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The novel gene TRIM44L from orange-spotted grouper negatively regulates the interferon response

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ABSTRACT

Accumulated evidence suggests that some of the tripartite motif (TRIM) -family proteins function as critical regulators of carcinogenesis, immunity, and antiviral functions. TRIM44 is an atypical TRIM family protein that lacks the entire RING domain and has been demonstrated to play a crucial role in cancer and viral infection. To our knowledge, the role of TRIM44 in fish still remains largely unknown. Here, we cloned and characterized a novel TRIM44-like gene from orange spotted grouper (EcTRIM44L). Sequence analysis indicated that EcTRIM44L encoded a 393 amino acid peptide, which shared 81.44% and 51.02% identity with large yellow croaker (*Larimichthys crocea*) and zebrafish (*Danio rerio*), respectively. However, EcTRIM44L only exhibited 24.69% identity with the TRIM44 protein of humans (*Homo sapiens*). Moreover, EcTRIM44L contained two conserved domains, including a B-Box domain and a coiled-coil domain, but not a RING domain. Using fluorescence microscopy, we observed green fluorescence in the cytoplasm of the EcTRIM44L-EGFP transfected grouper spleen (GS) cells. As the infection proceeded, EcTRIM44L transcription was significantly up-regulated in red-spotted grouper nervous necrosis virus (RGNNV) infection, suggesting that EcTRIM44L might be involved in fish virus infections. The *in vitro* overexpression of EcTRIM44L significantly enhanced RGNNV replication, as demonstrated by the accelerated cytopathic effect (CPE) progression induced by RGNNV, as well as the increased expression of coat protein (CP) and RNA-dependent RNA polymerase (RdRp). The overexpression of EcTRIM44L significantly decreased the level of interferon (IFN) related signaling molecules and pro-inflammatory cytokine expression, suggesting that EcTRIM44L affected virus replication by negatively regulating the IFN response. In addition, the melanoma differentiation-associated protein 5 (MDA5) and mitochondrial antiviral-signaling protein (MAVS), but not mediator of IRF3 activation (MITA)-evoked IFN response was negatively regulated by EcTRIM44L. Together, for the first time, our results indicate that EcTRIM44L negatively regulates the interferon response against grouper RNA virus infection.

1. Introduction

Tripartite motif (TRIM) proteins play an important role in a wide range of biological processes, including cellular proliferation, differentiation, development, apoptosis, oncogenesis, and innate immunity [1,2]. In response to a viral infection, TRIM proteins differentially regulate the host immune response and affect virus replication. For example, TRIM30α negatively regulates TLR-mediated NF-κB activation [3], and TRIM5 both constitutively promotes innate immune signaling and acts as a pattern recognition receptor specific for the retrovirus capsid lattice [4]. Moreover, TRIM21 activates the proinflammatory response, which results in the secretion of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [5]. In addition, TRIM8 negatively regulates the TLR3-and TLR4-mediated innate immune and inflammatory responses, which reveals an additional mechanism for the termination of TLR3/4-mediated inflammatory responses [6].

TRIM44 is an atypical TRIM family protein which contains a zinc-finger domain but lacks the RING finger domain, and functions as a deubiquitinase [7,8]. TRIM44 has also been identified as a prognostic factor that promotes cellular proliferation and migration, and inhibits apoptosis in a diversity of tumors [9,10]. In addition, human TRIM44

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has been demonstrated to act as a positive regulator of the virus-triggered immune response by enhancing the stability of virus-induced signaling adaptor (VISA) [11].

Grouper (*Epinephelus* spp.) is one of the most important economic marine fish species that are widely cultured in tropical and subtropical regions, particularly in south-east Asia. However, cultured groupers are frequently suffered from outbreaks of devastating viral diseases caused by Singapore grouper iridovirus (SGIV) and red-spotted grouper nervous necrosis virus (RGNNV) in recent years [12–14]. To better understand the potential immune defense mechanisms by grouper against viral infection, numerous genes involved in interferon response have been cloned and their roles in the response to viral infection have been characterized [15–21]. Interestingly, TRIM family members (e.g., TRIM25, TRIM16L, TRIM8, TRIM32, and TRIM62) exerted different roles during SGIV and RGNNV infection [15,22–25]. For example, TRIM25 was found to positively regulate the interferon response, whereas TRIM62 negatively regulated the antiviral immune response against RGNNV infection [25]. Based on transcriptome analyses of grouper, we also found several EST sequences which shared identity with TRIM44 from other species [26]. However, whether grouper TRIM44 homologs also exert regulatory roles on the host interferon and inflammatory response during viral infection remains unknown.

Recently, a novel TRIM gene from orange spotted grouper (EcTRIM44L) was cloned and characterized. The role of EcTRIM44L in response to fish virus infection and the potential mechanisms underlying the function of EcTRIM44L were investigated in grouper spleen (GS) cells. Our data will provide novel insights into the function of fish TRIM genes against viral infection.

2. Materials and methods

2.1. Fish, viruses, and cells

Orange-spotted groupers, *E. coioides* (50 g − 60 g), were purchased from Hainan Province, China and housed in a recirculating seawater system. Grouper spleen (GS) cells were cultured at 28 °C in Leibovitz's L15 medium containing 10% fetal bovine serum (FBS, Gibco) [27]. The viruses used in this study (including SGIV and RGNNV) were prepared as previously described [27,28].

2.2. Cloning of EcTRIM44L and bioinformatics analysis

According to the assembly of EST sequences from the grouper spleen transcriptome [29], we cloned the full-length cDNA of EcTRIM44L by PCR amplification, which was verified with DNA sequencing. The EcTRIM44L amino acid sequence was subjected to a BLAST analysis against the NCBI database. Multiple sequences of EcTRIM44L homologs were analyzed using ClustalX1.83 software, and edited with the GeneDoc program. A phylogenetic tree was constructed using Mega 6.0 software.

2.3. EcTRIM44L expression patterns

To clarify the EcTRIM44L distribution pattern, we extracted the total RNA from different grouper tissues, including the head, kidney, heart, liver, spleen, intestine, muscle, brain, skin, gill, stomach, fin, and kidney, as previously mentioned [16]. Additionally, we used quantitative real-time PCR (qPCR) to examine the level of EcTRIM44L expression.

