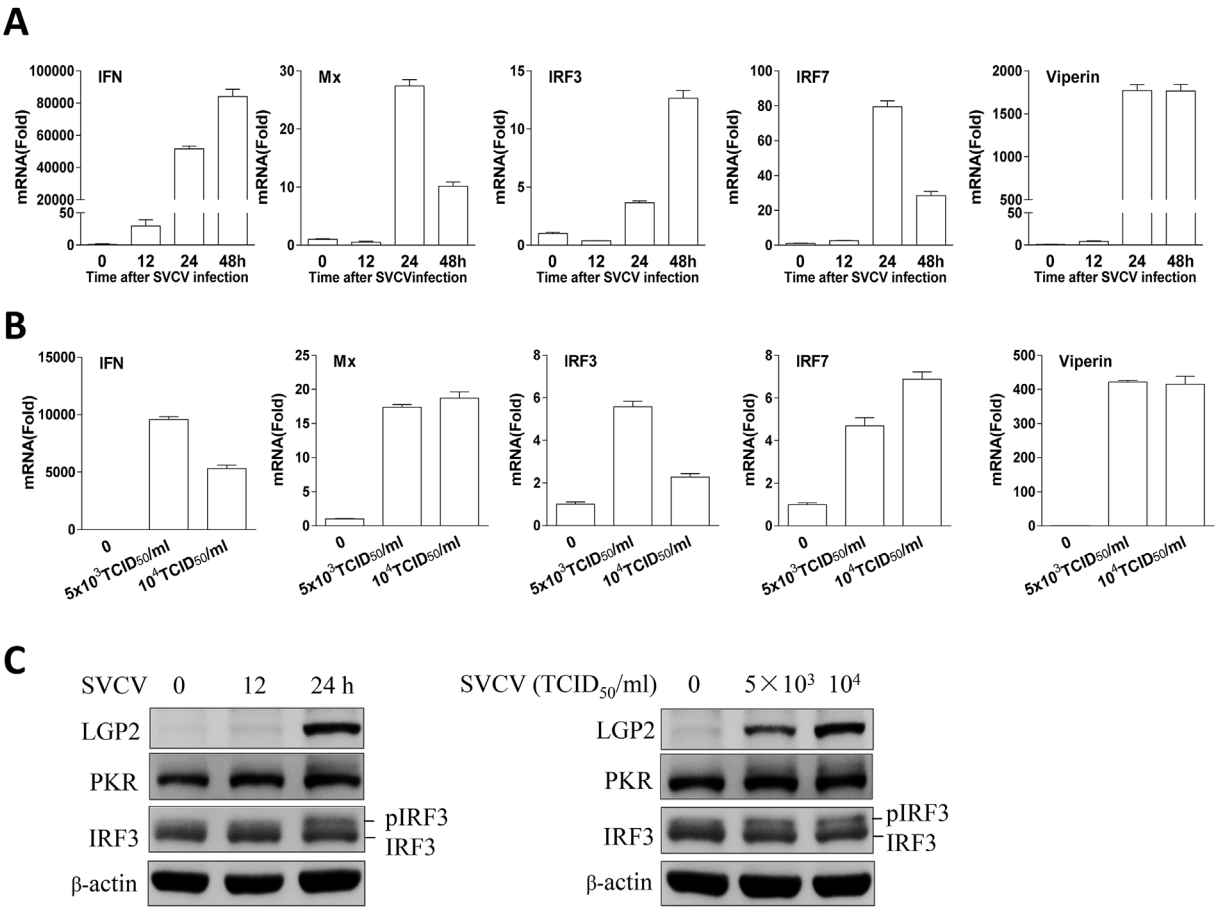

Fig. 2. Induction of fish IFN and ISGs in EPC cells by SVCV infection.
(A and B). IFN and ISGs are transcriptionally induced in EPC cells by SVCV infection. EPC cells seeded overnight in 3.5 cm dishes were infected with SVCV (final viral titer at 10^4TCID$_{50}$/ml) for different time points (A), or with increasing titers of SVCV (5×10^3TCID$_{50}$/ml or 10^4TCID$_{50}$/ml) for 24 h (B). Cells were harvested for total RNA extraction, reverse transcription and RT-qPCR analysis of IFN and ISGs expression. The expression value was expressed as relative to the corresponding expression level of β-actin according to the $2^{(-\Delta\Delta C(T))}$method. Error bars represent SD obtained by measuring each sample in triplicate.
(C). LGP2, IRF3 and PKR protein are upregulated in EPC cells by SVCV infection. EPC cells seeds overnight in 3.5 cm dishes were infected with 10^4TCID$_{50}$/ml of SVCV for different time points or with 5×10^3TCID$_{50}$/ml or 10^4TCID$_{50}$/ml of SVCV for 24 h. Western blotting was used to analyze the expression of LGP2, IRF3 and PKR proteins using corresponding Abs.

