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Short communication

The two TRIM25 isoforms were differentially induced in *Larimichthys crocea* post poly (I:C) stimulation

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ABSTRACT

In this study, we identified and characterized a tripartite motif containing 25 (TRIM25) gene homologue, LcTRIM25, from large yellow croaker (*Larimichthys crocea*). Two isoforms of LcTRIM25, which were generated via alternative splicing, were identified via a molecular analysis of cDNA clones. The long isoform of LcTRIM25 (termed as LcTRIM25-L) contained the full open reading frame of the gene, encoded a protein of 698 amino acid residues, and possessed 11 exons. The short isoform of LcTRIM25 (termed as LcTRIM25-S) contained 9 exons and encoded a protein of 665 amino acid residues. The two LcTRIM25 isoforms contained a conserved Really Interesting New Gene (RING) domain, a B-box2 domain, a Coiled-coil domain (CCD), and variable C-terminal PRY/SPRY domains. Phylogenetic analysis showed that the two LcTRIM25 isoforms of the large yellow croaker was clustered together with their counterparts from other teleost fish. The Real-time PCR analysis showed that the LcTRIM25-L and LcTRIM25-S isoforms were both ubiquitously expressed in nine examined tissues in the large yellow croaker, with predominant expressions in the liver. The expression levels of the two isoforms of LcTRIM25 were rapidly and significantly upregulated *in vivo* after poly (I:C) stimulation in peripheral blood, head kidney, spleen and liver. Moreover, LcTRIM25-L and LcTRIM25-S showed differential expression post poly (I:C) stimulation. LcTRIM25 may have a dual role in innate immunity via alternative gene splicing. These results indicated that LcTRIM25 is likely to be involved in antiviral immune responses.

1. Introduction

In vertebrates, the innate immune response acts as the first line of host defense against invading pathogens [1]. Upon infection, microbial components, known as pathogen-association molecular patterns (PAMPs), are recognized by several cellular pattern recognition receptors (PPRs) [2]. To date, three classes of PRRs have been identified: Toll-like receptors (TLRs), Nucleotide oligomerization domain (NOD)-like receptors (NLRs) and Retinoid acid-inducible gene-I (RIG-I)-like receptors (RLRs) [3–7]. RLRs are involved in the sensing RNA virus invasion in the cytoplasm, and the expression of RLRs (such as RIG-I) are regulated by many molecules, including viruses, the viral dsRNA mimic poly(I:C), and type I interferon (IFN) [2]. Notably, the ubiquitination of caspase activation and recruitment domains (CARDs) of RIG-I by the tripartite motif-containing 25 (TRIM25) protein, an E3 ubiquitin ligase enzyme, is critical to initiate downstream signaling pathways and type I IFN induction [8].

TRIM proteins participate in multiple biological processes , including cell differentiation, cell cycle regulation, apoptosis [9,10]. Moreover, TRIM proteins primarily function as ubiquitin ligases to regulate the innate immune response to infection [11]. The first member of the TRIM family, *Xenopus* nuclear factor 7 (XNF7), was identified by Reddy et al. (1991) [12]. XNF7 was thought to exert an important nuclear function during early development [12]. To date, more than 60 TRIM proteins have been described in humans and mice [9]. The TRIM25 gene was found to be essential for RIG-I-mediated IFNβ production and antiviral activity in response to RNA virus infection [13]. TRIM proteins contain a Really Interesting New Gene (RING) domain, two B-boxes domains, a coiled-coil domain (CCD) and a variable C-terminal PRY/SPRY domains [14,15]. The RING domain is involved in zinc binding and can promote ubiquitin conjugation to target proteins [16]. The B-boxes domains also promote the E3 ubiquitin ligase activity of TRIM proteins [17,18]. The coiled-coil domain is involved in dimerization of TRIM ligases [19] and the PRY/SPRY domains can activate downstream antiviral signaling through interaction with CARDs of RIG-I, leading to IFN production [20].

Many members of the TRIM family have been studied in teleost fish. For instance, more than 80 TRIM genes were identified in the *Danio rerio* genome [21]. A TRIM25 homolog has been reported in many fish, such as *Salmo salar* [22], *Rhodeus uyekii* [23], *Epinephelus coioides* [24]

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and *Oreochromis niloticus* [25]. Evidence from several studies showed that fish TRIM25 could be induced by viruses and the viral mimic poly (I:C) [22,24,26]. The *in vitro* overexpression of TRIM25 could inhibit virus replication and enhanced the expression levels of interferon signaling genes in cultured fish cells [24]. These studies indicated the pivotal role of TRIM25 in the innate immune response to viral infection in teleost fish.

The large yellow croaker, *Larimichthys crocea*, is an economically important marine fish species in China [27] and has been breeding for three decades, with the annual yield of 3-billion fry and yearly output over 160-thousand tones in the last 3 years. In recent years, the mariculture of the large yellow croaker has suffered from many diseases caused by bacterial, parasites, and viruses [28], resulting in huge economic losses. Chen et al. (2003) reported that the mortality rates of viral disease caused by iridovirus in maricultured large yellow croaker could reach as high as 75% [29]. Therefore, to establish effective measures for disease control, a better understanding of mechanisms of the antiviral immune response of the large yellow croaker is urgently required. The results from genome sequencing of the large yellow croaker revealed a gene family expansion of TRIMs, with 54 TRIM genes being detected in the genome of this fish species [30]. However, little is known about the function of TRIM25 in the large yellow croaker. In the present study, we sequenced the coding region of the TRIM25 gene of the large yellow croaker, and studied its molecular evolution and expression profiles in this fish species.