To analyze the EcTRIM44L expression profiles against fish virus infection, GS cells were seeded into 24-well cell culture plates, and then infected with SGIV or RGNNV. The cells were collected at 6 h, 12 h, 24 h, 36 h, and 48 h post infection (p.i.), and subjected to further qPCR analysis.

Table 1
Primers used in this study.

Primer names	Sequence5′-3′
EcTRIM44L-ORF-F	ATGGACCACAAAGGGGAACC
EcTRIM44L-ORF-R	CTTGTCGTCATCGTCTTTGTAG
EcTRIM44L-3.1-Flag-HindIII-F	CCC*AAGCTT*CGATGGACCACAAAGGGGAACC
EcTRIM44L-3.1-Flag-BamHI-R	CG*GGATCC*CTTGTCGTCATCGTCTTTGTAG
EcTRIM44L-C1-HindIII-F	CCC*AAGCTT*CGATGGACCACAAAGGGGAACC
EcTRIM44L-C1-BamHI-R	CG*GGATCC*CTTGTCGTCATCGTCTTTGTAG
EcTRIM44L-RT-F	ACTTGGCACCAAAAGAGACTCC
EcTRIM44L-RT-R	TGTCACTGTGTCCCTCTTCCCA
Actin- RT-F	TACGAGCTGCCTGACGGACA
Actin- RT-R	GGCTGTGATCTCCTTCTGCA
RGNNV CP-RT-F	CAACTGACAACGATCACACCTTC
RGNNV CP-RT-R	CAATCGAACACTCCAGCGACA
RGNNV RdRp-RT-F	GTGTCCGGAGAGGTTAAGGATG
RGNNV RdRp-RT-R	CTTGAATTGATCAACGGTGAACA
SGIV MCP- RT-F	GCACGCTTCTCTCACCTTCA
SGIV MCP- RT-R	AACGGCAACGGGAGCACTA
SGIV VP19-RT-F	TCCAAGGGAGAAACTGTAAG
SGIV VP19-RT-R	GGGGTAAGCGTGAAGAC
EcTNFα-RT-F	GTGTCCTGCTGTTTGCTTGGTA
EcTNFα-RT-R	CAGTGTCCGACTTGATTAGTGCTT
EcIL-1β-RT-PF	AACCTCATCATCGCCACACA
EcIL-1β-RT-PR	AGTTGCCTCACAACCGAACAC
EcIL-8-RT-PF	GCCGTCAGTGAAGGGAGTCTAG
EcIL-8-RT-PR	ATCGCAGTGGGAGTTTGCA
EcMXI-RT-F	CGAAAGTACCGTGGACGAGAA
EcMXI-RT-R	TGTTTGATCTGCTCCTTGACCAT
EcISG15-RT-F	CCTATGACATCAAAGCTGACGAGAC
EcISG15-RT-R	GTGCTGTTGGCAGTGACGTTGTAGT
EcIRF3-RT-F	GACAACAAGAACGACCCTGCTAA
EcIRF3-RT-R	GGGAGTCCGCTTGAAGATAGACA
EcIRF7-RT-F	CAACACCGGATACAACCAAG
EcIRF7-RT-R	GTTCTCAACTGCTACATAGGG
EcIFP35-RT-F	TTCAGATGAGGAGTTCTCTCTTGTG
EcIFP35-RT-R	TCATATCGGTGCTCGTCTACTTTCA
EcISG56-RT-F	CAGGCATGGTGGAGTGGAAC
EcISG56-RT-R	CTCAAGGTAGTGAACAGCGAGGTA

2.4. Plasmid construction

To clarify the molecular function of EcTRIM44L *in vitro*, EcTRIM44L was cloned into pEGFP-C1 and pcDNA3.1-Flag vectors, respectively. Corresponding recombinant plasmids were identified by DNA sequencing, including pEGFP-EcTRIM44L and Flag-EcTRIM44L.

2.5. Cellular transfection and luciferase assay

To detect the localization and function of EcTRIM44L *in vitro*, GS cells were seeded into 24-well plates and cultured overnight. Transfection was performed using the transfection reagent, Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions [29]. Briefly, the expression plasmids were mixed with Lipofectamine 2000 and then added to the cells for another 6 h incubation. After replacing the cells with fresh medium, cells were cultured at 28 °C for further study.

To determine the interferon promoter activity evoked by EcTRIM44L, GS cells were co-transfected with plasmids expressing EcTRIM44L with plasmids expressing MAVS, mediator of IRF3 activation (MITA), or melanoma differentiation-associated protein 5 (MDA5), and reporter plasmids expressing firefly luciferase promoter and renilla luciferase (internal control). Cells were collected and lysed at 48 h post-transfection, and a luciferase assay was performed using the Dual-Luciferase Reporter Assay system (Promega) as described earlier.

2.6. Fluorescence microscopy

To examine the subcellular localization of EcTRIM44L in grouper cells, pEGFP-C1 and pEGFP-EcTRIM44L plasmids were transfected into

