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A novel focal adhesion kinase from *Marsupenaeus japonicus* and its response to WSSV infection

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ABSTRACT

Focal adhesion kinase (FAK) is a cytoplasmic protein-tyrosine kinase involved in integrin-mediated signal transduction which regulates multiple cell functions in mammalian cells. In contrast to the well document of FAK in mammalian cells, the properties of FAK in crustacean have not been reported yet and even none of their gene or protein sequences is known to date. Here, we report for the first time the cloning of FAK from *Marsupenaeus japonicus* (designated as MjFAK) and the identification of its involvement in the virus infection and host defense. Sequence analysis displayed that MjFAK shared strong similarity to FAK family protein-tyrosine kinase, including conserved tyrosine phosphorylation sites, PTK domain and FAT domain. Immunofluorescence staining analysis showed that MjFAK was located prominently at the cell periphery and partly in cytoplasm and nucleus. Notably, considerable high content of MjFAK and MjFAK (pY399) were found in shrimp, which differs greatly from the low level of endogenous FAK and pFAK in the mammalian cells. It implies that pMjFAK may play a significant role in shrimp. Moreover, pMjFAK increased at the early infection stage, and the hemocyte adhesion activity of fibronectin also increased significantly accompanying with its phosphorylation. These results suggest that pMjFAK may not only promote the WSSV infection, but also participate in the defense mechanism via the enhancement of the immune-cell adhesion. Our data provide a clue to recognize the FAK-mediated signaling connection in the control of immunity and virus infection in crustaceans, which will be helpful to shrimp viral disease control.

1. Introduction

Preventing diseases caused by virus, especially by white spot syndrome virus (WSSV) is the greatest challenge to shrimp aquaculture. Recently, investigations on shrimp defense mechanism have achieved great progress, however, it mainly focused on immune factors such as phenoloxidase, antimicrobial peptide, lectin, PmAV and hemocyanin [1–5]. However, signaling molecules relative to WSSV infection are rarely documented [6]. In the previous study, we have shown shrimp integrin binds to some RGD-containing WSSV proteins and may function as a cellular receptor for WSSV [7], but how its downstream signal molecules work during WSSV infection is still unclear.

The focal adhesion kinase (FAK), downstream to integrin, is a member of non-receptor protein-tyrosine kinase. It can be activated upon adhesion of cells to the extracellular matrix (ECM) such as fibronectin, lamin through membrane integrin [8]. For mammalian cells, the integrin mediating activation of FAK is initiated by auto-phosphorylation on Tyr397, the ensuing phosphorylation of FAK on Tyr576 and Tyr577 is required for full catalytic FAK activation. The phosphorylation on Tyr925 forms a SH2 binding site, promoting FAK binding to adaptor protein Grb2, which mediates the activation of downstream targets like ERK/MAP kinase cascade [9]. Interestingly, FAK phosphorylation is annexed by several viruses, serving for their entry or capsids delivery [10–12]. These findings imply an association between FAK phosphorylation and virus infection.

In contrast to the well document of FAK in mammalian cells, the studies of FAK in invertebrate mainly concentrate on DFAK56, a *Drosophila* homolog of FAK, exhibiting a high overall amino acid similarity with human FAK [13–15]. As is the case for the mammalian FAK, the tyrosine phosphorylation of DFAK56 is

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Abbreviations: WSSV, white spot syndrome virus; FAK, focal adhesion kinase; PTK, protein-tyrosine kinase; FAT, focal adhesion target; ECM, extracellular matrix; FN, fibronectin; pFAK, phosphorylated FAK.

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enhanced by plating on ECM proteins and is suggested to function in adhesion-dependent signaling transduction during *Drosophila* development [14]. Despite the new findings that the invertebrate FAK is involved in controlling morphogenesis and physiological processes [16,17], however, the mechanism of FAK in immunity against virus infection is still poorly understood.

In the present study, *MjFAK* was isolated from *M. japonicus* for the first time. Its phosphorylation and the hemocyte adhesion were also analyzed after WSSV challenge. Our results revealed that the expression/phosphorylation of MjFAK post-infection was enhanced early and decreased at late phase; moreover, the cell adhesion corresponded to such change, suggesting that MjFAK may engage in shrimp immunity against virus infection.

2. Materials and methods

2.1. Isolation of cDNAs encoding MjFAK

Polymerase chain reaction was performed using degenerated primers FAKF1 and FAKR1 (Table 1) corresponding to the conserved amino acid sequences GQFGDV and WMFGVC in mammalian FAKs, respectively. A *M. japonicus* hepatopancreas and hemocyte mixed cDNA library constructed into pAD-GAL4-2.1 phagemids was taken as the template. Amplified cDNAs (~550 bp) were subcloned into pMD18-T vector (Takara) for sequencing.

