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Fish TRIM8 exerts antiviral roles through regulation of the proinflammatory factors and interferon signaling

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ABSTRACT

The tripartite motif (TRIM)-containing proteins usually exert important regulatory roles during multiple biological processes. TRIM8 has been demonstrated to be a RING domain-containing E3 ubiquitin ligase which plays critical roles in inflammation and cancer. In this study, a TRIM8 homolog from grouper, Epinephelus coioides (EcTRIM8) was cloned, and its effects on fish virus replication were investigated. The full-length EcTRIM8 cDNA encoded a polypeptide of 568 amino acids with 92% identity to TRIM8 homolog from large yellow croaker (Larimichthys crocea). Sequence alignment analysis indicated that EcTRIM8 contained conserved RING finger, B-box and coiled-coil domain. Expression patterns analysis showed that EcTRIM8 was predominant in kidney, gill, fin, liver, spleen and brain. After challenging with Singapore grouper iridovirus (SGIV) or polyinosin-polycytidylic acid (poly I:C), the EcTRIM8 transcript was significantly increased at the early stage of injection. Under fluorescence microscopy, we observed different distribution patterns of EcTRIM8 in grouper spleen (GS) cells, including punctate fluorescence evenly situated throughout the cytoplasm and bright aggregates. The ectopic expression of EcTRIM8 in vitro significantly inhibited the replication of SGIV and red spotted grouper nervous necrosis virus (RGNNV), evidenced by the obvious reduction in the severity of cytopathic effect (CPE) and the significant decrease in viral gene transcription and protein synthesis. Moreover, the transcription of the proinflammatory factors and interferon related immune factors were differently regulated by EcTRIM8 during SGIV or RGNNV infection. In addition, overexpression of EcTRIM8 significantly increased the transcription of interferon regulator factor 3 (IRF3) and IRF7, and enhanced IRF3 or IRF7 induced interferon-stimulated response element (ISRE) promoter activity. Together, our results firstly demonstrated that fish TRIM8 could exert antiviral function through the regulation of the expression of proinflammatory cytokines and interferon related transcription factors in response to fish viruses.

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1. Introduction

The tripartite motif (TRIM) protein family was composed of multidomain ubiquitin E3 ligases characterized by the presence of three conserved domains, RING (Really Interesting New Gene), B-Box and coiled-coil (RBCC) [1]. Increased reports demonstrated that TRIMs exerted crucial roles in multiple biological processes, including cell growth, apoptosis, differentiation, carcinogenesis and antiviral immunity [2–5]. Recently, a great deal work focused great attention on the potential roles of TRIM proteins in the antiviral immune response against DNA or RNA viruses [5,6]. TRIM56 functioned as an antiviral host factor that confers resistance to yellow fever virus (YFV), dengue virus serotype 2 (DENV2), and human coronavirus (HCoV) OC43 through overlapping and distinct molecular determinants [7]. TRIM6 was found to interact with IKKs and promoted induction of IKKs-dependent IFN-stimulated genes (ISGs) [8]. In addition, TRIM25, TRIM13, and TRIM44 were also
demonstrated to exert critical roles in antiviral immune response [5,6,9]. However, all these research efforts so far on the roles of TRIMs in antiviral immunity were mainly from the mammalian studies.

TRIM8, an important member of TRIM family, has been demonstrated to serve as a critical regulator in carcinogenesis and inflammation. In vitro, TRIM8 could interact with protein inhibitor of activated STAT3 (PIAS3), and the overexpression of TRIM8 abolished the negative effect of PIAS3 on signal transducer and activator of transcription 3 (STAT3), either by degradation of PIAS3 or exclusion of PIAS3 from the nucleus [10]. The ectopic expression of TRIM8 also activated NF-κB and mediated TNFα- and IL-1β-induced activation of NF-κB and K63-linked polyubiquitination of TAK1 [11]. Moreover, TRIM8 was found to physically interact with p53, impairing its interaction with murine double minute 2 (MDM2), suggested that TRIM8 could be proposed as a novel therapeutic target to enhance p53 tumor suppressor activity [12]. In addition, TRIM8 was able to interact with suppressor of cytokine signaling-1 (SOCS1), and its expression decreased the repression of interferon gamma signaling mediated by SOCS-1 [13]. Although molecular functions of mammalian TRIM8 were explored in multiple biological processes, the roles of its corresponding homolog in fish still remained largely unknown. Moreover, the potential roles of TRIM8 during virus infection also remained uncertain in both mammals and fish.

