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Original article

miRNA regulation of JAK/PI3K/AKT pathway in white spot syndrome virus-infected shrimp: A computational and expression analysis

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Abstract

Background: White spot syndrome virus (WSSV) serve as a serious threat for shrimp aquaculture industry. Shrimp immune responses involve several pathways, including the JAK/STAT signaling cascade, which is vital for cellular communication and immune regulation. While this pathway is well-studied in vertebrates, its function and miRNA-mediated regulation in shrimp remains unclear.

Objective: To investigate expression of JAK/PI3K/AKT pathway genes and identify miRNAs regulating these genes during WSSV infection in *Penaeus vannamei (P. vannamei)*.

Methods: Hemocytes from WSSV-infected *P. vannamei* were collected at 6, 24, and 48 hours post-infection (hpi). Expression of *PvJAK*, *PvPI3K*, *PvAKT*, *PvmTOR*, and *PvDomeless* were analyzed via quantitative reverse transcription polymerase chain reaction. Bioinformatics tools were used to predict miRNAs targeting these genes for further validation.

Results: WSSV infection significantly upregulated JAK/PI3K/AKT pathway genes, indicating their role in the antiviral response. In silico analysis identified 20 miRNAs potentially targeting these genes, 14 for *PvJAK*, 9 for *PvDomeless*, and 2 each for *PvAKT* and *PvPI3K*. Expression analysis showed five *PvJAK*-targeting miRNAs (Pva-miR-18, Pva-miR-71, Pva-miR-101, Pva-miR-146-5b, and Pva-miR-1) were downregulated post-infection. *In vitro* analysis suggested *PvJAK* is a direct target of Pva-miR-18.

Conclusion: This study revealed that WSSV can regulate JAK/PI3K/AKT pathway during infection in *P. vannamei*. Among the miRNAs identified, Pva-miR-18 may regulate *PvJAK* expression. Cumulatively, we suggests the potential regulatory of JAK/PI3K/AKT pathway through miRNA.

Keywords: JAK/PI3K/AKT pathway, miRNA, *Penaeus vannamei*, white spot syndrome virus, WSSV.

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The global shrimp aquaculture sector witnessed robust expansion in 2021, marked by an 8.9% rise in shrimp production compared to the previous year (GOAL, 2021). Nonetheless, this progress is significantly hindered by various pathogens, including bacterial, fungal, and viral agents. [1, 2] Among these diseases, infection caused by the White spot syndrome virus (WSSV) stands out as a severe threat, resulting in a 100% mortality rate in shrimp within 3 to 7 days of infection. This poses a considerable detriment to the shrimp aquaculture sector. [3] Thailand, a key player in global shrimp exports, faces a notable risk of WSSV outbreaks due to the intensive shrimp farming practices prevalent in the country. [4]

To defend against foreign pathogens, shrimps depend on various immunological effectors and pathways. The pathways that play important role in the shrimp immunity includes apoptosis pathway, [5, 6] phagocytosis, nuclear factor-κB (NF-κB) pathway, [7] and JAK/STAT pathway. [8] These pathways genes are post-transcriptionally regulated by small non-coding microRNAs (miRNAs) which are initially synthesized in the nucleus from the genome by Drosha and Dicer nucleases to produce pre-miRNA which is further exported to the cytoplasm through nuclear pores by exportin-5. [9] These miRNAs regulates the expression of their target host or viral genes by binding to the 32 untranslated regions (32 UTRs) of the target mRNAs or 52 untranslated regions (52 UTR) or even within the coding sequence of their target mRNAs. [10] The viral miRNAs in the WSSV-infected kuruma shrimp *Marsupenaeus japonicus* may target virus transcripts and facilitate virus infection. [11] The early gene *wsv477* of WSSV which is involved in early DNA replication and virus proliferation, is targeted by shrimp miR-7 in its 32 UTR. [12]

JAK/STAT signaling pathway regulates various cellular functions including apoptosis, hematopoiesis and adipogenesis. [13] Roles of JAK/STAT pathway in the antiviral immunity is extensively analyzed in mammals and Drosophila. [14, 15] Because of its role in antiviral defense, viruses may target JAK/STAT pathway as a strategy to evade host immune responses. Specifically, the V protein of the simian virus is recognized for its ability to selectively degrade the host *STAT1*. In shrimp, the JAK/STAT pathway has been recognized as a pivotal contributor to the innate antiviral response, with key components like *Domeless*, *JAK* and *STAT* already identified. [16-18] Previous studies underscored that the inhibition of *PvJAK* led to heightened mortality rates and increased viral loads, shows its crucial role in antiviral immunity against WSSV. [16] Other interesting aspect of this pathway is its interconnect with other cellular pathways like PI3K/AKT/mTOR pathway. [19, 20] Due to the preseance of SH2 domain in STAT and PI3K, the activation and phosphorylation of the tyrosine residues of JAKs leads to the binding and activation of the SH2 domain of STAT and PI3K proteins to facilitate their functions. [20] The PI3K/AKT/mTOR pathway is considered as a crucial pathway to regulate various cellular functions includes proliferation, invasion, adhesion and migration. Within human melanoma cells, PI3K exerts a regulatory influence by negatively impacting STAT activity. [21] These interactions position the JAK/STAT pathway as a central hub for cellular communication.