2. Materials and methods

2.1. Taxonomic coverage

We amplified and sequenced the coding region of TRIM25 gene in large yellow croaker (Larimichthys crocea). The new sequences were deposited in GenBank with the accession numbers MH998008 and MH998007.

2.2. Extraction, amplification and sequencing

The total RNA was extracted from spleen tissues (stored at −80 °C) of the large yellow croaker using the Trizol reagent (Invitrogen, USA), and then the total RNA was dealt with RNase-free DNase I (Takara, Japan). According to manufacturer's the protocol, 5 μg of total RNA was reverse-transcribed into cDNA using SuperScript™ III Reverse Transcriptase kit (Invitrogen, USA). A pair of primers was designed to amplify the TRIM25 gene from the large yellow croaker (Table 1). Polymerase Chain Reaction (PCR) was performed using Premix Ex Taq™ (Takara, Japan), with the following conditions: pre-denaturation at 95 °C for 5 min, 32 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min 10 s), and a final extension at 72 °C for 10 min; the PCR product was then kept at 12 °C. The PCR product was isolated using a 1% agarose gels, purified using a Gel Extraction Kits (Tiangen, China), cloned into the pGEM-T easy vector (Promega, USA), and sequenced using the dye-terminator kits (Applied Biosystems, USA) on an ABI 3730 DNA sequencer. The TRIM25 coding sequence of the large yellow croaker was termed LcTRIM25 and used for subsequent analysis.

Table 1
PCR primer sequences for LcTRIM25 from *Larimichthys crocea*.

Primers	Sequences (5′-3′)
Gene construction	
TRIM25-F	AGGACTCATCATGGCTACCATG
TRIM25-R	TGCATCATTTATGTGCATATGCT
TRIM25-L-RT-F	GGAGGTACTGGATCTCAGCC
TRIM25-L-RT-R	GAGTCGGGGTAGTTTTCGTG
TRIM25-S-RT-F	AGAACAATCAGATCTTCAATGGC
TRIM25-S-RT-R	GGCTCCTGTGCATGCTG
β-actin-RT-F	GCGACCTCACAGACTACCTC
β-actin-RT-R	GTAGGTGGTCTCGTGGAT

2.3. Sequence analysis and phylogenetic reconstruction

The nucleotide sequences of TRIM25 were aligned using ClustalX, and the coding sequences were translated into amino acids using MEGA 7.0. The conserved protein domains were predicted using the Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/). The amino acid sequences identity and similarity percentages of TRIM25 proteins were calculated using Matrix Global Alignment Tool (MatGAT v2.02). The genomic sequence of LcTRIM25 was retrieved from the whole genome database of the large yellow croaker, and the corresponding genomic DNA and cDNA were aligned using bl2seq (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to predict the exon/intron boundaries. Furthermore, to identify whether the genes surrounding TRIM25 were conserved in vertebrates, gene synteny was analyzed using Genomicus v93.01 [31] and these gene were confirmed using the ensemble database and genomics in NCBI.

A phylogenetic tree was constructed based on the TRIM25 amino acid sequences of different species using neighbor-joining (NJ) methods in MEGA 7.0, which is a bottom-up (agglomerative) clustering method for the creation of phylogenetic trees [32].

2.4. Expression of TRIM25 gene in normal and poly(I:C)-challenged tissues

The expression of the LcTRIM25 gene in most of the tissues tested from the large yellow croaker was detected using quantitative real-time PCR (qPCR). The healthy large yellow croaker (~100 g body weight) used for the research were obtained by artificially breeding in Zhoushan in Zhejiang province, China. These healthy fish were placed in a tank maintained at 25 °Cand fed at least twice a week before poly(I:C) treatment. After the healthy large yellow croakers were killed under anaesthesia, their brain, head kidney, spleen, liver, intestine, heart, skin, muscle and peripheral blood were collected and immediately stored at −80 °C. Three untreated individuals were used for replication.

In addition, the expression changes of LcTRIM25 in major immune organs post poly(I:C) stimulation were assessed using qPCR. Sterile poly (I:C) stock (Sigma-Aldrich, USA) was dissolve in phosphate-buffered saline (PBS) at 1 mg/ml. Each fish was challenged by intraperitoneal injection of poly (I:C) solution at a dose of 0.25 mg per 100 g body weight. The control group was injected with an equal volume of sterile PBS solution. The peripheral blood, head kidney, spleen and liver were obtained from the immune challenged fish at 0 (PBS-injected), 3, 6, 12, 24 and 48 h after poly(I:C) stimulation. For each group, three individuals were used for replication.

The qPCR analysis of the TRIM25 gene expression level was performed using SYBR® Premix Ex Taq™ (Takara, Japan) on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Specific primers for two TRIM25 isoforms were designed (Table 1). Moreover, the housekeeping gene β-actin was used as the internal reference to normalize the expression of the LcTRIM25 gene and was detected using specific primers β-actin-RT-F/R (Table 1). The dissociation curve was analyzed at the end of qPCR to determine the target specificity.

2.5. Statistical analysis

For qPCR results, we used the $2^{-\Delta\Delta CT}$ to analyze relative gene expression, and we interpreted the differences between the experimental group and the control group using analysis of variance (ANOVA). Then, the data were analyzed about Duncan's multiple comparison. All data were analyzed by using the mean ± standard error (SE); the difference between each group was considered significant at the 95% level.

