Fish & Shellfish Immunology 57 (2016) 68–78

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Negative regulation of the innate antiviral immune response by TRIM62 from orange spotted grouper

Ying Yang [a, b, c, 1], Youhua Huang [a, b, c, 1], Yepin Yu [a, b, c], Sheng Zhou [a, b, c], Shaowen Wang [a, b, c], Min Yang [a, b, c], Qiwei Qin [a, b, c, d, **], Xiaohong Huang [a, b, c, *]

[a] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, China
[b] Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, China
[c] University of Chinese Academy of Sciences, Beijing, China
[d] College of Marine Sciences, South China Agricultural University, Guangzhou, 510642, China

ARTICLE INFO

Article history:
Received 26 June 2016
Received in revised form
7 August 2016
Accepted 13 August 2016
Available online 15 August 2016

Keywords:
TRIM62
Grouper
RGNNV
Antiviral
Interferon

ABSTRACT

Increased reports uncovered that mammalian tripartite motif-containing 62 (TRIM62) exerts crucial roles in cancer and innate immune response. However, the roles of fish TRIM62 in antiviral immune response remained uncertain. In this study, a TRIM62 gene was cloned from orange spotted grouper (EcTRIM62) and its roles in grouper RNA virus infection was elucidated in vitro. EcTRIM62 shared 99% and 83% identity to bicolor damselfish (*Stegastes partitus*) and human (*Homo sapiens*), respectively. Sequence alignment indicated that EcTRIM62 contained three domains, including a RING-finger domain, a B-box domain and a SPRY domain. In healthy grouper, the transcript of EcTRIM62 was predominantly detected in brain and liver, followed by heart, skin, spleen, fin, gill, intestine, and stomach. Subcellular localization analysis indicated that bright fluorescence spots were observed in the cytoplasm of EcTRIM62-transfected grouper spleen (GS) cells. During red-spotted grouper nervous necrosis (RGNNV) infection, overexpression of EcTRIM62 significantly enhanced the severity of CPE and increased viral gene transcriptions. Furthermore, the ectopic expression of EcTRIM62 significantly decreased the transcription level of interferon signaling molecules, including interferon regulatory factor 3 (IRF3), IRF7, interferon-stimulated gene 15 (ISG15), melanoma differentiation-associated protein 5 (MDA5), myxovirus resistance gene MXI, and MXII, suggesting that the negative regulation of interferon immune response by EcTRIM62 might directly contributed to its enhancing effect on RGNNV replication. Furthermore, our results also demonstrated that overexpression of EcTRIM62 was able to differently regulate the expression levels of pro-inflammation cytokines. In addition, we found the ectopic expression of EcTIRM62 negatively regulated MDA5-, but not mediator of IRF3 activation (MITA)-induced interferon immune response. Further studies showed that the deletion of RING domain and SPRY domain significantly affected the action of EcTRIM62, including the enhancing effect on virus replication and regulation of interferon immune response. Thus, our studies firstly demonstrated that EcTRIM62 negatively regulated the innate antiviral immune response against fish RNA viruses.

1. Introduction

Tripartite motif-containing (TRIM) proteins have been demonstrated to play critical roles in a broad range of biological processes [1–6]. Recent studies have successively uncovered their crucial actions in innate antiviral immune response [1,2,6]. TRIM38 overexpression not only significantly decreased LPS- and poly(I:C)-induced IFN-β promoter activation in different cells, but also greatly attenuated poly(I:C)-induced expression of IFN-β and chemokine

* Corresponding author. Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, China.
** Corresponding author. Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, China.
E-mail addresses: qinqw@scsio.ac.cn (Q. Qin), huangxh@scsio.ac.cn (X. Huang).
[1] Authors contribute equally to this paper.

http://dx.doi.org/10.1016/j.fsi.2016.08.035
1050-4648/

(C-C motif) ligand 5 (CCL5) [6]. TRIM56 overexpression enhanced poly (dA:dT)- and poly (I:C)-stimulated IFN-β promoter activation, but also increased IFN-β promoter activity induced by mediator of IRF3 activation (MITA) or interferon-β promoter stimulator 1 (IPS-1) [7]. In addition, human TRIM25 was also demonstrated to interact with retinoic acid inducible gene I (RIG-I), and this interaction effectively evoked a marked increase in RIG-I downstream signaling activity, including IFN-β or NF-κB promoter activity [4]. Although the detailed roles of several TRIM proteins in antiviral immune response have been explored, the potential mechanisms underlying the function of the fish TRIM proteins still remained largely unknown.

