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Perspective

Analysis on the expression and function of syndecan in the Pacific white shrimp *Litopenaeus vannamei*

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ABSTRACT

Syndecan is considered to be a multifunctional protein which functions as a cell surface receptor involved in cell adhesion, migration, cytoskeleton organization and differentiation. Previous bioinformatic analysis has revealed that syndecan in shrimp might interact with white spot syndrome virus (WSSV). In the present study, we experimentally studied the function of syndecan in shrimp immunity. The syndecan from *Litopenaeus vannamei* (*LvSDC*) was cloned and analyzed. The full-length cDNA of *LvSDC* was 1005 bp, consisting of 59 bp 5′-UTR, 253 bp 3′-UTR, and 693 bp open reading frame encoding 230 amino acids. LvSDC consisted of an extracellular domain (ED), a transmembrane domain (TM) and a cytoplasmic domain (CD). TM and CD shared high similarities with those of syndecan proteins from other species. *LvSDC* was ubiquitously expressed in all tested tissues, with the highest level in Oka. After WSSV challenge, the transcription level of *LvSDC* in Oka was apparently up-regulated. Recombinant LvSDC protein and its rabbit polyclonal antibody were prepared for detecting the location of LvSDC in hemocytes using immunocytochemistry approach. Data showed that LvSDC mainly located at the cell membrane and the cytoplasm of hemocytes. After silencing of LvSDC with siRNA, the WSSV copy numbers and mortality of shrimp after WSSV infection were both significantly decreased. These data provide useful information for understanding the immune mechanism of shrimp to WSSV infection.

1. Introduction

The Pacific white shrimp *Litopenaeus vannamei* is one of the most important aquatic economic species in China. White spot syndrome virus (WSSV) is the most dangerous pathogen which results in high mortality of farmed Penaeid shrimp with a cumulative mortality up to 100% within 3–7 days (Liu et al., 2009). WSSV shows a wide tissue distribution in shrimp, which can infect almost all the vital organs of shrimp (Lo et al., 1997). Knowledge about the receptors of WSSV in shrimp would be very helpful for understanding the infection mechanism of WSSV and further developing strategy for virus disease control.

Some proteins located on the cell membrane in shrimp have been suggested to be potential receptors for WSSV infection through interacting with the envelope proteins of WSSV. The GTP-binding protein Rab7 in *Marsupenaeus japonicas* showed direct interaction with the envelope protein VP28 of WSSV (Sritunyalucksana et al., 2006). The beta-integrin from *M. japonicas* could directly interact with envelope protein VP187 of WSSV (Li et al., 2007). One chitin-binding protein (PmCBP) from *Penaeus monodon* was suggested to be a cellular receptor or co-receptor for WSSV through interaction with the envelope protein VP53A of WSSV (Chen et al., 2009). One C-type lectin from *Litopenaeus vannamei* was reported to have strong affinity to WSSV through interacting with several envelope proteins of WSSV (Zhao et al., 2009). Syndecan from *Fenneropenaeus chinensis* was predicted to have potential interaction with collagen-like protein of WSSV through bioinformatic analysis (Sun et al., 2014). Those reports showed that WSSV might have multiple receptors to mediate its infection to shrimp.

Syndecan, belonging to the type I transmembrane protein family, is considered to be a multifunctional protein which can function as cell surface receptor involved in adhesion, migration, cytoskeleton organization and differentiation (Carey, 1997; Rapraeger, 2001; Yoneda and Couchman, 2003). They can bind to a wide variety of soluble and insoluble extracellular molecules, such as extracellular matrix component, growth factors, cytokines, chemokines, to regulate the biological activity of the organisms (Park et al., 2000; Smith et al., 2006). The expression of syndecan can be enhanced during inflammatory response (Zhang et al., 1999; Zhou et al., 2003). Syndecan can mediate the interaction between bacteria and the

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epithelial cells, and its over-expression can increase the invasion of *Neisseria gonnorrhoeae* (Freissler et al., 2000). Some other pathogenic bacteria including *Listeria monocytogenes*, *Streptococcus pyogenes* and *Staphylococcal sp*., can utilize syndecan for their binding to the cell surface (Kim et al., 2004). Syndecan was also reported to be an attachment receptor for hepatitis E virus (HEV), papillomavirus and some other viruses (Bartlett and Park, 2010; Kalia et al., 2009; Spillmann, 2001). However, most knowledge comes from studies in mammalian. There is still no experimental evidence for the role of syndecan in virus infection to crustaceans.

In the present study, the full length cDNA of syndecan was isolated from Penaeid shrimp *L. vannamei*, and its function was analyzed. The data will help us to understand the role of syndecan in the innate immunity of shrimp.

2. Materials and methods

2.1. Animals and sample collection

2.1.1. Shrimp for cDNA cloning, tissue distribution and siRNA interference

Shrimp with an average weight of 25.1 ± 2.5 g were used for tissue dissection and WSSV infection experiment. Shrimp with an average weight of 2.08 ± 0.75 g were used for siRNA interference assay. Shrimp were obtained from a local shrimp farm and maintained in filtered sea water at temperature (25 °C) with continuous aeration in the fiberglass tanks. During the acclimation, the shrimp were fed with artificial food pellet three times a day and the water was changed daily. Then six shrimp were randomly selected to be tested by a WSSV-specific PCR amplification as described previously (Sun et al., 2013).

