Fish & Shellfish Immunology 60 (2017) 141–149

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

The immunomodulation of a maternal translationally controlled tumor protein (TCTP) in Zhikong scallop *Chlamys farreri*

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form
15 November 2016
Accepted 17 November 2016
Available online 18 November 2016

Keywords:
Chlamys farreri
TCTP
Maternal
Larval development
Innate immune response
Histamine-releasing

ABSTRACT

Translationally controlled tumor protein (TCTP) is initially described as a highly conserved protein implicated in cell growth, and it is subsequently confirmed to play important roles in mediating the innate immune response, especially the inflammatory. In the present study, the full-length cDNA sequence of a TCTP from Zhikong scallop *Chlamys farreri* (designed as *Cf*TCTP) was cloned by rapid amplification of cDNA ends (RACE) technique based on the expression sequence tag (EST) analysis. It was of 1230 bp with an open reading frame (ORF) of 543 bp encoding a polypeptide of 180 amino acids. The deduced amino acid sequence contained a conserved TCTP signature sequence (from I^{47} to E^{58}) and it shared 26.1%–48.9% similarities with previously identified TCTPs. *Cf*TCTP was clustered with the TCTP from *Argopectehs irradias* in the phylogenetic tree and was designated into a single branch of mollusk with TCTP from *Ruditapes philippinarum*. The mRNA transcripts of *Cf*TCTP were constitutively expressed in all the tested tissues, including haemocytes, muscle, mantle, gill, hepatopancreas, kidney and gonad, with the highest expression level in hepatopancreas. The mRNA expression level of *Cf*TCTP in oocytes and fertilized eggs kept at a higher level, and was down-regulated from 2-cell embryos to the lowest level in gastrula. Then it was up-regulated in trochophore and dropped down in the late veliger larvae to the similar level as that in oocytes. After pathogen-associated molecular patterns (PAMPs) stimulation, the expression of *Cf*TCTP mRNA in haemocytes was increased at 3 or 6 h, and fall down to the normal level at 24 h. The recombinant protein of *Cf*TCTP could induce the release of histamine from BT-549 cells. All these results indicated that *Cf*TCTP was a pro-inflammatory factor and it could be maternally transferred from female gonad to oocytes and offspring, and play pivotal role in the embryonic development and immune protection of scallops.

1. Introduction

The translationally controlled tumor protein (TCTP) was named due to its high expression level in tumor cells at translational level [1], and initially described as a factor related to cell growth in mouse ascites and erythroleukemic cells [2–4]. Structurally, TCTP is similar to the Mss4/Dss4 (mammalian suppressor of Sec4) family members and forms a structural superfamily [5]. There are two signature regions, termed TCTP-1 and TCTP-2, in TCTP proteins. Presently, TCTP is defined as a family of highly conserved proteins existed in a large variety of eukaryotes [6,7], and plays an important role in abundant biological processes including cell growth [6], anti-apoptotic activities [8,9] and cell cycle progression [10], as well as response to extracellular signals and cellular conditions [11].

It has been reported that TCTP is an important participant in series of immune responses [12]. In vertebrate, TCTP is known as IgE-dependent histamine-releasing factor (HRF) due to its function in histamine-releasing and anti-apoptotic activities, thus it is regarded as an important pro-inflammatory factor [13]. For instance, TCTP in *Plasmodium falciparum* could induce histamine release from human basophils and IL-8 secretion in eosinophils [12]. TCTP has also been reported to involve in the recovery of oxidative due to its function of histamine-releasing in *Lampetra japonica* [14]. In fish *Scophthalmus maximus*, TCTP was involved in

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http://dx.doi.org/10.1016/j.fsi.2016.11.043
1050-4648/

the albino skin and the expression level of *Sm*TCTP in albino skin was higher than that in normal skin [15].

Many TCTP homologues and their roles in innate immune response have been identified and documented in invertebrates. In shrimps *Litopenaeus vannamei*, *Penaeus indicus* and *Marsupenaeus japonicas*, the expression of TCTP genes were found to be related to the WSSV infection [16–20]. In mollusca *Venerupis philippinarum*, the transcripts of TCTP was significantly decreased from 6 h to 12 h after *Vibrio anguillarum* challenge, followed by an up-regulation at 48 h [21], suggesting its potentially protective function of in the antimicrobial response. Accumulating evidences have suggested that invertebrate TCTPs are also involved in a series of anti-stress responses. Recently, the involvement of TCTP in anti-stress activities was also drawn great attention. *Bm*TCTP could function as an anti-apoptotic protein and protect cells form oxidative damage in *Brugia malayi* [22]. TCTP from *Eriocheir sinensis* was found to function as an anti-metal stress factor [23]. In *Mytilus galloprovincialis*, *Mg*TCTP was involved in the mediation of cadmium stress [24]. TCTP is a house-keeping, ubiquitously expressed and fairly abundant protein [25]. However, whether molluscan TCTP is involved in pro-inflammatory responses by histamine-releasing activities remains unknown.

