Fish and Shellfish Immunology 82 (2018) 378–385

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Short communication

Biological function of a gC1qR homolog (*EcgC1qR*) of *Exopalaemon carinicauda* in defending bacteria challenge

Jiquan Zhang[a,b,c], Yujie Liu[a], Yanyan Li[a], Naike Su[a], Yaru Zhou[a], Jianhai Xiang[b,c], Yuying Sun[a,*]

[a] College of Life Sciences, Hebei University, Baoding, Hebei, 071002, China
[b] Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266000, China
[c] Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China

ARTICLE INFO

Keywords:
Exopalaemon carinicauda
Globular heads of C1q
CRISPR/Cas9
Knock-out

ABSTRACT

The gC1qR is a ubiquitously expressed cell protein that interacts with the globular heads of C1q (gC1q) and many other ligands. In this study, one gC1qR homolog gene was obtained from *Exopalaemon carinicauda* and named *EcgC1qR*. The complete nucleotide sequence of *EcgC1qR* contained a 774 bp open reading frame (ORF) encoding EcgC1qR precursor of 257 amino acids. The deduced amino acid sequence of *EcgC1qR* revealed a 55-amino-acid-long mitochondrial targeting sequence at the N-terminal and a mitochondrial acidic matrix protein of 33 kDa (MAM33) domain. The genomic organization of *EcgC1qR* gene showed that *EcgC1qR* gene contained five exons and four introns. *EcgC1qR* could express in all of the detected tissues and its expression was much higher in hepatopancreas and hemocytes. The expression of *EcgC1qR* in the hepatopancreas of prawns challenged with *Vibrio parahaemolyticus* and *Aeromonas hydrophila* changed in a time-dependent manner. The expression of *EcgC1qR* in prawns challenged with *V. parahaemolyticus* was up-regulated at 6 h ($p < 0.05$), and significantly up-regulated at 12 h and 24 h ($p < 0.01$), and then returned to the control levels at 48 h post-challenge ($p > 0.05$). At the same time, the expression in *Aeromonas*-challenged group was significantly up-regulated at 6, 12 and 24 h. The recombinant EcgC1qR could inhibit the growth of two tested bacteria. In addition, we successfully deleted *EcgC1qR* gene through CRISPR/Cas9 technology and it was the first time to obtain the mutant of gC1qR homolog gene in crustacean. It's a great progress to study the biological function of *gC1qR* in crustacean in future.

1. Introduction

As economically important species, many crustaceans are cultured and the majority of them are shrimp and prawns. In recent years, the outbreak of diseases has significantly compromised shrimp aquaculture [1]. The complement system performs a critical function in host defense and inflammation [2]. In 1994, Ghebrehiwet et al. [3] firstly isolated a novel cell surface protein from Raji cells, which could bind to the globular "heads" of C1q molecules and designated gC1qR.

The gC1qR is a ubiquitously expressed cell protein that interacts with the globular heads of C1q (gC1q) and many other ligands [4]. It has been reported that crustacean gC1qR plays an important role in defensing attack of virus and bacteria. The firstly reported crustacean gC1qR gene (*PlgC1qR*) was from the freshwater crayfish *Pacifastacus leniusculus* and it had antiviral activity against white spot syndrome virus (WSSV) [5]. Li et al. [6] found that recombinant gC1qR from *Fenneropenaeus chinensis* could bind to *Staphylococcus aureus* in a concentration-dependent manner and it might be involved in defending against bacterial infections in shrimp. Yang et al. [7] firstly identified and characterized the C1q subcomponent binding protein (*PmC1qBP*) from *Penaeus monodon* and found that *PmC1qBP* was involved in shrimp immune responses to pathogenic infections. Ye et al. [8] reported that *MrgC1qR* from *Macrobrachium rosenbergii* might function as a pathogen-recognition receptor (PRR). Huang et al. [2] reported the first gC1qR in crab and speculated that *EsgC1qR* was involved in the innate immunity of Chinese mitten crab, *Eriocheir sinensis*.

Exopalaemon carinicauda, an economically important species in China, had an advantage over other shrimp of prawns in basic research. It can be maintained with reproductive capacity all the year round in the laboratory environment with an about 60-day reproduction cycle. Its genome draft had been performed and the assembly covers more than 95% of coding regions [9]. In addition, we had successfully performed the site-specific genome editing in *E. carinicauda* via CRISPR/Cas9 [10], and it can be used as a feasible means for the study of

* Corresponding author.
E-mail address: sunyuying125@163.com (Y. Sun).

https://doi.org/10.1016/j.fsi.2018.08.046
Received 30 May 2018; Received in revised form 15 August 2018; Accepted 21 August 2018
Available online 23 August 2018

Table 1
Primers mentioned in the paper.

Primers	Sequences (5′-3′)	Sequence information
RT-EcgC1qRF	CCAAGTGTTTTAGGAGGTCT	Real-time PCR
RT-EcgC1qRR	ACAAAGTGGAAGATGGGAAT	Real-time PCR
18S-F	TATACGCTAGTGGAGCTGGAA	Real-time PCR
18S-R	GGGGAGGTAGTGACGAAAAAT	Real-time PCR
9k-EcgC1qRF	GCTACGTACATCATCACCATCACCACAGTCTATTTAGCCGTGCCCTCA	Construct the expression vector, introducing a restriction enzyme site for *SnaB* I and a 6 × His-tag
9k-EcgC1qRR	GCGCGGCCGCTTAGAAGTAACCCTCTCCCAAAGGATC	Construct the expression vector, introducing a restriction enzyme site for *Not* I
5′AOX1	GACTGGTTCCAATTGACAAGC	Confirm the insert target gene
3′AOX1	GCAAATGGCATTCTGACATCC	Confirm the insert target gene
EcgC1qR-gRNA	CAGGACAATAGCGCGAAGTT	sgRNA target site for *EcgC1qR*
EcgC1qR-detF	TATTTAGCCGTGCCCTCATG	Detection primers
EcgC1qR-detR	TGTGTGGATGCCGTGAATAC	Detection primers

Note: F and R stand for forward primers and reverse ones, respectively.

