SpgC1qR interacts with WSSV VP28 exhibiting antiviral activity

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ABSTRACT

Although human gC1qR is a multi-ligand binding protein with diverse biological functions, the functions of invertebrate gC1qR homologues remain largely unknown. In the present study, we characterized a novel gC1qR homologue, namely SpgC1qR, from mud crab Scylla paramamosain. SpgC1qR shared high identity and similar three-dimensional structure with human gC1qR. After challenge with White spot syndrome virus (WSSV), the transcripts of SpgC1qR were significantly increased, suggesting that SpgC1qR may be involved in antiviral immune response. To reveal the likely antiviral activity of SpgC1qR, the proliferation profile of WSSV in SpgC1qR-silenced crabs was examined. The result showed that knockdown of SpgC1qR by RNAi facilitated viral proliferation in vivo. This result was further confirmed by a SpgC1qR pre-incubation assay, in which pre-incubating WSSV particles with SpgC1qR dramatically suppressed viral replication. Moreover, a GST pull-down assay revealed that SpgC1qR specifically bound to the viral envelope protein VP28. These findings clearly demonstrated that SpgC1qR specifically interacted with viral envelope protein VP28 and restricted WSSV replication, suggesting that it played a crucial role in anti-WSSV immune response of mud crab. This study provided new insights into the antiviral mechanism mediated by SpgC1qR and the biological functions of invertebrate gC1qR homologues.

1. Introduction

The globular ‘head’ of complement component C1q receptor (gC1qR) is a multifunctional and multiligand binding protein, named after its ability to bind to the globular head of complement C1q sub-component. Human gC1qR was first isolated from Raji cells as a 33 kD glycoprotein [1], and it was also designated as P33, p32, C1qBP, or TAP on the basis of its diverse biological functions [2]. Studies have shown that human gC1qR is mainly distributed in the mitochondrial matrix, but it can also be detected on the surface of cells, especially under abnormal conditions of the host (disease or stress). This protein can be secreted into outside of cells in a soluble form [3] or expressed on the cell surface serving as a binding site for plasma and microbial proteins [4].

Human gC1qR is a multi-ligand binding protein involved in various ligand-mediated cellular responses. Studies have shown that it can bind various types of ligands and display a variety of biological functions, such as activating the complement system, participating in tumor angiogenesis, inducing apoptosis, and participating in pathogen infection and inflammatory reactions [2, 5, 6]. Among them, the positive and negative effects of gC1qRs on pathogen infection have become the focus of functional research. A few reports have shown that human gC1qR acts as receptors for many pathogens and promotes viral and bacterial infection. It binds to the core proteins of adenovirus and hepatitis B virus and promotes viral infection and proliferation [7, 8]. Knockdown of human gC1qR by RNA interference (RNAi) significantly reduces the binding and infection of hantavirus to human lung epidermal cells [9]. In addition, it has been reported that human gC1qR on the surface of cells can promote the infection of Staphylococcus aureus and the spores of Bacillus cereus by binding to certain bacterial components [10–12]. However, recent studies have demonstrated that some gC1qR homologues suppress bacterial infection. Human gC1qR competitively binds to hyaluronidase secreted by Streptococcus pneumoniae participating in antibacterial immunity [13]. Several crustacean gC1qRs interact with some bacterial components and play important roles in antibacterial immune responses [14–18].

White spot syndrome virus (WSSV), an enveloped double-stranded DNA virus, is a serious pathogen to many crustacean species [19–21]. WSSV belongs to a new virus family Nimaviridae under a new genus...
**2. Materials and methods**

*2.1. Reagents and chemicals*

TIANamp Marine Animals DNA Kit was obtained from Tiantan Biotech (Beijing, China). RNAiso Plus, First-Strand cDNA Synthesis Kit, and SYBR Premix Ex Taq Polymerase were purchased from TaKaRa Biotech (Beijing, China). GST Fusion Protein Purification Kit and Histagged Protein Purification Kit were purchased from GenScript (Nanjing, China).

