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A histone K-lysine acetyltransferase *CqKAT2A-like* gene promotes white spot syndrome virus infection by enhancing histone H3 acetylation in red claw crayfish *Cherax quadricarinatus*

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ABSTRACT

In contrast to that hypoacetylation of histones is associated with condensed chromatin and gene silencing, the hyperacetylation of histones can promote an "open chromatin" conformation and transcriptional activation, which is recruited by some viruses to enhance the viral genome replication in host cells. However, the function of histone acetylation modification in the infection of white spot syndrome virus (WSSV), one of the most virulent pathogens for crustaceans like shrimp and crayfish at present, is still unknown. Previously, we found that the transcript of a histone K-Lysine acetyltransferase *CqKAT2A-like* gene was down-regulated in a differentially expressed transcriptome library of the haematopietic tissue (Hpt) cells from red claw crayfish *Cherax quadricarinatus* upon WSSV infection at 12 hpi. To further reveal its possible role in anti-WSSV response, *CqKAT2A-like* gene was then identified with an open reading frame (ORF) of 2523 bp encoding 840 amino acids, which contained a conserved PCAF-N domain, acetyltransf1 domain and bromo domain. Gene expression analysis showed that *CqKAT2A-like* was distributed in all tissues examined with high presence in haemocyte and muscle, and the transcript was significantly down-regulated after WSSV infection in Hpt cells. Furthermore, the level of histone H3 acetylation (H3ac) was strongly reduced by gene silencing of *CqKAT2A-like*, which was accompanied with the significantly decreased gene expression of WSSV in Hpt cells, suggesting that *CqKAT2A-like* gene can promote the activity H3ac and the replication of WSSV. When the H3ac was induced by histone deacetyltransferase inhibitor TSA, the transcription of WSSV genes including both IE1 and VP28 genes was significantly increased, indicating that H3ac participated in WSSV infection in Hpt cells. Taken together, these data suggest that *CqKAT2A-like* gene might promote the replication of WSSV by regulating H3ac, which sheds new light on the pathogenesis of WSSV in crustaceans.

1. Introduction

Eukaryotic DNA is packaged as a protein-DNA complex called chromatin. The basic structure of chromatin is the nucleosome, which consists of core histone proteins (H2A, H2B, H3, and H4) wrapped by 147 bp of DNA (Luger et al., 1997). Chromatin is a dynamic structure that not only helps to package the entire eukaryotic genome into the confines of the nucleus but also regulates the accessibility of DNA for transcription, recombination, DNA repair and replication (Venkatesh and Workman, 2015). Through diverse epigenetic modifications, such as acetylation, phosphorylation, methylation, ubiquitylation and sumoylation, the chromatin is dynamically organized into regions of either loosely packaged actively transcribed chromatin (euchromatin) or highly condensed transcriptionally repressed chromatin (heterochromatin) (Bannister and Kouzarides, 2011; Smolle and Workman, 2013; Venkatesh and Workman, 2015). Histone acetylation modification is a relatively typical way of epigenetic modifications, and the acetylation of certain lysine (K) residues in histones H3 is generally an indicator of transcriptionally active chromatin (Mizzen and Allis, 1998). At present, more and more evidence has clarified the significance of epigenetic modification in viral gene transcription or virus productive infection. For example, the infection of influenza A virus was obviously attenuated when the histone acetylation was inhibited in A549 and 293T cells (Zhao et al., 2015). During parvovirus infection, the acetylation of histones on parvoviral DNA is essential for viral gene expression and the completion of the viral life cycle (Mantyla et al.,

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2016). Besides, Bovine herpesvirus 1 (BoHV-1) infection significantly reduced histone H3 acetylation, which was well correlated with the pronounced depletion of HATs in Madin-Darby bovine kidney (MDBK) cells (Zhu et al., 2018).

