RESEARCH ARTICLE

Molecular Characterization and Expression Analysis of *ptr01*, *ptr42*, and *ptr58* in Zebrafish (*Danio rerio*)

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Abstract

Tripartite motif (TRIM) proteins were shown to play an important role in innate antiviral immunity. FinTRIM (*ptr*) is a new subset of TRIM genes that do not possess obvious orthologs in higher vertebrates. However, little is known about its function. In this study, we used bioinformatic analysis to examine the phylogenetic relationships and conserved domains of zebrafish (*Danio rerio*) *ptr01*, *ptr42*, and *ptr58*, as well as qualitative real-time PCR to examine their expression patterns in zebrafish embryonic fibroblast (ZF4) cells and zebrafish tissues. Sequence analysis showed that the three finTRIMs are highly conserved, and all contain a RING domain, B-box domain, and SPRY-PRY domain. In addition, *ptr42* and *ptr58* had one coiled-coil domain (CCD), whereas *ptr01* had two CCDs. Tissue expression analysis revealed that the mRNA level of *ptr01* was the highest in the liver, whereas those of *ptr42* and *ptr58* were the highest in the gill; the expression of these finTRIMs was clearly upregulated not in the eyes, but in the liver, spleen, kidney, gill, and brain of zebrafish following spring viremia of carp virus (SVCV) infection. Similarly, the expression of these three finTRIM genes also increased in ZF4 cells after SVCV infection. Our study revealed that *ptr01*, *ptr42*, and *ptr58* may play an important role in antiviral immune responses, and these findings validate the need for more in-depth research on the finTRIM family in the future.

Keywords FinTRIM · Tissue distribution · Expression patterns · Bioinformatics analysis · Spring viremia of carp virus (SVCV) · Zebrafish

Introduction

Innate immunity is the body's immune defense function in the process of development and evolution (Fearon and Locksley 1996; Beutler 2004). Previous studies showed that innate immunity plays an important role in resistance against infection of pathogenic microorganisms, antitumor mechanism, self-tolerance maintenance, and specific immunity (Smyth *et al.* 2001; Germain 2004).

The tripartite motif (TRIM) protein family has attracted the attention of many researchers in recent years for various reasons. This family is known to include conserved domain-containing proteins existing in the cytoplasm. Members of the TRIM family contained a RING finger domain linked with one or two B-box domain and a predicted coiled-coil (CC) region, as well as a highly variable C-terminus, such as the PRY/SPRY domain, named the B30.2 domain or the NHL domain. The TRIM family is also known as the Ring finger, B box, coiled-coil (RBCC) family (Reddy and Etkin 1991; Reddy *et al.* 1992; Borden 1998; Raymond *et al.* 2001; Uchil *et al.* 2008). Studies

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showed that many TRIM proteins play an essential role in antiviral immune signaling pathways in mammals. TRIM5 α is the first TRIM protein found to play a role in viral infection. Particularly, the PRY-SPRY domain of TRIM5 α can bind directly to the human immunodeficiency virus (HIV) capsid protein and act as a direct antiviral agent (Matthew *et al.* 2004). In addition, the PRY-SPRY domain of TRIM5 α was shown to bind to encephalomyocarditis virus (EMCV) 3C protease and to the nucleoprotein of influenza virus, ultimately degrading viral proteins via the ubiquitin proteasome pathway (Barr *et al.* 2008; Eldin *et al.* 2009; Pietro *et al.* 2013). TRIM52 was reported to inhibit viral replication by degrading Japanese encephalitis virus (JEV)'s NS2A (Fan *et al.* 2016). In addition, except binding with viral proteins directly, the TRIM proteins TRIM11, TRIM22, TRIM28, and TRIM32 are also reported to inhibit virus replication by interfering viral transcription (Rajsbaum *et al.* 2014). In addition to participating in antiviral response directly, TRIM proteins have recently been shown to play an important role in regulating antiviral innate immune signaling pathway either positively, such as TRIM4, TRIM21, TRIM25, and TRIM56, or negatively, such as TRIM11, TRIM27, TRIM28, TRIM38, and TRIM59 (Rajsbaum *et al.* 2014).

Many studies have shown that, in addition to that of mammals, TRIM proteins play an important role in the antiviral immune system of fish. Fish TRIM8 exerts antiviral roles by regulating proinflammatory factors and interferon signaling (Huang *et al.* 2016b). TRIM32 and TRIM39 can inhibit the replication of iridovirus and nodavirus (Wang *et al.* 2016a, b; Yu *et al.* 2017), and some fish TRIM proteins play a negative regulatory role in antiviral signaling pathways. For example, TRIM16 negatively regulates interferon immune response and promotes the replication of DNA virus (Yu *et al.* 2016). On the other hand, orange-spotted grouper (*Epinephelus coioides*) TRIM62 promotes the replication of Redspotted grouper nervous necrosis virus (RGNNV), a positive single-stranded RNA (ssRNA) virus (Yang *et al.* 2016b).

