Virology 419 (2011) 84–89

Contents lists available at SciVerse ScienceDirect

Virology

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

An immediate-early protein of white spot syndrome virus modulates the phosphorylation of focal adhesion kinase of shrimp

Huasong Lu, Lingwei Ruan, Xun Xu *

Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration (SOA), No. 178 Daxue Road, Xiamen 361005, PR China

ARTICLE INFO

Article history:
Received 2 June 2011
Returned to author for revision 22 June 2011
Accepted 29 July 2011
Available online 9 September 2011

Keywords:
Shrimp
WSSV
WSV083
Focal adhesion kinase
Phosphorylation

ABSTRACT

WSSV interacts with integrin during infection of shrimps and modulate the focal adhesion kinase which is known as a regulator of several downstream signaling pathways. Viral protein kinases are thought to be important for virus infection by regulating the host signaling pathways. WSV083 is an immediate-early gene of white spot syndrome virus that contains a Ser/Thr protein kinase domain. So, does WSSV modulate FAK phosphorylation via the WSV083 molecule? In this study, co-transfection of WSV083 and MjFAK genes proceeded in insect cells revealed that the MjFAK phosphorylation and cell adhesion activity could be inhibited by the expression of WSV083. Kinase domain mutants of WSV083 lost its ability of inhibiting FAK phosphorylation. Moreover, silencing of FAK gene through RNAi accelerated the shrimp death rate upon WSSV challenge. These results demonstrate for the first time that modulation of FAK phosphorylation by WSV083 plays a critical role in the pathogenesis of WSSV infection.

Introduction

As an important pathogen of the shrimp aquaculture, white spot syndrome virus (WSSV) has caused serious economic losses over the world (Lighner and Redman, 1998; Bachere, 2000). Although this virus was intensively studied in the last decades, basic mechanism of its infection remains unclear. Therefore, the investigation on the Host/Pathogen relationships will provide deep insights into the pathogenesis caused by WSSV and promote our understanding of the virus control.

For survival and successful infection, viruses have evolved to sneak through the antiviral response and hijack the host system, but little is known about the mechanism (Chen and Gerlier, 2006). Virus encoded protein kinases have been reported in many previous studies, such as HSV UL13, VZV ORF47, EBV BGLF4 and KSHV ORF36, etc. (Smith and Smith, 1989). These proteins usually function through phosphorylating hosts and virus proteins to regulate cellular processes, including transcription, translation, protein degradation, and etc. (Edelman et al., 1987). Like other dsDNA virus, WSSV transcribes its genes in a temporal manner, which is classified into three stages: immediate-early (IE), early and late. The IE gene expression depends on the host cell machinery and occurs independently of any viral de novo protein (Liu et al., 2005). To date, *pk* gene and one of WSSV IE gene WSV083 were identified as WSSV-encoded protein kinases (Liu et al., 2001; Li et al., 2009). However, the functions of these kinase genes remain unclear.

Focal adhesion kinase (FAK) is a focal adhesion-associated protein involved in cellular adhesion and spreading processes. It functions as both a scaffold and a tyrosine kinase in cells and has been demonstrated to play a pivotal role in signaling pathways. In mammalian, several viruses have been identified to regulate FAK activity, serving for their entry or inducing apoptosis (Cicala et al., 1999; Cicala et al., 2000). We have previously demonstrated that the phosphorylation of *Marsupenaeus japonicus* FAK (designated as MjFAK) was changed during WSSV infection (Zhang et al., 2009), which implied that WSSV might have the ability of regulating FAK phosphorylation.

In this report, transient transfection experiments in High Five cells were used to study the relationship between WSV083 and MjFAK. RNAi silencing of FAK in shrimp was also performed to monitor the function of FAK during WSSV infection. Our results revealed that FAK plays an intimate role in the shrimp immune system and WSV083 may contribute to the virus pathogenicity by modulating the phosphorylation of FAK. These results could facilitate our overall understanding of molecular mechanism between virus and host interaction.

Results

Expression of MjFAK and WSV083i in High Five cells

High Five cells were transiently transfected with pIZ/V5-FAK; and cells transfected with pIZ/V5-His were served as control. Strong signals (Fig. 1, lanes 2, 4, 6 and 8) were detected when cells were transfected with pIZ/V5-FAK, while no signals were detected in the control group (Fig. 1, lanes 1, 3, 5 and 7). These experiments suggested that anti-FAK (phosphoY397) antibody and anti-FAK (phosphoY577) antibody could

* Corresponding author at: Third Institute of Oceanography, No. 178 Daxue Road, Xiamen, Fujian, PR China (361005). Fax: +86 592 2195296.
E-mail address: xmxuxun@yahoo.cn (X. Xu).

0042-6822/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.07.021

specially recognize shrimp FAK but not the endogenous FAK in High Five cells.