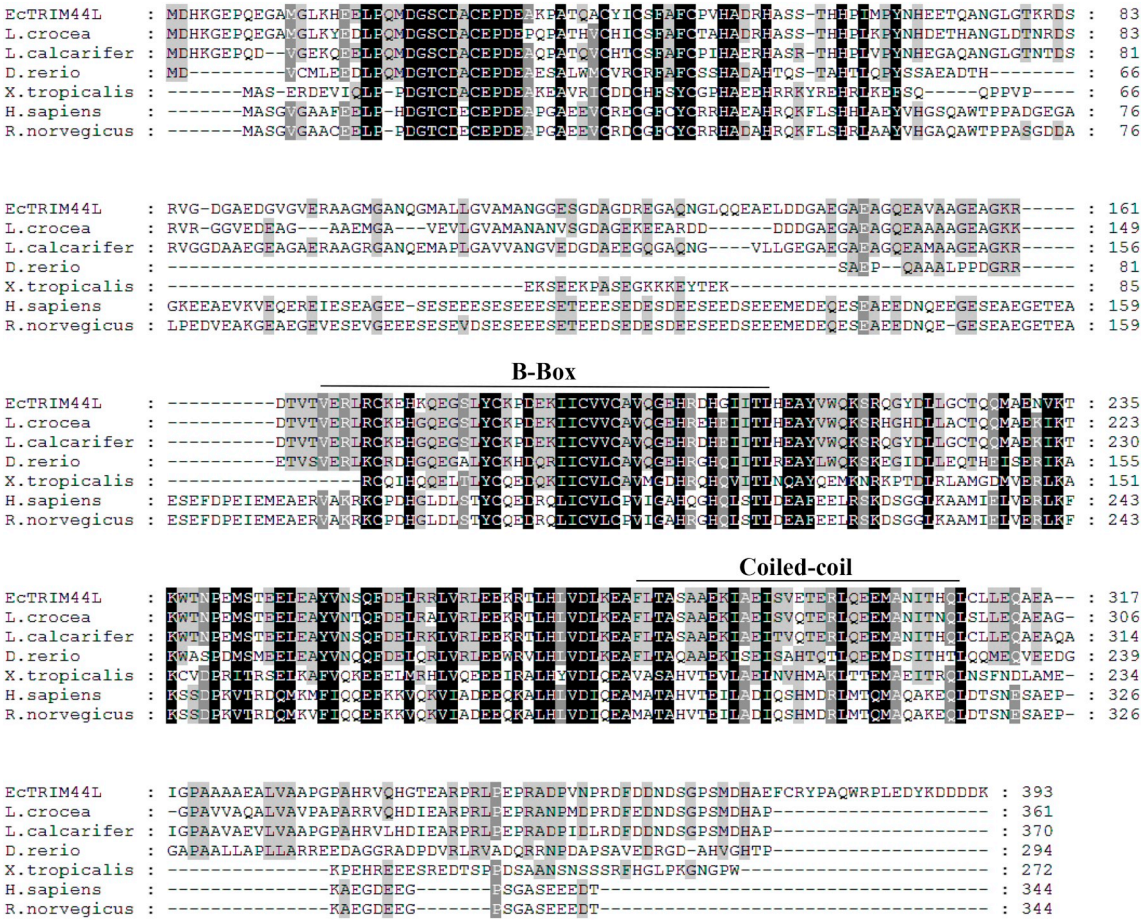

Fig. 1. Amino acid alignment of EcTRIM44L and other TRIM44 homologs from different species. The conserved domains, including B-Box and Coiled-coil domain, are underlined.

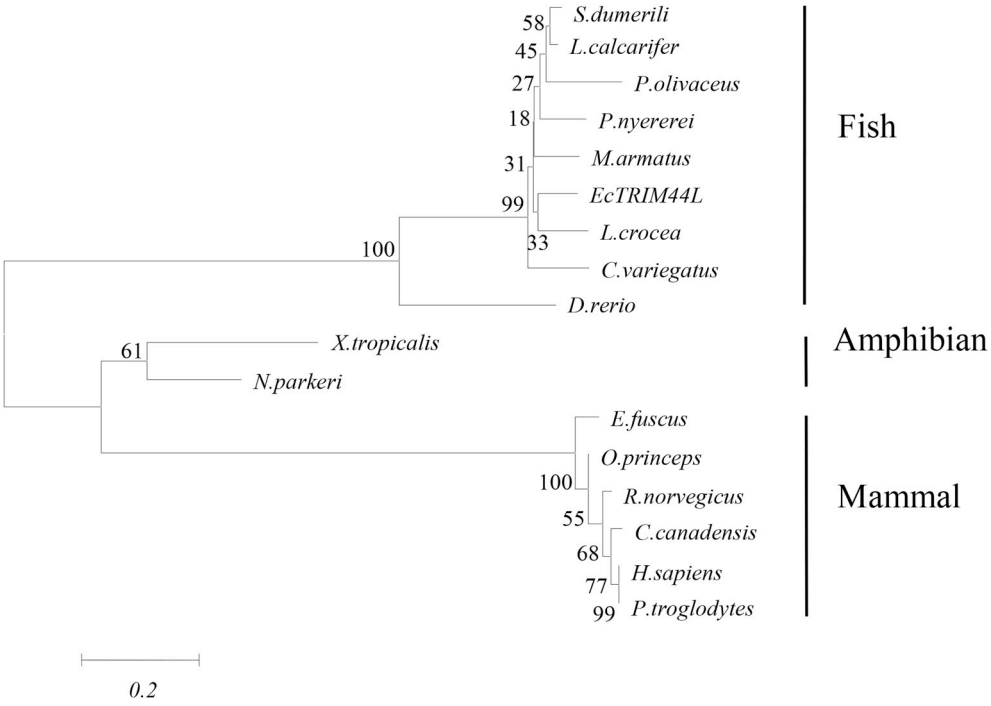

Fig. 2. Phylogenetic analysis of EcTRIM44L. A phylogenetic tree was constructed using MEGA 6.0 with the neighbor-joining (NJ) method. The bootstrap values are indicated at the branch points. The sequences of TRIM44-like genes used in this study were obtained from GenBank, and their accession numbers are listed as follows: *S. dumerili*, XP_022610248; *M. armatus*, XP_026167683; *L. calcarifer*, XP_018519596; *L. crocea*, XP_010731023; *D. rerio*, NP_001155221; *P. nyererei*, XP_005737727; *P. olivaceus*, XP_019934518; *C. variegatus*, XP_015231221; *H. sapiens*, NP_060053; *R. norvegicus*, NP_001013221; *X. tropicalis*, XP_002937339; *N. parkeri*, XP_018412965; *P. troglodytes*, XP_508888; *C. canadensis*, XP_020016408; *E. fuscus*, XP_008145221; *O. princeps*, XP_012782488.