A new fish TRIM family was identified in zebrafish (*Danio rerio*), and named finTRIM (*ftt*), which is a novel gene family unique to teleost fishes with no obvious orthologs in higher vertebrates. FinTRIMs are specifically induced by viruses and poly (I:C), and therefore, may be involved in the teleost antiviral natural immune processes (Van der Aa *et al.* 2009, 2012). Little is currently known about the role of finTRIM in the fish immune system. Recent studies have shown that FTR36 triggers the IFN pathway and mediates the inhibition of spring viremia of carp virus (SVCV) replication (Chen *et al.* 2019). Overexpression of *ftt83* promotes the infection of some RNA viruses, including Infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), and

SVCV. However, *ftt82* did not play any role in viral infection, although engaged in zebrafish vascular patterning (Chang *et al.* 2017; Langevin *et al.* 2017).

In our previous study, RNA sequencing (RNA-Seq) technology was employed to identify differentially expressed genes in zebrafish after SVCV infection (Wang *et al.* 2017). Three *ftt* genes, namely finTRIM family, member 1, 42, and 58 (*ftt01*, *ftt42*, and *ftt58*, respectively), were obviously upregulated, and thus selected based on transcriptome analysis results. This study aimed to: (1) characterize the molecular structure of the *ftt01*, *ftt42*, and *ftt58* genes of zebrafish; (2) analyze the spatial expression patterns of the three genes using quantitative real-time PCR; (3) investigate the mRNA expression dynamics of the three genes *in vivo* and *in vitro*. The results of this study will enhance our understanding of the potential function of these putative finTRIM proteins in the antiviral system of zebrafish.

Materials and Methods

Cell, Virus, and Zebrafish

Zebrafish embryonic fibroblast (ZF4) cells (ATCC CRL-2050) derived from 1-day-old zebrafish embryos were routinely maintained in a humidified chamber (28 °C, 5% CO₂) with Dulbecco minimal Eagle's/F-12 medium (Hyclone, Logan, UT, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, Melbourne, Australia), streptomycin (100 µg/mL), and penicillin (100 µg/mL) (Gibco). SVCV (ATCC: VR-1390) was used for infection; the virus titer was determined using a 50% tissue culture infectious dose (TCID₅₀) assay and calculated basing on the Reed-Muench method (Pizzi 1950). Adult wild-type (WT) AB strain male zebrafish were purchased from the China Zebra-fish Resource Centre (CZRC) to ensure a clear genetic background. The care, breeding, and feeding of zebrafish followed established protocols (<http://www.zfish.cn/inforscan/251.html>). Next, zebrafish were allowed to acclimate to a lower temperature by reducing the water temperature by 1 °C every 24 h until the water temperature reached 17 °C. Subsequently, they were maintained at this temperature for ≥ 7 days (d) before the virus infection assay (Ahne *et al.* 2002; Sanders *et al.* 2003; Novoa *et al.* 2006).

Tissues Harvest and SVCV Infection in Zebrafish

To examine the tissue distribution of *ftt01*, *ftt42*, and *ftt58*, 8 healthy zebrafish (4 males and 4 females) were sampled. The fishes were anesthetized with MS-222 (300 mg/L) before dissection. Brain, eyes, gill, heart, liver, spleen,

kidney, swim bladder, skin, and muscle tissues were collected for RNA isolation.

To examine the immune responses of those genes in different immune-related tissues after SVCV infection, 120 juvenile zebrafish were collected at the laboratory of Huazhong Agricultural University. In total, 20 zebrafish was randomly sampled, injected with MEM medium (HyClone, Logan, UT, USA), and used as a control group. The other group was injected intraperitoneally with 10 μ L of SVCV at a dose of 10^5 TCID₅₀, which was determined by our preliminary experiment in this study. In total, 10 fishes was randomly sampled from the experimental group at 0, 1, 2, 3, 4, and 5 days post-infection (dpi). The fishes were anesthetized with MS-222, and then the spleen, head kidney, spleen, liver, gill, brain, and eye were collected for RNA extraction.

SVCV Infection in ZF4 Cells

To examine the immune responses of the *ftir01*, *ftir42*, and *ftir58* genes in zebrafish ZF4 cells, the cells were infected with SVCV at 5 MOI and then collected by centrifugation at 6, 12, 24, and 36 h after infection.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from various tissues and cells using TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's protocol. One microgram of total RNA was used for reverse transcription reaction using PrimeScriptTM RT reagent Kit with a gDNA Eraser (TaKaRa). cDNA products were stored at -20°C .