*2.2. Mud crab challenge and tissue collection*

Mud crabs (approximately 150 g each) purchased from a farm in Chongming County (Shanghai, China) were temporarily cultured in aerated seawater in tanks (400 L) before conducting experiments. Healthy crabs were selected to investigate the tissue distribution of SpgC1qR homologues. The gills of mud crabs were sampled at 0, 6, 12, 24, 48, and 72 h post infection. Other tissues of healthy mud crabs, including the heart, hepatopancreas, gills, stomach, intestine, ovary and muscle, were cut, washed with sterile PBS, and collected for following total RNA extraction. The total RNAs from hemocytes and other tissues were applied to investigate tissue distribution of SpgC1qR. To examine the expression profile after viral challenge, healthy mud crabs were randomly selected and divided into two groups. Purified WSSV particles (1 × 10^7 copies) in 100 µL of PBS were injected into the base of the fifth leg of mud crabs, and an equal volume of PBS was served as a control. The gills of mud crabs were sampled at 0, 6, 12, 24, 48, and 72 h post injection. For each sample, at least three crabs were used to eliminate individual differences. Two other batches of RNA samples isolated previously at different times were used to eliminate the differences among batches.

*2.3. Total RNA isolation and cDNA synthesis*

The total RNAs from hemocytes and other tissues were isolated with RNAiso Plus reagent. The contaminated genomic DNA during total RNA extraction was removed with DNase I (Promega, USA). cDNA templates were synthesized with total RNAs following the manufacturer’s instructions of First-Strand cDNA Synthesis Kit.

*2.4. Bioinformatic analysis*

The similarity of SpgC1qR with other gC1qR homologues in invertebrates and vertebrates was analyzed using the online BLAST program (BLASTP) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Signal peptide prediction was carried out using SignalP [35]. The mitochondrial targeting sequence and putative domains were predicted by MITOPROT programs (http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html) and SMART (http://smart.embl-heidelberg.de/), respectively. ProtParam (https://web.expasy.org/protparam/) was used to predict and analyze the physical and chemical properties of SpgC1qR, including relative molecular weight (Mw), theoretical isoelectric point, cell attachment sequence (arginyl-glycyl-aspartic acid, RGD) and amino acid composition. Multiple sequence alignment was performed using ClustalX 2.0 (http://www.ebi.ac.uk/tools/clustalw2) and GENEDOC. The phylogenetic tree was constructed using MEGA 7.0 software [36]. The three-dimensional (3D) structure of SpgC1qR MAM33 (the domain of mitochondrial acidic matrix protein of 33 kDa) was predicted through the Phyre server [37] using the crystal structure of human gC1qR MAM33 (PDB ID: d1P32, chain A) as template. The predicted quaternary structure of SpgC1qR trimer was modeled by superimposing three individuals according to the structure of human gC1qR MAM33 trimer. The 3D structures of SpgC1qR MAM33 and quaternary structure of SpgC1qR MAM33 trimer were generated by Discovery Studio Visualizer.

*2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)*

Quantitative real-time PCR (qRT-PCR) was conducted to determine the expression level of SpgC1qR in a real-time thermal cycler Quantstudio 6 Flex (ABI, USA) following a previous protocol [38]. A pair of primers (RF and RR; Table 1) was designed to produce a 191 bp amplicon of SpgC1qR. Another pair of primers (18SRF and 18SRR; Table 1) was used to amplify a 121 bp fragment of 18S rRNA as reference. The total volume was 20 µL (10 µL of 2 × SYBR Premix Ex Taq, 2 µL of cDNA, and 4 µL of each primer). qRT-PCR was programmed as follows: 95 ℃ for 3 min, 40 cycles at 95 ℃ for 10 s and 60 ℃ for 60 s. The melt from 60 ℃ to 95 ℃. All tests were performed thrice with individual templates. The 2^–ΔΔCt method was used to calculate the relative expression level of SpgC1qR in different tissues, and the algorithm of 2^–ΔΔCt was utilized to analyze the expression profile after WSSV infection [39]. Unpaired t-test was used to determine significant differences (*, P < 0.05).