Meanwhile, western blotting analysis was employed to identify the expression of recombinant WSV083 in High Five cells. A V5 tag was fused to the C-terminal of WSV083; the result showed anti-V5 antibody could detect the fusion protein of WSV083 with molecular mass about 72 kDa (Fig. 2A, lane 2).

Inhibition of MjFAK tyrosine phosphorylation by WSV083 in vitro

To explore the function of WSV083, we analyzed the association between MjFAK and WSV083 in High Five cells. The pIZ/V5-FAK was co-transfected with pIZ/V5-WSV083, and pIZ/V5-FAK co-transfected with pIZ-GFP or pIZ/V5-His was served as control. Forty-eight hours after transfection, cells were harvested for the western blotting analysis. The results showed that the expression of WSV083 lead to a significant decrease in the tyrosine phosphorylation of MjFAK (Fig. 3A). Since WSV083 has a Ser/Thr kinase domain at C-terminal, further experiments were conducted to determine whether this domain was necessary for WSV083. Kinase domain deletion mutant vector pIZ/V5-WSV083-DM and kinase domain point mutant vector pIZ/V5-WSV083-PM were co-transfected with pIZ/V5-FAK, respectively. The western blotting analysis showed that both mutants lost the ability to inhibit FAK phosphorylation (Fig. 3B); which implied the structure, especially the kinase domain of WSV083 was important for its function. Moreover, the inhibition of MjFAK by WSV083 was further analyzed with immunofluorescence assay. As shown in Fig. 4, the signal of the cells that were co-transfected with pIZ/V5-FAK and pIZ/V5-WSV083 was extremely lower than other groups. Therefore, both the western blot analysis and immunofluorescence assay suggested that WSV083 could down-regulate the tyrosine phosphorylation of MjFAK.

Cell adhesion activity assay

Previous study has shown the relationship between the MjFAK phosphorylation and cell adhesion (Zhang et al., 2009), a process where FAK is intimately involved. Thus, we next utilized the cell adhesion assay to detect whether cell adhesion activity is inhibited upon WSV083 expression. Cells transfected with WSV083, pIZ/V5-His, WSV083 kinase mutants or none were applied to cell adhesion assay. As shown in Fig. 5, the adhesion of cells transfected with pIZ/V5-WSV083 was significantly decreased compared to other groups, which was consistent with the phosphorylation analysis. As FAK is involved in cellular adhesion process, so it is supposed that the inhibition of FAK phosphorylation by WSV083 also resulted in the decrease of cell adhesion.

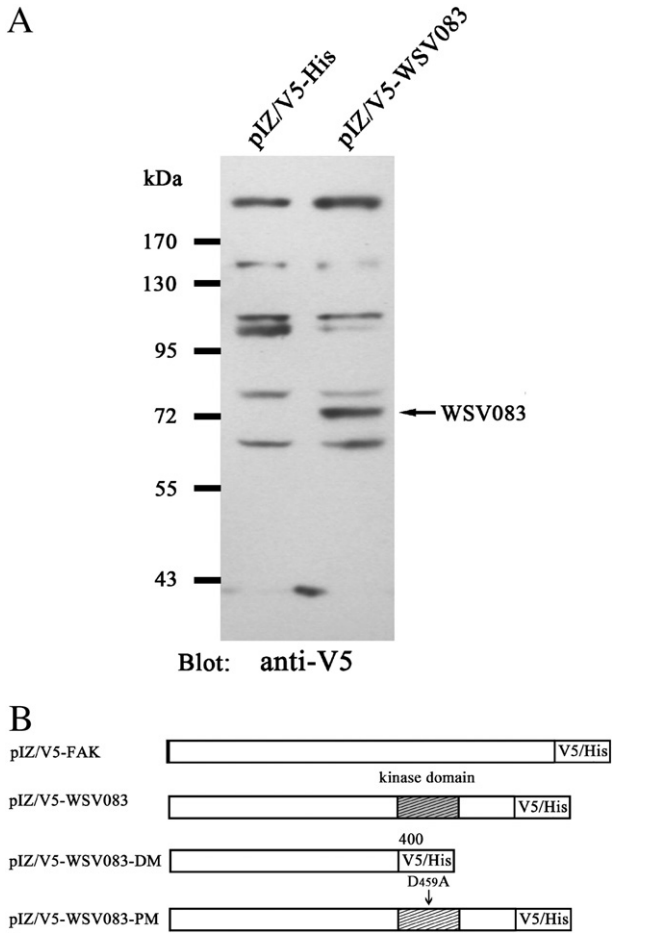

Fig. 2. Expression of WSV083. (A) Western blot analysis of High Five cells transfected with pIZ/V5-WSV083 using anti-V5 antibody. Cell lyses were harvested from High Five cells transfected with either pIZ/V5-His (lane 1) or pIZ/V5-WSV083 (lane 2). The arrows indicate the bands of WSV083. Standard molecular masses are presented in kilodaltons on the left. (B) Schematic diagram of the expression vectors used in transfection.