Groupers, Epinephelus spp. are commercially important farmed fish species in China and Southeast Asian countries. However, the outbreak of iridoviral and nodaviral diseases always caused heavy economic losses in grouper aquaculture [14–17]. As two important pathogens, Singapore grouper iridovirus (SGIV) and red spotted grouper nervous necrosis virus (RGNNV) usually caused extremely economic losses in grouper aquaculture [14,18]. As two important pathogens, Singapore grouper iridovirus (SGIV) and red spotted grouper nervous necrosis virus (RGNNV) usually caused extremely high mortality at different developmental stages of grouper [15,16]. Although several immune regulatory molecules have been illustrated to play crucial roles in grouper immune defense against fish virus infection [18–24], limited references focused on grouper TRIM family proteins [25]. Our previous reports demonstrated that grouper TRIM39 was able to inhibit grouper iridovirus and nervous necrosis virus infection in vitro [25]. However, no references focused on the roles of other grouper TRIMs during virus infection up to now.

In the present study, we cloned a TRIM8 homolog from marine fish and evaluated the roles of EcTRIM8 in response to fish virus infection. Our data will contribute greatly to understanding the function of TRIM genes in response to fish virus infection.

2. Materials and methods

2.1. Fish, cells and viruses

Orange-spotted groupers, Epinephelus Coioides (50–60 g) were purchased from a marine fish farm, Hainan Province, China. Groupers were kept in a laboratory recirculating seawater system at 25 °C for two weeks before use. Grouper spleen (GS) cells used in this study were grown in Leibovitz’s L15 medium containing 10% fetal bovine serum (FBS, Gibco) at 25 °C [26]. SGIV and RGNNV were prepared and stored at −80 °C until used.

2.2. Cloning of EcTRIM8 and bioinformatic analysis

According to the sequences of several ESTs of EcTRIM8 from grouper spleen transcriptome [27], the full length cDNA of EcTRIM8 was amplified using the primers listed in Table 1. The putative open reading frame (ORF) and deduced amino acid sequences of EcTRIM8 was analyzed using BLAST program in NCBI database. The conserved domains and motifs were predicted using SMART program (http://smart.embl-heidelberg.de/). Multiple sequences alignment of TRIM8s was performed using ClustalX1.83 software. The phylogenetic tree was constructed using Mega 4.0 software.

2.3. Expression profiles for EcTRIM8 in healthy and challenged grouper

To examine the distribution pattern of EcTRIM8 in different tissues from healthy orange-spotted grouper, including head kidney, heart, liver, spleen, intestine, muscle, brain, skin, gill, stomach and kidney, total RNA was extracted as described previously [22]. The transcripts of EcTRIM8 in different tissues were detected by quantitative real-time PCR (qRT-PCR) as described following. To evaluate the expression profiles of EcTRIM8 in response to different stimuli, groupers were injected with PBS, SGIV, poly I:C as described previously [22]. Briefly, fish was intraperitoneal injected 100 μl individually with different stimuli, including poly I:C (1 μg/ml) and SGIV (105 TCID50/ml). The fish was treated with PBS as external control. Poly I:C treated groupers were collected at 0, 3, 6, 12, 24, 48 h post injection, and SGIV treated groupers were collected at 0, 3, 12, 24, 48, 72 h post infection. At indicated time points, the spleen of different groups (n > 3) of challenged fishes were harvested for RNA extraction and qRT-PCR analysis.

2.4. Plasmid construction

To explore the characteristic and function of EcTRIM8 in vitro, the full length of EcTRIM8 was amplified and cloned into pEGFP-N3 or pcDNA3.1-Flag vector as described previously. All the primers were listed in Table 1, and the constructed plasmids (pEGFP-EcTRIM8 and pcDNA-EcTRIM8) were subsequently confirmed by DNA sequencing.

2.5. Cell transfection and reporter gene assay

Cell transfection was performed using Lipofectamine 2000 program (http://smart.embl-heidelberg.de/). Multiple sequences alignment of TRIM8s was performed using ClustalX1.83 software. The phylogenetic tree was constructed using Mega 4.0 software.