Generally, histone acetylation levels are regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Yang and Seto, 2007). For HATs, there are three major families: general control non-derepressible 5 (Gcn5)-related N-acetyltransferases (GNATs), p300/CBP and MYST proteins (Lee and Workman, 2007; Roth et al., 2001; Sterner and Berger, 2000), which are involved in a variety of biological functions. It is worth noting that histone lysine acetyltransferases KAT2A (also known as Gcn5) was initially identified as member of the GNATs, which positively regulates the transcription of amino acid biosynthetic genes in yeast (Brownell et al., 1996). Previous studies on KAT2A were mainly focused on KAT2A acetylate preferentially histone H3 and minor H4, leading to changes in chromatin structure. There are also many studies to prove that KAT2A plays a role in diverse biological processes, such as chromatin remodelling, transcriptional regulation, DNA replication, DNA repair, cell cycle progression and cell death (Kelly et al., 2009; Meritxell et al., 2014; Paolinelli et al., 2009; Xie et al., 2002). Furthermore, KAT2A is also associated with replication of some viruses. KAT2A participates in the integration step of human immunodeficiency virus-1 (HIV-1) replication cycle (Terreni et al., 2010). During adenovirus infection, KAT2A may act as a new negative regulator of transactivation by adenovirus early region 1A (adenovirus E1A) (Ablack et al., 2012). However, how KAT2A function in DNA virus infection has been rarely reported in invertebrate.

White spot syndrome virus (WSSV) is a rod-shaped, circular double-stranded DNA virus, and also is one of the most virulent pathogens that harms crustaceans culture like shrimp and crayfish (Peng et al., 2001). The genome of WSSV is around 300 kb (Escobedo-Bonilla et al., 2008; van Hulten et al., 2001; Yang et al., 2001), with a wide range of hosts in crustaceans, which can cause up to 90–100% cumulative mortality in cultured shrimp and crayfish within 3–7 days (Lightner, 1996; Escobedo-Bonilla et al., 2008). During the viral replication cycle, the WSSV genes can be roughly divided into three categories: immediate early (IE) genes, early genes and late genes (Marks et al., 2005). Previous studies have shown that WSSV IE genes can hijack host transcription factors to promote viral transcription, such as Relish, signal transducer and activator of transcription (STAT), activating transcription factor 4 (ATF4), x box binding protein 1 (XBP1), kruppel-like factor (KLF), c-Fos and c-Jun (Chang et al., 2012; Huang et al., 2010; Li et al., 2013, 2015; Liu et al., 2007). However, the effect of histone post-translation modification on the transcription of WSSV in host cells has not been reported. In previous histone-related research on WSSV infection, Wang et al. (2008) discovered that ICP11, a highly expressed protein encoded by WSSV in the early stage of viral infection, is a histone-binding DNA mimic, which can disrupt nucleosome assembly (Wang et al., 2008). To further reveal the histone modification related effect on WSSV infection, in this study, we used the methods of RNA interference and inhibitor for studying the mechanisms of histone K-lysine acetyltransferase KAT2A and the status of histone H3 acetylation, as well as their role in WSSV infection in red claw crayfish, *Cherax quadricarinatus*. For the first time we identified a histone K-lysine acetyltransferase KAT2A, which was found to promote the replication of WSSV in haematopietic tissue (Hpt) cells by enhancing the histone H3 acetylation (H3ac) in a crustacean the red claw crayfish. These data will contribute to understand the molecular mechanism of histone acetylation in antiviral immunity in invertebrate.

2. Materials and methods

2.1. Animals, Hpt cell cultures and virus

The healthy red claw crayfish *C. quadricarinatus* free of WSSV were purchased from Source Sentai Agricultural Science and Technology Co., Ltd of Zhangzhou, Fujian Province, China. The crayfish were acclimatized in freshwater tanks and the ambient temperature was 26 °C.

Hpt cells were prepared from haematopoietic tissue of intermolting male *C. quadricarinatus* and cultured as described by Söderhäll et al. (2003) and Liu et al. (2011) (Liu et al., 2011; Söderhäll et al., 2003). Hpt cells were cultured in 24-well plates (5×10^5 cells/500 μl) and 96-well plates (10^5 cells/100 μl) at 20 °C, and crayfish plasma containing crude astatine were added after cell attachment for about 30 min (Söderhäll et al., 2005). WSSV was kindly provided by Prof. Xun Xu (Third Institute of Oceanography, Ministry of Natural Resources, Xiamen, Fujian, China). The virus was prepared as described by Xie et al. (2005) and quantified by absolute quantification with PCR (Xie et al., 2005).

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from different tissues of crayfish using the TRIzol reagent (Roche, Mannheim, Germany), according to the manufacturer's instructions. RNase-Free DNase I (Ambion, USA) was used to eliminate genome DNA contamination in the extracted RNA. First strand cDNA synthesis was performed by using PrimeScript™ RT Reagent Kit (TaKaRa, Japan) following the manufacturer's instructions.