Bioinformatics Analysis

Phylogenetic trees were constructed by the neighboring-joining method by resampling (1000 replicates) using the MEGA7 software (Kumar *et al.* 2016). The number at each branch of the phylogenetic tree represents the bootstrap value (1000 replicates). The protein structures of the target genes were predicted using the Simple Modular Architecture Research Tool (SMART) (<http://smart.embl-heidelberg.de/>) (Letunic and Bork 2017); the multiple sequence alignment was performed with the CLUSTALW program (Thompson *et al.* 1994), and the 3D structural models of the *ftir01*, *ftir42*, and *ftir58* B30.2 domains, including PRY and SPRY, were built with the Swiss-model program using the high-resolution structure of mammalian TRIM25 as a template (PDB 4B8E) (Waterhouse *et al.* 2018). The Chimera program was used for structural analysis and B30.2 superposition (Pettersen *et al.* 2004).

Quantitative Real Time PCR and Statistical Analysis

Quantitative real time PCR (qRT-PCR) was used to detect the mRNA expression levels of the *ftir01*, *ftir42*, and *ftir58* genes in various tissues of the affected fish and ZF4 cells at different times post-infection using a 7300 RT-PCR system (Applied Biosystems, USA). The gene-specific primer pairs for qPCR were designed based on the coding DNA sequence of the *ftir01*, *ftir42*, and *ftir58* genes. In addition, the Primer-BLAST (Ye *et al.* 2012) was used to assess non-target amplification using the forward and reverse primer sequences and the entire NCBI nucleotide collection. The TATA-Box Binding Protein (TBP) gene was also used as an internal control gene (Liu *et al.* 2015). The PCR reaction mixture, at a total volume of 20 μ L, consisted of 10 μ L LightCycler[®]480 SYBR Green I Master (Roche, Germany), 6.4 μ L ddH₂O, 2 μ L cDNA (5 times dilution of the template), and 0.8 μ L of either gene-specific primer (10 μ mol/L). The primers used for qRT-PCR amplification of *ftir*TRIMs are listed in Supplementary Table S1. The qRT-PCR of each sample was performed in triplicate according to the following conditions: 95 $^{\circ}\text{C}$ for 10 min, followed by 40 cycles of 95 $^{\circ}\text{C}$ for 15 s, 58 $^{\circ}\text{C}$ for 20 s, and 72 $^{\circ}\text{C}$ for 20 s. At the end of each PCR reaction, amplification curve and melting curve analyses were performed to check the integrity of the reaction and the quality of the product, respectively. To compare the gene expressions of *ftir01*, *ftir42*, and *ftir58*, the $2^{-\Delta\Delta\text{CT}}$ method was adopted to calculate the relative expression levels of the target genes (Livak and Schmittgen 2001). All qPCR data were expressed as mean $\pm$ standard error (SE). One-way ANOVA and Duncan's *post hoc* test were conducted using the SPSS 17.0 software to examine the differences in relative gene expression among different samples. The *t* test was performed to examine the differences in gene expression level between the control and experimental groups after stimulation. A difference was considered statistically significant if $P < 0.05$ (*).

Results

Sequence Analysis and Phylogenetic Relationships of Zebrafish *ftir01*, *ftir42*, and *ftir58*, and Mammalian TRIM25

Amino acid alignment analysis showed that *ftir01*, *ftir42*, and *ftir58* shared 51.16%–71.79% identity; the full-length cDNA of *ftir01* encoded a polypeptide of 556 amino acids that shared 56.01%–54.03% identity with *ftir42* and *ftir58*. *Homo sapiens* TRIM25 shared 23%–25% amino acid

Fig. 1 **A** Multiple sequence alignment of zebrafish *frt01*, *frt42*, and *frt58*, and *Homo sapiens* TRIM25 amino acid sequences, as analyzed by ClustalW using default parameters. The conserved domains, including a RING-finger domain, B box/coiled-coil domain, and B30.2 domain (PRY and SPRY), were indicated with different colors. **B** Phylogenetic analysis. The Neighbor-Joining tree was constructed using MEGA7. A tree including zebrafish finTRIM genes and *Homo sapiens* TRIM25 was alone in the tree, which was separated from all zebrafish finTRIMs. The bootstrap values from 10,000 replicates were indicated at the branches.

Fig. 2 **A** Typical domain analysis of zebrafish *frt01*, *frt42*, and *frt58*, and *Homo sapiens* TRIM25 predicted by the SMART online server. The different domains are labeled and presented with different colors and shapes. **B** 3D structure of the B30.2 domains of *frt01*, *frt42*, and *frt58* were built with the Swiss-Model program using the high-

resolution structure of mammalian TRIM25 as a template (PDB 4B8E). Model superposition was performed with the Chimera program. Golden color represents the structure of the template, the B30.2 domain of mammalian TRIM25, and blue color represents the predicted structure of finTRIM proteins.