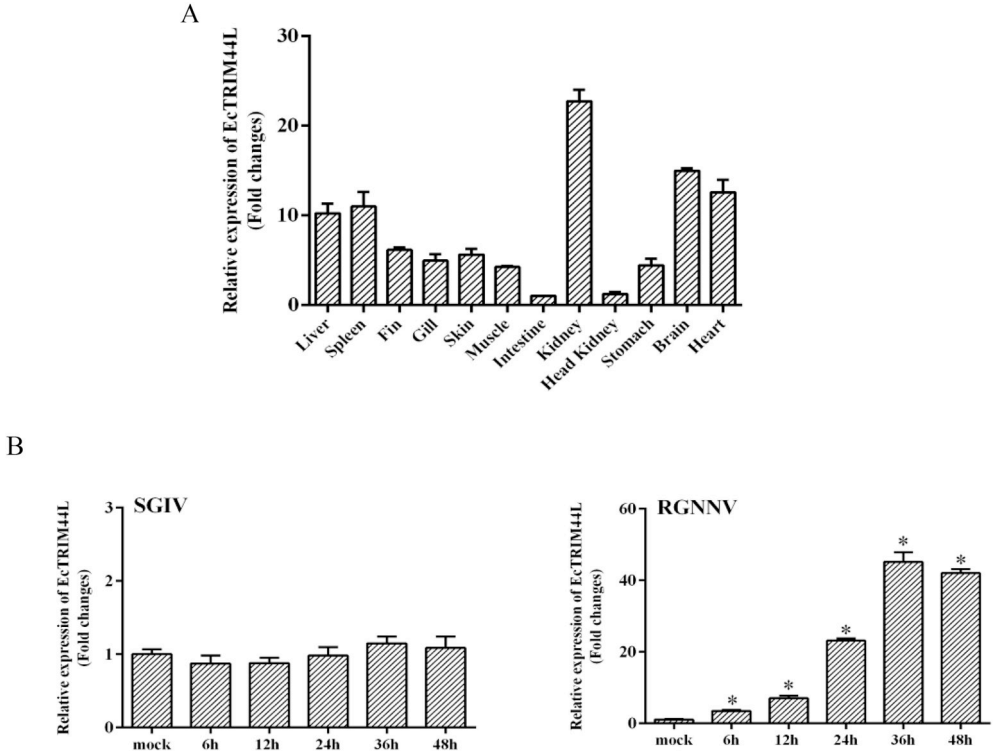

Fig. 3. Expression patterns of EcTRIM44L. (A) The EcTRIM44L expression profiles in various grouper tissues. (B) The level of EcTRIM44L expression in grouper cells in response to SGIV or RGNNV infection.

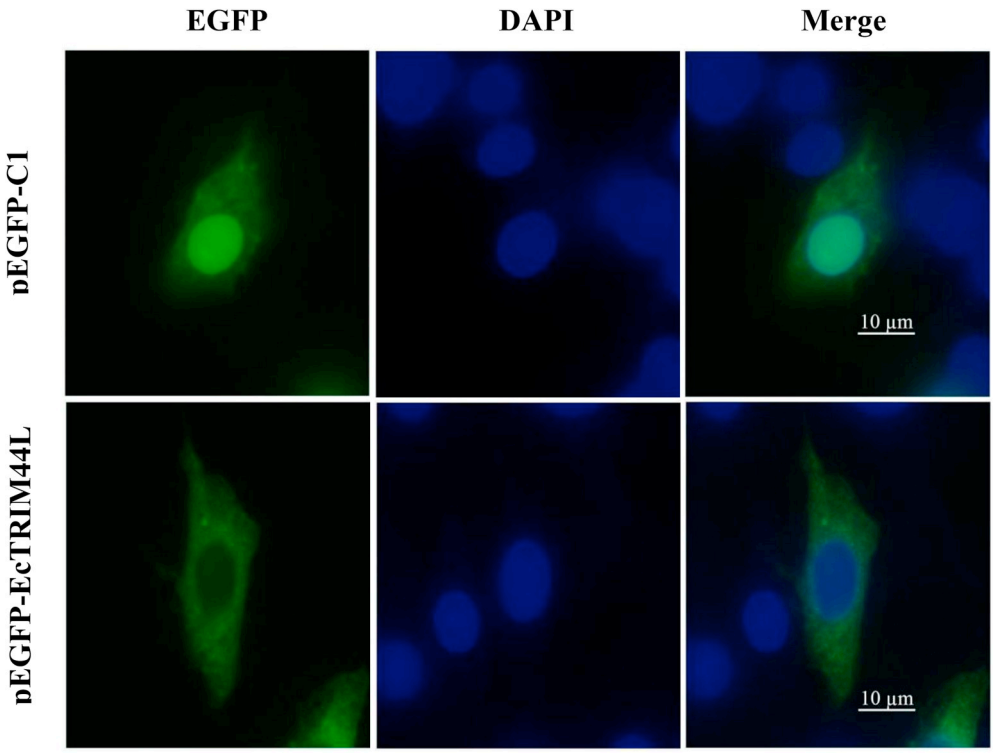

Fig. 4. Subcellular localization of EcTRIM44L in grouper cells. pEGFP-C1 and pEGFP-EcTRIM44L were transfected into GS cells, and then stained with DAPI. The fluorescence was observed under fluorescence microscopy.

GS cells as described above. At 48 h post-transfection, the cells were fixed and stained with 4,6-diamidino-2-phenylindole (DAPI). After washing with PBS, the samples were observed under fluorescence microscopy.

2.7. Immunofluorescence assay

GS cells were transfected with either control or EcTRIM44L plasmids for 12 h prior to RGNNV infection. The infected cells were