The missing 3′-terminal sequence (~2100 bp) was cloned by semi-nest PCR using specific primers FAKC1 (for the first round, Table 1) and FAKC2 (for the 2nd round, Table 1) coupling with vector primer VECC. Part of 5′-terminal sequence (~750 bp) was obtained by the same method using specific primers FAKN1 (for the first round, Table 1) and FAKN2 (for the 2nd round, Table 1) pairing with vector primer VECN. 5′-rapid amplification of cDNA ends method (5′-Full Race Kit, Takara) was applied to get the remaining 5′-terminal region (~1200 bp) from hemocyte RNA. The primers used in 5′-Race were also listed in Table 1.

2.2. Sequence analysis of MjFAK

Protein sequence alignment and analysis were performed using the DNA Software (Lynnon BioSoft). Database searches were performed using the BLAST programs and ScanProsite tool.

Table 1
Sequences of oligonucleotide primers used for amplification of MjFAK gene.

Name	Sequence (5′–3′)
FAKF1	NNNGG(A/C)CAGTTTGG(A/C/T)GA(C/T)GT, N = A, T, G, C
FAKR1	T(A/G)CANACNCC(A/G)AACATCCA, N = A, T, G, C
FAKC1	CTCCACAGCTCTCTCATACTTGG
FAKC2	CGGTGGGTTGAAGAACAGAG
FAKC3	CGCGGATCCGCCACAAGTGCATCGGAC (BamHI)
FAKC4	CCGGAATTCTCACACAGCGGAATGTTTTG (EcoRI)
FAKN1	ATGTATGGATGATCAAACTGTTGC
FAKN2	CACCTTACATGTCTTGATAGCCA
VECC	GCAGTAATACGACTCACTATAGGG
VECN	GAGATCGAATTAGGATCCTCTGC
FAK-F	GCCACAAGTGCATCGGAC
FAK-R	TCACACAGCGGAATGTTTTG
Actin-F	GACGGTCAGGTGATCACCAT
Actin-R	CGATTGATGGTCCAGACTCG
Primers used for 5′-Race	
FAK-RT	GGGTGACTGGAAT
FAK-GSP1	CAACCAGACCCTAGAGCACATC
FAK-GSP2	CCCTTGCCTAACAGTTCTAGGAAT
Inner primer	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
Out primer	CATGGCTACATGCTGACAGCCTA

2.3. Antibody

To generate mouse anti-MjFAK polyclonal antibody, the cDNA fragment encoding 3′-terminal last 200 amino acid residues of MjFAK protein was amplified with 5′-primer FAKC3 (BamHI site) and 3′-primer FAKC4 (EcoRI site). Glutathione S-transferase (GST)-MjFAK-C was then constructed by inserting such fragment into the pGEX-4T-2 expression vector. Expression of the GST-fused protein in *Escherichia coli* BL21 was induced with 0.2 mM isopropyl-β-D-thiogalacto-pyranoside (IPTG) and purification was performed with Glutathione Sepharose (GE Healthcare). Antibody against the purified GST-MjFAK-C (anti-MjFAK-C) was prepared by immunizing mouse. Anti-FAK (phosphoY397) and anti-FAK (phosphoY577) polyclonal rabbit antibodies were purchased from Abcam Inc. Monoclonal mouse anti-GST was purchased from Beyotime Institute of Biotechnology, China. Polyclonal mouse anti-actin was a gift from Prof. Feng Yang (The Third Institute of Oceanography, Xiamen, China).

2.4. Tissue-specific expression of MjFAK

Distribution of MjFAK mRNA in different tissues was measured by RT-PCR. Total RNA from various tissues was extracted using Trizol reagent (BBI). After treatment with DNase I, total RNA was primed with Random Hexamer Primer (Fermentas) and reverse-transcribed with SuperScriptIII (Invitrogen) for the first strand synthesis. Such cDNA was then subjected to PCR amplification. The MjFAK primers used for RT-PCR were FAK-F and FAK-R (Table 1). For internal control, specific primers Actin-F and Actin-R (Table 1) were designed according to *M. japonicus* actin (GenBank accession no. AB055975).

2.5. Shrimps and WSSV challenge

Wild *M. japonicus* (Crustacea, Decapoda) (about 10–12 cm) were purchased from a supermarket in Xiamen, China, and kept in 500-l tanks (at 25 °C) filled with air-pumped circulating sea water.