Table 1

| Name                  | Sequence (5′–3′)                      |
|-----------------------|---------------------------------------|
| EcTRIM8-F             | ATGGATGAAAGTGGGAGAAGACGTGCC           |
| EcTRIM8-R             | CTGGTCCTACATGTGGTTGTTT               |
| EcTRIM8-Flag-F        | TCAGTGACACATTGGGAAGTTGAAAGACTCGCA    |
| EcTRIM8-Flag-R        | ACATCTCAGCTGGTCTCCATATATGGTGCATG     |
| pc1-EcTRIM8-F         | AATTGACTCTTTGTCGTCCTACAGGGTGTTATG    |
| pc1-EcTRIM8-R         | TACCACCTGGCTCAGGCA                    |
| Actin-RT-F            | GCTGGTATGTACACCTGGTAA                 |
| Actin-RT-R            | CTATCTGCTCATACCTGGTAA                 |
| SGIV MCP-RT-F         | GACGTCCTCTTCCTACCTTC                 |
| SGIV MCP-RT-R         | AACGGCAACGGGACAGACTCT                 |
| SGIV VP19-RT-F        | TCCAGGAGGAAGTATTAAG                  |
| SGIV VP19-RT-R        | GGGGTAAGCTGGTAAGACT                   |
| SGIV ICP-18-RT-F      | ATCCAGTCTACCTGGTGG                   |
| SGIV ICP-18-RT-R      | CCGTCGCCCTGTTCATAT                   |
| RGNNV RdRp-RT-F       | GGGTGGAGACAGGTTTAAG                  |
| RGNNV RdRp-RT-R       | CTTAGTGTATACACCTGGTAA                 |
| RGNNV CP-RT-F         | CAATCGGACACCATACCCCTC                |
| RGNNV CP-RT-R         | CAATCGGACACCATACCCCTC                |
| EcIF3-F               | ATGGTTGTTAGCTGGGAGCTGGG               |
| EcIF3-R               | GAGGCGAGAAGACAGGGTGAGGAGGA           |
| EcIF7-F               | AAACCGGATACAAACCA                     |
| EcIF7-R               | TCTTCTACAGCTCATACATAGGGC             |
| EcTNFo-RT-F           | GCTGTCCTGGTCTGCGGTGTA                |
| EcTNFo-RT-R           | CATGTCGACCTGTATAGGTCGTT              |
| EcL1-1R-RT-PF         | AACGTCATGTAGCAACAGC                   |
| EcL1-1R-RT-PR         | ACTGGTCCTCAACACGACGAC                 |
| EcL1-2-RT-PF          | GGCGTCATGAGAAGGAGGCTGTA              |
| EcL1-2-RT-FR          | ATCCACGTGGGGAGATGCGA                 |

To explore the characteristic and function of EcTRIM8 in vitro, the full length of EcTRIM8 was amplified and cloned into pEGFP-N3 or pcDNA3.1-Flag vector as described previously. All the primers were listed in Table 1, and the constructed plasmids (pEGFP-EcTRIM8 and pcDNA-EcTRIM8) were subsequently confirmed by DNA sequencing.

2.5. Cell transfection and reporter gene assay

Cell transfection was performed using Lipofectamine 2000 program (http://smart.embl-heidelberg.de/). Multiple sequences alignment of TRIM8s was performed using ClustalX1.83 software. The phylogenetic tree was constructed using Mega 4.0 software.
Fig. 1. Amino acid alignment of TRIM8s from different species. The RING domain, B-BOX, and coiled-coil domain were indicated above the sequences.
reagent (Invitrogen) as described previously [22]. Briefly, GS cells were grown in 24-well plates, and then the mixture of Lipofectamine 2000 and plasmids was added for 6 h incubation. After replacing with the fresh medium, cells were cultured at 25 °C for further study.

To evaluate the effects of EcTRIM8 on the promoter activity of ISRE induced by IRF3 or IRF7, luciferase activity assays were carried out as described previously [22]. In brief, GS cells were co-transfected with 0.1 μg ISRE-Luc, and 0.4 μg EcIRF3/IRF7 or 0.4 μg EcTRIM8 as described above. A total of 0.05 μg pRL-TK was included to normalize the expression level. At 48 h post-transfection, cells were collected to measure the luciferase activities using a Victor X5 Multilabel plate reader (PerkinElmer).

2.6. Fluorescent microscopy

To determine the subcellular localization of EcTRIM8 in fish cells, pEGFP-N3 and pEGFP-EcTRIM8 were transfected into GS cells as described above. At 48 h post transfection, cells were washed with PBS, and then fixed using 4% paraformaldehyde. After staining

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Fig. 2. Phylogenetic analysis of EcTRIM8. A neighbor-joining tree was constructed based on the protein sequences of TRIM8-like genes from different species using MEGA 4.0 software. The bootstrap values were indicated at the branch points. All the sequences of TRIM8-like genes used in this study were obtained from GenBank database.