### Table 1

| Primers          | Sequence (5′–3′)                                      |
|------------------|------------------------------------------------------|
| Real-time PCR    |                                                      |
| RF               | TTTGCGGACCGGTGGTACTTG                                   |
| RR               | TGGGAGCTGGTGATGGCATCC                                  |
| 18S rRNA         | CAGACAAATACGTCACCAAC                                   |
| 18SR             | GACTTAAACAGGGAACCTTCA                                   |
| Protein expression |                                                      |
| SpgC1qRREF       | TACTACGAAATCTACAGGAGAATGGT                              |
| SpgC1qRER        | TACACTCGGCCACAGACTACCTGGGT                              |
| RNAI             | GCGTATACGACTTATATGGCTGTTGTTGCTCTAGGAGG                 |
| SpgC1qRF         | GCGTATACGACTTATATGGCTGTTGTTGCTCTAGGAGG                 |
| EGPFFI           | GCGTATACGACTTATATGGCTGTTGTTGCTCTAGGAGG                 |
| WSSV detection  | AGCTGGAGACACTCTGCCCTTC                                  |
| WSSVRR           | TTACTGCGGTCATGCGGAGA                                   |

Restriction enzyme sites were shown in bold and italics.

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**Whispovirus.** It contains a 305-kb circular genome DNA, which encodes ~180 predicted proteins [22, 23]. To date, more than 40 WSSV structural proteins have been identified using proteomic methods [24, 25]. VP28, VP26, VP24, and VP19 are the four major viral envelope proteins and they constitute the major content of the entire envelope. Among them, VP28 is crucial for virus entry [26]. Although current studies have shown that some immune components play important roles in antiviral immune defense of crustaceans [27–33], the exact molecular mechanisms on how the innate immune system resists WSSV infection remain elusive.

**Scylla paramamosain** (mud crab) is an economically important crustacean species widely distributed in China and Indo-West Pacific countries. In the current study, we characterized a gC1qR homologue, namely SpgC1qR, from S. paramamosain. We analyzed the similarity of SpgC1qR with human gC1qR and investigated its potential antiviral function. Our study revealed that SpgC1qR interacted with viral envelope protein VP28 and dramatically inhibited WSSV replication, suggesting that it played a crucial role in antiviral immune response of mud crab. This study provided new insights into the antiviral mechanism mediated by SpgC1qR and the biological functions of invertebrate gC1qR homologues.

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2.6. RNA interference (RNAi) of SpgC1qR

RNAi assay was performed using a previously reported method with slight modifications [40]. In brief, a partial SpgC1qR DNA fragment was amplified by PCR with primers linked with the T7 promoter (SpgC1qRIF and SpgC1qRR; Table 1), and then it was used as the template to produce double-stranded RNA (dsRNA) with an in vitro T7 transcription kit (Takara, Dalian, China). EGFp dsRNA (dsEGFP) was also synthesized as the negative control with the primers listed in Table 1 (EGFPF1 and EGFPR). Mud crabs (~20 g each) were randomly divided into two groups. Each crab was injected with dsSpgC1qR (40 μg) or with the same amount of dsEGFP at the base of the fifth leg. At 24 h post-injection, gills were collected for RNA extraction, and RNAi efficiency was then determined by qRT-PCR with the total RNA. Experiments were performed independently for three times. For each test, at least four crabs were used for each group. Unpaired Student’s t-test was used to determine significant differences (**, P < 0.01).