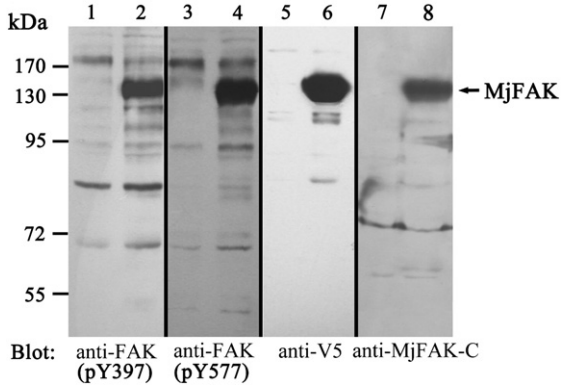

Fig. 1. Western blot analysis of MjFAK. Cell lysates were harvested from High Five cells transfected with either pIZ/V5-His (lanes 1, 3, 5 and 7) or pIZ/V5-FAK (lanes 2, 4, 6 and 8). Western blot analysis of the cell lyses using anti-FAK (phosphoY397) antibody (lanes 1 and 2), anti-FAK (phosphoY577) antibody (lanes 3 and 4), anti-V5 antibody (lanes 5 and 6) or anti-MjFAK-C antibody (lane 7 and 8). The arrows indicate the immunoreactive bands of MjFAK and its phosphorylated forms. Standard molecular masses are presented in kilodaltons on the left.

Silencing of FAK gene and its effects on WSSV challenge

We further tested whether MjFAK plays any role during WSSV invasion with RNAi depletion experiments. We first analyzed the effectiveness of RNAi directed against FAK. As shown in Fig. 6A, the shrimps injected with FAK dsRNA showed a decrease in mRNA levels of FAK at 48 h after the first injection, while the transcription of FAK was not changed in the GFP dsRNA-injected shrimps. Western blot analysis also showed that the expression of FAK was silenced (Fig. 6B).

Four groups of shrimps were utilized to investigate the impact of FAK gene on WSSV infection in vivo. In the WSSV challenge test groups, including dsRNA silencing for FAK or GFP, all shrimps reached 100% mortality by 7 days post-challenge (Fig. 6C). The mean time of death for the FAK dsRNA group (2.26 ± 0.64 days) was significantly shorter ($p < 0.05$) than the GFP dsRNA control group (3.20 ± 0.93 days). Meanwhile, the mortality of the former group was higher than the latter one especially at the early stage of virus infection (<3 days). These results indicated that silencing of FAK might promote the WSSV virulence and shrimp death upon virus infection.

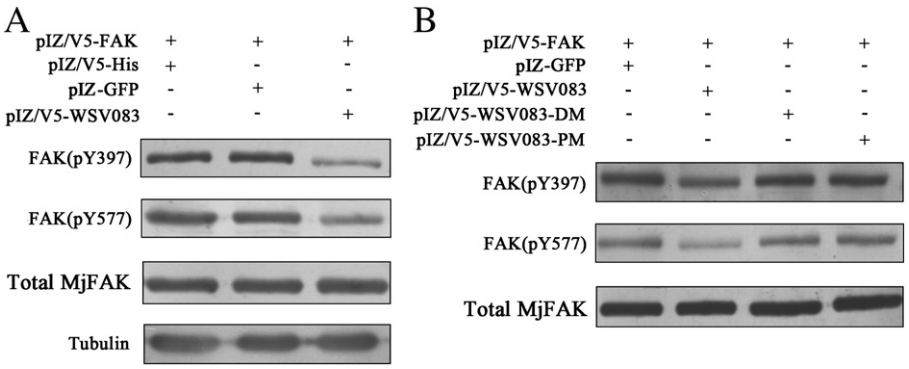

Fig. 3. Analysis of MjFAK tyrosine phosphorylation affected by WSV083. (A) Inhibition of tyrosine phosphorylation of MjFAK by WSV083. High Five cells were transfected with pIZ/V5-FAK in combination with pIZ/V5-WSV083, pIZ-GFP, or pIZ/V5-His, respectively. Western blot analysis of the cell lyses with anti-FAK (phosphoY397) antibody, anti-FAK (phosphoY577) antibody, anti-V5 antibody or anti-Tubulin antibody. (B) WSV083 mutants disable to inhibit FAK phosphorylation. Cells were transfected with pIZ/V5-FAK in combination with pIZ/V5-WSV083, pIZ-GFP, pIZ/V5-WSV083-DM or pIZ/V5-WSV083-PM, respectively. Western blot analysis of the cell lyses with anti-FAK (phosphoY397) antibody, anti-FAK (phosphoY577) antibody or anti-V5 antibody.