Fig. 3 Basal expression of *frt01*, *frt42*, and *frt58* mRNA in various tissues of zebrafish. The mRNA levels of the *frt01*, *frt42*, and *frt58* genes were normalized to those of TBP (internal control) in the same samples. The lowest expression was detected for *frt01* in the eye (**A**), for *frt42* in the swim bladder (**B**), and for *frt58* (**C**) in the skin, and

these were selected as calibration values. The relative expression of the *frt01*, *frt42*, and *frt58* genes in other tissues are represented as fold-changes compared to the calibration values. The results are expressed as mean $\pm$ SE (bars in the graph) from 6 fish.

Fig. 4 Expression pattern of *ftt01*, *ftt42*, and *ftt58* in the zebrafish tissues infected with SVCV. **A** Detection of the SVCV-G gene using RT-PCR in the eye, gill, spleen, kidney, brain, and liver samples from SVCV-infected zebrafish and the control group. At 1, 2, 3, 4, and 5 dpi, different tissues were collected and subjected to total RNA

extraction and real-time PCR analysis. The mRNA levels of *ftt01* (**B**), *ftt42* (**C**), and *ftt58* (**D**) were normalized to those of TBP, and the relative expression was represented as fold-changes compared to the mock group (M). The data are shown as mean $\pm$ SD, and statistical significances were determined by the Student's *t*-test. **P* < 0.05.

with the first group containing *ftt01* and its homologous gene, whereas the second group contained *ftt42*, *ftt58*, and other similar genes. As shown in Fig. 1B, *ftt42* showed the closest genetic relationship with *ftt58*, instead of *ftt01*.

Structure Characterization of Zebrafish *ftt01*, *ftt42*, and *ftt58*, and Mammalian *TRIM25*

To reveal the conserved and diverse domains in these genes, the structural drawings of *ftt01*, *ftt42*, and *ftt58*, and mammalian *TRIM25* were shown with boxes representing the distinct domains. The domain structures of finTRIMs were shown schematically with RING, B-box, coiled-coil, and B30.2 domain (PRY and SPRY). The results indicated that *ftt42*, *ftt58*, and *TRIM25* held a typical TRIM motif distribution, whereas *ftt01* had two coiled-coil motifs (Fig. 2A). The 3D structure of the B30.2 domains of *ftt01*, *ftt42*, and *ftt58* were predicted by the Swiss-model (Fig. 2B). For better presentation, different colors were

used in the figure. Golden color represents the structure of the template, the B30.2 domain of mammalian *TRIM25*, and blue color represents the predicted structure of fin-TRIM proteins. The results showed that the three finTRIMs shared high sequence similarity with mammalian *TRIM25*.

Quantitative Analysis of *ftt01*, *ftt42*, and *ftt58* mRNA Expression Levels in Zebrafish Tissues

qRT-PCR was used to quantify the three finTRIM genes in the brain, eyes, gill, heart, liver, spleen, kidney, swim bladder, skin, and muscle. As shown in Fig. 3, *ftt01*, *ftt42*, and *ftt58* were detected in all test tissues. *ftt01* was expressed at the highest level in the liver. *ftt42* and *ftt58* were expressed at the highest level in the gill, and highly expressed in several immune-related organs, such as the liver.

Fig. 5 Expression pattern of *ftr01*, *ftr42*, and *ftr58* in ZF4 cells infected with SVCV at 5 MOI. Gene expression was normalized to that of TBP, and is presented as fold-change compared to the

respective controls. The data are shown as mean $\pm$ SD, and statistical significances were determined by two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Quantitative Analysis of mRNA Expression Levels in Zebrafish Tissues after SVCV Infection

In our study, tissue-specific differences in the levels of the three finTRIMs were observed in the examined tissues following SVCV infection. RT-PCR was used to confirm whether zebrafish was infected with SVCV, and the results showed that virus was detected in all tissues (Fig. 4A), and in accordance with the result of our previous experiment, the amount of SVCV increased with time (Chen *et al.* 2019). As shown in Fig. 4B, the expression of *ftr01* mRNA in the kidney, spleen, and eye was upregulated from 1 to 5 dpi, and it reached the highest value at 5 dpi. In the liver and brain, the expression significantly increased from 0 to 4 dpi, and reached a peak value at 4 dpi before decreasing significantly at 5 dpi.

The expression of *ftr42* mRNA in the kidney, spleen, and eye was upregulated from 1 to 5 dpi, and it reached a peak value at 5 dpi. In the liver and brain, the expression was significantly upregulated from 1 to 4 dpi, with a peak value at 4 dpi. The expression was significantly downregulated afterwards at 5 dpi (Fig. 4C). The expression level of *ftr58* mRNA in the spleen and eye increased from 0 to 5 dpi, and reached a peak value at 5 dpi. In the kidney and brain, the expression significantly increased from 0 to 4 dpi, and reached a peak value at 4 dpi before significantly decreasing at 5 dpi. In the gill, the expression was significantly upregulated from 1 to 3 dpi, and it reached the highest value at 3 dpi; moreover, it slightly decreased at 4 dpi and increased again from 4 to 5 dpi. In the liver, the expression significantly increased from 1 dpi, and increased again from 3 to 5 dpi, with a slight decrease at 4 dpi (Fig. 4D).

