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An *Ns1abp-like* gene promotes white spot syndrome virus infection by interacting with the viral envelope protein VP28 in red claw crayfish *Cherax quadricarinatus*

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ABSTRACT

Influenza A virus non-structural-1A binding protein (named as Ns1abp) was originally identified as a host protein from human that bound to the viral NS-1 protein. In our previous study, the expression of an *Ns1abp-like* gene (denoted as *CqNs1abp-like* gene) was found to be up-regulated in a transcriptome library from the haematopoietic tissue (Hpt) cells of red claw crayfish *Cherax quadricarinatus* post white spot syndrome virus (WSSV) infection. To elucidate the role of *CqNs1abp-like* gene involved in WSSV infection, we cloned the *CqNs1abp-like* gene in which the open reading frame was 2232 bp, encoding 743 amino acids with two typical domains of one BTB (Broad-Complex, Tramtrack and Bric a brac) domain at N-terminal and six Kelch domains at C-terminal. The gene expression profile showed that the mRNA transcript of *CqNs1abp-like* gene was widely expressed in all the tested tissues with highest expression in nerve, relatively high expression in Hpt and lowest expression in eyestalk. Importantly, both the WSSV entry and the viral replication were significantly reduced in Hpt cells after gene silencing of *CqNs1abp-like* gene. By using protein pull-down assay, we found that the recombinant BTB domain, six Kelch domains and CqNs1abp-like intact protein were all bound to the WSSV envelope protein VP28, respectively, in which the BTB domain showed slightly less binding affinity than that of the six Kelch domains or the recombinant intact protein. Besides, the WSSV entry into Hpt cells was clearly decreased when the virus was pre-incubated with the recombinant BTB domain, six Kelch domains, or the recombinant CqNs1abp-like intact protein, respectively, suggesting that the *CqNs1abp-like* gene was likely to function as a putative recognition molecular towards WSSV infection in a crustacean *C. quadricarinatus*. Taken together, these data shed new light on the mechanism of WSSV infection and a putatively novel target on anti-WSSV infection in crustacean farming.

1. Introduction

White spot disease is one of the most common and damaging viral disease in shrimp aquaculture, which is caused by the white spot syndrome virus. WSSV is a double-stranded DNA virus (about 300 kb) with a large envelope (about 70–167 nm × 210–380 nm) (Escobedo-Bonilla et al., 2008). WSSV shows a rod- or elliptical-shaped like virus with a unique tail-like extension at one end by transmission electron microscopy analysis. A rod-shaped nucleocapsid is found inside the envelope. This virus has a very broad host range and strong pathogenicity among crustaceans, including shrimp, crab, lobster and freshwater crayfish (Jiravanichpaisal et al., 2001; Sundar Raj et al., 2012). The farmed shrimp infected with WSSV will usually die within 3–10 days (Wang et al., 2008). Since its first identification in 1993, WSSV has caused serious economic losses. Therefore, it is important to illuminate the mechanism of WSSV infection. Due to the lack of shrimp cell lines for the WSSV infection mechanism research, limited information has been uncovered from current understanding of the pathogenesis of WSSV. It is known that the haemocyte derives from the hematopoietic

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tissue (Hpt) and is finally released into the blood circulation in crayfish. Crayfish Hpt cell cultures has been well described by Söderhäll et al. (Jiravanichpaisal et al., 2006; Söderhäll et al., 2003), in which the Hpt cells are simple to be isolated and cultured in vitro, so it has been used as a good cell culture model for studying the process of WSSV infection (Huang et al., 2015; Jeswin et al., 2016; Söderhäll, 2013). Therefore, research on WSSV infection in Hpt cells will likely shed new light on the pathogenesis of this lethal virus in crustacean aquaculture.

Influenza A virus non-structural-1A binding protein (Ns1abp, also known as NS1-BP, Nd1 or KLHL39) was first identified from humans that interacted with the nonstructural NS1 protein of the influenza A virus (Wolff et al., 1998). The Ns1abp protein contains an N-terminal BTB (Broad-Complex, Tramtrack and Bric a brac) or POZ (poxvirus and zinc finger) domain and five Kelch-like tandem repeat elements of ~50 amino acids in Hela cells. In humans, the Kelch-repeat domain is the most prevalent substrate-binding domain and more than 95 BTB-Kelch family proteins have been found in human genome. However, it has not been well explored in crustacean. A recent study revealed that Ns1abp participated in regulating the alternative splicing of influenza A virus M1 mRNA to yield M2 mRNA by its interacting partner hnRNP K. M1 mRNA segments generate matrix protein M1 and ion channel protein M2 which are involved in virus trafficking, release and budding. Thereby Ns1abp plays an important role in influenza A viral gene expression during viral infection (Tsai et al., 2013). In consistent with its role in mRNA splicing, Ns1abp is localized in the nucleus and co-localizes with splicesome assembly factor SC35. The co-localization can be destroyed during viral infection and it then re-localizes throughout the nucleoplasm (Wolff et al., 1998). While in *Drosophila* and NIH3T3 cells, Ns1abp was found to be co-localized and physically associated with actin in the cytoplasm via its Kelch domain (Adams et al., 2000; Sasagawa et al., 2002). It has been suggested that Ns1abp functions as a stabilizer of actin filaments by binding to actin protein and may play a role in the dynamic organization of the actin cytoskeleton. However, it is unclear where it localizes during WSSV infection in red claw crayfish.