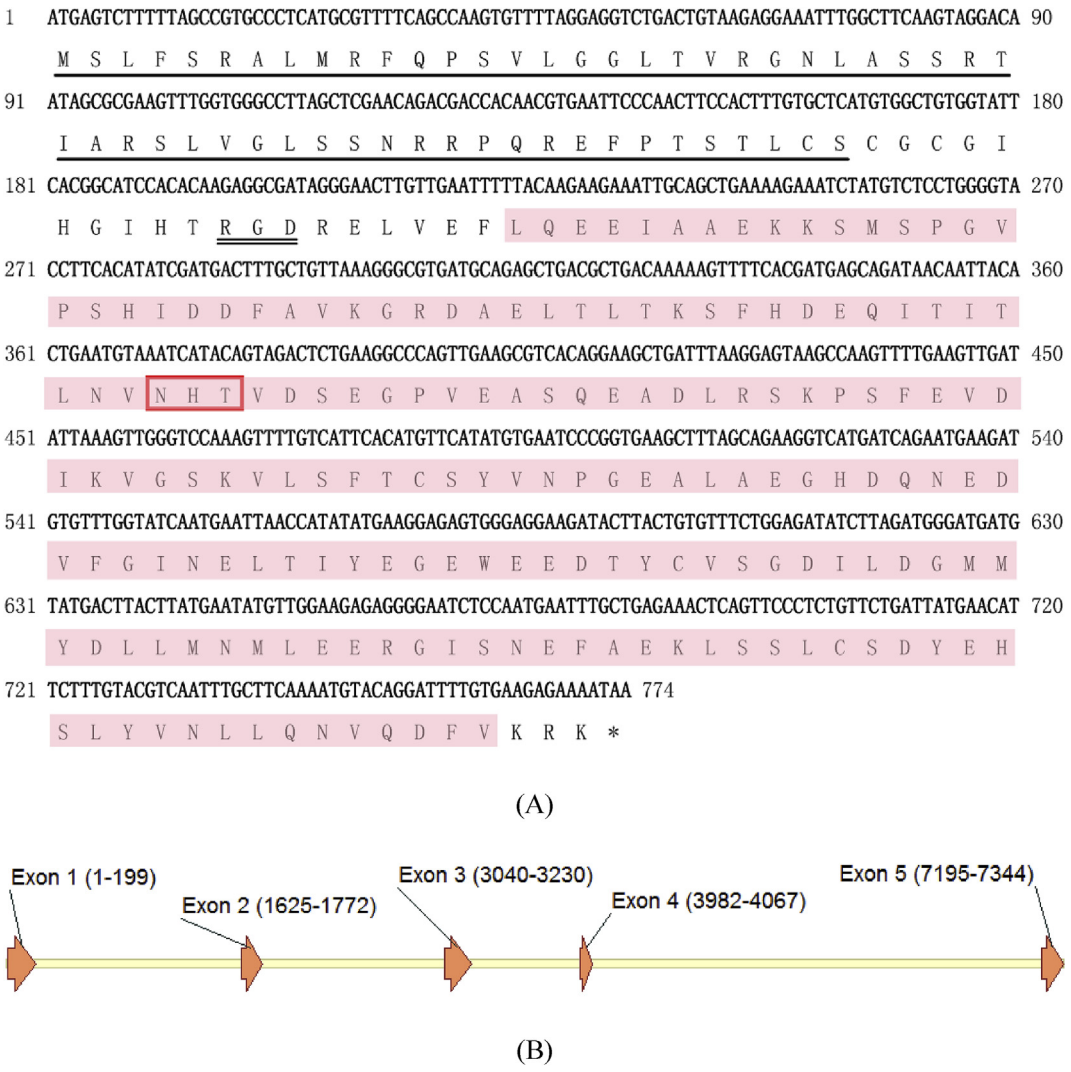

Fig. 1. (A) The nucleotide sequence and deduced amino acid sequence of *EcgC1qR*. The mitochondrial targeting sequence was underlined and the RGD motif was double underlined. The mitochondrial acidic matrix protein domain (MAM33) was shadowed in pink. The N-glycosylation sites are boxed in red. (B) The genomic structure of *EcgC1qR*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

important biological questions that cannot be easily addressed in other shrimp and prawns [11,12].

In this research, we firstly reported a gC1qR gene (*EcgC1qR*) in *E. carinicauda*. The expression profile of *EcgC1qR* in different tissues and its immune function against bacteria was analyzed. Furthermore, *EcgC1qR* was recombinantly expressed in *Pichia pastoris* and its potential function of recombinant *EcgC1qR* was also analyzed. In addition, we successfully deleted the *EcgC1qR* using CRISPR/Cas9 technology, which is a great progress to study the biological function of *gC1qR* in crustacean in future.