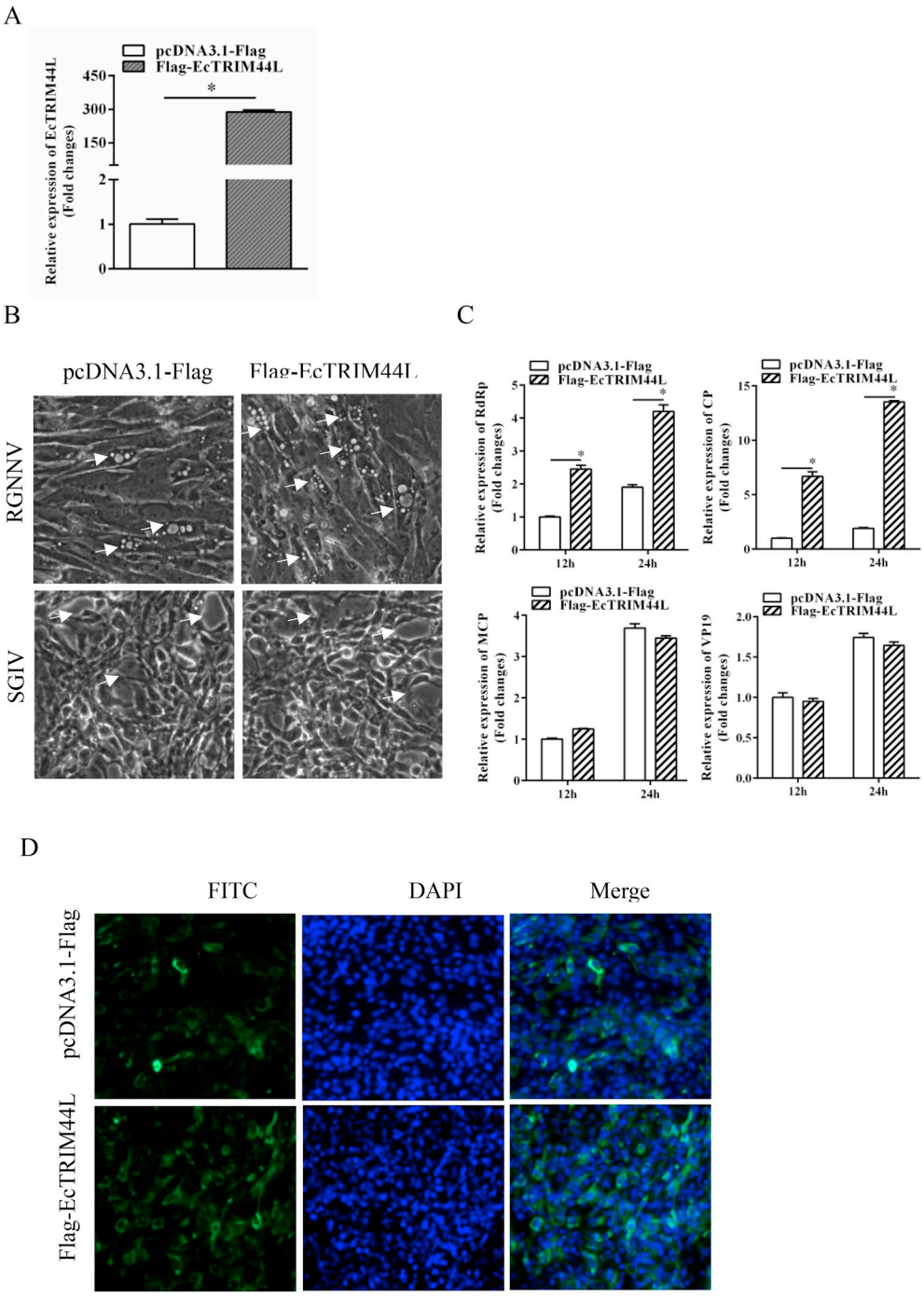

Fig. 5. The effect of EcTRIM44L-overexpression on fish virus replication. (A) The level of EcTRIM44L transcription in EcTRIM44L-overexpressing cells. (B) EcTRIM44L-overexpression significantly increased the severity of RGNNV-induced CPE in GS cells, and had no significant influence on the severity of CPE induced by SGIV in GS cells. The white arrows denote the vacuoles evoked by RGNNV and the rounding and aggregation of cells induced by SGIV. (C) The relative level of RGNNV CP and RdRp, as well as SGIV MCP and VP19 expression in infected EcTRIM44L overexpressing cells. (D) GS cells were transfected with either a control or EcTRIM44L plasmid for 12 h prior to RGNNV infection, and the infected cells were analyzed at 24 h p.i. by immunofluorescence assay (IFA) using an anti-CP antibody (green).

analyzed at 24 h p.i. with an immunofluorescence assay (IFA) using an anti-CP antibody (green) as described previously [30].

2.8. RNA extraction and qPCR

To determine the level of viral or host gene expression *in vitro*, the experimental grouper tissues or cells were collected at the indicated time points, and the total RNA was extracted using an SV Total RNA Isolation System (Promega) and reverse-transcribed with a ReverTraAce qPCR RT kit (Toyobo) as described previously [11]. qPCR was performed using the SYBR Green real-time PCR Kit (Toyobo) according to the manufacturers' instructions and was carried out following reverse transcription with QuantStudio5 applied biosystems (Thermo Fisher Scientific), as described previously. To evaluate the replication dynamics of RGNNV or SGIV, the expression level of viral genes, including RGNNV CP (coat protein), RdRp (RNA-dependent RNA polymerase), SGIV MCP (major capsid protein) and VP19 were detected. The expression level of genes involved in interferon response (e.g., IRF3, IRF7, ISG15, ISG56, IFP35, and myxovirus resistance gene MXI) and inflammatory response (e.g., IL-1β, IL-8, and TNFα) were also examined to assess the regulatory effect of EcTRIM44L on host immune responses.

Each qPCR analysis was performed at least in triplicate using the following cycling conditions: 94 °C for 5 min, followed by 45 cycles at 94 °C for 5 s, 60 °C for 10 s, and 72 °C for 15 s. The used primers were listed in Table 1. The expression level of target gene was normalized to that of β-actin and the data were calculated as the fold-expression compared to that of the empty vector.