While considerable research has been conducted on JAK/STAT and its interlinked pathway in various organisms, further investigations in invertebrates, such as shrimp, are needed to comprehensively understand their role in immunity and how miRNAs regulate their function. Building on this hypothesis, we aimed to unveil its expression patterns of JAK/PI3K/AKT pathway genes during WSSV infection. In addition, efforts were made to identify miRNAs that could target those genes during WSSV infection and modulate its expression.

Materials and methods

Shrimp and WSSV infection

For this experiment, *Penaeus vannamei (P. vannamei)* weighing 3 to 5 grams were selected. Initially, shrimp were acclimated in seawater tanks for 2-3 days and screened for any signs of disease before the commencement of the experiment. The WSSV stock for the infection experiment was prepared following the procedure outlined by Xie X, *et al*. [22] and stored at 80°C until needed. A 50 µL dose of diluted WSSV (1×10^6 copies/shrimp) was injected into the second abdominal segment of shrimp for the infection experiment. An equivalent volume of anticoagulant (MAS solution containing 27 mM sodium citrate, 336 mM NaCl, 115 mM glucose, and 9 mM EDTA, pH 7.0) was used to collect hemolymph from the ventral sinus, which was then centrifuged at 800×g for 10 min at 4°C to isolate the hemocytes. Total RNA was extracted from the hemocytes using the FavorPrep™ miRNA isolation kit (Favorgen) following the manufacturer's protocol.

Expression analysis of miRNA and gene by reverse transcription polymerase chain reaction (qRT-PCR)

Shrimp (n = 3) were infected with WSSV and hemocyte were collected after 6, 24 and 48 hpi. For the control group, shrimp (n = 3) were injected with 0.85% NaCl. Total RNA was extracted as described before. Using 100 ng of total RNA and the Mir-X™ miRNA First-Strand Synthesis Kit (Clonetech Laboratories), cDNA was synthesized which were later used as a template to validate the expression profile of shrimp miRNAs through qRT-PCR analysis. The expression analysis of six miRNAs was performed using the designed primers as shown in **Table 1.** The expression data were normalized to the *U6* gene. For the expression analysis of the target genes in the JAK/STAT pathway, cDNA was prepared using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). Relative expression level was calculated using elongation factor-1α (*EF-1α*) as an interanal control and the mathematical model of Pfaffl. [23]

Table 1. Primer used for qRT-PCR and cloning.