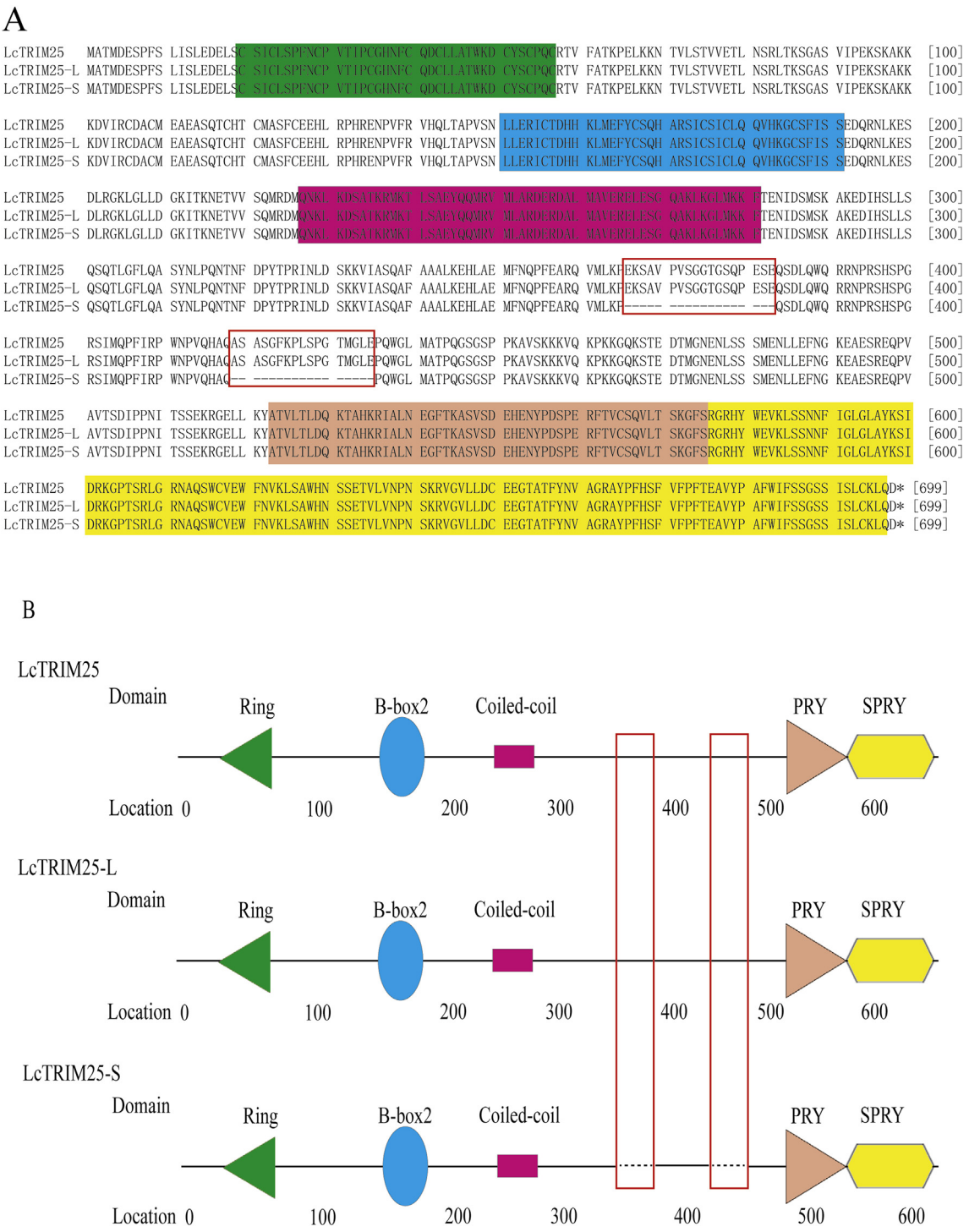

Fig. 1. Sequence analysis of the LcTRIM25 gene. (A) For LcTRIM25, LcTRIM25-L and LcTRIM25-S amino acid sequences, the different domains are shown in different colors. The red boxes represent the differences among LcTRIM25, LcTRIM25-L and LcTRIM25-S. The amino acid sequences of the LcTRIM25-L isoform were as same as those of LcTRIM25. (B) The structure domains of LcTRIM25, LcTRIM25-L and LcTRIM25-S were predicted with using SMART software. Different colors represent different protein domains, and the differences among the three sequences are marked by red boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Sequence analysis of the LcTRIM25 gene

The open reading frame of LcTRIM25 comprises 2097 nucleotides encoding a protein of 698 amino acid residues (Fig. 1A). During sequence analysis, two mRNA alternative splicing isoforms, LcTRIM25 long isoform (termed as LcTRIM25-L) and LcTRIM25 short isoform (termed as LcTRIM25-S), were identified in the large yellow croaker. The nucleotide numbers of LcTRIM25-L isoform and the LcTRIM25 cDNA were the same, both encoding a protein of 698 amino acid residues, whereas the open reading frame of LcTRIM25-S comprises 1998 nucleotides and encodes a protein of 665 amino acid residues (Fig. 1A). LcTRIM25-S contained 99 fewer nucleotides than LcTRIM25-L.

Table 2
Pairwise alignment of TRIM25 from *Larimichthys crocea* with other species.