2.3. Quantitative real-time PCR (qRT-PCR) and Western blotting

The SYBR premix (Roche, Mannheim, Germany) was used to perform real-time thermal cycle (Bio-Rad, USA) with a total volume of 20 μl, including 10 μl of SYBR® Green qPCR ReadyMix, 1 μl of cDNA, 7 μl of nuclease-free water, 1 μl of positive and reverse primers. The procedure of qRT-PCR was as following: 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each sample had three replicates. The obtained data was evaluated by using the $2^{-\Delta\Delta Ct}$ method as described previously (Kumar et al., 2016) and statistically analyzed; significant differences in the unpaired sample *t*-test were accepted at $p < 0.05$.

For Western blotting assay, protein samples were separated through 12% SDS-PAGE and then transferred to polyvinylidene fluoride membrane. The membrane was blocked by 5% skimmed milk in TBST-buffer (10 Mm Tris-HCl, pH 8.0, 150 mM NaCl, 1% Tween 20) for 2 h, and then incubated with 1:3000 diluted antiserum with tentative proteins (Cqβ-actin, VP28 and H3ac) in TBST of 1% skimmed milk for 1 h at room temperature or 4 °C overnight. After washing three times in TBST, the membrane was immunoblotting detected with HRP-conjugated goat anti-rabbit or anti-mouse IgG (1:5000 diluted in TBST). After incubation for 1 h at room temperature, the unbounded IgG was washed away and then followed by an enhanced chemiluminescence system was used for detection.

2.4. The cDNA cloning and sequence analysis

The open reading frame (ORF) of *CqKAT2A-like* cDNA sequence of *C. quadricarinatus* was isolated from a transcriptome library of Hpt cells post WSSV infection in our lab (Liu et al., 2011), and the primers (*CqKAT2A-like*-F, *CqKAT2A-like* –R; Table 1) were designed to amplify the cDNA of *CqKAT2A-like* gene. All amplified PCR products were gel-purified using a Gel Extraction Kit (Sangon Biotech, Co., Ltd., Shanghai, China). The similarity analysis of *CqKAT2A-like* gene sequence was conducted by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi/). The corresponding cDNA was conceptually translated, and the deduced proteins were predicted by using ExPASy (http://web.expasy.org/translate/). The domain architecture prediction of the proteins was performed by using SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de).

Table 1
Primers used in this study.

Primers	Sequence (5′-3′)
CqKAT2A-like-F	ATGGCATCAAGGGATAGTGAAG
CqKAT2A-like-R	CTATGTCTTCTCTTTGTTTTCCAAGA
CqKAT2A-like-qRT-F	AAATAGAGACTGAGGACCTGGGAG
CqKAT2A-like-qRT-R	GACGGAATGGAGTTTGAAGATGT
16S–F	AATGGTTGGACGAGAAGGAA
16S-R	CCAACTAAACACCCTGCTGATA
IE1-qRT-F	CTGGCACAACAACAGACCCTACC
IE1-qRT-R	GGCTAGCGAAGTAAAATATCCCCC
VP28-qRT-F	AAACCTCCGCATTCCTGT
VP28-qRT-R	GTGCCAACTTCATCCTCATC
ds*CqKAT2A-like*-F	TAATACGACTCACTATAGGGTTCTTCACTGCCTCAATCACTG
ds*CqKAT2A-like*-R	TAATACGACTCACTATAGGGTCATCTCCTTCTCGCTTCTCTC
dsGFP-F	TAATACGACTCACTATAGGGCGACGTAAACGGCCACAAGT
dsGFP-R	TAATACGACTCACTATAGGGTTCTTGTACAGCTCGTCCATGC

2.5. Tissue distribution analysis

The mRNA tissue distribution was analyzed by using qRT-PCR with the primers *CqKAT2A-like*-qRT-F and *CqKAT2A-like*-qRT-R (Table 1). The cDNA from Hpt, heart, nerves, gonads, muscle, haemocytes, intestines, gills, hepatopancreas, stomach, eyestalk and epithelial tissue was used in this assay. The 16S ribosomal gene was used as the control with the primers 16S–F and 16S-R (Table 1).