Quantitative Analysis of mRNA Expression Levels in ZF4 Cells after SVCV Infection

The relative expression of *ftr01*, *ftr42*, and *ftr58* mRNAs in ZF4 cells was detected after infection with SVCV at 5

MOI. As shown in Fig. 5A, the mRNA expression of *ftr01* was significantly upregulated between 6 h and 24 h post-injection time. The expression level of *ftr42* and *ftr58* mRNA was upregulated during the entire post-infection time (36 h), and it reached a peak value at 36 h (Figs. 4C and 5B).

Discussion

The TRIM family is an E3 ubiquitin ligase group that has attracted more and more attention in recent years owing to its effects on many important cellular processes, such as apoptosis, cell cycle regulation, differentiation, metabolic pathway, and cell response to viral infection (Meroni and Diez-Roux 2005). Increasing number of studies have shown that members of the TRIM family can regulate and coordinate innate immunity and antiviral responses (Chang 2008; Uchil *et al.* 2008, 2013; Versteeg *et al.* 2014). In addition, a few recent studies have indicated that the TRIM family play an important role in fish innate immunity; for example, orange-spotted grouper TRIM25 can inhibit the replication of Singapore grouper iridovirus (SGIV) and RGNNV through interferon immune response (Yang *et al.* 2016a), and common carp TRIM32 can inhibit the replication of SVCV (Wang *et al.* 2016a, b). However, little is currently known about the finTRIM family in terms of its biological function.

Bioinformatic analysis showed that *ftr01*, *ftr42*, and *ftr58* all had the RING domain, as in other RING motif-containing E3 ligases. The RING domain of TRIMs usually mediates interaction with ubiquitin-conjugating enzyme (E2) via zinc finger motifs (Ye and Rape 2009; Ebner *et al.* 2017; Esposito *et al.* 2017). Several studies have shown that the RING domain is essential for the antiviral activity of the TRIM members, including TRIM5 α , TRIM56, TRIM41, TRIM25, and *ftr83* (Zhang *et al.* 2013; Liu *et al.* 2014; Campbell *et al.* 2015; Yang *et al.* 2016a; Langevin *et al.* 2017). Several TRIM-like members, such as TRIM14

and TRIM16 lack a RING domain within the RBCC (Bell *et al.* 2012; Zhou *et al.* 2014), but fish TRIM16 has a RING domain while not possessing a B-Box domain (Yu *et al.* 2016). In this study, a B-Box domain existed in all three finTRIMs. The B-box domain is also crucial for TRIM proteins. A very recent study has indicated that the B-box domain is responsible for the self-association and protein–protein interactions TRIM, which can also promote the oligomerization of higher-order TRIM (Esposito *et al.* 2017). Analysis of the existing crystal structures of TRIM5 α , TRIM20, TRIM25, and TRIM69 revealed that TRIM dimers are formed by coiled-coil antiparallel helical structures (Li *et al.* 2014; Sanchez *et al.* 2014; Weinert *et al.* 2015; Dawidziak *et al.* 2017; Esposito *et al.* 2017).

Understanding the expression of genes in different tissues treated with different challenges is the basic method to examine the specific function of genes (Cui *et al.* 2010). In the present study, the expression of *frt01*, *frt42*, and *frt58* was detected in all tested tissues, and the overall expression patterns of the three finTRIMs were significantly higher in the liver and gill than in the other tissues tested. The head kidney, liver, gill, and hindgut of fish are generally considered as immune organs and central to the immune responses (Alvarez-Pellitero 2008). Thus, the expression levels of the three finTRIM genes are different in different tissues, indicating that they may exert their immune effect through tissue-specific mechanisms.