Fig. 3. The expression profiles of EcTRIM8 in healthy and challenged grouper. (A) The relative mRNA level of EcTRIM8 in different tissues from healthy groupers. After injection with poly I:C (B), and SGIV (C), the expression levels of EcTRIM8 in grouper spleen were detected using qRT-PCR.
with 4,6-diamidino-2-phenylindole (DAPI), cells were observed under fluorescence microscopy.

2.7. qRT-PCR analysis

To evaluate the effect of EcTRIM8 on fish virus replication, cells overexpressing EcTRIM8 and vector (pcDNA3.1-Flag) were incubated with SGIV (for 24, 48 h) or RGNNV (for 24, 48 h) and culture at 25 °C. At indicated time points, virus infected cells were collected for RNA extraction and qRT-PCR analysis.

In this study, all the qRT-PCR assays were performed as described previously [24]. Briefly, each assay was performed in triplicate using the cycling condition as follows: 94 °C for 5 min, followed by 45 cycles of 5 s at 94 °C, 10 s at 60 °C and 15 s at 72 °C. The relative expression level of virus genes including SGIV MCP (major capsid protein), VP19 (ORF019), ICP-18, RGNNV CP (coat protein) and RdRp (RNA-dependent RNA polymerase) and host genes including TNFα, IL-8, IL-1β, IRF3 (interferon regulatory factor 3) and IRF7 were analyzed as described previously [22,24]. The data were calculated as the folds based on the expression level of targeted genes normalized to β-actin. The Data were represented as mean ± SD, and the statistical significances were determined with Student’s t-test. The significance level was defined as p < 0.05(*).

2.8. Immune fluorescence assay

To evaluate the roles of EcTRIM8 overexpression on viral protein synthesis, immune fluorescence assay was performed using SGIV VP19 antibody or RGNNV CP antibody as described previously [24]. In brief, cells were seeded into 24-wells plate and then transfected with plasmids pcDNA3.1-Flag or pcDNA-EcTRIM8. Then cells were infected with SGIV or RGNNV at 24 h post transfection. After another 24 h incubation, cells were fixed and incubated with anti-VP19 (1:100) or anti-CP (1:100), followed by FITC-conjugated goat anti-mouse antibodies (Pierce). Finally, cells were stained with DAPI, and observed under fluorescence microscopy (Leica, Germany).

3. Results

3.1. Sequence characterization of EcTRIM8

Based on the EST sequences from grouper spleen transcriptome, we obtained the full length cDNA of EcTRIM8 in this study. EcTRIM8 encoded a 568-aa protein which shared 92% identity with TRIM8 homolog from large yellow croaker (Larimichthys crocea). Amino acid alignment indicated that EcTRIM8 contained a RING finger
domain in the N terminal, a B-BOX and a coiled-coil domain (Fig. 1). Phylogenetic analysis indicated that EcTRIM8 showed the nearest relationship to TRIM8 homolog of large yellow croaker (Fig. 2).

3.2. Tissue distribution and expression profiles of EcTRIM8

The mRNA expression level of EcTRIM8 in various tissues from healthy grouper was examined by qRT-PCR. As shown in Fig. 3A, the predominant expression of EcTRIM8 was found in the kidney, gill, fin, liver, spleen and brain.
To detect the expression level of EcTRIM8 after injection with poly I:C and SGIV, the transcript of EcTRIM8 in spleen was evaluated using qRT-PCR. Upon challenge with poly I:C or SGIV, the transcripts of EcTRIM8 were up-regulated at the early stage of injection. In detail, at 12 h post-injection, the expression levels of EcTRIM8 increased up to 6.5, and 2.6-fold compared to control group (Injection with PBS) in response to poly I:C, and SGIV challenge, respectively (Fig. 3).

3.3. The subcellular localization of EcTRIM8

To demonstrate the subcellular localization of EcTRIM8 in vitro, pEGFP-N3 or pEGFP-EcTRIM8 was transfected into GS cells and then stained with DAPI at 48 h post transfection. As shown in Fig. 4, the green fluorescence in pEGFP-EcTRIM8 transfected cells showed different distribution patterns, including fluorescent punctuates evenly situated throughout the cytoplasm and bright aggregates. Differently, in pEGFP-N3 transfected cells, the green fluorescence was distributed throughout the cells.