2.6. RNA interference and WSSV infection in Hpt cells

The sequence for dsRNA of *CqKAT2A-like* gene was amplified by the primers ds*CqKAT2A-like*-F and ds*CqKAT2A-like*-R linked to the T7 promoter (Table 1) and then used as templates for the synthesis of dsRNA by using the MegaScript kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Green fluorescent protein (GFP) served as the control and dsGFP RNA was synthesized in the same way, which was used as a control treatment and amplified by using the primers dsGFP-F and dsGFP-R (Table 1).

The RNA interference (RNAi) assay was performed as described in previous reports (Chen et al., 2016). For dsRNA transfection, 400 ng of dsRNA/well (24-well plates) and 100 ng of dsRNA/well (96-well plates) in RNase-free water was mixed with Cellfectin® II Reagent (Life Technologies, USA), maintained for 10 min at room temperature, appended with medium and added into the cell wells. Half of the medium was replaced with fresh medium to reduce the toxic effects of Cellfectin after 3 h. The dsRNA transfection was repeated once to improve RNAi efficiency at one day after the first transfection as described above.

WSSV infection was performed at 24 h after the second dsRNA transfection as mentioned above. WSSV infection was performed in 24-well plates and 96-well plates with MOI = 1 (multiple infection rate of 1) for determination of the transcription of WSSV genes. The cells in 24-well plates were collected with lysis after WSSV infection for 3, 6 and 12 h. The total RNA was extracted by using the GenElute™ Mammalian Total RNA miniprep kit (Sigma, USA) according to the protocol and cDNA synthesis was described in Section 2.2. Some genes, including the gene expression of *CqKAT2A-like*, viral gene IE1 and VP28 of WSSV were detected by qRT-PCR with specific primers (Table 1) after WSSV infection. For the cells in 96-well plates, the samples were collected and detected by Western blotting which was described in Section 2.3, and Cqβ-actin was used as the internal reference. The experiments were biologically repeated at least for three times.

2.7. Immunofluorescence assay

Hpt cells were cultured on the 96-well plate. After twice treatment with dsRNA, Hpt cells were fixed with 4% paraformaldehyde for 30 min followed by permeabilization with 0.2% Triton X-100 for 30 min. After blocking with 5% goat serum for 1 h, Hpt cells were washed with PBS for three times followed by incubation with the primary antibody H3ac (Merck, Germany, 1:300) diluted with 0.1% goat serum overnight at 4 °C. After washing with PBS for three times, Alexa Fluor 488 goat anti-rabbit IgG (Earthox, USA, 1:500) was added and incubated for 2 h at room temperature. The cell nucleus was stained with 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI) for 10 min at room temperature, and cell imaging was collected with an LSM 780 confocal fluorescence microscope (Zeiss, Germany).

2.8. Trichostatin A (TSA) inhibitor assay in Hpt cells

TSA is an efficient inhibitor of histone deacetyltransferase (HDAC) purchased from MedChemExpress (USA). Hpt cells were treated with TSA dissolved in dimethyl sulfoxide (DMSO) for 12 h. The same volume of DMSO was used as the control treatment. Then WSSV was inoculated into cell cultures with a MOI of 1. Subsequently, cells were collected for RNA extraction at 3, 6 and 12 hpi for RT-PCR accordingly and the proteins were prepared for Western blotting at 6 hpi, respectively, after WSSV infection.

3. Results and discussion

3.1. Gene cloning and bioinformatics analysis of CqKAT2A-like in red claw crayfish

Previously, we found that a down-regulation of *CqKAT2A-like* gene in transcription library from Hpt cells upon WSSV infection at 12 h (unpublished data), indicating that *CqKAT2A-like* gene was involved in cellular response against WSSV infection. However, the functional mechanism of *CqKAT2A-like* gene in WSSV infection was yet unknown which needs for further studies. In this regard, we then cloned the open reading frame (ORF) sequence of *CqKAT2A-like* gene (Genbank No: MN508816) according to a partial cDNA sequence from our transcriptome data. The ORF of *CqKAT2A-like* gene was 2523 bp, encoding 840 amino acids(Fig. 1A). The SMART analysis showed that the deduced *CqKAT2A-like* protein contains a P300/CBP-associated factor (PCAF), an acetyltransf-1 domain and a bromo domain (Fig. 1B). These three domains suggest that *CqKAT2A-like* gene may belong to histone acetyltransferase GCN5/PCAF family (Marmorstein and Roth, 2001). Multiple sequences alignment showed that *CqKAT2A-like* gene had 86% identity of amino acid sequence to *KAT2A* of *Penaeus vannamei* (XP_027221844.1) (Fig. 1C), around 54% with *Homo sapiens* (NP_066564.2), *Mus musculus* (NP_064388.2) and *Danio rerio* (XP_009297593.1), and 45% with *Drosophila persimilis* (XP_026845620.1). This molecular characteristic suggests that *CqKAT2A-like* gene may play a conservative role in crustacean and other species.