Purpose	Gene	Primer name	Primer sequence (5' -3')
mRNA expression analysis	*EF-1α*	EF-1α-FW	CGCAAGAGCGACAACTATGA
		EF-1α-RW	TGGCTTCAGGATACCAGTCT
	PvCDK1	PvCDK1-FW	CATCACGGCGACTCAGAGATTGA
		PvCDK1-RW	CATTGGCTGGAAGAGTGGACTTG
	PvmTOR	PvmTOR-FW	CATCGAACACTCGGGTCCTTTGA
		PvmTOR-RW	CATGAGGTGACAGCAAGTTCGGA
	Pvcaspase3	Pvcasp3-FW	AGTTAGTACAAACAGATTGGAGCG [33]
		Pvcasp3-RW	TTGTGGACAGACAGTATGAGGC [33]
	PvJAK	PvJAK-FW	CATGAGCTCTGGCTGAAGTCCGTAAAGAAGT
		PvJAK-RW	CATTCTAGATCTGTTCCACTGGCTCTTCA
	PvAKT	PvAKT-FW	CATGAGCTCTCTGTTCCACTGGCTCTTCA
		PvAKT-RW	CATTCTAGACAGGCGGTCATTTGTTTGGA
	PvPI3K	PvPI3K-FW	CATGAGCTC-GCGCCACTCTTGCTGAGATT
		PvPI3K-RW	CATTCTAGA-CAGTTTCATTGCCCTTCCGC
	PvDomeless	PvDomeless-FW	CATGAGCTCGGCAACAAGCTACCTCCGAT
		PvDomeless-RW	CATTCTAGATAGGCATCTGACATGCCA
	VP28	VP28-FW	AGGTGTGGAACAACACATCAAG
		VP28-RW	TGCCAACTTCATCCTCATCA
	U6	U6-FW	CTCGCTTCGGCAGCACA
		U6-RW	AACGCTTCACGAATTTGCGT
Cloning	Pva-miR-146-5b	F-miR 146_5b_Sac1	CATGAGCTCCCAACATTGGGACTACTGGTGT
		R-miR 146_5b_Xbal	CATTCTAGAAGTATCTGCCATTCCCCCTGA
	Pva-miR-18	F-miR 18_Sac1	CATGAGCTCTGCAGTAGGACCTGGATATACG
		R-miR 18_Xbal	CATTCTAGAAACTGGCTCATATCTGCTCAA
	Pva-miR-71	F-miR 71_Sac1	CATGAGCTCAAGCAAGACGACAGCCACAA
		R-miR 71_Xbal	CATTCTAGATTCACCAAAGGTAGGAGCTGC
miRNA expression analysis		Pva-mir-1	TGGAATGTAAAGAAGTATGGAG
		Pva-pmiR-18	CTTGGGAGGTGCTGCACTGCTA
		Pva-miR-1-3p	TGGAATGTAAAGAAGTATGGA
		Pva-miR-71	TGAAAGACATGGGTAGTGGGA
		Pva-pmiR-101	TCTTCTTTGGGCTCCTAGC
		Pva-miR-100	AGCCCGTAGATCCGAACTTG
		Pva-miR-146b-5p	TGAGAACTGAATTCCATAGGCGTT

Bioinformatic analysis of miRNA targeting genes of JAK-STAT/AKT pathway

The CU-MiR software [23] was employed to identify miRNAs that could potentially target the gene of interest in the JAK/PI3K/AKT pathway. This software scans mRNA targets to identify locations where seed sequences of miRNAs can bind complementarily. The criteria for predicting miRNA targets included the presence of a seed sequence (2 to 8 nucleotides) exhibiting complementarity to the mRNA at various locations such as the open reading frame (ORF), 3' UTR, and 5' UTR. Additionally, a minimum overall complementarity of 70% between the miRNA and its target mRNA was required. Subsequently, the identified miRNA target positions were analyzed using the RNAhybrid web tool to verify whether the miRNA/mRNA duplex could spontaneously form based on secondary structure predictions.

Validation of miRNA interaction with target gene in vitro

In vitro analysis of the interaction between the miRNAs of interest and their target genes was performed using a luciferase reporter assay. Gene-specific primers were designed to amplify the target *PvJAK* gene at the region where the predicted miRNA binding occurs. These gene fragments were amplified from the cDNA of unchallenged shrimp hemocytes. The pmirGLO vectors (Promega), pmirGLO-JAK-miR-18 and pmirGLO-JAK-miR-146-5p, containing the putative binding regions of Pva-miR-18 and Pva-miR-146-5p on the target *PvJAK* gene, were constructed. HEK-293 cells (1.0×10^5 cells/well) were seeded into a 24-well plate were co-transfected with 100 ng of pmirGLO-JAK-miR-18 or pmirGLO-JAK-miR-146-5p and 20 pmole of corresponding miRNA mimic or scramble. Control cells were transfected with the plasmid alone. Luciferase activity was measured at 48 h post-transfection using the Dual-luciferase® Reporter Assay System (Promega), and the hRluc-neo fusion (Renilla luciferase coding sequence) expression was measured to confirm successful vector transfection into the HEK cells.

Statistical analysis

MiRNAs and genes expression quantification was performed by qRT-PCR in triplicates according to Pfaffl, 2001. Subsequent statistical analyses employed a one-way analysis of variance. Data presentation includes means accompanied by standard deviations. The paired samples *t*-test was employed to assess the statistical significance of differences among means, with * and ** indicating significant differences at $P < 0.05$ and $P < 0.01$, respectively.