Discussion

Viral protein kinases have been suggested to play important roles in regulating a wide variety of viral infections (Kawaguchi et al., 1999; Izumiya et al., 2007; Hamza et al., 2004; Park et al., 2007). WSSV also encodes protein kinases: *pk* gene was the first identified WSSV-encoded protein kinase (Liu et al., 2001); one of WSSV IE genes WSV083 was predicted as a protein Ser/Thr kinase (Li et al., 2009). However, due to

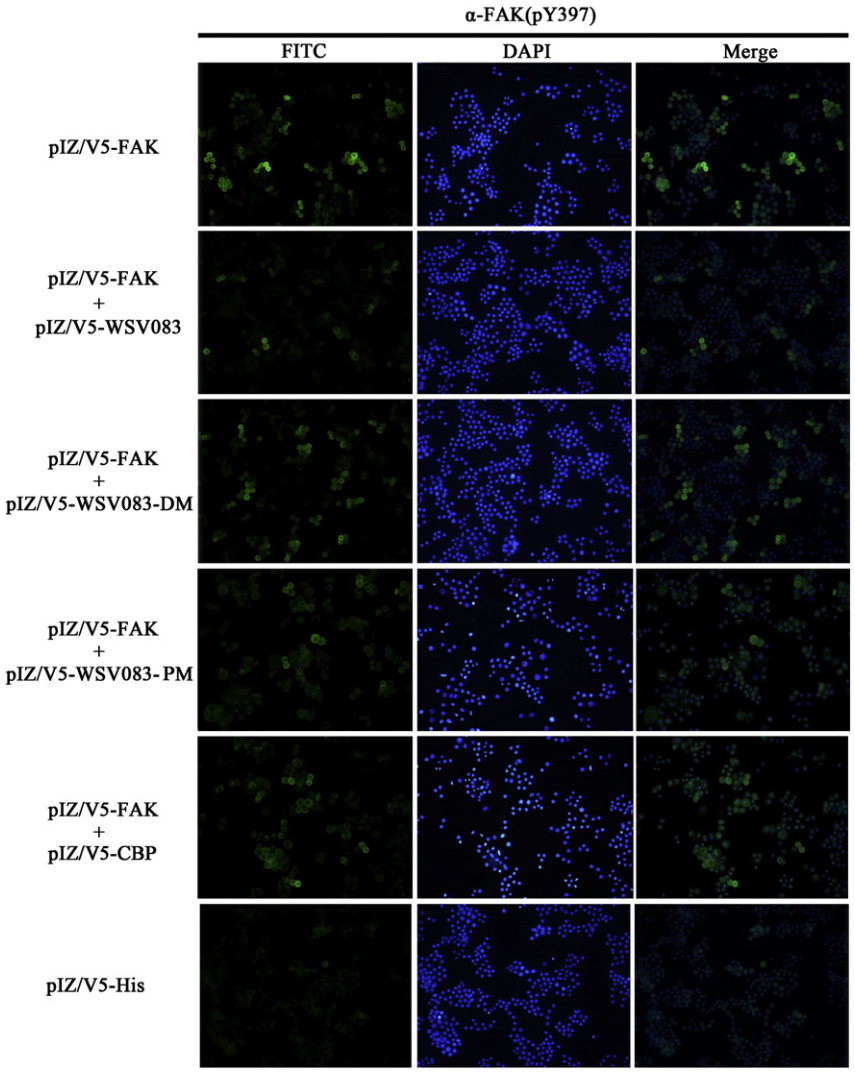

Fig. 4. Immunofluorescence assay of MjFAK phosphorylation. Cells were transfected with pIZ/V5-FAK alone or in combination with pIZ/V5-WSV083, pIZ/V5-WSV083-DM, pIZ/V5-WSV083-PM, pIZ/V5-CBP. After 48 h of transfection, anti-FAK (phosphoY397) antibody was used to detect the signal of FAK phosphorylation. Cells transfected with pIZ/V5-His were used as a negative control.

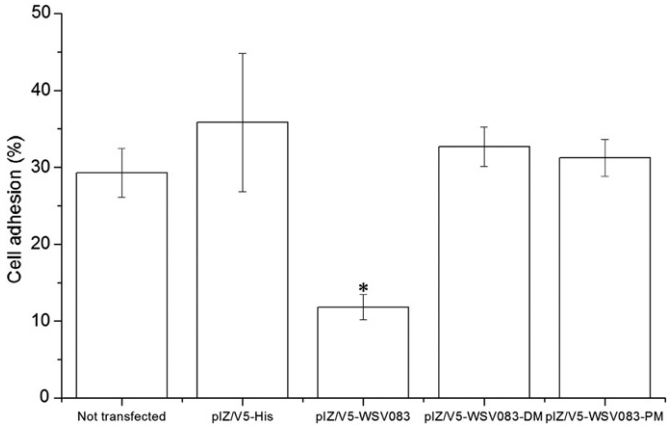

Fig. 5. Analysis of cell adhesion activity. Cells were transfected with pIZ/V5-WSV083, pIZ/V5-His, pIZ/V5-WSV083-PM, pIZ/V5-WSV083-DM, the cells that didn't transfect with plasmids were used as a control. After 48 h of transfection, cells were subjected to cell adhesion assay. The wells without coating and blocking were normalized to 100%. The relative percents were calculated as adhesion activity.