3.2. Tissue distribution and expression profile of CqKAT2A-like gene post WSSV infection in red claw crayfish

To reveal the possible role of *CqKAT2A-like* gene related to innate immunity, the tissue distribution of *CqKAT2A-like* gene was determined. As shown in Fig. 2A, *CqKAT2A-like* gene mRNA was expressed in all examined tissues with the highest expression in muscle followed by less expression in haemocyte, but lowest expression in intestines and gill tissue, suggesting that *CqKAT2A-like* gene was a constitutively and widely expressed gene in red claw crayfish. In consideration to that KAT2A has multiple functions, such as chromatin remodelling, transcriptional regulation, DNA replication, DNA repair,

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1     ATGGCATCAAGGGATAGTGAAGACACAGGCAGTGGTTCTGGTGCTTCAGGTTTGGTCCATCATCAACACCAAAACTCTCAGACTTCTAGTATGACAGCTGCAGTT
1      M  A  S  R  D  S  E  D  T  G  S  G  S  G  A  S  G  L  V  H  H  Q  H  Q  N  S  Q  T  S  S  M  T  A  A  V
106   GGAGCTGCAGGAGGAGGAGCAGGATCAGGAGGAGGGATGCCTGTTGGAGCAGTTGCTGCAGGCAGCTCTCAAGCAGCAGGGGCAGGAGAAGCAGCTGGGAGAGTT
36     G  A  A  G  G  G  A  G  S  G  G  G  M  P  V  G  A  V  A  A  G  S  S  Q  A  A  G  A  G  E  A  A  G  R  V
211   AGCAATGTACAGCGTATCCAGCAAAAGAAAGCACAAGTGAGAGCTTGGCCCCGGGACAAGAAGCTGGAGAAGCTGGCCATATATTCTTCATGTAGGGCTGATGAA
71     S  N  V  Q  R  I  Q  Q  K  K  A  Q  V  R  A  W  P  R  D  K  K  L  E  K  L  A  I  Y  S  S  C  R  A  D  E
316   AACTGCAGGTGTAATGGGTGGAAGAATCCAACACCACCAGCACACCCTGCACGGCCAGACACACCCCAGCCTGCTGCACCTCCGTCTCAGCCGTGTCGCAGCTGC
106    N  C  R  C  N  G  W  K  N  P  T  P  P  A  H  P  A  R  P  D  T  P  Q  P  A  A  P  P  S  Q  P  C  R  S  C
421   ACACATACCTTAGGTGACCATGTAAGTCACCTTGAATCTGCTGGAGATGATGATTTGGATCGCTTACTCTCCATCGTTGTTGACGTGGAGAATCTCTTCATTTGT
141    T  H  T  L  G  D  H  V  S  H  L  E  S  A  G  D  D  D  L  D  R  L  L  S  I  V  V  D  V  E  N  L  F  I  C
526   GTTCACCGAGAGGAGGATCCAGATACCAAGCAGGTGTATTCTTATCTCTTCCGCCGTCTTCGAAAATGCATTTTGCAAGTCAGTCCCCCAACGGTAGAAGGACCC
176    V  H  R  E  E  D  P  D  T  K  Q  V  Y  S  Y  L  F  R  R  L  R  K  C  I  L  Q  V  S  P  P  T  V  E  G  P
631   CTGGGCACCCCACCATTTGAACGACCTTCAATTTTCAAGGGTGTAACCAACTTCGTTCTCCACAAGTTTAATCATCTTCAACAAAAGGACTTCCAGATGATGAGT