Results

Expression of JAK/PI3K/AKT pathway genes

The role of the JAK/STAT signaling pathway and its interconnected pathways, such as the PI3K/AKT/mTOR pathway in regulation of viral infection has been demonstrated in vertebrates and certain model organisms. [14, 15] Herein, we determined the significance of JAK/PI3K/AKT pathway during WSSV infection, the expression of genes associated with this pathway such as *PvJAK*, *PvAKT*, *PvPI3K*, *PvDomless*, and *PvmTOR* were examined by using qRT-PCR in the hemocytes of WSSV-infected *P. vannamei* at 6, 24, and 48 hpi. Results revealed a significant upregulation of *PvJAK* and *PvDomless* expression at 24 and 48 hpi compared to the control group injected with 0.85% NaCl upon WSSV infection. Additionally, the expression of *PvPI3K* was significantly upregulated at 6, 24, and 48 hpi, while *PvAKT* showed a significant upregulation at 48 hpi compared to the control group. Although *PvmTOR* initially exhibited downregulation at 6 and 24 hpi, its expression was upregulated at 48 hpi and significantly upregulated. The observed alterations in gene expression suggest the potential involvement of the JAK/PI3K/AKT pathway in the innate immune response against WSSV infection in shrimp **(Figure 1)**.

Prediction of miRNA target

MiRNAs regulate the expression of target genes through complementary base-pairing. [9] A growing body of evidence supports the crucial role of miRNAs in diverse biological processes, including development, cellular differentiation, proliferation, apoptosis, hematopoiesis, and the immune system. [5, 6] To investigate miRNA-mediated regulation of the JAK/STAT pathway, we utilized CU-Mir, [23] an in-house developed program for miRNA target prediction. Specifically, we focused on predicting miRNAs that likely control the genes in the JAK/PI3K/AKT pathway, such as *PvJAK*, *PvPI3K*, *PvDomeless*, and *PvAKT*. Our analysis identified a total of 20 miRNAs capable of targeting these genes as shown in **Table 2.** Given that a single gene can be regulated by multiple

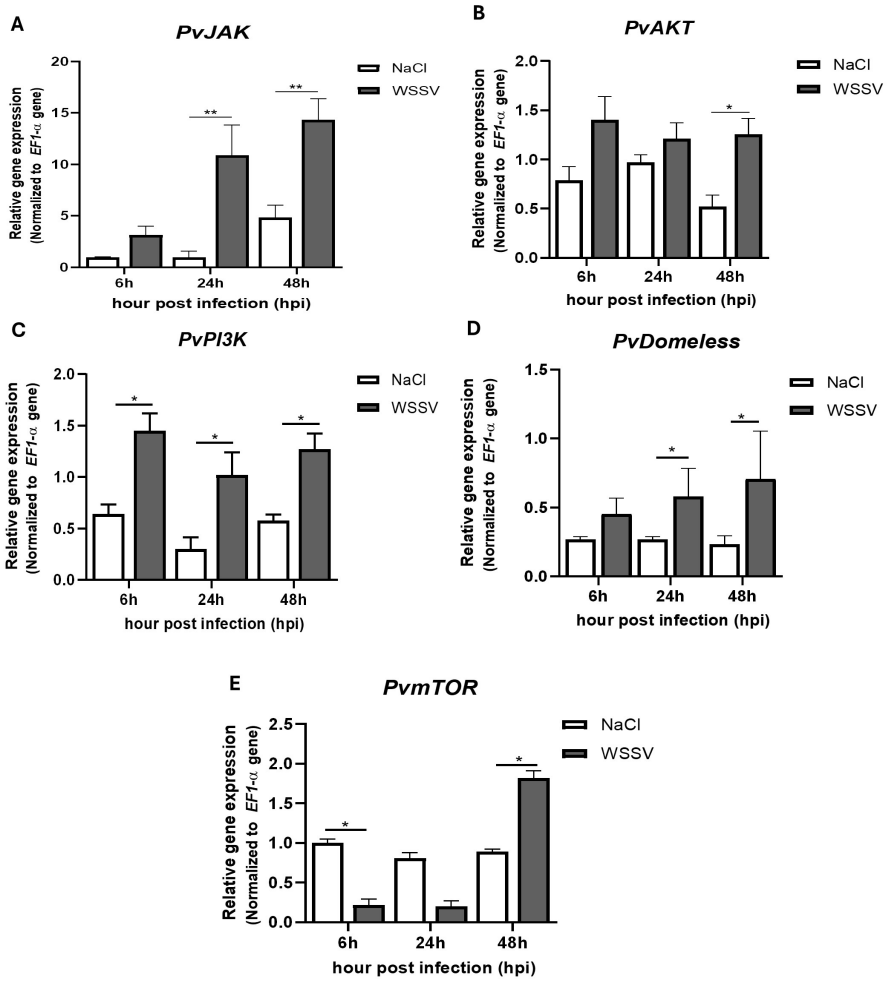


Figure 1. Expression analysis of JAK/PI3K/AKT pathway genes. qRT-PCR was conducted to determine the expression levels of **(A)** *PvJAK*, **(B)** *PvAKT*, **(C)** *PvPI3k*, **(D)** *PvDomeless*, and **(E)** *PvmTOR* in the hemocytes of WSSV-infected shrimp at 6, 24, and 48 hpi. Relative expression levels of these genes were calculated using *EF-1α* as an internal control. All experiments were performed in three biological replicates. The bar graphs are the data presented as means ± standard deviation (SD) of mean. * and ** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively

miRNAs, we also identified some miRNAs that might target more than one gene. Our findings revealed 14 miRNAs potentially targeting the *PvJAK* gene, while 9 miRNAs were predicted for *PvDomeless*, and 2 miRNAs for both *PvAKT* and *PvPI3K*. The interactions between miRNAs and their target mRNAs were visualized using Cytoscape software **(Figure 2)**. Additionally, we predicted the secondary structure of miRNAs targeting the *PvJAK* gene using RNA hybrid software **(Table 2).** The results revealed that Pva-miR-1 and Pva-miR-18 target the *PvJAK* at 5' UTR, Pva-miR-101, Pva-miR-71 and Pva-miR-146-5b target *PvJAK* at ORF region while Pva-miR-101 target 3' UTR.

miRNA expression analysis after WSSV infection by qRT-PCR

To verify the role of the identified miRNAs targeting *PvJAK* in WSSV infection, we conducted qRT-PCR analysis to examine the expression levels of six miRNAs in the hemocytes of WSSV-infected shrimps. The selected miRNAs included Pva-miR-1, Pva-miR-18, Pva-miR-71, Pva-miR-146-5b, Pva-miR-100, and Pva-miR-101. Taking *U6* as the internal reference, we assessed the relative expression levels of these miRNAs in shrimp hemocytes at 6, 24, and 48 hpi **(Figure 3).** After WSSV infection, five out of six miRNAs show significantly downregulated in the expression. The expression level of Pva-miR-18 **(Figure 3A)** significantly decreased at 6 and 48 hpi,

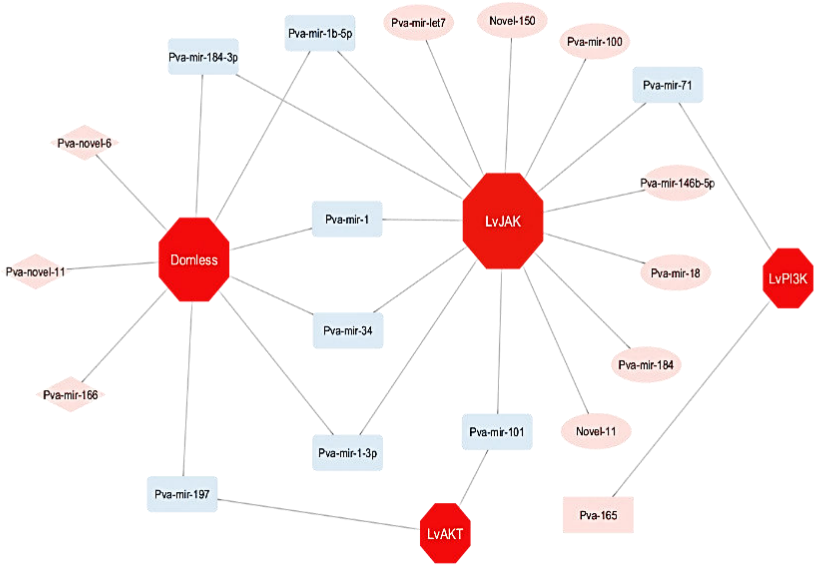


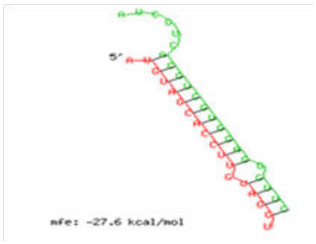
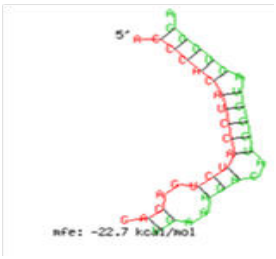
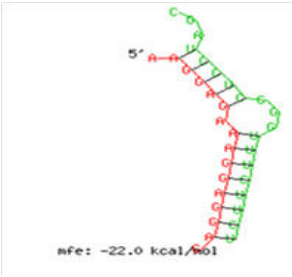
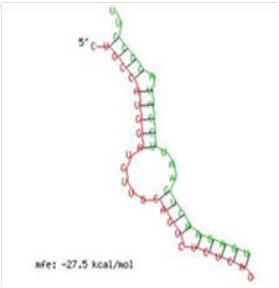
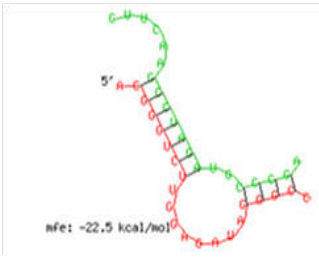
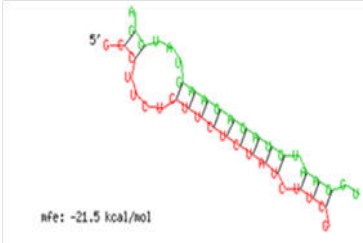
Figure 2. Prediction of miRNAs targeting genes in the JAK/PI3K/AKT pathway. The Cu-MIR program was used to predict complementary binding between miRNAs and genes of the JAK/PI3K/AKT pathway. Subsequently, the potential spontaneous formation of a simple secondary structure between miRNA and target mRNA was validated using RNAhybrid software. In the visual representation, miRNAs capable of binding with more than one gene are indicated in blue, while those binding to only one gene are represented in violet. The red color signifies the target gene, and the size of the node reflects the number of interactions between these genes and miRNAs.