ΔTM, MITA-CT, TBK1-K38M, IRF3-DN, IRF7-DN). As anticipated, SVCV infection upregulated the expression of LGP2, PKR and IRF3 proteins (Fig. 4). However, such induction of LGP2 protein was markedly inhibited by overexpression of the dominant negative mutants of fish RLR signaling molecules, individually (Fig. 4). Same results were also observed for the induction of IRF3 and PKR, although overexpression of MITA-CT did not resulted a very obviously decrease in the levels of IRF3 and PKR proteins (Fig. 4). The phosphorylation form of fish IRF3 was

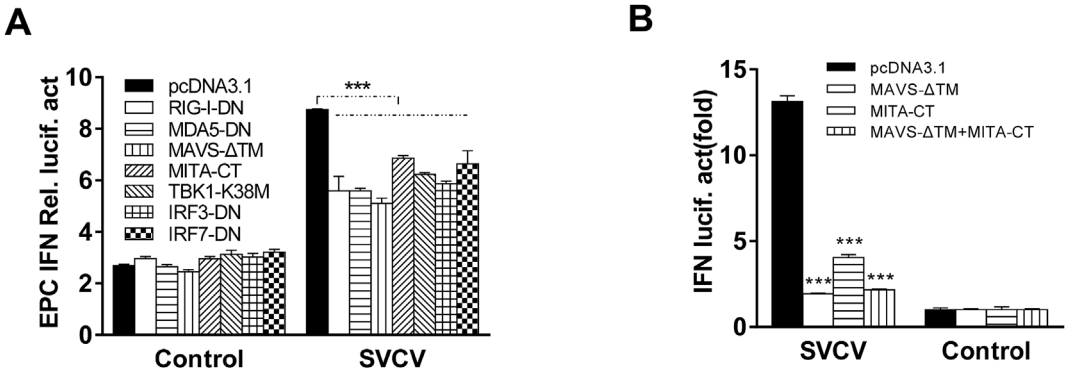

Fig. 3. SVCV-triggered activation of IFN promoter was inhibited by overexpression of dominant negative mutants of RLR signaling molecules.
EPC cells seeded overnight in 24-well plates were transfected with EPC IFNpro-luc (200 ng), Renilla luciferase plasmid (pRL-TK, 20 ng), together with the indicated dominant negative mutants plasmids (200 ng) individually (A) or with either or both of MAVS-ΔTM and MITA-CT (B). At 24 h post-transfection, the cells were infected with SVCV (10^5TCID$_{50}$/ml) for another 24 h and harvested for luciferase activity assays. Control cells were mock treated with the same volume of FCS-free 199 medium only. $*p < 0.05$, $***p < 0.01$, Student's *t*-test.