the lack of suitable cell line from shrimp, the functions of these viral kinase genes remain unknown to date. Our previous study found that shrimp FAK was involved in WSSV infection, and its activity was diminished at the late stage. All those prompt us to explore whether WSV083 could affect FAK activity and modulate its response to virus infection.

Recently, several studies have shown that the insect cell line can be used in the shrimp research (Yan et al., 2010; Zhi et al., 2010). In this study, transient transfection experiments were performed in High Five cells. The western blot and immunofluorescence assay indicated

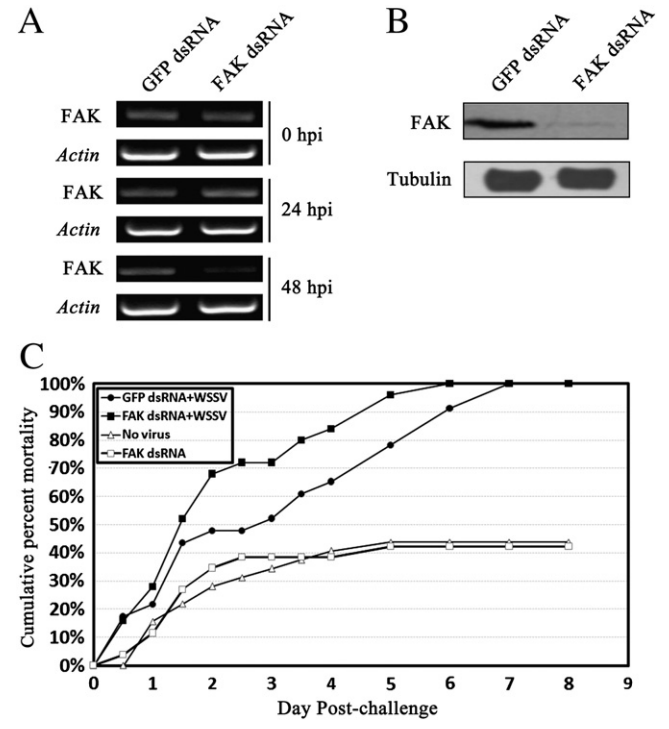

Fig. 6. Gene silencing of shrimp FAK promote WSSV virulence. (A) The shrimp were injected with either 10 μg of FAK dsRNA or GFP dsRNA. At different hours post injections, RT-PCR was performed to assay mRNA level of FAK and reference gene *Actin* using total RNA from the shrimp hemocytes. PCR products were run on a 2% agarose gel and stained with ethidium bromide. (B) Protein level of FAK in hemocytes was detected at 72 h post injection by Western blot. (C) Cumulative percent mortality of shrimp challenged with WSSV. Shrimps were pre-injected with FAK dsRNA or GFP dsRNA prior WSSV challenge. Control groups were treated with PBS or dsRNA FAK only. After WSSV injection, mortality was recorded over a period of 8 days.

that WSV083 could decrease MjFAK activity by suppressing its tyrosine phosphorylation. In mammalian, the phosphorylation of FAK could be regulated by many other signaling molecules because it always functions as a versatile protein in the cell signal network. Previous work has demonstrated that increased FAK phosphorylation at Ser-843 repressed its phosphorylation at Tyr-397 (Jacamo et al., 2007), suggesting a mechanism of cross-talk between different FAK phosphorylation sites. As WSV083 is a Ser/Thr protein kinase, one can speculate that WSV083 may use similar strategy to inhibit FAK tyrosine phosphorylation by enhancing its serine phosphorylation.

For invertebrates, cell adhesion and spreading, the processes in which FAK is involved, are critical for the function of their immune system. Our findings showed that the adhesion of cells transfected with pIZ/V5-WSV083 was significantly decreased which correlated with their inhibition of FAK activity in vivo. This result implied WSSV may utilize WSV083 to affect the host immune system. So, it is reasonable to speculate that after virus dominates in host cell, the virus encoded protein WSV083 could inhibit cell adhesion and related kinase activity to evade the host immune response.

To investigate the biological role of WSV083 in inhibiting FAK phosphorylation, RNAi technology using FAK dsRNA was employed to make sure whether knockdown of FAK gene would result in the enhancement of WSSV virulence. Our experiments demonstrated that silencing of FAK caused accelerated mortality from WSSV challenge, which supports the hypothesis that WSV083 can promote the viral pathogenicity by inhibiting FAK activity.