Species	TRIM25	
	Length	S/I
Larimichthys crocea	699	−/−
Epinephelus coioides	735	80.2/68.6
Oreochromis niloticus	663	78.5/65.4
Cynoglossus semilaevis	615	68.9/55.7
Danio rerio	655	68.6/52.1
Sinocyclocheilus rhinocerous	662	69.8/52.4
Cyprinus carpio	683	70.1/51.9
Gallus gallus	634	53.4/34.8
Mus musculus	635	51.9/34.2
Rattus norvegicus	644	52.3/35
Homo sapiens	631	52/34

Similarity (S) and Identity (I) percentages of *Larimichthys crocea* TRIM25 with other orthologs were calculated using MatGAT v2.0.

Compared with LcTRIM25-L, LcTRIM25-S lacked two amino acid sequences, residues 366 to 383 and residues 419 to 435 (Fig. 1), which comprise the CC-PRY/SPRY linker between the CC domain and PRY/SPRY domain, which may result in different three-dimensional structures between LcTRIM25-L and LcTRIM25-S. Protein domain prediction revealed that LcTRIM25 contains four highly conserved domains, a RING domain (residues from 20 to 57), a B-box2 domain (residues from 151 to 191), a Coiled-coil domain (residues from 227 to 281), C-terminal PRY/SPRY domains (residues from 523 to 697) (Fig. 1). The protein domain of LcTRIM25-L isoform is the same as LcTRIM25, the PRY/SPRY domain of LcTRIM25-S is from residues 490 to 664 due to the lack of CC-PRY/SPRY linker (Fig. 1B).

The lengths of the TRIM25 protein sequences in the examined fish were ranged from 615 AA (*Cynoglossus semilaevis*) to 735 AA (*Epinephelus coioides*) (Table 2). Among these species, the maximum identity of LcTRIM25-L was shared with *E. coioides* TRIM25 (68.6%) (Table 2). The TRIM25 proteins of *Homo sapiens* (631 AA), *Rattus norvegicus* (644 AA), and *Mus musculus* (635 AA) were found to be 35%, 34% and 34.2% identical to LcTRIM25-L, respectively (Table 2).

The genomic structure of the LcTRIM25 gene was examined and compared with those of six species (*Danio rerio, Gallus gallus, Xenopus laevis, Mus musculus, Rattus norvegicus* and *Homo sapiens*) (Fig. 2). The genomic sequence of LcTRIM25 comprised 5212 nucleotides. The LcTRIM25-L isoform embraced 11 exons spaced by 10 introns, whereas the LcTRIM25-S isoform had 9 exons spaced by 8 introns (Fig. 2). Thus, LcTRIM25-S lacked two exons (the fifth and the seventh exons) compared with LcTRIM25-L (Fig. 2). Meanwhile, the intron-exon junction sequences were conserved: the 5′-terminus was GT and 3′-terminus was AG, which conformed to the GT-AG principle. Except for *Xenopus Laevis*, the TRIM25 gene of the large yellow croaker shared highly similar in exon and intron numbers with the other five species. The *Danio rerio* TRIM25 gene had the same number of exons as the LcTRIM25-L isoform, and the other four species (*Gallus gallus, Mus musculus, Rattus norvegicus* and *Homo sapiens*) had the same number as the LcTRIM25-S isoform (Fig. 2). The TRIM25 gene from *Xenopus Laevis* contained 7 exons spaced by 6 introns (Fig. 2). The characteristics of TRIM25 gene indicated that these species may have suffered to different selection pressure from the environment and the evolution of the TRIM25 gene had led to low levels of conservatism in distant species [21,33].

3.2. Evolutionary analysis and phylogenetic tree construction of LcTRIM25 gene

To better understanding the evolution of TRIM25, these genes around TRIM25 were identified to revel the conservatism of the TRIM25 gene in fish, amphibians, birds and mammals (Fig. 3). The synteny analysis of LcTRIM25 and the two LcTRIM25 isoforms was the same. The synteny analysis found that the genes immediately upstream and downstream of TRIM25 were relatively consevred. The immediately downstream gene of TRIM25 was DGKE (encoding diacylglycerol kinase epsilon) in the large yellow croaker and in five other species (*Danio rerio, Gallus gallus, Mus musculus, Rattus norvegicus* and *Homo sapiens*). However, in *Xenopus laevis*, the upstream and downstream genes of TRIM25 were different from those in the other species (Fig. 3): DNAjc5.L was immediately downstream of TRIM25 and the upstream gene encoded zinc finger protein 512B (Fig. 3). Moreover, COIL was immediately upstream of TRIM25 in *Gallus gallus, Mus musculus, Rattus norvegicus* and *Homo sapiens* (Fig. 3). In *Danio rerio*, Rab11fip4b was the upstream gene of TRIM25 (Fig. 3). Unfortunately, the upstream region of LcTRIM25 was unable to be obtained in the large yellow croaker genomic database; therefore, whether the Rab11fip4b gene is the upstream gene of LcTRIM25 still needs to be determined in the large yellow croaker.

To observe the genetic relationship and evolutionary direction of the TRIM25 gene in vertebrates, we collected published TRIM25 sequences from GeneBank and constructed phylogenetic trees. The phylogenetic tree was constructed based on TRIM25 amino acid sequences from 12 species, from bony fish to human, using the neighbor-joining (NJ) approach to be separated into four distinct groups (fish, amphibian, avian and mammalian branches) (Fig. 4). TRIM25 phylogenetic tree analysis found that all the fish species were clustered together in the same subgroup with high nodal support. The two isoforms of LcTRIM25 had highly homology and the closest relationship with *E. coioides* from Perciformes (Fig. 4). In addition, TRIM25 amino acid sequences from amphibians, avians, and mammals were all placed on their appropriate branches in the phylogenetic tree (Fig. 4). The results indicated that the amino acid sequences of TRIM25 from different species could be clustered to their corresponding subgroup (fish, amphibian, avian and mammalian branches).