3.4. Ectopic expression of EcTRIM8 inhibited fish virus replication

To elucidate the roles of EcTRIM8 during fish virus infection, we firstly examined the CPE progression and viral gene transcription of SGIV and RGNNV in infected EcTRIM8-expressing cells, respectively. In control cells, SGIV or RGNNV infection induced CPE occurred at 12 h p.i., evidenced by cell rounding and vacuoles. At 24 h p.i., the complete CPE induced by virus infection could be observed under microscopy. As shown in Fig. 5A, in EcTRIM8 overexpressing cells, the severity of CPE induced by SGIV and RGNNV obviously weakened compared to control cells (vector transfected cells) at 24 h p.i. Furthermore, we evaluated the effect of EcTRIM8 overexpression on viral gene transcription. Our results showed that the transcription levels of RGNNV CP and RdRp were significantly decreased in EcTRIM8 overexpressing cells compared to control vector transfected cells (Fig. 5B). Meanwhile, EcTRIM8 overexpression also reduced the transcripts of SGIV MCP, VP19 and ICP-18 significantly (Fig. 5C).

In addition, we also determine the effect of EcTRIM8 overexpression on viral protein synthesis. Consistently, in SGIV-infected cells, the virus assembly sites which were labeled by anti-VP19 were obviously reduced in EcTRIM8 overexpressing cells. In RGNNV infected cells, the green fluorescence density of CP antibody labeled cells were also obviously decreased in EcTRIM8 overexpressing cells (Fig. 6). Thus, EcTRIM8 overexpression significantly inhibited fish virus replication in vitro.

3.5. Overexpression of EcTRIM8 regulated the expression of proinflammatory cytokines during virus infection

To elucidate the potential roles of EcTRIM8 during fish virus infection, we determined the changes in gene expression level of several proinflammatory cytokines. As shown in Fig. 7, we found that the expression level of cytokines, including TNFα, IL-1β, and IL-8 were all significantly increased in EcTRIM8 overexpressing cells. After SGIV infection, the expression level of TNFα and IL-1β were both increased significantly at 48 h p.i., and overexpression of EcTRIM8 enhanced the up-regulation of their expression. Although the expression of IL-8 was significantly decreased at 48 h p.i. in control cells, the overexpression of EcTRIM8 rescued the inhibitory
effect of SGIV infection on IL-8 expression at this time point. During RGNNV infection, the expression level of TNFα, IL-1β and IL-8 were all increased at 24 and 48 h p.i.. Notably, the overexpression of EcTRIM8 enhanced RGNNV-induced expression of IL-8, but weakened RGNNV-induced TNFα and IL-1β expression.

3.6. Overexpression of EcTRIM8 regulated interferon signaling during virus infection

Except the proinflammatory cytokines, we also evaluated the effects of EcTRIM8 overexpression on the expression of interferon signaling molecules during SGIV or RGNNV infection. As shown in Fig. 8, overexpression of EcTRIM8 significantly increased the expression of IRF3 and IRF7 compared to the control cells. During SGIV infection, although the expression of IRF3 and IRF7 was decreased with the infection time increased, the relative expression levels of them in EcTRIM8 overexpressing cells were significantly increased compared to control cells. In contrast, during RGNNV infection, the expression level of IRF3 and IRF7 were increased at 24 and 48 h p.i.. Moreover, the overexpression of EcTRIM8 significantly enhanced the expression of IRF7, but not IRF3. Meanwhile, the ectopic expression of EcTRIM8 significantly induced the ISRE promoter activity (23.00 ± 1.98 folds) compared to the control vector transfected cells. Further studies showed that both IRF3 and IRF7 was able to increase ISRE promoter activity, and the EcTRIM8 overexpression could positively regulated IRF3 and IRF7 induced ISRE promoter activity (Fig. 9). Thus, we proposed that EcTRIM8 might regulate IFN response differently during fish DNA virus and RNA virus infection.

4. Discussion

Mammalian TRIM proteins have been demonstrated to play critical roles in antiviral immunity [5,6]. Although the expression profiles of fish TRIM genes in response to poly I:C or other stimuli was uncovered, the actions of fish TRIMs in virus infection were still largely unknown. Our previous reports on grouper transcriptomic studies suggested that several TRIM genes might be involved in SGIV infection [27]. In this study, EcTRIM8 was cloned and its detailed roles were investigated. Sequence analysis indicated that the obtained EcTRIM8 homolog shared the highest identity with large yellow croaker. Amino acid alignment analysis showed that EcTRIM8 contained the conserved RING, B-BOX, and coiled-coil domains like mammalian TRIM8, suggesting that EcTRIM8 might be functional in various biological process. In considering that previous studies on TRIM8 mainly focused on its roles in inflammation and cancer [11,12], we further investigated the potential function of EcTRIM8 in virus infection. Here, we found that the transcription of EcTRIM8 in grouper spleen was increased after challenge with SGIV or poly I:C, suggested that EcTRIM8 might play crucial roles in fish immune response against viruses infection. In addition, our results showed that EcTRIM8 displayed different