2.7. Profiles of WSSV proliferation affected by RNAi of SpgC1qR or by pre-incubation with rSpgC1qR

Mud crabs (~20 g each) were randomly divided into two groups (15 crabs per group), and each crab was injected with dsRNA (dsSpgC1qR or dsEGFP) to knockdown the expression of SpgC1qR. After silencing SpgC1qR, each crab was injected with purified WSSV virions (2 × 10^9 copies). The dsEGFP-treated mud crabs were also injected with the same amount of WSSV serving as the control. Gills were sampled to extract genomic DNA for WSSV quantification at 0, 24, 48, and 72 h post-WSSV injection. The WSSV copies in gills at each time point were calculated according to a previously developed method [41]. The profile of WSSV proliferation affected by pre-incubation with rSpgC1qR was also investigated. Mud crabs (~100 g each) were randomly divided into two groups (25 crabs per group). Prepared WSSV virions (1 × 10^5 copies) were pre-incubated with rSpgC1qR (200 μg/mL), or with GST protein at room temperature for 30 min, and then injected into each crab in the corresponding groups. The gills were sampled at 0, 24, 48, 72, and 96 h post-injection to isolate genomic DNA for calculating the virus copies by qPCR through the same method. Unpaired Student’s t-test was used to determine significant differences (**, P < 0.01).

2.8. Recombinant expression and purification of SpgC1qR

Based on SpgC1qR cDNA sequence, a pair of primers, SpgC1qRIF and SpgC1qRR (Table 1), were designed to amplify the sequence encoding the mature peptide. After digestion with two restriction enzymes, the obtained DNA fragment was inserted into a pGEX-4T-1 expression vector. Subsequently, the recombinant vector was transformed into competent Escherichia coli BL21(DE3) cells for over-expression. After induction expression with isopropyl-β-d-thiogalactoside (IPTG) at 28 °C, the bacteria pellets were collected through centrifugation and were re-suspended in PBS containing 0.1% Triton X-100 for probe sonication lysis. The recombinant SpgC1qR (rSpgC1qR) and GST were respectively purified using glutathione Sepharose 4B chromatography following the instructions of the manufacturer. Protein concentration was determined by the Bradford method. The His-tagged VP28 of WSSV was overexpressed and purified with Ni-NTA His-Binding Resin by using the bacterial strains developed in our previous study.

2.9. GST pull-down assay

GST pull-down assay was performed according to a previously reported method with slight modifications [42]. A total of 150 μL of glutathione–Sepharose 4B resin (50% beads slurry) after washing with PBS was incubated with a mixture of GST-fused SpgC1qR (15 μg) and the recombinant viral protein VP28 (rVP28; 15 μg) for 2 h at room temperature. GST served as the control. After incubation, the samples were washed thoroughly with PBS, and then the proteins were eluted by adding PBS containing 10 mM reduced glutathione. The eluates were subjected to 12.5% SDS-PAGE and analyzed after staining the gel with Coomassie blue.

2.10. Ethical statement

Mud crabs used in this study are not protected or endangered species. Besides, no genetically modified organism used in the study. According to the national regulation (Fisheries Law of the People’s Republic of China), no permission was required to collect the animals and no formal ethics approval was required for this study.

3. Results

3.1. cDNA cloning of SpgC1qR

RACE-PCR analysis was used to obtain the full-length cDNA sequence of SpgC1qR in mud crab S. paramamosain. The complete sequence of 1204 bp possessed an open reading frame of 822 bp encoding a 273-amino-acid protein, a 5’ untranslated region (UTR) of 90 bp, and a 3’ UTR of 292 bp with a poly(A) tail. Fig. 1A shows the nucleotide and deduced amino acid sequences. No putative signal peptide was predicted, and a mitochondrial acidic matrix protein of 33 kDa (MAM33) domain (residues 84–270) was found in the deduced protein sequence. Using the MITOPROT program, a 59-amino-acid-long mitochondrial targeting sequence was found at the N-terminus of this deduced protein sequence. An RGD motif or cell adhesion sequence between mitochondrial targeting sequence and MAM33 domain was also found. The mature SpgC1qR protein had a theoretical Mw and pl of 23.7 kDa and 4.47, respectively.