TRIM62, a member of TRIM family, has attracted much attention due to its crucial roles in cancer and innate immune response [8,9]. TRIM62 not only exerted important roles in the regulation of apical–basal polarity and acinar morphogenesis, but also functioned as a chromosome 1p35 tumor suppressor and negative regulator of TGF-β driven epithelial-mesenchymal transition [10–12]. In response to fungal infection, TRIM62 was able to bind caspase recruitment domain-containing protein 9 (CARD9) and facilitated K27-linked poly-ubiquitination of CARD9 which was essential for CARD9 activity. Moreover, TRIM62-deficient mice showed increased susceptibility to fungal infection [8]. Interestingly, silence of EcTRIM62 significantly inhibited HIV release in both HEK293 cells and HeLa cells, but exerted the contrary effects on MLV release in these two cells [9], suggesting that TRIM62 played complicated roles in response to different viruses.

Groupers, Epinephelus spp. are potentially important and economically valuable aquaculture species in Southeast Asian countries including China [13]. In recent years, the outbreak of viral diseases evoked by Singapore grouper iridovirus (SGIV) and red spotted grouper nervous necrosis usually (RGNNV) always caused heavy economic losses in grouper aquaculture [14–16]. Our previous studies showed that several grouper TRIM proteins played different roles in SGIV and RGNNV infection [5,17–19]. Among them, EcTRIM39 inhibited virus replication by altering the cell cycle progression, while EcTRIM25 and EcTRIM8 increased the interferon immune response and inhibited SGIV and RGNNV replication [5,17,19]. In contrast, overexpression of EcTRIM13 in vitro significantly enhanced RGNNV replication through regulating MDA5-induced interferon immune response [18]. Whether other TRIM proteins were involved in fish virus replication still remained unknown.

In the current study, we cloned a TRIM62 homolog from orange spotted grouper and explored the molecular actions of EcTRIM62 in response to fish RNA virus infections. Our study provided the first evidence that fish TIRM62 exerted the negative regulation on the interferon immune response against virus infection.

2. Material and methods

2.1. Fish, cells and viruses

Orange-spotted groupers, *E. Coioides* were purchased from Hainan Province and then kept in a laboratory recirculating seawater system. Grouper spleen (GS) cells were grown in Leibovitz's L15 medium containing 10% fetal bovine serum (FBS, Gibco) at 25 °C [20]. Red-spotted grouper nervous necrosis virus (RGNNV) was prepared as described previously [21].

2.2. Cloning of EcTRIM62 and sequence analysis

After assembling the EST sequences of EcTRIM62 from grouper spleen transcriptome [22], we obtained the full length of EcTRIM62 using PCR amplification. The sequence analysis of EcTRIM62 was performed using BLAST program, and the conserved domains were predicted using SMART program. Multiple sequences alignment was carried our using ClustalX1.83 software and the data was edited using GeneDoc program. Phylogenetic analysis was performed using MEGA 4.0 software.

2.3. Expression patterns for EcTRIM62 in grouper

To determine the tissue distribution pattern of EcTRIM62 in normal orange-spotted grouper, RNA extraction was carried out from different tissues including head kidney, trunk kidney, heart, intestine, liver, spleen, brain, skin, gill, and fin, as described previously [23]. The relative expression level of EcTRIM62 was detected by quantitative real-time PCR (qRT-PCR) as described following.