In summary, our findings demonstrated that FAK was involved in WSSV infection and its activity was inhibited by the viral protein kinase WSV083 to enhance the virus virulence. However, because of lack of cell line and transfection system in shrimp, the infection mechanism is still unclear. The present work provides us a further understanding on how viruses manipulating host biological processes and also provides some important insights into new approaches to control shrimp diseases.

Materials and methods

Shrimp culture and WSSV virions

M. japonicus (Crustacea, Decapoda) were purchased from a supermarket in Xiamen, China, and stocked individually in tanks (80 l) filled with air-pumped sea water. The virus was proliferated in crayfish *Procambarus clarkii*. Intact WSSV virions were purified as described previously (Xie et al., 2005).

Antibodies

Anti-FAK (phosphoY397) and anti-FAK (phosphoY577) polyclonal rabbit antibodies were purchased from Abcam Inc. Anti-α-tubulin antibody was purchased from Sigma USA. Monoclonal rabbit anti-V5 antibody was purchased from Millipore Inc. Polyclonal mouse anti-MjFAK-C antibody was generated by Mingchang Zhang (The Third Institute of Oceanography, Xiamen, China).

Cell culture and transfection

High Five cells (Invitrogen) were grown in SFX medium (Hyclone) at 27 °C. For DNA transfection, cells were seeded overnight, and then plasmids were transfected by using CellfectinII (Invitrogen) according to the manufacturer's instructions. For immunofluorescence assay, plasmids were transfected to High Five cells on coverslips in each well.

Plasmids construction

The pIZ/V5-His vector (Invitrogen) for expression in High Five cells was used in the cloning of MjFAK and WSV083. *M. japonicus*

hepatopancreas and hemocyte mixed cDNA library constructed into pAD-GAL4-2.1 phagemids was taken as the template for MjFAK cloning. For western blot analysis, a V5 tag was introduced to the C-terminal of MjFAK by PCR primers: MjFAKF and MjFAKR (Table 1). The PCR product was digested with *Kpn*I and ligated to *Kpn*I digested pIZ/V5-His expression vector (designated as pIZ/V5-FAK). Expression vector WSV083, designated as pIZ/V5-WSV083 was constructed with primers WSV083F and WSV083R (listed in Table 1). Expression vector pIZ/V5-WSV083-DM, which has a kinase domain deletion from aa 401 to 581, was constructed with primers WSV083F and WSV083DM. Point mutation expression vector pIZ/V5-WSV083-PM had a site mutant at position 459 (a putative ATP binding site). Primers WSV083F and WSV083PMR, WSV083PMF and WSV083R were used to amplify two fragments of WSV083 separated at position 459 (g and c, mutation site), then these two fragments were taken as templates for the second round PCR using the primers WSV083F and wsv083R. The PCR products were finally digested with *Kpn*I and *Xho*I and inserted into pIZ/V5-His plasmid to form WSV083PM construct. A schematic diagram of the expression vectors is shown in Fig. 2C. The Chitin-binding protein expression vector pIZ/V5-CBP presented from X.F. Yan was used as control (The Third Institute of Oceanography, Xiamen, China). The sequences of all vectors were confirmed by DNA sequencing.

Western blot analysis

At 48 h post transfection, High Five cells were harvested and washed with PBS twice prior lysis. For western blot analysis, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris–HCl [pH 7.4],150 mM NaCl, 1% Triton X-100,1%sodium deoxycholate, 0.1% SDS, 1 mM EDTA; added with 1 mM PMSF, 1×PIC, 25 mM glycerol phosphate, 1 mM Na_3VO_4 and 10 mM NaF) for 30 min at 4 °C. Then the supernatant was vortexed with SDS-PAGE loading buffer and analyzed on 8% SDS-PAGE gel. After the proteins were transferred onto nitrocellulose membrane (Bio-Rad, USA), the membrane was then blocked with TBST (TBS pH 7.4, containing 0.1% Tween-20) containing 5% BSA for 1 h at room temperature. Western blot was firstly performed with primary antibody anti-MjFAK-C (1:1000), anti-FAK (phosphoY397) (1:2000), anti-FAK (phosphoY577) (1:2000), anti-tubulin (1:5000), anti-V5 (1:1000) and anti-GFP (1:1000), respectively. After incubating with second antibody, signals were detected with Super-Signal West Pico Chemiluminescent Substrate (PIERCE, USA).