For WSSV challenge, shrimps were randomly tested to be WSSV-free by PCR [18]. Each shrimp was injected with 100 μl virions suspension (approximately 1×10^5 virions) in the lateral area of the third abdominal segment. Shrimps injected with PBS were set as a negative control. At different time points (0, 2, 6, 12, 24, 48, and 72 h post-infection), hemocytes of four individuals were withdrawn randomly using 2 ml syringe preloaded with Alsevier solution (sodium citrate 0.8%, citric acid 0.05%, glucose 1.87%, NaCl 0.42%, pH 6.0).

2.6. Western blotting and immunofluorescence

Hemocytes collected in Alsevier solution were centrifuged at $1000 \times g$ for 3 min immediately. The protein samples were then separated by 8% SDS-PAGE followed by western blotting using anti-MjFAK-C (1:1000), anti-FAK (phosphoY397) (1:5000), anti-FAK (phosphoY577) (1:1000), and anti-actin (1:1000). For immunofluorescence, reactions were carried out at room temperature. Hemocytes suspended in Alsevier solution were allowed to attach onto glass slides. The cells were fixed for 20 min in 4% parafromaldehyde, and then treated with 0.2% TritonX-100 for 10 min. After blocking with 5% bovine serum albumin (BSA) for 60 min, the cells were incubated for 60 min with anti-MjFAK-C (1:500 in 5% BSA) or anti-FAK (phosphoY397) (1:500 in 5% BSA) or anti-FAK (phosphoY577) (1:200 in 5% BSA) or anti-GST (1:200 in 5% BSA). Thereafter, the cells were washed five times for 5 min each in PBS and incubated for another 60 min with Alexa488-coupled goat anti-mouse or goat anti-rabbit IgG antibody (both

used 1:1000 in 5% BSA, Invitrogen). For nucleus staining, 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Invitrogen) was applied. After washing three times for 10 min each in PBS, the cover glasses were mounted with mounting media (Boster). Immunofluorescence was imaged with confocal microscopy (Leica SP2).

2.7. Cell adhesion assay

The cell adhesion assay was performed as described previously [19]. The ELISA plates were coated with a serial amount of fibronectin (FN) (Chemican), and then subjected to cell adhesion activity of FN. To compare the adhesion activities of hemocytes between WSSV-treated shrimps and PBS treatment, the same amount of hemocytes (5×10^6 cells/ml) at 12 and 72 h post-injection was determined by cells counter. In addition, the $OD_{590\ nm}$ value of hemocytes in wells without coating and BSA blocking were set as 100% adhesion. The data were statistically analyzed by ANOVA test using SPSS (SPSS Inc., Chicago, IL). Differences were considered statistically significant at P value less than 0.05.

2.8. Analysis of virus load

Viral loads of WSSV-challenged shrimps at different time points were analyzed by PCR as described before [18]. DNA templates were extracted from a mixture of equal amount of hemocytes from shrimps used for Western blot. These templates were used to amplify WSSV-specific DNA fragments. *Actin* gene was set as a control of template amount.

3. Results

3.1. cDNA isolation of MjFAK

Degenerated oligonucleotide primers were first used to amplify cDNAs from the hepatopancreas and hemocytes mixed cDNA library. Amplified cDNA fragments were cloned into plasmids and sequenced. Database search showed that a cDNA fragment shared a high homology with the protein kinase domain (PTK) of FAK. Semi-nest PCR and 5′-Race PCR were applied to get the 5′- and 3′-remaining regions. Thus, the full length of cDNA sequence was obtained.

3.2. Sequence analysis of MjFAK

The whole cDNA sequence of MjFAK is 4613 bp (GenBank accession no. EU927416), which contains a long open reading frame encoding a protein of 1028 amino acid residues with predicted molecular mass of 114,895 Da. MjFAK, like other FAKs, contains a conserved PTK, flanked by large N- and C-terminal sequences (Fig. 1A). Alignment of the amino acid sequence of the MjFAK PTK with those of database showed that MjFAK PTK shared 67.8% identity to human FAK and 65.3% identity to DFAK56, respectively (Fig. 1B). The focal adhesion targeting (FAT) sequence that localizes FAK to focal adhesions in vertebrates is conserved in the C-terminal regions of MjFAK (48.9% identity to human FAK and 45.2% identity to DFAK56) (Fig. 1B).