3.2. Similarity, phylogenetic and 3D analyses

BLASTP search analysis revealed that SpgC1qR was highly identical (more than 60%) with most crustacean gC1qR homologs. For example, it shared 88% identity with Portunus trituberculatus PtgC1qR (accession number, AYV97197.1), 74% with Eriocheir sinensis EsgC1qR (ANN46490.1), 74% with Penaeus vannamei PvgC1qR (AFY05650.1), and 72% with Macrobrachium rosenbergii MrgC1qR (AJE28351.1). SpgC1qR also shared more than 40% identity with most of the other reported gC1qR homologues. In addition, the alignments of the representative crustacean gC1qRs showed that the amino acids in MAM33 domain were quite conserved (Fig. 2A). By contrast, the short amino acid sequences between mitochondrial targeting sequences and MAM33 domains were not very conserved, which vary greatly in length though they all had a common RGD motif (Fig. 1S). The high similarities among gC1qR homologues suggested that they might have similar biological functions. In the conserved RGDs and MAM33 domains, the conserved RGDs and MAM33 domains may be responsible for their similar functions.

A phylogenetic tree was constructed with SpgC1qR and other gC1qRs from both invertebrates and vertebrates to analyze the evolutionary relationship among them. The phylogenetic tree demonstrated the arthropod gC1qRs could form one big meaningful group with the high knot value of 99, and the vertebrate gC1qRs including fishes, tropical clawed frog, and mammals were clustered into another big meaningful group. Furthermore, in the arthropod cluster, the crustacean gC1qRs formed a unique subgroup with the knot value of 100. This phylogenetic tree indicated that SpgC1qR had a much closer evolutionary relationship with other crustacean gC1qRs than insect and vertebrate gC1qRs (Fig. 1B).

Similarity analysis showed that SpgC1qR MAM33 shared 32.8% identity with that of human gC1qR. Given that the identity between these two peptides was not very high, Phyre server was recommended to predict the 3D model. Fig. 2B shows that the predicted 3D model of SpgC1qR MAM33 comprised seven consecutive antiparallel β-strands.
flanked by one \( N\)-terminal and two \( C\)-terminal \( \alpha\)-helices (very short \( \beta\)-strand or \( \alpha\)-helices was ignored), which is similar to the 3D structures of human gC1qR MAM33. Besides, similar to the quaternary structure of human gC1qR MAM33, three monomers of Sp\( g\)C1qR MAM33 also form a trimer with a doughnut-shaped structure (Fig. 2 C). This finding suggests that Sp\( g\)C1qR may have similar biological activities with human gC1qR.

3.3. Sp\( g\)C1qR was highly expressed in ovary and upregulated after WSSV challenge

QRT-PCR was conducted to investigate tissue distribution of Sp\( g\)C1qR. Fig. 3 A shows that Sp\( g\)C1qR was expressed in all examined tissues and it showed the highest expression level in the ovary, followed by the hepatopancreas, heart, hemocytes, intestine and gills; the expression level of Sp\( g\)C1qR in the stomach or muscle was much lower than that in other tested tissues. Given that the gill is an important immune tissue and a target tissue for WSSV invasion, the time-course expression profile of Sp\( g\)C1qR in gills after WSSV challenge was further analyzed. We found that Sp\( g\)C1qR was significantly upregulated from 48 to 72 h post injection, and it reached the maximum level at 48 h post injection (Fig. 3 B). This result suggested that Sp\( g\)C1qR may participate in the antiviral immunity of mud crab.

3.4. Sp\( g\)C1qR knockdown facilitated WSSV proliferation

To clarify the potential antiviral activity exerted by Sp\( g\)C1qR on viral replication, mud crabs were initially injected with dsSp\( g\)C1qR to silence Sp\( g\)C1qR and then challenged with WSSV. As shown in Fig. 4 A, Sp\( g\)C1qR was successfully knocked down by injecting dsSp\( g\)C1qR because the expression level of this gene at 24 h after injection with dsSp\( g\)C1qR was only about 30% of that in the control crabs treated with dsEGFP. Subsequently, the proliferation profiles of WSSV in dsRNA-treated crabs were investigated by analyzing viral-genome copies (DNA virus) through qPCR. Results showed that the quantities of WSSV in the gills of Sp\( g\)C1qR-silenced crabs were much higher than those in control crabs at 48, and 72 h post-viral infection (Fig. 4 B). Collectively, these results clearly demonstrated that knockdown of Sp\( g\)C1qR significantly facilitated WSSV proliferation in vivo, suggesting that Sp\( g\)C1qR was a critical antiviral factor.