3.3. Expression of LcTRIM25 gene in normal tissues

The expression of LcTRIM25 was examined in nine normal tissues (muscle, skin, brain, intestine, peripheral blood, heart, spleen, head kidney, and liver) of healthy large yellow croaker using qPCR. The results showed that the two LcTRIM25 isoforms were expressed in all examined tissues, but with a relatively low expression level; the highest expression of the two LcTRIM25 isoforms was found in the liver (about 3-fold higher) (Fig. 5). Similarly, the TRIM25 gene in the Korean rose bitterling *Rhodeus uyekii* was expressed ubiquitously in all tested tissues and was highly expressed in the ovary, spleen, and liver [23]. Besides, the TRIM25 gene in the orange spotted grouper was ubiquitously expressed but at a relatively low expression level [24]. The TRIM25 expression divergence in normal tissues may reflect its different expression patterns. Moreover, the expression level of LcTRIM25-L and LcTRIM25-S were different in the same tissues, such as in the head kidney and intestine (Fig. 5).

3.4. Expression of LcTRIM25 gene after poly(I:C) stimulation

To clarify the role of LcTRIM25 in the antiviral immune response, the expression level of LcTRIM25 at different time points in response to poly(I:C) challenge in major immunity tissues (peripheral blood, spleen, head kidney and liver) was analyzed using qPCR. The expression levels of the two LcTRIM25 isoforms changed significantly after viral dsRNA mimic poly(I:C) stimulation (Fig. 6). Their expression rapidly increased at the early stage of stimulation in the major immune organs (peripheral blood, head kidney, spleen, and liver) (Fig. 6). In peripheral blood, the expression level of LcTRIM25-L was higher than LcTRIM25-S, with a peak at 24 h for LcTRIM25-L and at 12 h for LcTRIM25-S (Fig. 6A). Moreover, the expression level of LcTRIM25-S was higher than LcTRIM25-L, in all other tissues (head kidney, spleen and liver) of the large yellow croaker (Fig. 6B, C and D). The expression of both

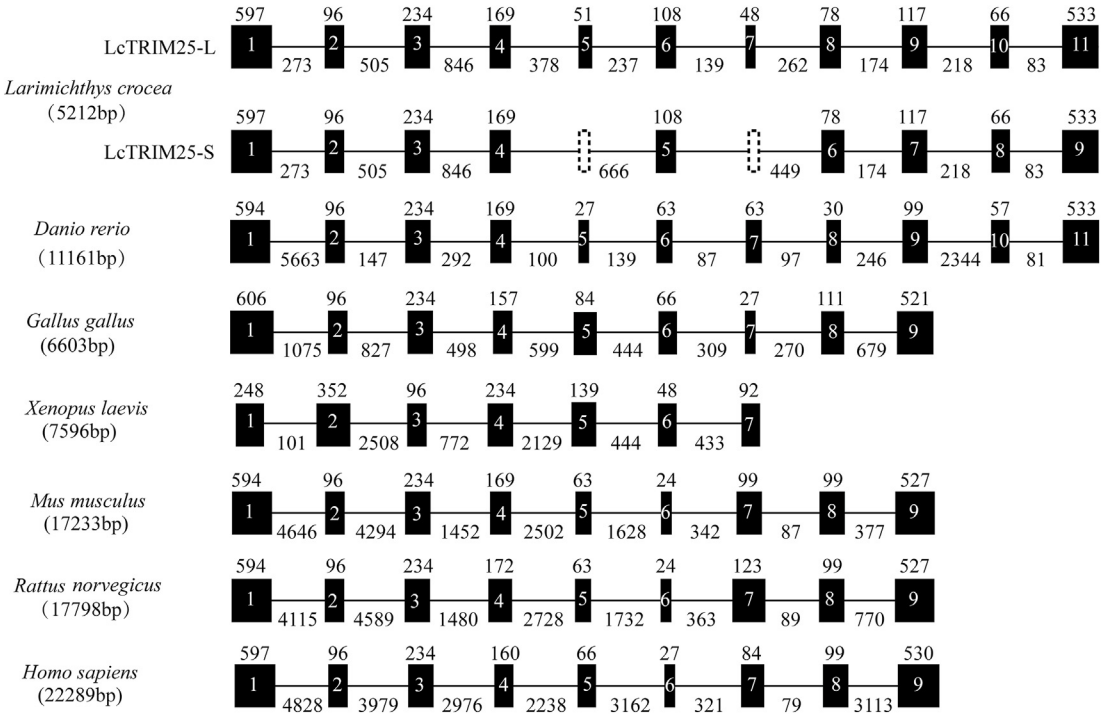

Fig. 2. The genomic structure comparison of homologous genes in vertebrate. The comparison of the genomic structure between LcTRIM25 and other TRIM25 homologous is shown. In addition, the differences between the genomic structures of LcTRIM25-L and LcTRIM25-S are shown. The genomic sequences were: *Larimichthys crocea* (NW_017609304), *Danio rerio* (NC_007117), *Gallus gallus* (NC_006105), *Xenopus Laevis* (NW_016683825), *Mus musculus* (NC_000077), *Rattus norvegicus* (NC_005109), *Homo sapiens* (NC_000017).