while at 24 hpi, the expression level remained unchanged. For Pva-miR-71 **(Figure 3B)** and Pva-miR-101 **(Figure 3E)**, downregulation was observed at 48 hpi, with no significant change at 6 and 24 hpi. Meanwhile, the expression level of Pva-miR-146-5b **(Figure 3C)** sharply decreased at 6 and 48 hpi but remained unchanged at 24 hpi. Pva-miR-1 **(Figure 3F)** exhibited significant downregulation at 24 and 48 hpi. In addition, Pva-miR-100 **(Figure 3D)** showed no change after 6 and 48 hpi, but interestingly, its expression was upregulated at 24 hpi. Furthermore, Pva-miR-1, Pva-miR-18, Pva-miR-71, Pva-miR-146-5b, and Pva-miR-100 exhibited a negative correlation with *PvJAK* expression **(Figure 1A)**, suggesting that these miRNAs might target the *PvJAK* genes during WSSV infection.

Confirmation of miRNAs interaction with their target PvJAK gene by dual-luciferase reporter assay

Due to their lower minimum free energy (mfe), Pva-miR-18 and Pva-miR-146-5b were selected for further validation with the target region of the PvJAK gene. We constructed pmirGLO-JAK-miR-18 and pmirGLO-JAK-miR-146-5b plasmids containing DNA fragments corresponding to the putative Pva-miR-18 and Pva-miR-146-5b-binding regions of *PvJAK*, respectively. These reporter plasmids were co-transfected into HEK293-T cells with either a Pva-miR-18 mimic and Pva-miR-18 scramble or Pva-miR-146-5b mimic and Pva-miR-146-5b scramble. Introduction of the Pva-miR-18 mimic resulted in approximately a 25% reduction in luciferase activity in cells transfected with pmirGLO-PvJAK, as depicted in **Figure 4A**, compared to the corresponding control. This reduction in firefly luciferase activity suggests the binding of Pva-miR-18 to the cloned miRNA target sequence. Conversely, the presence of the Pva-miR-146-5b mimic **(Figure 4B)** had no effect on luciferase activity, indicating that *PvJAK* may not be its target. These findings suggest that *PvJAK* could be a specific target gene of Pva-miR-18.

Table 2. Predicted the secondary structure of miRNAs that targeting the *PvJAK* gene by using RNA hybrid software.

Target gene	miRNA	Secondary structure	Location of target sequence
PvJAK	Pva-miR-18		5'UTR
	Pva-miR-71		ORF
	Pva-miR-101		ORF
	Pva-miR-146-5b		ORF
	Pva-miR-100		3'UTR
	Pva-miR-1		5'UTR