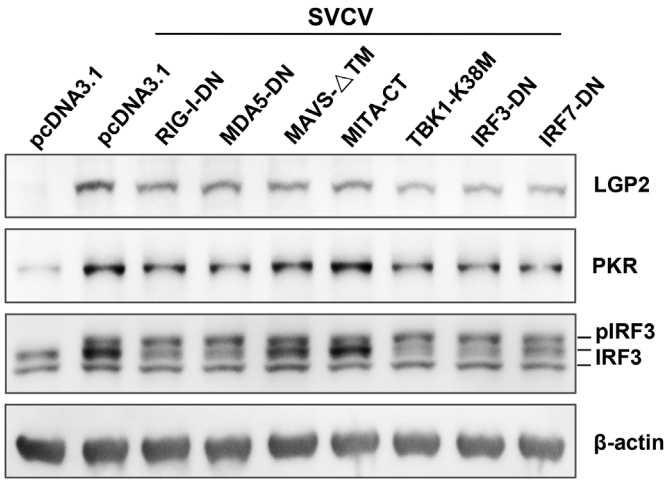

Fig. 4. SVCV-induced expression of ISG proteins was blocked by overexpression of the dominant negative mutants of RLR signaling molecules. EPC cells seeded overnight in 24-well plates, were transfected with empty vector plasmid pcDNA3.1 or each of dominant negative mutants plasmids (200 ng), including RIG-I-DN, MDA5-DN, MAVS-ΔTM, MITA-CT, TBK1-K38M, IRF3-DN and IRF7-DN. At 24 h post-transfection, the cells were infected with SVCV (10^5TCID$_{50}$/ml) for another 24 h or added with the same volume of FCS-free 199 medium as control. Western blotting was used to analyze the expression of LGP2, IRF3 and PKR proteins using corresponding antibodies. The results were representative of three independent experiments.

observed exclusively in SVCV-infected cells and was obviously diminished when these dominant negative mutants were transfected. These results indicated that SVCV-induced expression of ISG proteins is blocked by knockdown of pivotal components involved in RLR signaling.

4. Discussion

Extensive studies have shown that IFN response, characteristic of the expression of IFN and ISGs, is initiated in teleost fish and cultured fish cells in response to virus infection [5,6]. Infection of fish cells with DNA viruses or RNA viruses results in similar production of fish IFN, which is believed to in turn induce the expression of fish ISGs through JAK-STAT signal pathway [5,6]. Since most of the genes involved in RLR signaling pathway- or other signaling pathway-mediated IFN response are typical ISGs, it is not hard to understand the upregulation of these genes at mRNA and/or protein levels in response to both DNA and RNA virus infection. These fish genes, presented in the current study, include three RLR receptors genes RIG-I, MDA5 and LGP2, adaptor gene MITA, transcription factors IRF3 and IRF7, and some antiviral effector genes including Mx and PKR. However, it is not easy to make a conclusion that these genes should be involved in the initiation of fish IFN response due to their expression features in response to viral infection. That is, although the upregulation of ISGs sufficiently indicates successful initiation of IFN response in a given virus-infected fish cells, what signal pathway has been exactly triggered by this virus infection needs to be further investigated.

In mammals, gene knockout studies have suggested that mouse RLR-directed IFN signaling is mainly triggered by RNA viruses but cGAS-directed signaling involved in DNA virus-triggered IFN immunity [1]. In the present study, we provided evidence supporting that RLR-directed IFN signaling has been triggered in EPC cells by SVCV infection. Similar to our previous results [15], fish IFN promoters are robustly activated by SVCV infection (Fig. 1) and consistently, IFN gene and some pivotal ISGs are transcriptionally induced, including Mx, PKR, viperin, IRF3 and IRF7 (Fig. 2A and B). Using the antibodies specific to fish LGP2, IRF3 and PKR, we found that these three fish IFN-inducible proteins [8,10,15], especially LGP2, are significantly upregulated in SVCV-infected cells (Fig. 2C). More importantly, IRF3 protein phosphorylation is detected in response to virus infection (Figs. 2C and 4). Fish IRF3, unlike its mammalian orthologues, is a typical ISG, and the phosphorylated IRF3 form displays a better potential to induce IFN expression than the intact IRF3 form [8]. These results suggest a successful initiation of IFN antiviral response observed in SVCV-infected EPC cells.