LcTRIM25 isoforms peaked at 12 h in head kidney (Fig. 6B). The expression level of LcTRIM25-S was approximately two times as high as LcTRIM25-L in the spleen and the expression of both LcTRIM25 isoforms peaked at 6 h (Fig. 6C). After poly(I:C) the expression of the two LcTRIM25 isoforms peaked at 12 h in the liver (Fig. 6D).

4. Discussion

The TRIM family of proteins has been divided into two groups: One with multiple C-terminal domains, which are usually more conserved in distant species; and the other with faster evolution and lower amino acid sequence conservation in distant species, which is termed the structural Class IV subgroup [33]. Class IV TRIM proteins have different

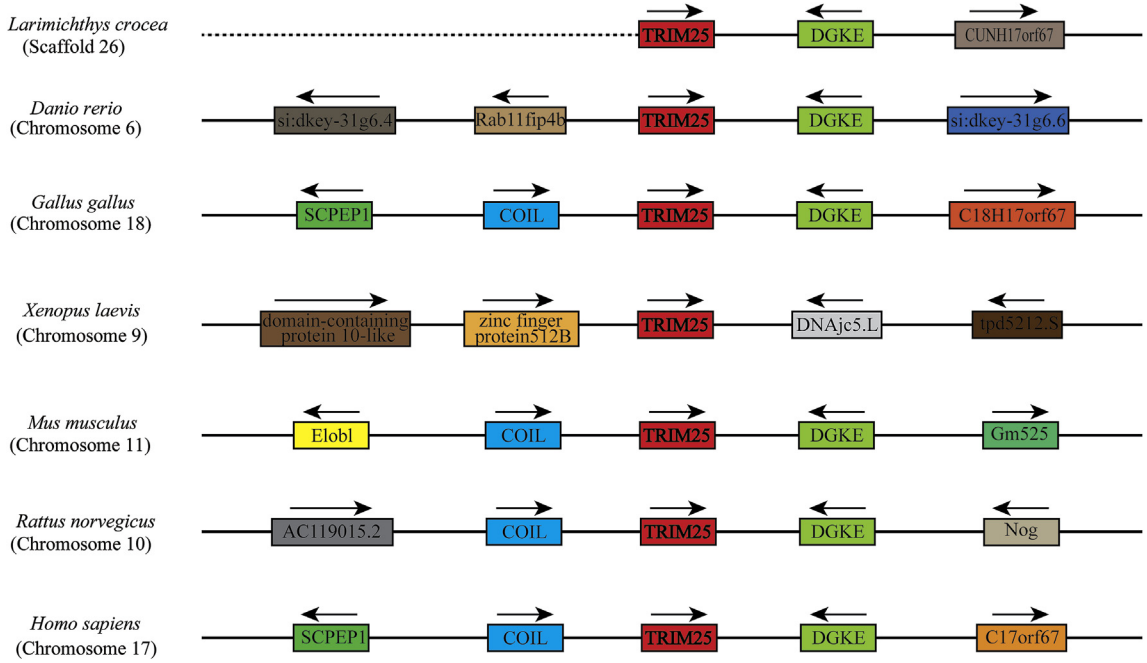

Fig. 3. Analysis of gene synteny among vertebrate. The liner structure of LcTRIM25 was analyzed among selected vertebrates to detect homology. The synteny analysis between LcTRIM25 and the two LcTRIM25 isoforms produced the same results.

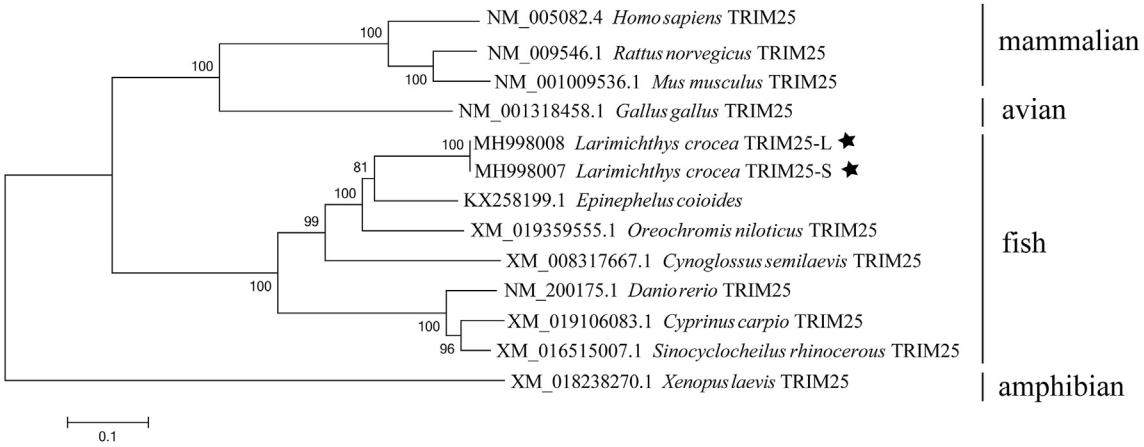

Fig. 4. Phylogenetic trees of TRIM25 sequences. The complement amino acid sequence of TRIM25 from 12 species were aligned with using MEGA 7.0, and the phylogenetic trees were constructed using the neighbor joining (NJ) method. The 12 different species were divided into four classes: mammalian, avian, fish and amphibian. The Genbank accession numbers of homologous genes from selected species shown on the trees.