211    L  G  T  P  P  F  E  R  P  S  I  F  K  G  V  T  N  F  V  L  H  K  F  N  H  L  Q  Q  K  D  F  Q  M  M  S
736   GACTTGGCCAAGATGTTTCTTCACTGCCTCAATCACTGGCGTCTAGAAACTCCATCTGCCAGACGAGCTCACACAACTGTTGAAGAAAGTTCAGCTTATAAAGTC
246    D  L  A  K  M  F  L  H  C  L  N  H  W  R  L  E  T  P  S  A  R  R  A  H  T  T  V  E  E  S  S  A  Y  K  V
841   AATTATACAAGGTGGCTGTGTTTTAGCTATGTCCCACAACTGTGTGACTCACTCCCTCACCATGAAACTACAACAATATTTGGAAGGACTTTCCTGCGTGCTGTT
281    N  Y  T  R  W  L  C  F  S  Y  V  P  Q  L  C  D  S  L  P  H  H  E  T  T  T  I  F  G  R  T  F  L  R  A  V
946   TTCCACACTTTACGAAAGCAATTGCTTGATAAATTTCGAGCTGAGAAAGATAAAATGCCCGCTGAGAAGAAGCTGCTGCTTCTTAATCATTTTCCCAGATTCTTG
316    F  H  T  L  R  K  Q  L  L  D  K  F  R  A  E  K  D  K  M  P  A  E  K  K  L  L  L  L  N  H  F  P  R  F  L
1051  TCAATGCTTGAAGAAGAAGTTTACTCAAATACTTCTCCAATTTGGGATACTGACTACCGGCCTCAGTTACCTCCACACTTACATAACCAGGCTGCTGAAAGAGGA
351    S  M  L  E  E  E  V  Y  S  N  T  S  P  I  W  D  T  D  Y  R  P  Q  L  P  P  H  L  H  N  Q  A  A  E  R  G
1156  AGTACTGCTGCTCGGGGTGAATTTGAGAGGCTGTCAGTGCAGCCAGGGGAGGGTCAGTACACCACTGTTAGTCTGACTCCTGCTACAAGAAGACAGCGCCTTGAT
386    S  T  A  A  R  G  E  F  E  R  L  S  V  Q  P  G  E  G  Q  Y  T  T  V  S  L  T  P  A  T  R  R  Q  R  L  D
1261  GACAGAGGAGAGAAGCGAGAAGGAGATGATTTGTCAGAGAGCATGATCAATAAGAGAATAAAAATAGAGACTGAGGACCTGGGAGAGGAAACAGTGGCCGAGGTT
421    D  R  G  E  K  R  E  G  D  D  L  S  E  S  M  I  N  K  R  I  K  I  E  T  E  D  L  G  E  E  T  V  A  E  V
1366  GTTGCTACTATTACTGACCCAAGGAAGATGATTGGTCCAGAAGTTCTTTTCTCAGAGAATGCTGCTAGAGATGAGGCTGCCAAGCTAGAGGAACGAAAGGGTAAT
456    V  A  T  I  T  D  P  R  K  M  I  G  P  E  V  L  F  S  E  N  A  A  R  D  E  A  A  K  L  E  E  R  K  G  N
1471  ATAGAATTCCACGTCATTGGTAACTCCCTCACCCAGAAGGTTTCCAAACAGACAATGCTGTGGTTGATTGGTCTTCAGAATGTATTTTCTCATCAGCTTCCACGA
491    I  E  F  H  V  I  G  N  S  L  T  Q  K  V  S  K  Q  T  M  L  W  L  I  G  L  Q  N  V  F  S  H  Q  L  P  R
1576  ATGCCCAAAGACTATATAACACGACTTGTATTTGATCCGAAACATTGTACATTGGCATTAATCAAGGAGAATCGGCCGATTGGAGGTATATGCTTCCGGATGTTT
526    M  P  K  D  Y  I  T  R  L  V  F  D  P  K  H  C  T  L  A  L  I  K  E  N  R  P  I  G  G  I  C  F  R  M  F
1681  GCTGCCCAGGGATTTACAGAAATTGTTTTCTGTGCAGTTACATCCAATGAGCAAGTGAAAGGTTATGGTACCCACATGATGAATCACTTGAAAGATTATCATGTC