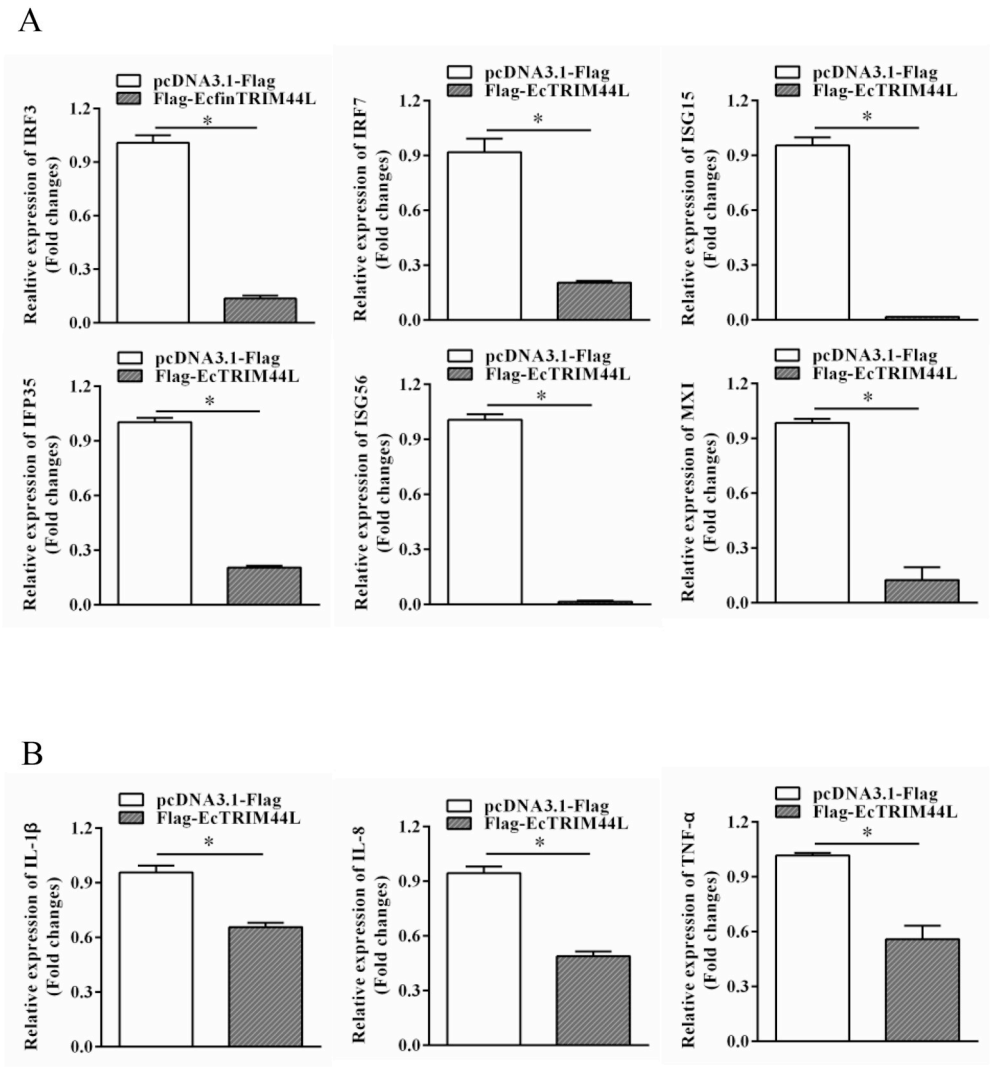

Fig. 6. EcTRIM44L overexpression inhibited the interferon and inflammatory response. (A) EcTRIM44L-overexpression decreased the level of interferon-related cytokine and effector expression. (B) The ectopic expression of EcTRIM44L down-regulated the level of pro-inflammatory factor expression.

2.9. Statistical analysis

The data are expressed as the mean ± SD and statistical significance was determined using a Student's *t*-test and established at $p < 0.05$ (*).

3. Results

3.1. Characteristics of EcTRIM44L

EcTRIM44L encodes a 393 amino acid peptide which shares 81.44% and 51.02% identity with the large yellow croaker (*Larimichthys crocea*) and zebrafish (*Danio rerio*), respectively. However, EcTRIM44L only shows 24.69% identity to TRIM44 protein of humans (*homo sapiens*). Amino acid alignment indicates that EcTRIM44L and TRIM44 homologs from other species all contained two conserved domains, including a B-BOX domain and a coiled-coil domain, but not a RING domain (Fig. 1). Phylogenetic tree shows the closest relationship between EcTRIM44L and that of large yellow croaker, and all the TRIM44 homologs from fish are clustered into one group, which is separated from amphibians and mammals (Fig. 2).

3.2. EcTRIM44L expression patterns

Using qPCR, EcTRIM44L transcripts were detected in all of the tissues isolated from healthy grouper. As shown in Fig. 3A, EcTRIM44L was predominantly expressed in the liver, spleen, kidney, brain, and heart. To examine the expression patterns of EcTRIM44L in response to viral infection *in vitro*, the level of EcTRIM44L transcription was detected in GS cells infected with RGNNV or SGIV. As shown in Fig. 3B, the level of EcTRIM44L expression was not significantly changed during SGIV infection. In RGNNV-infected cells, the level of EcTRIM44L expression was increased significantly from 6 h p.i. and reached the peak (~45 folds) at 36 h p.i. compared to that of the mock-infected cells.

3.3. EcTRIM44L encodes a cytoplasmic protein

To demonstrate the subcellular localization of EcTRIM44L *in vitro*, pEGFP-EcTRIM44L and pEGFP-C1 were transfected into GS cells and fluorescence was observed using fluorescence microscopy. As shown in Fig. 4, green fluorescence was distributed throughout the cytoplasm and nucleus in the pEGFP-C1-transfected cells. In contrast, green fluorescence was only observed in the cytoplasm in EcTRIM44L-transfected cells. Thus, EcTRIM44L was proposed to encode a cytoplasmic protein.