By proteomic analysis, VP28 has been identified as one of the major envelope proteins of WSSV, and it can interact with other viral structural proteins like VP26 and VP24 of WSSV (Xie et al., 2006). In our previous study, we also found that VP28 plays an important role in the entry of WSSV into host cells (Chen et al., 2016). Besides, VP28 can be recognized by several host cell proteins, such as GABARAP and Laminin receptor in a crustacean red claw crayfish *Cherax quadricarinatus* (Chen et al., 2016; Liu et al., 2018). Hence, the relative quantification of VP28 can be regarded as a good indication of WSSV infection and we then chose this gene/protein for the indication of WSSV infection in our present study. Previously, we found that the transcript of *CqNs1abp-like* gene was responsive to WSSV infection in the Hpt cells from red claw crayfish. Whereas, whether *CqNs1abp-like* gene affected the WSSV entry and further the viral replication in crustacean was not clear. To further investigate the contribution of *Ns1abp-like* gene in WSSV infection in a crustacean, we then cloned the full-length open reading frame (ORF) of *CqNs1abp-like* from red claw crayfish *C. quadricarinatus*, and determined its gene expression profile and potential functions involved in WSSV infection. Importantly, WSSV entry and replication was significantly reduced by gene silencing of *Ns1abp-like* gene. The CqNs1abp-like protein-WSSV interaction assay indicated that *CqNs1abp-like* protein was likely to act as an interacting partner, which bound to one of the WSSV envelope proteins VP28 and thus promoted the WSSV infection in red claw crayfish Hpt cells.

2. Material and methods

2.1. Animals and tissue collection

Healthy red claw crayfish, *C. quadricarinatus* were purchased from Source Sentai Agricultural Science and Technology Co., Ltd of Zhangzhou, Fujian Province, China, and acclimatized in aerated freshwater at 26 °C for at least one week before experiments. After that, crayfish tissues were collected. Haemocyte was obtained with a sterile steel needle (16#) from the animal's abdomen and mixed with anticoagulant solution (NaCl 510 mM; glucose 100 mM; citric acid 200 mM; Na-citrate 30 mM; EDTA-2Na 10 mM; pH 7.3) (1:1) on the ice followed by centrifugation at 1000×*g* for 10 min at 4 °C. Hpt cells were prepared from Hpt of *C. quadricarinatus* and cultured as described by Söderhäll et al. and Liu et al. (Liu et al., 2011; Söderhäll et al., 2003). Other tissues (stomach, gonad, muscle, nerve, intestine, heart, hepatopancreas, gill, epithelium and eyestalk) were sampled from three random individuals for total RNA isolation.

2.2. Total RNA extraction and first strand cDNA synthesis

Total RNA from tissues as described above was isolated using Trizol reagent (Roche, Mannheim, Germany) according to the manufacturer's instructions. The extracted RNA was evaluated with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) and analyzed by 1.0% agarose gel electrophoresis. RNA was reversely transcribed to cDNA by PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, code No. RR047A) following the manufacturer's instructions.

2.3. Gene cloning of the full-length ORF sequence of CqNs1abp-like gene

Two partial *CqNs1abp-like* gene cDNA sequences of *C. quadricarinatus* were isolated from a transcriptome library of Hpt cells post WSSV infection in our lab (unpublished data). These two sequences contained the 5′-end part with the initial codon "ATG" and the following 687 base pairs, and the 3′-end part with the stop codon "TAA" and the upstream of 1104 base pairs according to the BLAST result of NCBI web site, but which was lacking of the middle part of full-length of ORF sequence. PCR primers (Ns1abp-F/Ns1abp-R, Table 1) were then designed to confirm these two partial sequences and fix gap sequence of *CqNs1abp-like* gene by using the Hpt cell cDNA as template. The ORF of *CqNs1abp-like* gene cDNA (primers: Fl- Ns1abp-F/Fl- Ns1abp-R) was obtained by combination of these two parts together and the PCR amplification conditions were as follows: 3 min at 94 °C; 30 cycles of 94 °C for 30 s, 65 °C for 30 s (decrease 0.3 °C per cycle) and 72 °C for 2 min; and 72 °C for 5 min. All PCR products were gel-purified using a Gel Extraction Kit (Sangon Biotech, Co., Ltd., Shanghai, China), and the expected DNA fragments were ligated into a pMD18-T vector (TaKaRa). The vectors were transformed into *E. coli* DH5α cells. Positive clones containing inserts of an expected size were sequenced at Xiamen Borui Biotech Company, China.

2.4. Bioinformatics analysis

The similarity analysis of *CqNs1abp-like* gene sequence was conducted using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi/), and the domain architecture prediction of the proteins was performed with SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de). The 3D structure of CqNs1abp-like protein was constructed by using SWISS-MODEL server (http://swissmodel.expasy.org/).