Fig. 7. Overexpression of EcTRIM8 altered the expression of proinflammatory cytokines during virus infection. The relative expression level of TNFα (A), IL-8 (B), and IL-1β (C) were determined using qRT-PCR analysis.
distribution patterns in transfected cells, including punctate fluorescence and bright aggregates. Although punctate fluorescence was also observed in mammalian TRIM8 transfected cells, the aggregates were not described in the published reference [28]. Whether the specific localization patterns of EcTRIM8 were associated with its function was still uncertain.

Increased reports demonstrated that many TRIM proteins exerted critical roles in innate antiviral immunity [5–7]. TRIM56 functioned as an antiviral host factor that confers resistance to YFV, DENV2, and HCoV OC43 through overlapping and distinct molecular determinants [7]. In vitro, ectopic expression of feline TRIM25 in HEK293T cells reduced viral protein levels leading to the inhibition of feline leukemia virus (FeLV) release at the late stage of virus infection [29]. Although TRIM8 has been demonstrated to play critical roles in inflammation and carcinogenesis [30], the roles of TRIM8 in virus infection still remained uncertain. In our study, overexpression of EcTRIM8 significantly reduced the severity of CPE evoked by SGIV or RGNNV infection. At the transcription level, viral genes including SGIV MCP, VP19, ICP-18, RGNNV CP and RdRp genes were all significantly inhibited by ectopic expression of EcTRIM8. At the protein level, the protein synthesis of SGIV VP19 and RGNNV CP protein were both significantly decreased in EcTRIM8 overexpressing cells. Thus, we proposed that TRIM8 was also an important antiviral factor in response to virus infection.

It has been reported that multiple signaling molecules were mediated or modified by TRIM proteins in innate immune response or virus infection [31,32]. TRIM13 inhibited MDA5 activity during encephalomyocarditis virus (EMCV) infection [5], while TRIM56 overexpression enhanced IFN-β promoter activation after double-stranded DNA stimulation [33]. Overexpression of TRIM21 resulted in more degradation of DDX41 and less production of interferon-β (IFN-β) in response to intracellular dsDNA [32], and knockdown of TRIM38 expression resulted in augmented activation of IRF3 and enhanced expression of IFN-β [34]. In our study, the ectopic expression of EcTRIM8 in vitro not only regulated the expression of pro-inflammatory cytokines, but also altered the expression of interferon related signaling molecules or EcTRIM8, and the ISRE promoter activity was determined using reporter gene assay.

**Fig. 8.** Overexpression of EcTRIM8 regulated the expression of interferon related transcription factors during virus infection. The relative expression level of IRF7 (A) and IRF3 (B) were determined using qRT-PCR analysis.

**Fig. 9.** Overexpression of EcTRIM8 regulated IRF3- and IRF7-induced interferon response. GS cells were transfected with empty vector, IRF3/IRF7, or EcTRIM8, and the ISRE promoter activity was determined using reporter gene assay.
regulated by the ectopic expression of EcTRIM8. Given that interferon signaling molecules usually played crucial roles against RNA virus infection, and group ISG15 and IRF3 have been demonstrated to exert antiviral function in response to RGNNV infection [22,35], we proposed that the positively regulatory roles on interferon signaling might directly contribute to the antiviral action of EcTRIM8 against RGNNV infection. In contrast, the interferon related molecules including both grouper IRF3 and ISG15 were not able to inhibited SGIV infection in vitro [22,35], we speculated that the inhibitory effect of EcTRIM8 on SGIV replication might be mainly due to its regulatory roles on the pro-inflammatory cytokines during virus infection. Together, EcTRIM8 exerted antiviral roles against iridovirus and nodavirus infection via different potential mechanisms. In summary, we cloned a novel TRIM8 homolog from fish and investigated its roles in fish virus infection. We found that overexpression of EcTRIM8 in vitro inhibited fish virus replication, including iridovirus and nodavirus. Moreover, overexpression of EcTRIM8 was able to regulate the expression of pro-inflammatory cytokines and interferon related signaling molecules during fish virus infection. Our results will provide new insights into understanding the function of TRIM8 against fish virus infection.

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