Further assays showed that SVCV infection-triggered IFN response is markedly inhibited by function blockade of RIG-I and MDA5 individually, as evidenced by luciferase assays (Fig. 3) and western blots (Fig. 4), indicating that both RIG-I and MDA5 should have participated in the recognition of SVCV infection. Using the dominant-negative mutants of downstream RLR signaling molecules including MAVS, MITA, TBK1, IRF3 and IRF7, an obvious blockade is consistently observed (Figs. 3 and 4). Combined with the findings that rainbow trout MDA5 can bind to synthetic dsRNA poly(I:C) by pull-down assays [7], and SVCV is a negative RNA virus [13,14], these results suggest that similar to mammals, RLR signaling pathway participates in RNA virus infection-initiated fish IFN response for establishment of host antiviral state.

Despite of the conservation of IFN antiviral response in vertebrate, fish seems to have unique features. While mammalian cGAS-MITA pathway is essential for sensing DNA virus infection [3], zebrafish cGAS orthologue is dispensable for HSV-1 (dsDNA virus) infection *in vivo* [28]. Moreover, silencing of zebrafish MITA rather than zebrafish MAVS markedly attenuates HSV-1-induced antiviral responses [28], highlighting the relevance of fish MITA, but not of MAVS, in DNA virus-induced IFN response. However, *in vitro* evidence has shown that fish MITA is essential for RLR-triggered IFN response [9,11,12]. In the present study, overexpression of either fish MAVS-ΔTM or MITA-CT, the dominant negative mutants of MAVS and MITA, impaired the activation of fish IFN promoters by SVCV (Fig. 3) and the upregulation of IFN-inducible protein LGP2 by SVCV (Fig. 4). The expression of IRF3 and PKR proteins are also significantly blocked by MAVS-ΔTM but not obviously by MITA-CT (Fig. 4). The distinct blockade effect of MAVS-ΔTM and MITA-CT might be due to their differential potentials to regulate the expression of IRF3 and PKR by SVCV, because MAVS-ΔTM exhibits a better inhibition than MITA-CT. However, the detailed mechanisms wait for further investigation. Despite of these differences, these results indicate that both fish MAVS and MITA are required for SVCV-triggered IFN expression. Interestingly, overexpression of MAVS-ΔTM and MITA-CT together did not lead to much more severe attenuation of SVCV-triggered fish IFN promoter activation than overexpression of either (Fig. 3B). Similar results are observed in intracellular poly(I:C)-induced fish IFN promoter activation, where the IFN promoter activation is not synergistically induced by cotransfection of fish MAVS and MITA and also is not severely attenuated by cotransfection of MAVS-ΔTM and MITA-CT [12]. In line with these results, fish MITA exhibits an ability to protect fish cells against RNA virus infection [9,21]. Although the detailed mechanisms are not fully known, it is obvious that there is function crosstalk of MITA and MAVS in mammals [2]. For example, MITA-knockout mouse is partially impaired in induction of IFN when infected with Sendai virus and VSV, two RNA viruses [29,30]; cGAS-MITA pathway is crucial for DNA virus infection, but genetic ablation of murine cGAS reveals an unappreciated contribution to the innate control of an RNA virus (WMV) [31].

A third member of RLR family, LGP2, can recognize both dsRNA and ssRNA but exhibits controversial function [1,2]. Our recent results have shown that fish LGP2 displays a function-switch in response to SVCV infection. In the early phase of SVCV infection, LGP2 facilitates SVCV-triggered fish IFN promoter activation as a positive regulator of IFN response but in the later phase of SVCV infection, it switches to inhibit SVCV-induced IFN response as a negative regulator [15]. These results also indicate that fish LGP2 is involved in SVCV-triggered fish IFN response.

Acknowledgments

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