Tyrosine phosphorylation sites within FAK have been found to play critical roles in signal transduction pathway. A major auto-phosphorylated site at Y^{397}AEI for human FAK and Y^{430}AEI for DFAK56 binding to the SH2 domain of Src after phosphorylation is also highly conserved in MjFAK (Y^{399}AEI) (Fig. 1A). The tyrosine phosphorylated sites at QS$Y^{578}Y^{579}$KAS in MjFAK are corresponding to ST$Y^{576}Y^{577}$KAS in human FAK or QS$Y^{635}Y^{636}$HST in DFAK56 (Fig. 1A), which is essential for maximal FAK kinase activity [9]. For the tyrosine phosphorylated site Y^{925}ENV of human FAK or Y^{954}CAT of DFAK56 is slightly divergent when comparing to Y^{895}EST of MjFAK (Fig. 1A), which locates in the FAT sequence and binds to the Grb2 SH2/SH3 adaptor protein. Furthermore, proline-rich motifs in C-terminal region, binding to the SH3 domain, have been taken as a characteristic element of FAK. MjFAK contains only one such proline-rich motif P^{711}PKPSRV corresponding to the first proline-rich motif P^{712}PKPSRP in human FAK (Fig. 1A). Taken these sequence data together, we conclude that MjFAK is a novel homolog of focal adhesion kinase in shrimp.

3.3. Tissue distribution of MjFAK

The tissue distribution of *MjFAK* mRNA was studied by RT-PCR. The results showed that *MjFAK* expressed in all analyzed tissues including hepatopancreas, hemocyte, gill, heart, muscle, and intestine (Fig. 2).

3.4. Subcellular localization of MjFAK in hemocyte

Western blotting was employed to identify MjFAK in shrimp hemocyte. Anti-FAK-C antibody could recognize MjFAK with a molecular mass about 130 kDa in hemocyte (Fig. 3A, lane 1). In addition, anti-FAK (phosphoY397) and anti-FAK (phosphoY577) could also identify the phosphorylated MjFAK of hemocyte specifically (Fig. 3A, lane 2 and lane 3), suggesting that the tyrosine residues MjFAK (Y399) and MjFAK (Y579) could be phosphorylated in hemocyte.

To explore the subcellular localization of MjFAK in hemocyte, hemocytes were withdrawn and allowed to attach on glass slide for immunofluorescence. Confocal laser scanning microscopy identified that MjFAK distributed mainly at the periphery of hemocyte, which may represent focal adhesions of the cells (Fig. 3B, a). Similarly, the distribution of phosphorylated MjFAK including MjFAK (pY399) and MjFAK (pY579) had the same distribution pattern (Fig. 3B, b and c). Control experiments did not cause any signals (Fig. 3B, d). Interestingly, part of MjFAK including its phosphorylated forms was localized in nucleus (Fig. 3B, a–c), indicating that MjFAK possibly function in the transcriptional regulation.

3.5. Phosphorylation analysis of MjFAK response to WSSV

MjFAK in shrimp hemocyte can be phosphorylated on both Tyr399 and Tyr579 as shown in Fig. 3A and B, which indicates that MjFAK activation may also depend on phosphorylation of major tyrosine sites. In order to determine whether phosphorylated MjFAK could be regulated by WSSV infection or not, WSSV-free *M. japonicus* were challenged with purified WSSV. Hemocytes of four individuals at various time points post-infection were used for Western blotting analysis. The phosphorylated MjFAK increased at 2 h after WSSV infection, and reached its peak at 12 h. At subsequent time points, the phosphorylated level decreased dramatically at 48 h, such trend coincided with total MjFAK protein expression level (Fig. 4A, as indicated by MjFAK (pY399), MjFAK (pY579) and total MjFAK). While in PBS injection experiment, the level of MjFAK expression and phosphorylation remained similar at various time points post-injection (data not shown). On the other hand, the viral loads of the infected shrimps remained at a low level within the first 6 h post-infection, increased abruptly at 12 h and maintained at a high level at the following time points (Fig. 4B). Taken these data together, it is reasonable to assume that WSSV infection upregulate the MjFAK expression, leading to increase the phosphorylation level of MjFAK.