To further understand the role of finTRIM genes in SVCV infection, we investigated the expression of *frt01*, *frt42*, and *frt58* at different post-infection times in SVCV-infected zebrafish. Similar to *TRIM39* (Wang *et al.* 2016a, b), the three finTRIM genes were significantly upregulated in the late stage of infection, but to different degrees. However, there was no significant difference in the expression levels of these genes during the early stage of infection (≤ 24 h). We speculated that the virus titers in zebrafish were low during the early stages of SVCV infection, which had not caused large-scale immune response *in vivo*. This observation has also been reported recently for the *TRIM16* gene (Yu *et al.* 2016). A host's immune response is activated to remove an invading pathogen, but excessive immune response is also unfavorable for the body, as shown by the results of this study; *frt01* and *frt42* expression in the liver of SVCV-infected fish reached a peak at 4 dpi and then dropped from day 4 to 5 post-infection. This decrease can effectively protect the body from excessive immune response. Such as the transcription of zebrafish ISGs, including IRF7, Mx were upregulated at the early stage of SVCV infection and downregulated at the later stage (Gong *et al.* 2018). Because many upregulated genes, such as *TRIM8*, *TRIM13* and *TRIM25*, appeared to play a critical role in the fish immune system following a viral infection (Huang *et al.*

2016a, b; Yang *et al.* 2016a), it is reasonable to believe that these finTRIM genes in zebrafish may also play an important role in the immune system. In addition, we have also analyzed the expression of finTRIM genes in SVCV-infected ZF4 cells, and showed that all these three genes were upregulated during the 24 h of SVCV infection. In a previous study, *frt51* and *frt84* are also upregulated in SVCV-infected ZF4 cells (Luo *et al.* 2017), providing another supportive evidence that the finTRIM family may participate in antiviral innate immunity.

In conclusion, we analyzed the sequence and characterized the structure of *frt01*, *frt42*, and *frt58* by bioinformatics, and showed that the expression of *frt01*, *frt42*, and *frt58* increased significantly after SVCV infection. The study results will serve as the baseline information and research ground for future studies to understand the essential role and mechanisms of these finTRIM proteins in fish innate immunity against viral infections.

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Author Contributions LX, LW and KM conceived the project, wrote and revised the manuscript. KM performed the experiments. LW, KM and ZZ did data analysis. LY, LX checked and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with Ethics Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement All animal procedures were conducted strictly in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All the animals used for viral infection were not endangered or protected species. Zebrafish were employed in *in vivo* experimental tests under the approval of the Animal Ethics Committee of Huazhong Agricultural University (HZAU). The infection and dissection experiments were performed under anesthesia with 3-aminobenzoic acid ethyl ester methane sulfonate (MS-222) (Sigma, USA) to minimize suffering in the fishes.

References

- Ahne W, Bjorklund HV, Essbauer S, Fijan N, Kurath G, Winton JR (2002) Spring viremia of carp (svc). *Dis Aquat Organ* 52:261–272
- Alvarez-Pellitero P (2008) Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. *Vet Immunol Immunopathol* 126:171–198