evolutionary directions, and the B30.2 domain of Class IV comprises the PRY/SPRY domains [34]. In the present study, LcTRIM25 contained four structure domains (RING, B-box2, CCD and C-terminal PRY/SPRY domains) (Fig. 1). Sequence analysis of TRIM25 in the large yellow croaker identified two splice isoforms, the shorter of which LcTRIM25-S, lacked the fifth and the seventh exons. The number of exons in the TRIM25 gene was different among different species (fish, avian, amphibian and mammalian) (Fig. 2), and the results suggested that the gene is quickly evolving in fish and tetrapods. Among the species analyzed, the genes immediately downstream and upstream of TRIM25 are relatively conserved (Fig. 3). In addition, the phylogenetic analysis showed that LcTRIM25 gene was most similar to the TRIM25 gene of *E. coioides* from Perciformes, but had poor amino acid conservation with more distant species (Fig. 4). Overall, the conservation of the TRIM25 sequence among mammals, birds, amphibians, and fish was poor. These results indicated that each species might be subjected to different environmental selection pressures, leading to different evolutionary directions and lower level of amino acid sequence conservation among the different species [35,36]. These results also showed that TRIM25 of the large yellow croaker belonged to the Class IV TRIM proteins [33].

The Class IV TRIM proteins are involved in the antiviral immune responses to induce IFN production [13,37,38]. Moreover, many TRIM genes undergo mRNA alternative splicing, which suggests regulatory diversity [39,40]. In the present study, sequencing of the LcTRIM25 cDNA reveled two splicing isoforms, LcTRIM25-L and LcTRIM25-S (Fig. 1), which were ubiquitously expressed in nine normal tissues of healthy large yellow croaker (Fig. 5) and were rapidly and significantly upregulated *in vivo* after poly (I:C) stimulation (Fig. 6). The results suggest that the two isoforms of LcTRIM25 are involved in antiviral immunity.

Interestingly, LcTRIM25-S isoform was predominantly upregulated in the head kidney, spleen, and especially, the liver. However, the LcTRIM25-L isoform was significantly upregulated in peripheral blood (Fig. 6). These results suggested that the immune functions of LcTRIM25-L and LcTRIM25-S were distinct in different organs and that the temporal expression pattern of TRIM25 varied among the organs

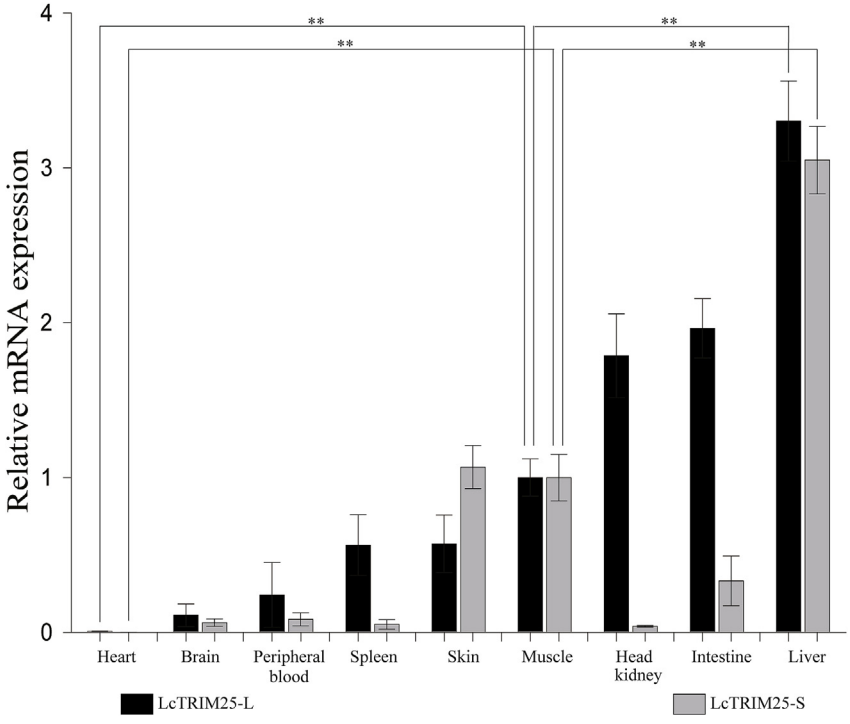

Fig. 5. Expression of the LcTRIM25 gene in healthy tissues of *Larimichthys crocea*. The expression level of LcTRIM25 was detected in nine healthy tissues of *Larimichthys crocea* using real-time PCR and the expression level was different among different tissues. Experiments data are shown as the mean ± SE from three independent experiments. Asterisks indicate significant differences (**$P < 0.01$ and *$P < 0.05$).