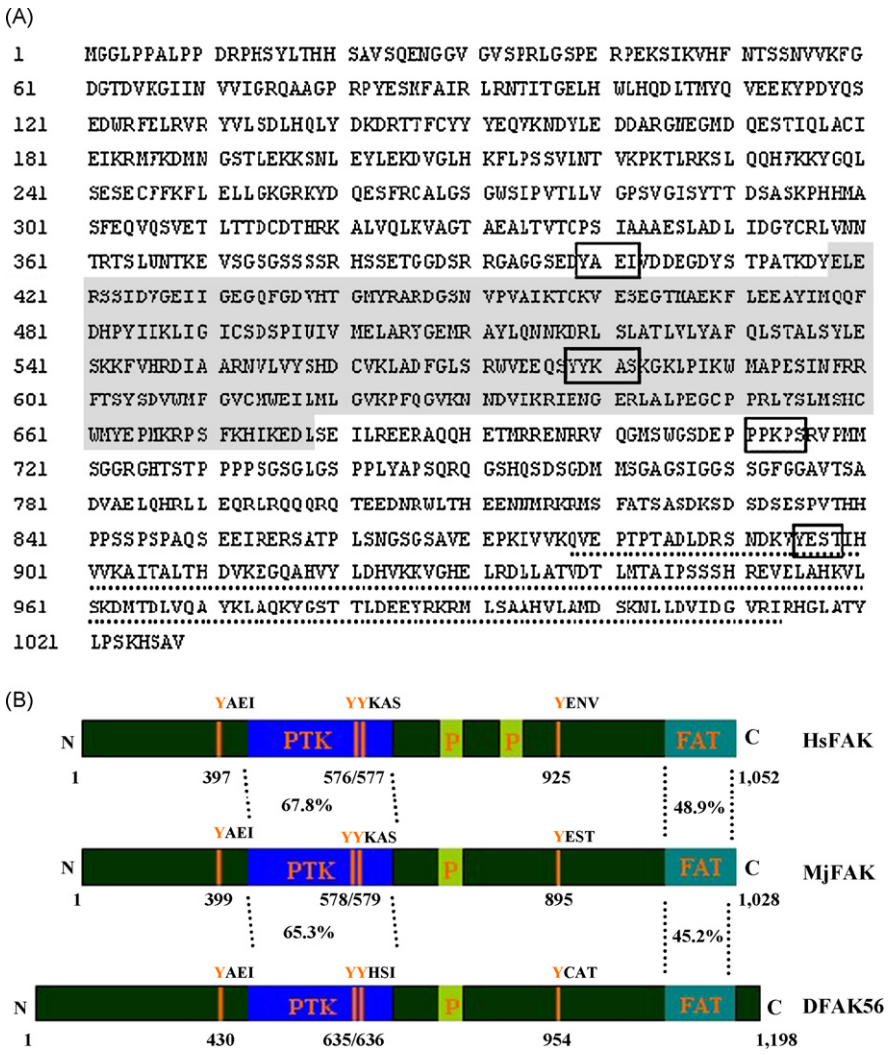

Fig. 1. Molecular characterization of MjFAK. (A) Deduced amino acid sequence of MjFAK. The central PTK is shaded, FAT domain is underlined. The putative tyrosine phosphorylation sites Y^{399}AEI, $Y^{578}Y^{579}$KAS, Y^{895}EST, and a proline-rich region (PPKPS) are boxed. (B) Schematic comparison of MjFAK with human FAK (HsFAK) and *Drosophila* FAK (DFAK56). Structural features such as Tyr phosphorylation sites, proline-rich region, PTK and FAT domain are conserved among MjFAK, HsFAK and DFAK56. The percentages of amino acid identities are shown for the PTK and FAT domain.

3.6. Hemocyte adhesion activity analysis

To better understand that the change of MjFAK level during WSSV infection may influence the hemocyte adhesion in shrimp, we first checked the hemocyte adhesion activity under various concentration of bovine FN. The cell adhesion activity in non-coating and blocking wells were normalized to 100%. As shown in Fig. 5A, FN was demonstrated to increase the hemocyte adhesion significantly in a dose-dependent manner. Under the concentration 0.2 μg/ml, the hemocyte adhesion almost reached its maximal activity. Moreover, we found shrimp hemocyte adhesion on FN also required supplemented calcium (data not shown).

Since the time points at 12 and 72 h were just consistent with the MjFAK maximal upregulation and downregulation, respectively, both of the time points were selected to study cell adhesion. Comparing to PBS-injected group, the hemocyte adhesion of WSSV treated shrimp was enhanced by 34.5% at 12 h post-infection (Fig. 5B) and subsequently decreased by 29.9% at 72 h (Fig. 5C). This phenomenon implies that WSSV infection could enhance hemocyte adhesion at early stage, and then attenuated at later phase.