- Barr SD, Smiley JR, Bushman FD (2008) The interferon response inhibits hiv particle production by induction of trim22. *PLoS Pathog* 4:e1000007
- Bell JL, Malyukova A, Holien JK, Koach J, Parker MW, Kavallaris M, Marshall GM, Cheung BB (2012) Trim16 acts as an e3 ubiquitin ligase and can heterodimerize with other trim family members. *PLoS ONE* 7:e37470
- Beutler B (2004) Innate immunity: an overview. *Mol Immunol* 40:845–859
- Borden KL (1998) Ring fingers and b-boxes: zinc-binding protein-protein interaction domains. *Cell Biol* 76:351–358
- Campbell EM, Weingart J, Sette P, Opp S, Sastri J, O'Connor SK, Talley S, Diazgriffero F, Hirsch V, Bouamr F (2015) Trim5 α -mediated ubiquitin chain conjugation is required for inhibition of hiv-1 reverse transcription and capsid destabilization. *J Virol* 90:1849–1857
- Chang TH (2008) Trim family proteins and their emerging roles in innate immunity. *Nat Rev Immunol* 8:849–860
- Chang HW, Wang WD, Chiu CC, Chen CH, Wang YS, Chen ZY, Liu W, Tai MH, Wen ZH, Wu CY (2017) Ftr82 is critical for vascular patterning during zebrafish development. *J Mol Sci* 18:156
- Chen B, Huo S, Liu W, Wang F, Lu Y, Xu Z, Liu X (2019) Fish-specific finTRIM FTR36 triggers IFN pathway and mediates inhibition of viral replication. *Fish Shellfish Immunol* 84:876–884
- Cui Z, Liu Y, Luan W, Li Q, Wu D, Wang S (2010) Molecular cloning and characterization of a heat shock protein 70 gene in swimming crab (*portunus trituberculatus*). *Fish Shellfish Immunol* 28:56–64
- Dawidziak DM, Sanchez JG, Wagner JM, Ganser-Pornillos BK, Pornillos O (2017) Structure and catalytic activation of the trim23 ring e3 ubiquitin ligase. *Proteins* 85:1957–1961
- Ebner P, Versteeg GA, Ikeda F (2017) Ubiquitin enzymes in the regulation of immune responses. *Crit Rev Biochem Mol Biol* 52:425–460
- Eldin P, Papon L, Oteiza A, Brocchi E, Lawson TG, Mechti N (2009) Trim22 e3 ubiquitin ligase activity is required to mediate antiviral activity against encephalomyocarditis virus. *J Gen Virol* 90:536–545
- Esposito D, Koliopoulos MG, Rittinger K (2017) Structural determinants of trim protein function. *Biochem Soc Trans* 45:183–191
- Fan W, Wu M, Qian S, Zhou Y, Chen H, Li X, Qian P (2016) Trim52 inhibits japanese encephalitis virus replication by degrading the viral ns2a. *Sci Rep* 6:33698
- Fearon DT, Locksley RM (1996) The instructive role of innate immunity in the acquired immune response. *Science* 272:50–53
- Germain RN (2004) An innately interesting decade of research in immunology. *Nat Med* 10:1307–1320
- Gong XY, Zhang QM, Gui JF, Zhang YB (2018) SVCV infection triggers fish IFN response through RLR signaling pathway. *Fish Shellfish Immunol* 86:1058–1063
- Huang Y, Yu Y, Yang Y, Yang M, Zhou L, Huang X, Qin Q (2016a) Fish trim8 exerts antiviral roles through regulation of the proinflammatory factors and interferon signaling. *Fish Shellfish Immunol* 54:435–444
- Huang Y, Yang M, Yu Y, Yang Y, Zhou L, Huang X, Qin Q (2016b) Grouper trim13 exerts negative regulation of antiviral immune response against nodavirus. *Fish Shellfish Immunol* 55:106–115
- Kumar S, Stecher G, Tamura K (2016) Mega7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Langevin C, Alekseeva E, Houel A, Briolat V, Torhy C, Lunazzi A, Levraud JP, Boudinot P (2017) Ftr83, a member of the large fish-specific fintrim family, triggers ifn pathway and counters viral infection. *Front Immunol* 8:617
- Letunic I, Bork P (2017) 20 years of the smart protein domain annotation resource. *Nucleic Acids Res* 46:D493–D496
- Li Y, Wu H, Wu W, Zhuo W, Liu W, Zhang Y, Cheng M, Chen YG, Gao N, Yu H (2014) Structural insights into the trim family of ubiquitin e3 ligases. *Cell Res* 24:762–765
- Liu B, Li NL, Wang J, Shi PY, Wang T, Miller MA, Li K (2014) Overlapping and distinct molecular determinants dictating the antiviral activities of trim56 against flaviviruses and coronavirus. *J Virol* 88:13821–13835
- Liu L, Zhu B, Wu S, Lin L, Liu G, Zhou Y, Wang W, Asim M, Yuan J, Li L (2015) Spring viraemia of carp virus induces autophagy for necessary viral replication. *Cell Microbiol* 17:595–605
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods* 25:402–408
- Luo K, Li Y, Xia L, Hu W, Gao W, Guo L, Tian G, Qi Z, Yuan H, Qiaoqing X (2017) Analysis of the expression patterns of the novel large multigene trim gene family (fintrim) in zebrafish. *Fish Shellfish Immunol* 66:224–230
- Matthew S, Owens CM, Perron MJ, Michael K, Patrick A, Joseph S (2004) The cytoplasmic body component trim5alpha restricts hiv-1 infection in old world monkeys. *Nature* 427:848–853
- Meroni G, Diez-Roux G (2005) Trim/rbcc, a novel class of 'single protein ring finger' e3 ubiquitin ligases. *BioEssays* 27:1147–1157
- Novoa B, Romero A, Mulero V, Rodríguez I, Fernández I, Figueras A (2006) Zebrafish (*danio rerio*) as a model for the study of vaccination against viral haemorrhagic septicemia virus (vhsv). *Vaccine* 24:5806–5816
- Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) Ucsf chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25:1605–1612
- Pietro AD, Kajasterudnitski A, Oteiza A, Nicora L, Towers GJ, Mechti N, Vicenzi E (2013) Trim22 inhibits influenza a virus infection by targeting the viral nucleoprotein for degradation. *J Virol* 87:4523–4533
- Pizzi M (1950) Sampling variation of the fifty per cent end-point, determined by the reedmuench (behrens) method. *Hum Biol* 22:151–190
- Rajsbaum R, García-Sastre A, Versteeg GA (2014) Trimmunity: the roles of the trim e3-ubiquitin ligase family in innate antiviral immunity. *J Mol Biol* 426:1265–1284
- Reddy BA, Etkin LD (1991) A unique bipartite cysteine-histidine motif defines a subfamily of potential zinc-finger proteins. *Nucleic Acids Res* 19:6330
- Reddy BA, Etkin LD, Freemont PS (1992) A novel zinc finger coiled-coil domain in a family of nuclear proteins. *Trends Biochem Sci* 17:344–345
- Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Luzi L, Riganelli D, Zanaria E, Messali S, Cainarca S (2001) The tripartite motif family identifies cell compartments. *EMBO J* 20:2140–2151
- Sanchez JG, Okreglicka K, Chandrasekaran V, Welker JM, Sundquist WI, Pornillos O (2014) The tripartite motif coiled-coil is an elongated antiparallel hairpin dimer. *Proc Natl Acad Sci U S A* 111:2494–2499
- Sanders GE, Batts WN, Winton JR (2003) Susceptibility of zebrafish (*danio rerio*) to a model pathogen, spring viremia of carp virus. *Comp Med* 53:514–521
- Smyth MJ, Godfrey DI, Trapani JA (2001) A fresh look at tumor immunosurveillance and immunotherapy. *Nat Immunol* 2:293–299
- Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment

- through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Uchil PD, Quinlan BD, Chan WT, Luna JM, Mothes W (2008) Trim e3 ligases interfere with early and late stages of the retroviral life cycle. *PLoS Pathog* 4:e16
- Uchil PD, Hinz A, Siegel S, Coenen-Stass A, Pertel T, Luban J, Mothes W (2013) TRIM protein-mediated regulation of inflammatory and innate immune signaling and its association with antiretroviral activity. *J Virol* 87:257–272
- Van der Aa LM, Levraud JP, Yahmi M, Lauret E, Briolat V, Herbomel P, Benmansour A, Boudinot P (2009) A large new subset of TRIM genes highly diversified by duplication and positive selection in teleost fish. *BMC Biol* 7:7
- Van der Aa LM, Jouneau L, Laplantine E, Bouchez O, Van KL, Boudinot P (2012) FinTRIMs, fish virus-inducible proteins with E3 ubiquitin ligase activity. *Dev Comp Immunol* 36:433–441
- Versteeg GA, Benke S, Garcíasastre A, Rajsbaum R (2014) Intrinsic immunity: positive and negative regulation of immune signaling by tripartite motif proteins. *Cytokine Growth Factor Rev* 25:563–576
- Wang W, Huang Y, Yu Y, Yang Y, Xu M, Chen X, Ni S, Qin Q, Huang X (2016a) Fish trim39 regulates cell cycle progression and exerts its antiviral function against iridovirus and nodavirus. *Fish Shellfish Immunol* 50:1–10
- Wang Y, Li Z, Lu Y, Hu G, Li L, Zeng L, Yong Z, Liu X (2016b) Molecular characterization, tissue distribution and expression, and potential antiviral effects of trim32 in the common carp (*Cyprinus carpio*). *Int J Mol Sci* 17:E1693
- Wang Y, Zhang H, Lu Y, Wang F, Liu L, Liu J, Liu X (2017) Comparative transcriptome analysis of zebrafish (*Danio rerio*) brain and spleen infected with spring viremia of carp virus (SVCV). *Fish Shellfish Immunol* 69:35–45
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L (2018) Swiss-model: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46:W296–W303
- Weinert C, Morger D, Djekic A, Grütter MG, Mittl PRE (2015) Crystal structure of trim20 c-terminal coiled-coil/b30.2 fragment: implications for the recognition of higher order oligomers. *Sci Rep* 5:10819
- Yang Y, Huang Y, Yu Y, Yang M, Zhou S, Qin Q, Huang X (2016a) Ring domain is essential for the antiviral activity of trim25 from orange spotted grouper. *Fish Shellfish Immunol* 55:304–314
- Yang Y, Huang Y, Yu Y, Zhou S, Wang S, Yang M, Qin Q, Huang X (2016b) Negative regulation of the innate antiviral immune response by trim62 from orange spotted grouper. *Fish Shellfish Immunol* 57:68–78
- Ye Y, Rape M (2009) Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol* 10:755–764
- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL (2012) Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform* 13:134
- Yu Y, Huang X, Zhang J, Liu J, Yin H, Ying Y, Jia C, Huang Y, Qin Q (2016) Fish trim16l exerts negative regulation on antiviral immune response against grouper iridoviruses. *Fish Shellfish Immunol* 59:256–267
- Yu Y, Huang X, Liu J, Zhang J, Yin H, Ying Y, Huang Y, Qin Q (2017) Fish trim32 functions as a critical antiviral molecule against iridovirus and nodavirus. *Fish Shellfish Immunol* 60:33–43
- Zhang S, Guo JT, Wu JZ, Yang G (2013) Identification and characterization of multiple trim proteins that inhibit hepatitis b virus transcription. *PLoS ONE* 8:e70001
- Zhou Z, Jia X, Xue Q, Dou Z, Ma Y, Zhao Z, Jiang Z, He B, Jin Q, Wang J (2014) Trim14 is a mitochondrial adaptor that facilitates retinoic acid-inducible gene-i-like receptor-mediated innate immune response. *Proc Natl Acad Sci U S A* 111:E245–E254