3.5. The mature peptide of Sp\( g\)C1qR was successfully expressed in a soluble form

To investigate the possible biological activity of Sp\( g\)C1qR, the mature peptide of Sp\( g\)C1qR was recombinantly expressed in E. coli. After the bacterial cells were lysed and centrifuged, rSp\( g\)C1qR was detected in the supernatants, indicating that it was expressed in a soluble form. Accordingly, the protein was further purified through affinity chromatography. The predicted MW of rSp\( g\)C1qR was 50.4 kDa, which approximately agreed with the size of the major band that appeared in the corresponding purified protein lane (Fig. 5 A). In addition, GST was expressed and purified in the same way, and used as the control (Fig. 5 B).

3.6. Sp\( g\)C1qR protein exhibited strong inhibitory activity on WSSV replication

To further verify the antiviral role of Sp\( g\)C1qR in the process of WSSV infection, the proliferation profile of WSSV in gills after the pre-incubation of viral particles with rSp\( g\)C1qR or GST protein was analyzed. Compared with WSSV particles pre-incubated with GST protein, the virus pre-incubated with rSp\( g\)C1qR dramatically inhibited WSSV proliferation at 48, 72, and 96 h post-injection because the pre-incubation of virus with rSp\( g\)C1qR resulted in much lower virus copies at 48, 72, and 96 h post-infection (Fig. 5 C). This result suggested that Sp\( g\)C1qR played an important role in the antiviral activity of mud crab.
SpgC1qR interacted specifically with the WSSV structural protein VP28

Considering that SpgC1qR suppressed WSSV proliferation, we speculated that this molecule may interact with certain components on WSSV particles. Thus, a GST pull-down assay was performed to test the binding ability of SpgC1qR to a major structural protein VP28 given that the latter is the most important envelope protein of WSSV mediating viral infection. Results showed that rSpgC1qR could specifically bind to rVP28, but GST protein did not have any apparent binding activity to this viral protein (Fig. 6A and B). This finding clearly demonstrated that SpgC1qR specifically interacted with VP28, implying that VP28 is a target component that could be recognized by SpgC1qR.

4. Discussion

Though human gC1qR is a multi-ligand binding protein with diverse biological functions [2], the biological functions of invertebrate gC1qR homologues remain largely unknown. In the present study, we found that SpgC1qR shared high identity and similar 3D structure with human gC1qR. The expression of SpgC1qR was significantly elevated after WSSV infection. Knockdown of SpgC1qR by RNAi facilitated viral proliferation in vivo; pre-incubating WSSV particles with rSpgC1qR dramatically suppressed viral replication. Moreover, SpgC1qR specifically bound to the viral envelope protein VP28. These findings revealed that SpgC1qR played a crucial role in anti-WSSV immune responses.

Though SpgC1qR was defined as a receptor protein for complement subcomponent C1q in this study, the fact that invertebrate animals lack the classical complement system of high animals indicates that SpgC1qR may have some extra biological functions different from mammalian gC1qRs. In the present study, we found that crustacean gC1qRs displayed a more distant evolutionary relationship with vertebrate gC1qRs, but these crustacean gC1qRs themselves shared very high similarities and had close evolutionary relationships. Considering that most reported crustacean gC1qRs possess similar antibacterial activities [14–17], we speculated that their similar antibacterial abilities might be due to their conserved sequences and closer evolutionary relationships among them. The distant evolutionary relationship of vertebrate gC1qRs
from crustacean ones suggested that the former might have evolved certain biological functions quite different from that of the latter. Herein, human gC1qR exhibiting diverse activities is a good example. Interestingly, we found that SpgC1qR responded to WSSV infection participating in antiviral immunity. We speculated that crustacean gC1qRs might have a similar antiviral function because crustacean gC1qRs shared high similarities with each other. This ability is also different from human gC1qR, which facilitates the infection of several kinds of viruses [7–9].