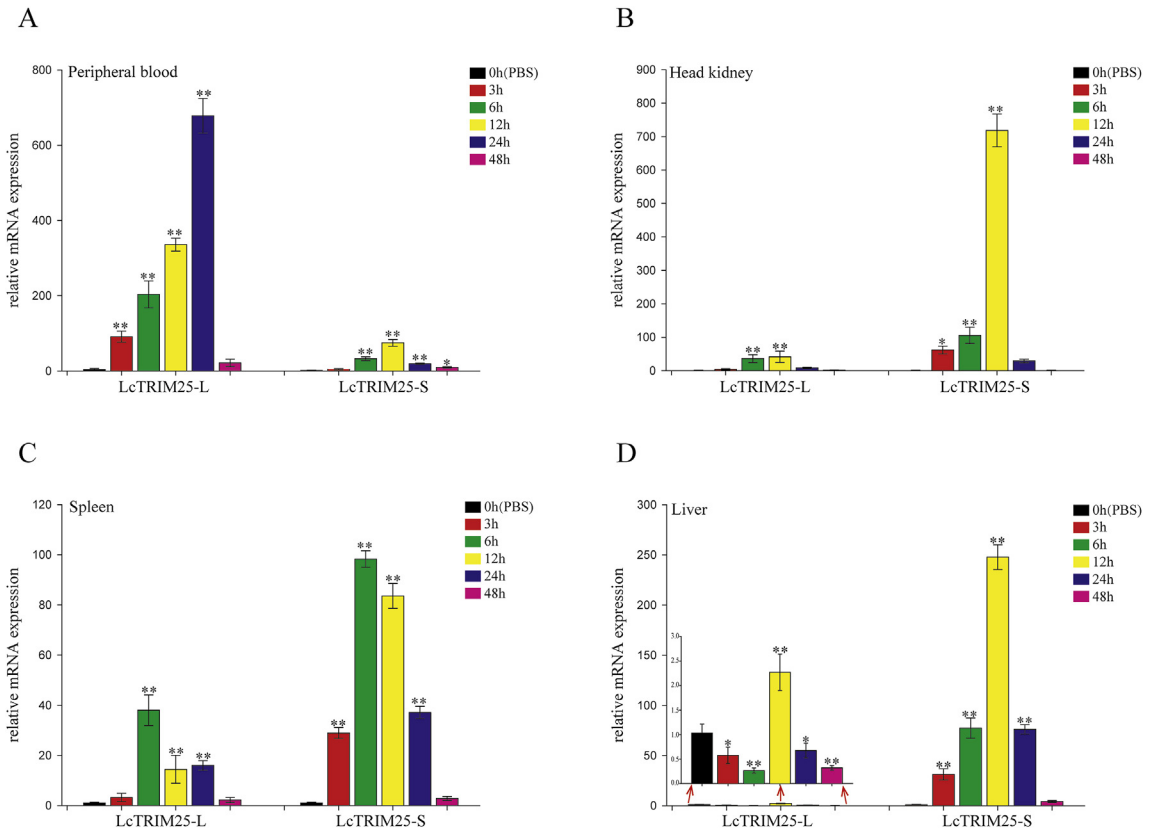

Fig. 6. Expression analysis of the LcTRIM25 gene in major immune tissues after poly(I:C) challenge. After injection of poly(I:C), the expression level of LcTRIM25 was analyzed in peripheral blood, head kidney, spleen, and liver at 0, 3, 6, 12, 24, and 48 h. (A) The expression analysis of LcTRIM25-L and LcTRIM25-S in peripheral blood of *Larimichthys crocea*, (B) in head kidney, (C) in spleen and (D) in liver. To further understand the expression pattern of LcTRIM25-L in the liver after poly (I:C) stimulated, which was magnified. Experiments data are shown as the mean ± SE from three independent experiments. Asterisks indicate significant differences (**$P < 0.01$ and *$P < 0.05$).

tested.

The differential expression of LcTRIM25-L and LcTRIM25-S post poly(I:C) stimulation suggested that LcTRIM25 may have a dual role in the antiviral immune response. A previous study found that the ubiquitin-like protein HLA-F adjacent transcription 10 (FAT10) could inhibit the formation of antiviral particles by binding to RIG-I-TRIM25 to form an inhibitory complex in mammals, which attenuated the RIG-I-mediated antiviral response [10,41]. The E3 ubiquitin ligase TRIM25 stabilized FAT10 on the inhibitory complex. However, the FAT10 protein is unique to mammals [42]. According to the differential expression of the two LcTRIM25 isoforms, we speculated that there are factors similar to FAT10 in teleost fish. The two isoforms of LcTRIM25 may selectively interact with FAT10 analogues to form an inhibitory complex in different immune tissues to attenuate antiviral immunity, resulting from the differential expression of LcTRIM25-L and LcTRIM25-S in different tissues. In addition, the mechanism of the linear ubiquitin assembly complex (LUBAC) in the negative regulation of the RIG-I antiviral signaling pathway was reported [43]. LUBAC consists of two RING-IBR-RING (RBR)-containing E3 ligases HOIL-1L (RBCK1) and HOIP [43,44]. The HOIL-1L/HOIP LUBAC suppresses RIG-I antiviral signaling pathway by inducing TRIM25 degradation and inhibiting the interaction between TRIM25 and RIG-I, resulting in the avoidance of excessive inflammation. The two isoforms of LcTRIM25 might interact with HOIL-1L/HOIP LUBAC in the late stage of poly (I:C) stimulation to induce TRIM25 degradation and thus avoid an excessive immune response.

In summary, the present study identified two splice isoforms of LcTRIM25 in the large yellow croaker. The sequence analysis suggested that LcTRIM25 is poorly conserved and is evolving rapidly. Transcriptional analysis revealed the ubiquitous expression of LcTRIM25 in nine examined tissues, with predominant expression in the liver. LcTRIM25 expression was rapidly and significantly upregulated *in vivo* after poly(I:C) stimulation. Moreover, LcTRIM25-L and LcTRIM25-S showed differential expression post poly(I:C) stimulation. The results of the present study provide the basic for further study of the regulatory mechanism of E3 ubiquitin ligase TRIM25 in the large yellow croaker.

Notes

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