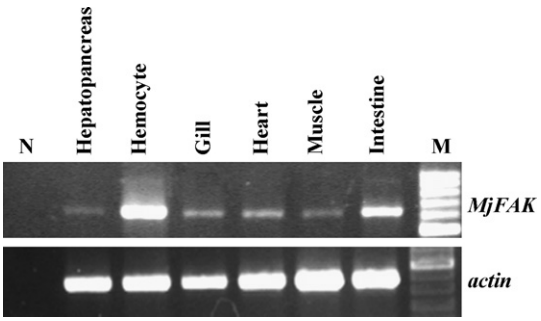

Fig. 2. RT-PCR analysis of *MjFAK* distribution in various tissues. N: negative control. M: 100-bp ladder.

4. Discussion

FAK, a member of non-receptor tyrosine kinase, is extensively addressed as pivotal roles in cell adhesion, anti-apoptosis, actin assembly, tumor formation and progression [8,20–22]. The previous finding that shrimp integrin may function as a cellular receptor for WSSV prompts us to explore how FAK responses to WSSV infection.

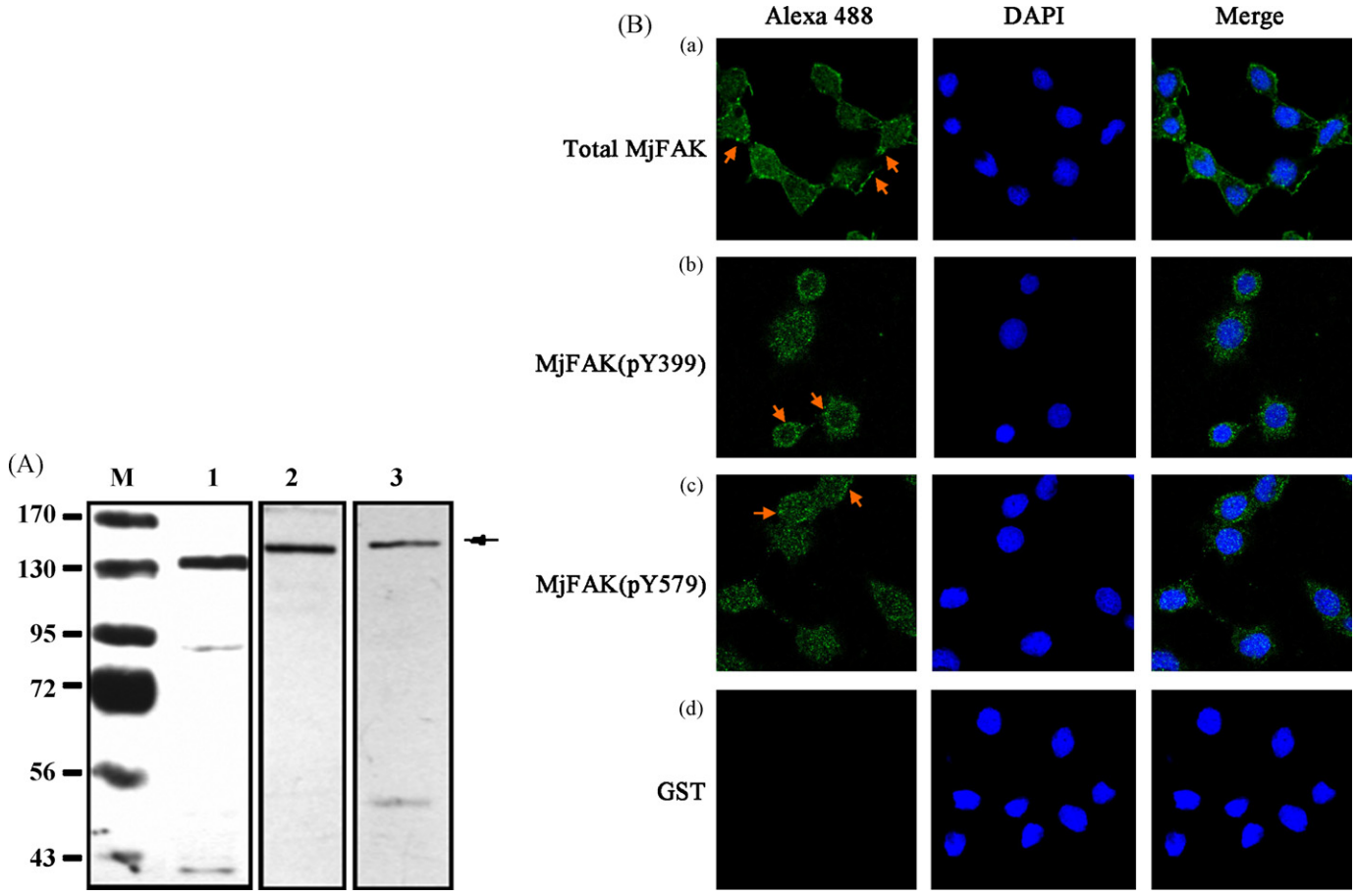

Fig. 3. Identification of MjFAK and its phosphorylation in hemocyte. (A) Hemocyte withdrawn in Alsevier solution was centrifuged and resolved by 8% SDS-PAGE, Western blotted and tested with mouse anti-MjFAK-C (lane 1, recognize total MjFAK), rabbit anti-FAK (phosphor Y397) (lane 2, recognize MjFAK (pY399)) and rabbit anti-FAK (phosphor Y577) (lane 3, recognize MjFAK (pY579)). The arrows indicate the immunoreactive bands of total MjFAK and its phosphorylated forms. Standard molecular masses are indicated in kilodaltons on the left. (B) Immunofluorescent analysis of MjFAK localization. Hemocytes in Alsevier solution were allowed to attach on glass slide, washed with PBS and incubated with mouse anti-MjFAK-C, rabbit anti-FAK (phosphor Y397) and rabbit anti-FAK (phosphor Y577) to visualize total MjFAK (a), MjFAK (pY399) (b) and MjFAK (pY579) (c), respectively. Mouse anti-GST antibody was used as a negative control (d). The red arrows indicate its possible localization at focal contacts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

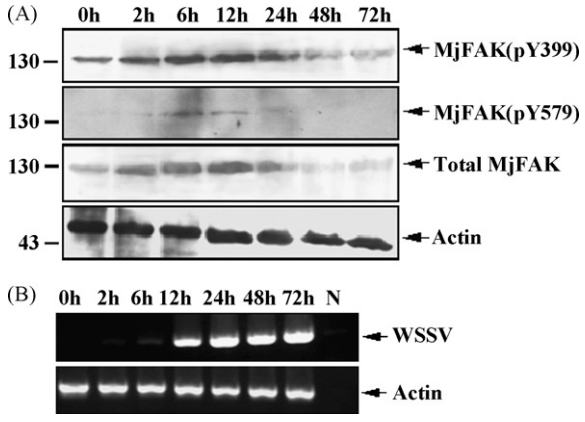