Immunofluorescence assay

At 48 h post transfection, High Five cells on coverslips were washed with PBS twice and fixed with 4% paraformaldehyde for 10 min at room temperature, then permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature. After blocking with 5% BSA for 60 min, the cells were incubated for 60 min with anti-FAK (phosphoY397) (1:200). Thereafter, the cells were washed for five times for 5 min each with PBS and incubated for another 60 min with FITC-coupled goat anti-rabbit IgG antibody (used in 1:50). For nucleus staining, 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Invitrogen) was applied. After washing for three times for 10 min each with PBS, the cover glasses were mounted with mounting media (Boster). Immunofluorescence was imaged with Confocal microscopy (LeicaSP2) or Laser microscopy (Leica).

Cell adhesion assay

The cell adhesion assay was performed as described previously (Lin et al., 2006). The ELISA plates were coated with 0.2 μg/ml of fibronectin (FN) (Chemican), and then incubated at 4 °C overnight. To explore the effect of WSV083 upon cell adhesion activities, the same amount of cells transfected with pIZ/V5-WSV083, pIZ/V5-His, pIZ/V5-WSV083-PM, pIZ/V5-WSV083-DM or none respectively were subjected to cell adhesion activity of FN. In addition, The OD value of wells without coating and BSA blocking was calibrated as 100% adhesion. The data were statistically analyzed using a paired Student's *t*-test.

Construction of dsRNA and RNA silencing of FAK

DsRNA was synthesized using Promega T7 RiboMAX™ Express RNAi System following the instructions. In brief, dsRNA transcription template of shrimp FAK and the control GFP gene were prepared by PCR, with primers listed in Table 1. To obtain dsRNA, 1 μg sense and antisense template were incubated with Enzyme Mix T7 Express at 37 °C for 2 h, the two products were mixed and denatured at 70 °C for 10 min and cooled to room temperature. After RNase and DNase treatment, dsRNA was precipitated, dissolved in DEPC water and stored in −80 °C.

To ensure the silencing effect on FAK expression, FAK dsRNA and GFP dsRNA were separately injected into twelve shrimps in each group. At different time points (0, 24 and 48 h post-injection), hemocytes of three

Table 1
Summary of primers used in this study.

Name	Sequence (5′-3′)
For construction of plasmids [a]	
WSV083F	GG**GGTACC**ACTATGGGGGGACCCACT
WSV083R	CCG**CTCGAG**CGTTTCTTTACTTTAAACAGGTT
WSV083DM	CCG**CTCGAG**CGGAAAGAGTCTGTTAT
WSV083PMF	AATGATTGcCTTGGGACTCTCATACC
WSV083PMR	GGTATGAGAGTCCCAAGgCAATCATT
WSV083Flag	GG**GGTACC**ATGGACTACAAGGACGACGATGACAAGATGGGGGGACCCACT
MjFAKF	GG**GGTACC**ATGGGCGGCCTTCCGCCAGC
MjFAKR	GG**GGTACC**CACAGCGGAATGTTTTGAAGG
For construction of dsRNA [b]	
dsRNAFAKF	CCACCGCAAGGCCCTTGTAC
dsRNAFAKFT7	*GGATCCTAATACGACTCACTATAGG*CCACCGCAAGGCCCTTGTAC
dsRNAFAKR	ATGGATGATCAAACTGTTGC
dsRNAFAKRT7	*GGATCCTAATACGACTCACTATAGG*ATGGATGATCAAACTGTTGC
dsRNAGFPF	GTGCCCATCCTGGTCGAGCT
dsRNAGFPFT7	*GGATCCTAATACGACTCACTATAGG*GTGCCCATCCTGGTCGAGCT
dsRNAGFPR	TGCACGCTGCCGTCCTCGAT
dsRNAGFPRT7	*GGATCCTAATACGACTCACTATAGG*TGCACGCTGCCGTCCTCGAT

[a] Nucleotides in bold indicate restriction sites introduced for cloning.
[b] Nucleotides in italic indicate T7 RNA polymerase promoter used for transcription.

random individuals were used for RNA extraction and RT-PCR analysis. Western blot was also performed at 72 h post injection to estimate the expression level of FAK.

WSSV challenge experiment

After acclimatization for 2 days, 25–32 shrimps in each group were treated twice with total 10 μg of various dsRNA (24 h between the two injection) by intramuscular injection into the 2nd abdominal segment 2 days prior to WSSV challenge. The dsRNAs used were FAK dsRNA and GFP dsRNA. Each shrimp was injected with 100 μl virions suspension (approximately 10^6 virions) and the control groups were injected with PBS. After WSSV injection, accumulative mortality was recorded over a period of 6–8 days.

Acknowledgments

We thank M.C. Zhang for providing the *M. japonicus* hepatopancreas and hemocyte mixed cDNA library. This work was supported by the earmarked fund for Modern Agro-industry Technology Research System and the National Natural Science Foundation of China (31001125 and 30830084).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.virol.2011.07.021.