Zhikong scallop *Chlamys farreri* is a dominant aquaculture species of Mollusca in China, and the mechanism of its immune defense response has attracted increasing attention [26–31]. The inflammatory response is an integral part of the innate immune reaction to neutralize infectious agents and initiate repair to the damaged tissue which is indispensable for the scallops to eliminate none-self [32]. In the present study, an EST sequence from *C. farreri* was found to be homologous to the previously identified TCTP from *Labeo rohita* [33]. The main objectives of this study were (1) to clone the full-length cDNA of TCTP gene from *C. farreri* (designed as *Cf*TCTP), (2) to detect its spatial mRNA transcription in different tissues, and the temporal expressions in the embryos at early developmental stages and after the stimulation of pathogen-associated molecular patterns (PAMPs), (3) to validate the possible function of *Cf*TCTP protein in the immune response, hopefully provide new insights into the innate immune system of scallops.

2. Materials and methods

2.1. Scallop and immune stimulation

Adult scallops *C. farreri* with an average shell length of 55 mm were collected from a local farm in Qingdao, Shandong province, China, and kept in the aerated seawater at 15 °C for two weeks before processing.

Seven kinds of tissues, including gonad, muscle, mantle, gill, kidney and hepatopancreas, were collected from six adult scallops as parallel samples. Hemolymph from the six individuals was collected and immediately centrifuged at 800×*g*, 4 °C for 10 min to harvest the haemocytes. All these samples were stored at −80 °C for subsequent RNA extraction.

Two hundred and forty scallops were employed for PAMPs stimulation experiment, and they were randomly divided into six groups. The scallops in the five treatment groups received an injection of 50 μL phosphate buffered saline (PBS buffer, 0.14 mol/L sodium chloride, 3 mmol/L potassium chloride, 8 mmol/L disodium hydrogen phosphate dodecahydrate, 1.5 mmol/L potassium phosphate monobasic, pH 7.4), lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 (Sigma-Aldrich, 0.5 mg/mL in PBS), peptidoglycan (PGN) from *Staphylococcus aureus* (Sigma-Aldrich, 0.8 mg/mL in PBS), β-Glucan (GLU) from *Saccharomyces cerevisiae* (Sigma-Aldrich, 1.0 mg/mL in PBS) and polyinosinic-polycytidylic acid sodium salt (poly (I: C), Sigma Aldrich, 1 mg/mL in PBS), respectively. The rest untreated scallops were employed as blank control. After injection, the scallops were returned to the tanks and six individuals were randomly collected at 3, 6, 9, 12, 24 and 48 h post injection. The haemolymphs were collected and then centrifuged at 800×*g*, 4 °C for 10 min to harvest the haemocytes and stored at −80 °C before use.

All embryos and larvae were sampled from a farm in Rongcheng, China [34]. The samples from different stages were identified microscopically, as oocytes, fertilized eggs, 2-cell embryos, 4-cell embryos, 8-cell embryos, 16-cell embryos, 32-cell embryos, morula (6 h post-fertilization, hpf), blastula (11 hpf), gastrula (18 hpf), trochophore (22 hpf), early D-hinged larvae (2 day post-fertilization, dpf), early veliger larvae (4 dpf), mid-veliger larvae (7 dpf) and late veliger larvae (23 dpf). Six duplicate samples were collected in each stage. All the samples were collected with six repeat sampling after addition of 1 mL TRIzol reagent (Invitrogen) for subsequent RNA extraction.

2.2. RNA isolation and cDNA synthesis

Total RNA was extracted from tissues and the haemocytes of *C. farreri* using Trizol reagent according to the manufacture's protocol (Invitrogen). First-strand cDNA synthesis was performed using the DNase I (Promega) treated total RNA as template and adaptor primer-oligo (dT) as primer (Table 1). The reactions were incubated at 42 °C for 1 h, terminated by heating at 95 °C for 10 min.

2.3. cDNA library construction, EST analysis and full-length cDNA cloning

An EST of 662 bp (cl138ct148cn157) highly similar to the identified TCTP from *Labeo rohita* [33,35] was selected for further cloning the full-length cDNA of TCTP from *C. farreri*. Two gene-specific primers, *Cf*-TCTP-Race-F1 and *Cf*-TCTP-Race-F2 were designed to clone the 3'sequence of *Cf*TCTP by rapid amplification of cDNA ends (RACE) technology based on the EST. PCR amplification was performed in a TP-600 PCR Thermal Cycler (Takara, Japan). The DNA was gel-purified with MiniBest Agrose Gel DNA Extraction Kit Ver. 4.0 (9762, Takara, Japan) and then cloned into the pMD19-T simple vector (3271, Takara, Japan) and sequenced by M13-47 and RV-M primers.

2.4. Sequence analysis of *Cf*TCTP

The searches for nucleotide and protein sequences similarity were conducted with the BLAST algorithm at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.gov/blast). The EditSeq module of DNAStar Lasergene software suite 11.0.0 was used to analyze the deduced amino acid sequences of *Cf*TCTP, and the protein motif features of *Cf*TCTP were predicted by Simple Modular Architecture Research Tool (SMART) 7.0 (http://smart.embl-heidelberg.de/). ClustalW multiple alignment program 2.1 (http://www.ch.embnet.org/software/ClustalW.html) and multiple alignment show program 2.0 (http://www.bioinformatics.org/sms2/) was used to perform the multiple sequence alignment of TCTP proteins. 3D structures of *Cf*TCTP and TCTP from *Mus musculus* (NP_033455) were predicted by using I-TASSER methods. A phylogenic neighbor-joining tree was constructed with MEGA 6.06 software. To derive confidence value for the phylogeny analysis, bootstrap trials were replicated 1000 times.