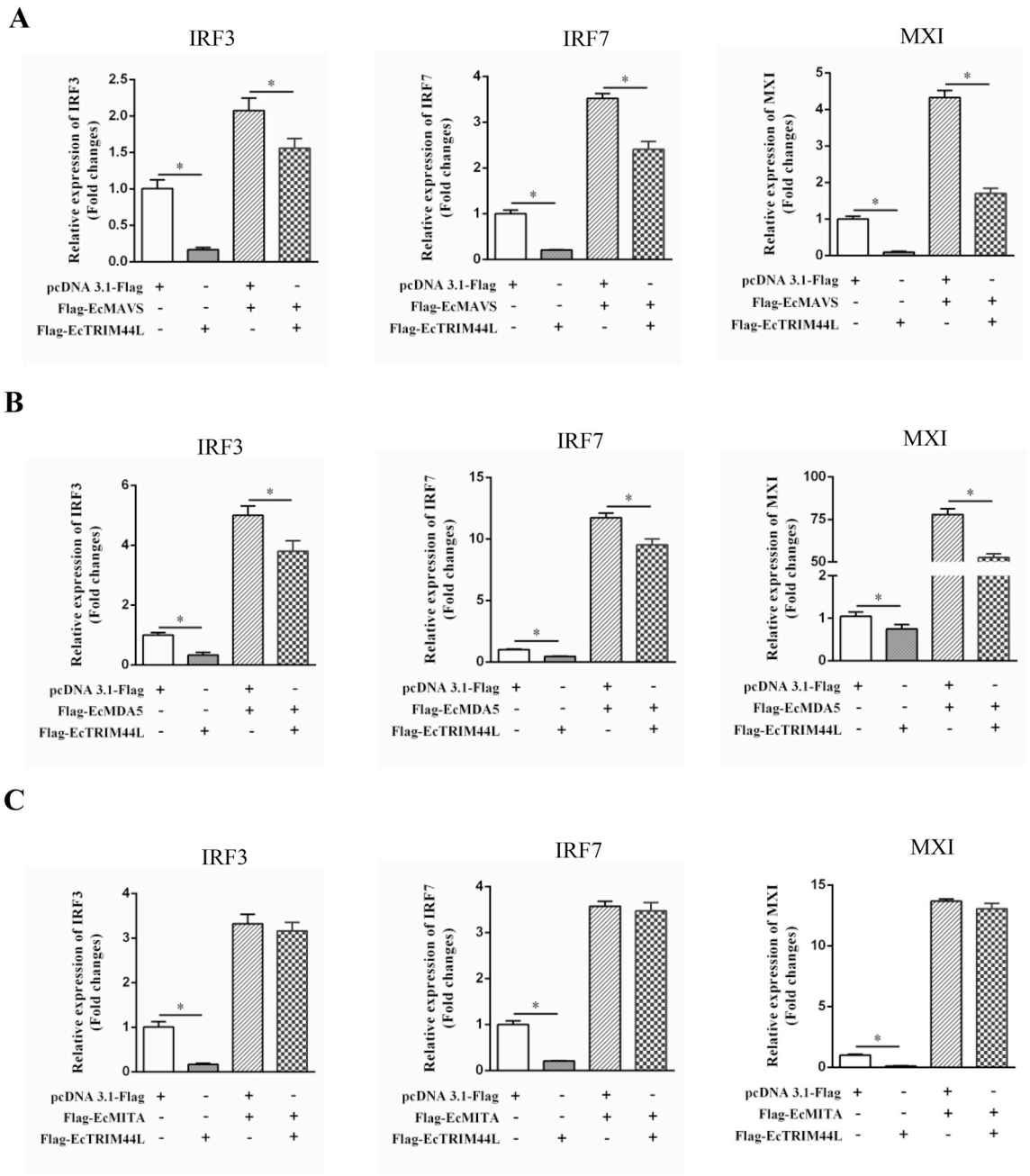

Fig. 7. EcTRIM44L co-transfection with MAVS, MDA5, or MITA. EcTRIM44L co-transfection with MAVS down-regulated the level of interferon-related signaling molecule expression (A). EcTRIM44L co-transfection with MDA5 down-regulated the level of interferon-related signaling molecule expression (B). EcTRIM44L co-transfection with MITA did not affect the level of interferon-related signaling molecule expression (C).

3.4. The effect of EcTRIM44L on grouper viral infection

To assess the role of EcTRIM44L during viral infection *in vitro*, we examined the process of CPE progression and viral replication in EcTRIM44L-overexpressing cells upon SGIV or RGNNV infection. As shown in Fig. 5A, the level of EcTRIM44L transcription in EcTRIM44L-transfected cells was significantly increased compared to the control vector transfected cells, suggesting that EcTRIM44L was successfully overexpressed in transfected cells. The severity of RGNNV-induced CPE was significantly enhanced in EcTRIM44L-overexpressing cells when compared with the control vector transfected cells (Fig. 5B); however, no obvious changes were observed during SGIV infection. Consistently, the level of viral gene expression, including RGNNV CP and RdRp, was significantly increased in RGNNV-infected EcTRIM44L-overexpressing cells compared to those in the control vector transfected cells (Fig. 5C). No significant alterations in the levels of SGIV MCP and VP19 expression were detected in infected EcTRIM44L-overexpressing cells compared to the control vector transfected cells. The immunofluorescence assay results also verified that EcTRIM44L significantly promoted the protein synthesis of the RGNNV CP (Fig. 5D). Thus, it was speculated that EcTRIM44L may function as a pro-viral factor in response to fish RNA virus infection.

3.5. The ectopic expression of EcTRIM44L down-regulated the expression of interferon and inflammatory signaling molecules

To clarify the potential mechanism of the antiviral action of EcTRIM44L, qPCR was used to examine the levels of host interferon

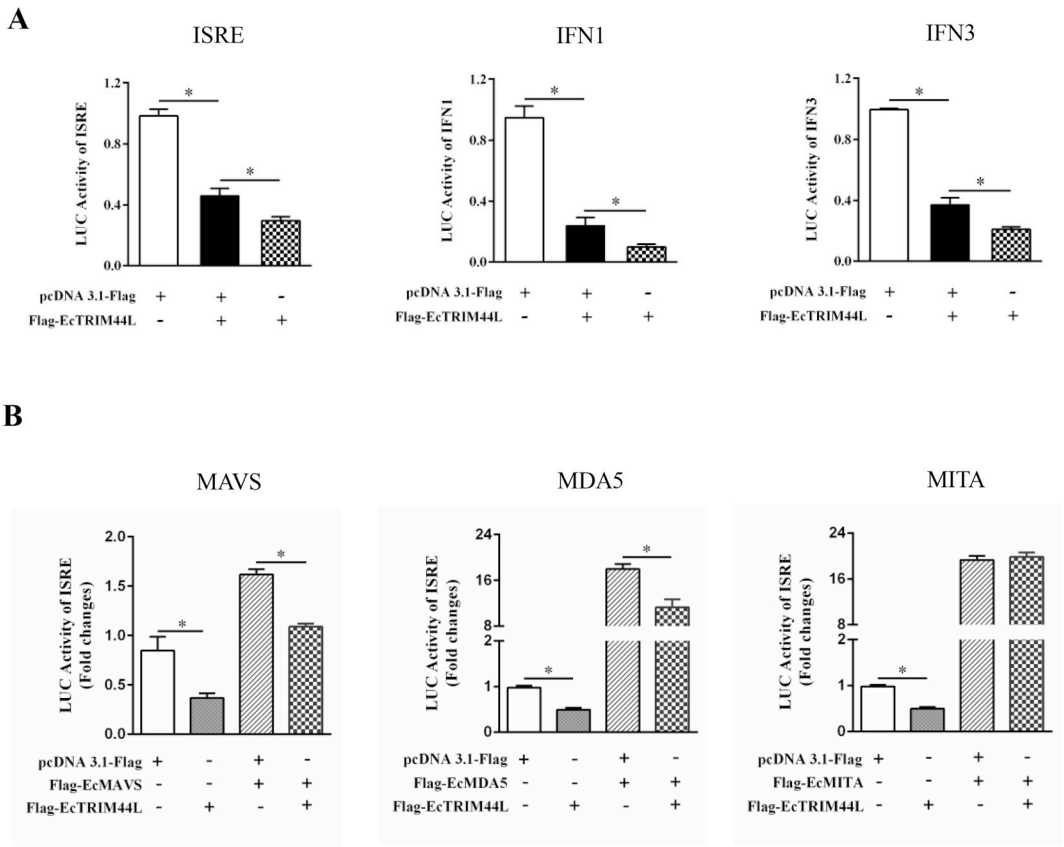

Fig. 8. EcTRIM44L overexpression down-regulated the ISRE promoter activities induced by MDA5 or MAVS, but had no significant influence on MITA-induced the ISRE promoter activities. (A) The ectopic expression of EcTRIM44L inhibited ISRE, IFN-1, and IFN-3 promoter activities. (B) The overexpression of EcTRIM44L reduced the MAVS-or MDA5-induced activity of the ISRE promoter, but had no significant influence on MITA-induced the ISRE promoter activities.