Human gC1qR exhibits a variety of biological functions by binding to different ligands. In addition to interacting with the globular ‘head’ domain of the plasma complement component C1q to activate complement system, human gC1qR interacts with viral proteins of certain viruses [7–9]. Through binding to the targeted viral components, human gC1qR promotes viral infection and maintains virus persistence. Besides, human gC1qR interacts with some bacterial components. It functions as a crucial immune component preventing bacterial infection by inhibiting hyaluronidase activity [13], or promotes bacterial infection by enhancing its binding ability to fibrinogen [11] or by facilitating the attachment of exosporium to host cells [12]. Considering that SpgC1qR had a distant evolutionary relationship from human gC1qR, it is hard to precisely predict the possible function of SpgC1qR. Thus, the possible biological function was examined in this study. We found that SpgC1qR specifically interacted with a viral envelope protein VP28. Different from human gC1qR promoting the infection of most viruses by binding to certain viral proteins, our study revealed that SpgC1qR suppressed WSSV proliferation. To verify this conclusion, we first showed that knockdown of SpgC1qR remarkably facilitated WSSV replication, and then demonstrated that pre-incubating WSSV particles with rSpgC1qR significantly suppressed viral proliferation. These findings suggested that SpgC1qR was an important antiviral immune molecule.

WSSV is one of the most devastating pathogens in crustacean aquaculture [19–21]. Although current researches revealed that some immune molecules play essential roles in anti-WSSV immune responses [27–33], the exact immune mechanisms of how the innate immune system of crustaceans defends against WSSV infection remain elusive. Envelope proteins play a crucial role in viral infection and are regarded as the first-line molecules to interact with the host. VP28, the most abundant envelope protein of WSSV, is involved in the systemic infection of shrimp by WSSV [26]. The structural study revealed that VP28...
was anchored to the outer surface of WSSV as a trimer through the N-terminal transmembrane regions, which makes its mushroom-like structure (trimer) protrude outside the envelope, and facilitates its interaction with certain components of host [43]. Besides, although human gC1qR is a mitochondrial matrix protein located predominantly in the mitochondrial matrix, this protein can be detected in the nucleus and/or cell surface under abnormal circumstances [4,44], such as microbial infection leading to cells lysis. Thus, the gC1qR on the cell surface can serve as a receptor for bacterial and viral proteins. Considering that gC1qR family members show relatively conserved overall structure, we speculated that SpgC1qR might possess similar basic biological characteristics to human gC1qR. This means that SpgC1qR may be present on the surface of cells when WSSV infection occurs, especially at the late stage of viral infection. Moreover, SpgC1qR has an RGD motif located outside the MAM domain of mature peptide, which is regarded as the ligand for a cell surface protein integrin. The interaction of the RGD motif with integrin would also enhance the binding of SpgC1qR to cell surface. This study showed SpgC1qR was highly expressed in the late stage of viral infection, which would lead to more SpgC1qR present on the surface of cells or in hemolymph to resist the invasion of WSSV particles. In addition, reports have shown that human gC1qR molecules are present in a form of doughnut-shaped structure [45]. Based on predicting 3D structure and superimposing three SpgC1qR monomers, we found that SpgC1qR also adopted a form of trimers with a doughnut-shaped structure, which had a central concave surface facilitating its interaction with the protruding structure of VP28. However, the detailed interaction mechanism of these two different trimers needs further study.

In conclusion, in this study, we identified a novel gC1qR homologue, SpgC1qR, and characterized its antiviral function and likely antiviral mechanism. A possible antiviral model mediated by the interaction of SpgC1qR with the major envelope protein VP28 was proposed. This study provided new insights for understanding the antiviral mechanism as well as the design and development of new antiviral drugs.

Declaration of Competing Interest

The authors declare no conflict of interest.
Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j/fsirep.2022.100052.

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