2.1.2. Tissue collection

For tissue distribution analysis, six healthy shrimp were randomly selected for tissue sampling, including hemocytes, intestine, hepatopancreas, gill, heart, muscle, nerve, lymphoid organ (Oka) and stomach. Hemolymph from six healthy shrimp was collected from the ventral sinus located at the first abdominal segment using a syringe with equal volume of anticoagulant-modified Alsever solution (Rodríguez et al., 1995). Hemocytes were isolated from hemolymph by centrifugation at 800 *g*, 4 °C, for 10 min and preserved in liquid nitrogen immediately for RNA extraction. All the tissue samples were stored at –80 °C immediately for RNA extraction used for gene expression analysis.

For WSSV challenge experiment, two groups including WSSV group and their control group (PBS) were set. In WSSV group, each shrimp was injected with 10 μl quantified WSSV (10^4 copies); in PBS group, each shrimp was injected with 10 μl PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) as control. The Oka tissues of 6 shrimp were collected from each group at 0, 0.5, 3, 6, 12, 24 and 48 h post injection.

2.2. Preparation of total RNA and cDNA synthesis

Total RNA from different tissues were extracted using RNAiso Reagent (Takara, Japan) following the manufacturer's protocol. The concentration, purity and integrity of RNA were detected by NanoDrop 1000 spectrophotometer (Thermo Fish Scientific Inc, USA) or electrophoresis on 1% agarose gel. Before cDNA synthesis, contaminant DNA was removed from the total RNA by RNase-free DNase (Promega, USA). The cDNA synthesis was performed at 37 °C for 1 h and 35 min with 1 μg RNA, 1× M-MLV buffer, 0.125 mM dNTP, 10 μM HEX random primers (Takara, Japan), 20 U RNasin, 200 U M-MLV (Takara, Japan) in a total volume of 25 μl.

2.3. Cloning and sequencing of LvSDC

The cDNA sequence of *LvSDC* was acquired from the transcriptome database of *L. vannamei* in our lab (Wei et al., 2014). One pair of specific primers SDC-F and SDC-R were designed to confirm the predicted cDNA sequence. PCR amplification was performed using the synthesized cDNA from Oka as the template: 1 cycle of 94 °C for 5 min, 35 cycles including denaturation at 94 °C for 40 s, annealing at 56 °C for 40 s, and extension at 72 °C for 40 s; followed by 1 cycle of 72 °C for 10 min. The PCR products were subcloned into plasmid vector pMD19-T simple (Takara, China) and transformed into competent *Escherichia coli* TOP10 cells (Tiangen, China) for sequencing. The sequences of all primers used in this section were shown in Table 1.

2.4. Bioinformatic analysis of LvSDC

The open reading frame (ORF) of *LvSDC* was identified by ORF Finder at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Translation of nucleotide sequence, isoelectric point and molecular weight prediction were carried out using the Translate software (http://web.expasy.org/translate/). Signal peptide was analyzed using the program SignalP (http://www.cbs.dtu.dk/services/SignalP/), and N-linked glycosylation sites were detected with the NetNGlyc 1.0 Server. The MegAlign program within DNASTAR was employed for multiple alignments. Phylogenetic tree was constructed on the basis of the deduced amino acid sequences using Neighbor-joining (NJ) within MEGA version 5.0 (Tamura et al., 2011) and 1000 bootstrap values. The secondary structure of SDC protein was predicted using the software from Swiss model server (http://swissmodel.expasy.org). Functional domains and protein binding sites of LvSDC were analyzed by Conserved Domains Database (CDD) search program (http://www.ncbi.nlm.nih.gov/cdd).

2.5. Quantitative real-time PCR

Analysis on the temporal and spatial expression of *LvSDC* were performed on an Eppendorf Mastercycler® ep realplex real-time PCR System (Eppendorf, Germany). 18S rRNA was used as reference gene which was described previously (Chi et al., 2013). Specific PCR primers designed for real time PCR detection were also shown in Table 1. The expected fragments of LvSDC and 18S rRNA gene were 182 bp and 166 bp in length, respectively. The effectiveness of each pair of primers was analyzed following the method described by Freeman et al. (1999). The PCR programs of 18S rRNA were as follows: 94 °C for 5 min, 40 cycles of 94 °C for 15 s, 56 °C for 20 s and 72 °C for 20 s. Melting curves analysis was performed over a

Table 1
Information of primers used for gene cloning and real time PCR.

Primers	Sequences (5′–3′)
LvSDC-RT-F	CGACAAGGAGGTCTATGCC
LvSDC-RT-R	GAGCGAGGGGAAAGAGAAC
SDC-F	ACGACGACACAGAAGACGACTAC
SDC-R	CAACTTTGAAGGAGGAATGGGA
SDCexpF	GCCGAATTCCACAAGGTTTCAGCAGACT
SDCexpR	CCGCTCGAGGACCTCCTTGTCGTTTGGAG
18S-F	TATACGCTAGTGGAGCTGGAA
18S-R	GGGGAGGTAGTGACGAAAAAT
VP28-F	AAACCTCCGCATTCCTGTGA
VP28-R	TCCGCATCTTCTTCCTTCAT
siRNA-1	GCAUUGGAGUCUUGGACAUTT
siRNA-2	GCGGUCCUUACGAUACUUATT
siRNA-3	GCAUCUUGGCAGCAUUUAUTT
NC	UUCUCCGAACGUGUCACGUTT