To clarify the expression profiles of EcTRIM62 in innate immunity, groupers were injected with SGIV or poly I:C as described previously [23]. In brief, the spleen of injected groupers were collected at 3, 6, 12, 24, 48, 72 h post injection. The relative expression level of EcTRIM62 was examined using qRT-PCR analysis.

2.4. Plasmid construction

To identify the molecular function of EcTRIM62 in vitro, the full length of EcTRIM62 and RING (R)/SPRY (S) domain mutants including EcTRIM62-ΔR and EcTRIM62-ΔS were all subcloned into pEGFP-C1 and pcDNA3.1-flag vector using the primers in Table 1. The constructed plasmids (pEGFP-EcTRIM62, pEGFP-EcTRIM62-ΔR,

Table 1
Primers used in this study.

Name	Sequence (5′–3′)
EcTRIM62-ORF-PF	ATGGCTTGCTGCTTGAAGGA
EcTRIM62-ORF-PR	AAGGCGTACAGTGTTGATTCGC
EcTRIM62-Flag-F	TAAGGTACCGAATGGCTTGCTGCTTGAAGGA
EcTRIM62-Flag-R	ACATCTCGAGAAGGCGTACAGTGTTGATTCGC
EcTRIM62-ΔR-Flag-F	TAAGGTACCGAATGCGGAGGACCTTCACCGACC
EcTRIM62-ΔS-Flag-R	AATCTCGAGCGACATGAAGCCCTCTGCG
C1-EcTRIM62-F	TAACTCGAGCTATGGCTTGCTGCTTGAAGGA
C1-EcTRIM62-R	AATGGATCCAAGGCGTACAGTGTTGATTCGC
C1-EcTRIM62-ΔR-F	CTCGAGCTATGCGGAGGACCTTCACCGACC
C1-EcTRIM62-ΔS-R	AATGGATCCCGACATGAAGCCCTCTGCG
EcTRIM62-RT-F1	GTGGCCTACGGGAATCTCCAT
EcTRIM62-RT-R1	TGCTGCCCTTGCGACTGACT
EcTRIM62-RT-F2	TCAGTATCTACCAGGACCCCGT
EcTRIM62-RT-R2	CGTGGTCCTTGCAGGGGTAG
EcTRIM62-RT-F3	ATCAAATGAGACCCAAACTCCTG
EcTRIM62-RT-R3	TGTAGTACAATCTAGGATCTTGAGCACA
Actin- RT-F	TACGAGCTGCCTGACGGACA
Actin- RT-R	GGCTGTGATCTCCTTCTGCA
CP- RT-F	CAACTGACAACGATCACACCTTC
CP- RT-R	CAATCGAACACTCCAGCGACA
RdRp- RT-F	GTGTCCGGAGAGGTTAAGGATG
RdRp- RT-R	CTTGAATTGATCAACGGTGAACA
EcIRF3-RT-F	GACAACAAGAACGACCCTGCTAA
EcIRF3-RT-R	GGGAGTCCGCTTGAAGATAGACA
EcIRF7-RT-F	CAACACCGGATACAACCAAG
EcIRF7-RT-R	GTTCTCAACTGCTACATAGGGC
EcIL6-RT-F	CTCTACACTCAACGCGTACATGC
EcIL6-RT-R	TCATCTTCAAACTGCTTTTCGTG
EcTNFa-RT-F	GTGTCCTGCTGTTTGCTTGGTA
EcTNFa-RT-R	CAGTGTCCGACTTGATTAGTGCTT
EcIL-1β-RT–PF	AACCTCATCATCGCCACACA
EcIL-1β-RT-PR	AGTTGCCTCACAACCGAACAC
EcIL8-RT-F	GCCGTCAGTGAAGGGAGTCTAG
EcIL8-RT-R	ATCGCAGTGGGAGTTTGCA
EcISG15-RT-F	CCTATGACATCAAAGCTGACGAGAC
EcISG15-RT-R	GTGCTGTTGGCAGTGACGTTGTAGT
EcMDA5-RT-F	ACCTGGCTCTCAGAATTACGAACA
EcMDA5-RT-R	TCTGCTCCTGGTGGTATTCGTTC