immune and inflammatory factor transcription in EcTRIM44L-over-expressing cells. As shown in Fig. 6A, the transcripts of IRF3, IRF7, ISG15, ISG56, IFP35, and MXI were all significantly down-regulated in EcTRIM44L-overexpressing cells compared to the control vector transfected cells. In particular, the expression levels of IRF3 and IRF7 in EcTRIM44L-overexpressing cells were decreased to 13.7% and 20.5% compared to the control vector transfected cells, respectively. In addition, EcTRIM44L overexpression also significantly decreased the transcription of pro-inflammatory factors, including TNFα, IL-1β, and IL-8. Thus, our data indicated that EcTRIM44L negatively regulated the host interferon and inflammatory response.

3.6. EcTRIM44L negatively regulated the MDA5-and MAVS-evoked interferon response

To explore whether the MDA5, MITA, or MAVS signaling molecules were associated with EcTRIM44L, we investigated the regulatory role of EcTRIM44L on immune response induced by MDA5, MITA, or MAVS, respectively. The level of expression of the above immune-related genes was detected in cells co-transfected with EcTRIM44L and MDA5/MITA/MAVS. As shown in Fig. 7, compared to the empty vector, the over-expression of MITA, MDA5, or MAVS alone increased the expression of interferon-related signaling molecules (e.g., IRF3, IRF7, and MXI). However, co-transfection with EcTRIM44L significantly decreased the expression of these immune genes evoked by MDA5 or MAVS, but not MITA. Thus, we speculated that EcTRIM44L significantly weakened the MDA5-or MAVS-induced interferon immune response, but not that of MITA.

In addition, the effects of EcTRIM44L overexpression on interferon promoter activity (IFN-1, IFN-3, and ISRE) were determined using a dual-luciferase reporter assay. In the EcTRIM44L-transfected cells, the promoter activities of ISRE, IFN-1, and IFN-3 were all significantly decreased compared to those in empty vector transfected cells, and the inhibitory effects were concentration-dependent (Fig. 8A). Moreover, the activation of the ISRE promoter by MDA5 or MAVS were both significantly reduced in the co-transfected cells compared to the control vector transfected cells. In contrast, MITA-induced ISRE activation was not substantially affected by EcTRIM44L overexpression (Fig. 8B). Thus, EcTRIM44L was proposed to promote virus replication by negatively regulating the MDA5-or MAVS-induced interferon immune response.

4. Discussion

It has been demonstrated that numerous TRIM family members exert critical functions in virus infection [5,6,31,32]. For mammals, TRIM proteins are involved in antiviral immunity through an astonishing diversity of mechanisms, including the modulation of immune signaling and regulation of autophagy during viral infection [33]. Recently, several fish TRIM genes have also been found to be differentially regulated in response to fish virus infection and play a critical role in the innate antiviral immune response [31,33,34]. In the present study, a TRIM44 homolog from orange-spotted grouper was investigated, and the sequence analysis indicated that TRIM44 homologs from different fish species all contained two conserved domains, including a B-Box domain and coiled-coil domain. It is important to note that EcTRIM44L shared 81.44% identity with that of large yellow croaker (*Larimichthys crocea*), but only a 24.69% identity with the human TRIM44 protein. However, whether fish TRIM44 exerts a similar function as mammalian TRIM44 remains uncertain.

The appropriate subcellular localization of proteins is critical because it provides a physiological context for their activity [35]. The

TRIM family members display different subcellular localization, including filaments, scattered, or dotted distribution in the cytoplasm or nucleus [36,37]. In this study, we found that EcTRIM44L formed cytoplasmic expression in grouper cells, which is similar to grouper TRIM16L but different from grouper TRIM32, finTRIM82, and TRIM35 [22,24,31,38]. Given that fish TRIM proteins play different roles in the antiviral immune response (e.g., finTRIM82, TRIM62, TRIM35, and TRIM32) [24,25,31,38], we firstly evaluated the potential effects of EcTRIM44L on fish virus replication. Our results showed that the overexpression of EcTRIM44L significantly increased viral gene transcription during RGNNV infection, but had no significantly effect on SGIV infection. Therefore, we speculate that EcTRIM44L acts as an pro-viral factor in response to fish RNA virus infection, but not to DNA virus infection. Our previous studies have also demonstrated that grouper TRIM62 and TRIM35 significantly enhance the level of RGNNV gene transcription *in vitro* [25,38].

Interferon-related cytokines or effectors are now recognized as key components of the innate immune response against viral infection [39]. Our previous studies also demonstrated that interferon related genes, such as IRF3, IRF7, and ISG15 exerted antiviral activities against RGNNV or SGIV replication [16,17,40]. Here, we found that EcTRIM44L overexpression significantly decreased the transcription of interferon-related cytokines or effectors, including IRF3, IRF7, ISG15, MXI, IFP35, and ISG56. Consistently, EcTRIM44L overexpression was found to significantly decrease interferon promoter activity. Furthermore, the ectopic expression of EcTRIM44L significantly weakened the MDA5-or MAVS-induced interferon response, as evidenced by the decreased expression of interferon-related cytokines or effectors and interferon promoter activity. In contrast, the MITA-induced interferon immune response was not affected by EcTRIM44L, although MITA has been reported to exhibit antiviral activity against SGIV infection [19]. Thus, we speculated that the pro-viral roles of EcTRIM44L on RGNNV might be due to the negative regulatory effect on MDA5-or MAVS-induced interferon response.

In summary, this study investigated a novel TRIM44 gene and its role during grouper virus infection. EcTRIM44L encoded a cytoplasmic protein and functioned as a crucial pro-viral factor in response to RGNNV infection. Moreover, EcTRIM44L overexpression significantly decreased the MDA5-or MAVS-induced interferon immune response. Together, our results not only demonstrate for the first time that grouper TRIM44L promotes viral replication by negatively regulating the host interferon response, but also provide novel insight into understanding the role of TRIM proteins in the innate immune response during vertebrate evolution.

Acknowledgements

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