Fig. 4. Analysis of MjFAK expression and phosphorylation after WSSV challenge. (A) Hemocyte samples from different time points after virus challenge were resolved by 8% SDS-PAGE, and then subjected to Western blot with anti-FAK (phosphoY397), anti-FAK (phosphoY577) anti-MjFAK-C, respectively. To assess the amount of proteins in each immunoreaction, the same amount of proteins were blotted and probed with anti-Actin antibody. Standard molecular masses are indicated in kilodaltons on the left. (B) Viral loads of infected shrimps were detected by PCR, using hemocyte samples from different time points (0–72 h) post-infection.

In this paper, MjFAK was characterized as the first FAK family protein-tyrosine kinase in crustaceans. Sequence alignment displayed that MjFAK shared a high overall amino acid similarity with human FAK and DFAK56. While analyzing its phosphorylation, both Tyr399 and Try579 of MjFAK were found to be phosphorylated in vivo, which suggests the MjFAK activation depends on the same mode of regulation as mammalian FAK, the Tyr399 auto-phosphorylation is required for MjFAK activation and the Tyr579 phosphorylation is essential to the regulation of its catalytic kinase activity. The subcellular distribution of MjFAK in focal adhesions, cytoplasm and nucleus implies that MjFAK not only plays a role in mediating signal transduction at focal adhesions, but also translocates to nucleus to regulate transcription as mammalian FAK does [23,24]. The difference between them is that shrimp maintains high FAK expression, while in mammalian, high content of FAK is correlated with different types of tumor [22]. Notably, the phosphorylated level of Tyr579 is especially lower than that of Tyr399 (as shown at 0 h in Fig. 4A), but its dominant expression was found in a small proportion of cells (Fig. 3B, c). Possibly, the level of MjFAK (pY579) varies in different types of hemocyte.