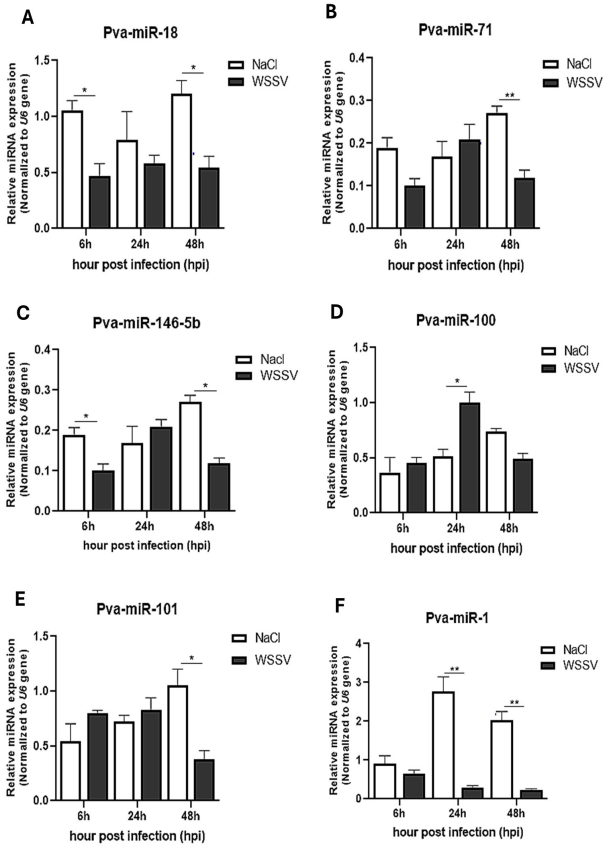


Figure 3. miRNAs relative expression level analysis in the WSSV-infected shrimp hemocyte. qRT-PCR was used to conduct the expression analysis of **(A)** Pva-miR-18, **(B)** Pva-miR-71, **(C)** Pva-miR-146-5b, **(D)** Pva-miR-100, **(E)** Pva-miR-101, and **(F)** Pva-miR-1 at 6, 24, and 48 hpi. The internal control use for this experiment was *U6* for these miRNAs to calculate the relative expression level. The bar graphs are the data presented as means ± standard deviation (SD). All experiments were performed in three replicates (n = 3). The * and ** represent the significant difference at $P < 0.05$ and $P < 0.01$, respectively.

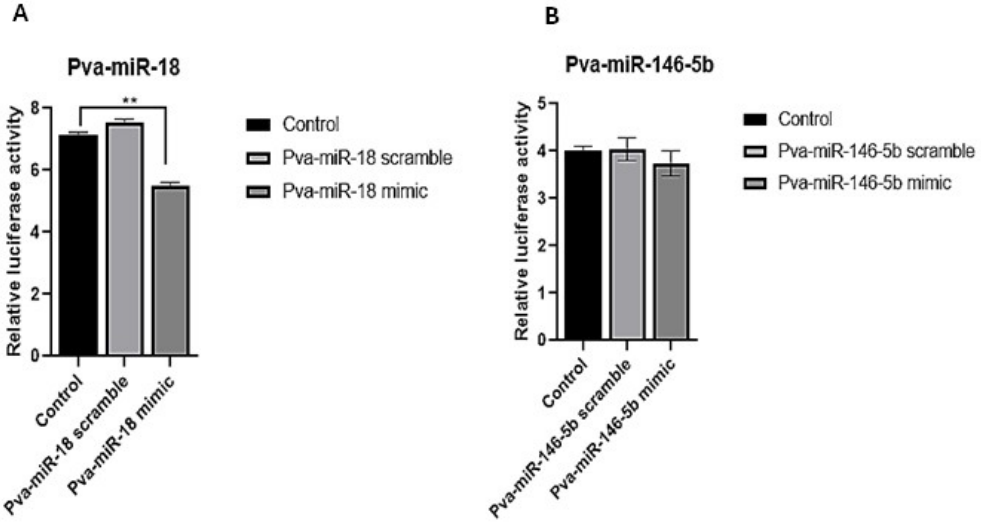


Figure 4. The confirmation of the interaction between miRNAs and the target sequence of the *PvJAK* gene. Luciferase activity in HEK-293 cells was assessed following transfection with either **(A)** Pva-miR-18 mimic or Pva-miR-18 scramble and **(B)** Pva-miR-146-5b mimic or Pva-miR-146-5b scramble, in conjunction with the pmirGLO-JAK-miR-18 or pmirGLO-JAK-miR-146-5b plasmid respectively. The presented data represents the mean ± standard deviation (SD) derived from three independent experiments. ** indicates a significant difference in the relative luciferase activity ($P < 0.01$).