References

Bachere, E., 2000. Shrimp immunity and disease control. Aquaculture 191, 3–11.
Chen, M., Gerlier, D., 2006. Viral hijacking of cellular ubiquitination pathways as an anti-innate immunity strategy. Viral Immunol. 19, 349–362.
Cicala, C., Arthos, J., Ruiz, M., Vaccarezza, M., Rubbert, A., Riva, A., Wildt, K., Cohen, O., Fauci, A.S., 1999. Induction of phosphorylation and intracellular association of CC chemokine receptor 5 and focal adhesion kinase in primary human CD4 + T cells by macrophage-tropic HIV envelope. J. Immunol. 163, 420–426.
Cicala, C., Arthos, J., Rubbert, A., Selig, S., Wildt, K., Cohen, O.J., Fauci, A.S., 2000. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4(+) T cells. Proc. Natl. Acad. Sci. U. S. A. 97 (3), 1178–1183.
Edelman, A.M., Blumenthal, D.K., Krebs, E.G., 1987. Protein serine/threonine kinases. Annu. Rev. Biochem. 56, 567–613.
Hamza, M.S., Reyes, R.A., Izumiya, Y., Wisdom, R., Kung, H.J., Luciw, P.A., 2004. ORF36 Protein kinase of Kaposi's sarcoma herpesvirus activates the c-Jun N-terminal kinase signaling pathway. J. Biol. Chem. 279 (37), 38325–38330.
Izumiya, Y., Izumiya, C., Van Geelen, A., Wang, D.H., Lam, K.S., Luciw, P.A., Kung, H.J., 2007. Kaposi's Sarcoma-associated herpesvirus-encoded protein kinase and its interaction with K-bZIP. J. Virol. 81, 1072–1082.
Jacamo, R., Jiang, X., Lunn, J.A., Rozengurt, E., 2007. FAK phosphorylation at Ser-843 inhibits Tyr-397 phosphorylation, cell spreading and migration. J. Cell. Physiol. 210 (2), 436–444.
Kawaguchi, Y., Matsumura, T., Roizman, B., Hirai, K., 1999. Cellular elongation factor 1delta is modified in cells infected with representative alpha-, beta-, or gammaherpesviruses. J. Virol. 73, 4456–4460.
Li, F., Li, M.Y., Ke, W., Ji, Y.C., Bian, X.F., Yan, X.M., 2009. Identification of the immediate-early genes of white spot syndrome virus. Virology 385, 267–274.
Lighner, D.V., Redman, R.M., 1998. Shrimp diseases and current diagnostic methods. Aquaculture 164, 201–220.
Lin, C.Y., Hu, K.Y., Ho, S.H., Song, Y.L., 2006. Cloning and characterization of a shrimp clip domain serine protease homolog (c-SPH) as a cell adhesion molecule. Dev. Comp. Immunol. 30, 1132–1144.
Liu, W.J., Yu, H.T., Peng, S.E., Chang, Y.S., Pien, H.W., Lin, C.J., Huang, C.J., Tsai, M.F., Huang, C.J., Wang, C.H., Lin, J.Y., Lo, C.F., Kou, G.H., 2001. Cloning, characterization, and phylogenetic analysis of a shrimp white spot syndrome virus gene that encodes a protein kinase. Virology 289 (2), 362–377.
Liu, W.J., Chang, Y.S., Wang, C.H., Kou, G.H., Lo, C.F., 2005. Microarray and RT-PCR screening for white spot syndrome virus immediate-early genes in cycloheximide-treated shrimp. Virology 334 (2), 327–341.
Park, J., Lee, M.S., Yoo, S.M., Seo, T., 2007. A novel protein encoded by Kaposi's sarcoma-associated herpesvirus open reading frame 36 inhibits cell spreading and focal adhesion kinase activation. Intervirology 50, 426–432.
Smith, R.F., Smith, T.F., 1989. Identification of new protein kinase related genes in three herpesviruses, herpes simplex virus, varicella-zoster virus, and Epstein-Barr virus. J. Virol. 63, 450–455.
Xie, X.X., Li, H.Y., Xu, L.M., Yang, F., 2005. A simple and efficient method for purification of intact white spot syndrome virus (WSSV) viral particles. Virus Res. 108, 63–67.
Yan, F., Xia, D., Lv, S., Qi, Y., Xu, H., 2010. Functional analysis of the orf390 gene of the White Spot Syndrome Virus. Virus Res. 151 (1), 39–44.
Zhang, M.C., Wang, H.F., Li, D.F., Xu, X., 2009. A novel focal adhesion kinase from *Marsupenaeus japonicus* and its response to WSSV infection. Dev. Comp. Immunol. 33, 533–539.
Zhi, B., Tang, W., Zhang, X., 2010. Enhancement of shrimp antiviral immune response through caspase-dependent apoptosis by small molecules. Mar Biotechnol, NY. (Epub ahead of print).