The increase in the amount of MjFAK protein after WSSV infection is accompanied by an increase in its Tyr399 and Tyr579

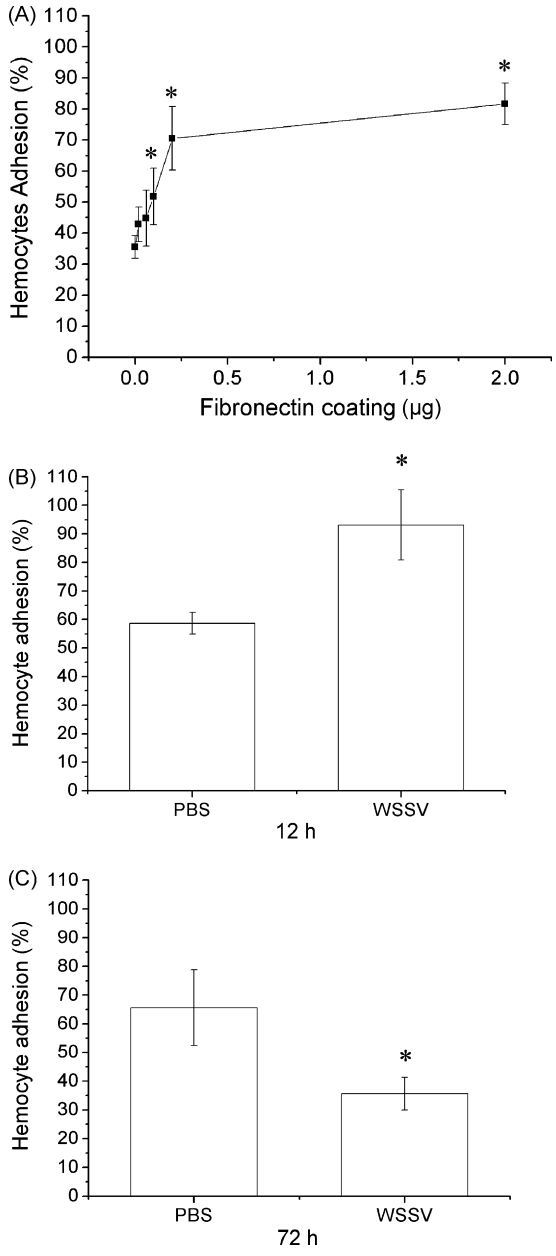

Fig. 5. Hemocyte adhesion activity of FN post-WSSV infection. (A) The ELISA plates were coated with a serial of different concentrations of FN (0, 0.02, 0.04, 0.06, 0.1, 0.2, and 2.0 μg), respectively. Then these wells were applied to cell adhesion assay. The wells without coating and blocking were normalized to 100%, and the ratios of various concentration values to such wells were shown. (B and C) Hemocyte adhesion activity of 0.2 μg FN post-infection at 12 and 72 h, PBS injection was taken as a control. The number of cells used was counted and diluted to 5×10^6 cells/ml. The bar represents the S.D. of the mean ($n = 4$) and * indicates a significant difference of $P < 0.05$.

phosphorylated levels (Fig. 4A). Recent investigation has indicated that integrin interplays with some viral RGD-containing proteins and mediated WSSV infection [7]. So it is likely that the virus utilizes the interaction between viral RGD-containing proteins and surface integrin, leading to the MjFAK/phosphorylation upregulation, which in turn facilitates the entry of virus and the delivery of genome to nucleus. However, further experiments should be carried out to explore whether the RGD-containing viral proteins could trigger MjFAK or not.

It has been suggested that WSSV infection block apoptosis induced by actincmycin D in primary cells [25]. Although WSSV499 (an anti-apoptosis protein encoded by WSSV ORF390) can target Pmcaspase and acts as a direct caspase inhibitor [26], such action takes place at the caspase downstream signaling pathway. Since FAK overexpression suppresses apoptosis in Hela cells and is presumed to inhibit an upstream signal pathway triggering the activation of caspase 3 [20], it can be deduced that WSSV may utilize the same mechanism by which elicits the increasing of MjFAK to inhibit hemocyte apoptosis for viral propagation. This may be an explanation for that WSSV infection causes shrimp hemocyte apoptosis but with a relatively low percentage [27,28].

From the host defense point of view, cell adhesion in invertebrate is essential to the cellular immune responses of encapsulation and nodule formation [29]. Here, hemocyte adhesion of FN is found to be correlated with MjFAK phosphorylation, which is consistent with previous reports that FAK phosphorylation involves in cell adhesion [30,31]. We suppose that, during WSSV infection, especially in the early stage, the MjFAK expression/phosphorylation increases to promote cell adhesion, as virus dominates in host cell, MjFAK is downregulated, eliciting a significant decrease in its hemocyte adhesion activity.

Finally, we conclude that WSSV carries out its infection by the activation of MjFAK; while host, simultaneously, makes a protective effect against viral infection by increasing cell adhesion. It implies that MjFAK may integrate signals from virus pathogen stimuli into different cellular outcomes via distinct downstream pathway. Although the mechanism of MjFAK activation and cell adhesion enhancement is still unclear, the present work provides us a further understanding of the relationship between virus infection and host defense.

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