Discussion

Much like other invertebrates, shrimp rely on their innate immune system, swiftly responding to foreign pathogens through intracellular signaling cascades which leads to the activation of both cellular and humoral immune responses. [24] The main players in the innate humoral response are three immune signaling pathways: Toll-like receptor pathway, immune deficiency pathway, and JAK/STAT pathway. [25, 26] The JAK/STAT signaling pathway demonstrates evolutionary conservation across a diverse spectrum of species, ranging from *Dictyostelium discoideum* to humans. In vertebrates, this pathway is typically activated indirectly through cell death and stress signals mediated by cytokines or growth factors. However, in shrimp, the activation of JAK/STAT pathway takes place directly through lectin, which cross-links microbial pathogens with the cell surface receptor Dome. This activation serves a pivotal role in the shrimp's antibacterial immune responses by enhancing the expression of specific antimicrobial peptides (AMPs). [18] Previous studies involving genetic knockouts in mice have highlighted the predominant role of the mammalian JAK/STAT signaling pathway in immune responses, particularly in antiviral defense. [26] The transcriptional profile of Drosophila infected with Drosophila C Virus suggests that the JAK/STAT pathway may also respond to viral infections in invertebrates. [27] Our expression analysis of JAK/PI3K/AKT pathway genes aligns with these findings, supporting the notion that this pathway may be responsive to WSSV infection in shrimp. Together, these observations indicate that, alongside the Toll and Imd pathways, the JAK/STAT pathway might constitute a third evolutionarily conserved pathway contributing to innate immunity in invertebrates.

Furthermore, the JAK/STAT signaling pathway exhibits the ability to crosslink with other cell-signaling pathways, such as the PI3K/AKT/mTOR pathway. [21] In mammals, stimulation of *PI3K* by extracellular signals induces the translocation of *Akt* from the cytoplasm to the cell membrane. Subsequently, phosphorylation of two specific residues (Ser308 and Thr473) on *Akt* that localized at the cell membrane resulting in the complete activation of *Akt*. *Akt* plays a pivotal regulatory role in various cellular processes, notably apoptosis, proliferation, and differentiation. [28] Moreover, the PI3K/AKT pathway can activate the mTOR pathway, which participates in the synthesis of diverse cyclins, including cyclin D1, thereby promoting the cell growth. [29] This cumulative evidence suggests that the JAK/STAT pathway can interconnect with other pathways such as mTOR and MAPK, regulating downstream functions like the cell cycle and apoptosis. However, the specific role of the JAK/STAT pathway and its interconnected pathways in shrimp remains unclear. Additionally, limited information is available regarding the mechanism through which WSSV regulates the cell cycle and apoptosis in shrimp. In this study, we investigated the function of the JAK/PI3K/AKT interconnected pathway in shrimp in response to WSSV infection. Upon WSSV infection, the expressions of *PvJAK*, *PvAKT, PvDomeless*, *PvPI3K*, and *PvmTOR* were significantly upregulated compared to the control group. These elevated transcription levels during WSSV challenge suggest a substantial role for the JAK/PI3K/AKT pathway in the shrimp response to viral infection.

In recent years, a growing body of evidence indicates a pivotal role for miRNAs in regulating genes associated with both host organisms and pathogenic viruses during viral infections. [11] Numerous studies have been conducted on the effect of miRNAs on viral pathogenesis in mammals, and the results indicate that the regulation of cellular responses and the outcome of virus infections are strongly influenced by both virus-induced cellular miRNAs and virus-encoded miRNAs. [30] By using bioinformatics analysis, we identified some differentially expressed miRNAs (DEMs) capable of targeting genes within the JAK/PI3K/AKT pathway. From a total of 20 miRNAs, we specifically focused on those targeting *PvJAK* for expression analysis. Our findings reveal that 5 miRNAs exhibit a negative expression correlation with *PvJAK*, suggesting their potential as target miRNAs. The *in vitro* experiment result of interaction between Pva-miR-18 and *PvJAK* suggests that Pva-miR-18 may target *PvJAK* during WSSV infection. The upregulation of expression further indicates a potential regulatory mechanism of *PvJAK* during viral infection. This discovery aligns with previous studies that have identified miRNAs as crucial regulators of host-virus interactions in WSSV-infected shrimp, as demonstrated by HuangT*et al.*[31] Also, the differential expression of specific miRNAs in response to viral infection in shrimp shows the dynamic and intricate nature of host-virus interaction. [32]

Conclusion

The exploration of the molecular mechanisms of shrimp antiviral responses is necessary for understanding of the specific role played by the interconnected JAK/STAT pathway in the context of WSSV infection. This study revealed that WSSV can regulate JAK/PI3K/AKT pathway during infection. Additionally, among predicted miRNA, *PvJAK* might be regulated by Pva-miR-18 as suggested by an *in vitro* assay. Cumulatively, we suggests the potential regulatory of JAK/PI3K/AKT pathway through miRNA . It is crucial to emphasize that further investigations, incorporating functional assays and detailed characterization of the Pv-miR-18 and *PvJAK* interaction, are essential for validating and fully comprehending the implications of this regulatory relationship.

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Conflict of interest statement

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Data sharing statement

All data generated or analyzed during the present study are included in this published article. Further details are available for noncommercial purposes from the corresponding author on reasonable request.

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