561    A  A  Q  G  F  T  E  I  V  F  C  A  V  T  S  N  E  Q  V  K  G  Y  G  T  H  M  M  N  H  L  K  D  Y  H  V
1786  AAGAATAATGTGCTTCACTTTCTCACCTTTGCTGATGAGTTTGCCATAGGGTACTTCAAGAAACAAGGGTTTAGCAAGGATATCCAACTGTCACGCTCTGTCTAC
596    K  N  N  V  L  H  F  L  T  F  A  D  E  F  A  I  G  Y  F  K  K  Q  G  F  S  K  D  I  Q  L  S  R  S  V  Y
1891  CAAGGTTACATCAAAGATTATGAGGGAGCAACACTTATGGGCTGTGAATTGAATCCAAACATTGTCTACACTGAGTTTACTGCTGTAATCAGAAGGCAGAAGGAG
631    Q  G  Y  I  K  D  Y  E  G  A  T  L  M  G  C  E  L  N  P  N  I  V  Y  T  E  F  T  A  V  I  R  R  Q  K  E
1996  ATCATTAAAAAACTGATAGAGAGGAAACAGAGTGAAATTCGTAAGGTGCATCCGGGGCTGACTTGCTTCAGAGATGGGGTGAGAGAAATTCCCATTGAAAGTATC
666    I  I  K  K  L  I  E  R  K  Q  S  E  I  R  K  V  H  P  G  L  T  C  F  R  D  G  V  R  E  I  P  I  E  S  I
2101  CCAGGAATTCGAGAAGCAGGCTGGAAGGGCCCAGCAACTCCCTCCCGTTCCTCCAGATCTCCTACCCATGAGCAGCAGCAGGACCCTGAATGTTTACACAGCATG
701    P  G  I  R  E  A  G  W  K  G  P  A  T  P  S  R  S  S  R  S  P  T  H  E  Q  Q  Q  D  P  E  C  L  H  S  M
2206  CTTAAGACACTTCTCTGCAATGTCAAGAGTCATGCTGCTGCCTGGCCGTTTCAAGTTCCTGTCGACCCAAATGAAGTGCCTGATTATTATGACCATATTAAATAT
736    L  K  T  L  L  C  N  V  K  S  H  A  A  A  W  P  F  Q  V  P  V  D  P  N  E  V  P  D  Y  Y  D  H  I  K  Y
2311  CCAATGGACTTGAAGACCATGACTGAACGCTTGAAAGCTAACTATTACGTAAATGCCCGACTCTTCAACGCTGATATGTTACGAATCTTCAAAAACTGTCGCTTT
771    P  M  D  L  K  T  M  T  E  R  L  K  A  N  Y  Y  V  N  A  R  L  F  N  A  D  M  L  R  I  F  K  N  C  R  F
2416  TATAATCACCCTGAGACAGAATATTATAAATGTGCAAATAACCTTGAAAAATACTACATAAACAAAGTGAAAGAACTTGGACTCTTGGAAAACAAAGAGAAGACA
806    Y  N  H  P  E  T  E  Y  Y  K  C  A  N  N  L  E  K  Y  Y  I  N  K  V  K  E  L  G  L  L  E  N  K  E  K  T
2521  TAG
841    *
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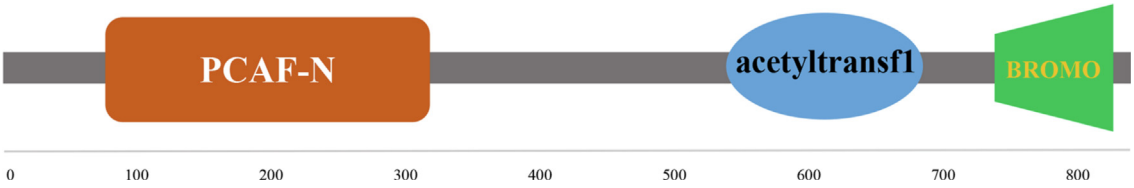
Fig. 1. The bioinformatics analysis of *CqKAT2A-like gene*. (A) The cDNA sequence and the deduced amino acid sequence of *CqKAT2A-like gene* from the red claw crayfish *Cherax quadricarinatus*. The associated domains are marked as: PCAF-N (horizontal lines), acetyltransf 1 (wavy lines), bromo (shadows). (B) Predicted protein domain structure of *CqKAT2A-like gene*. *CqKAT2A-like* protein contains PCAF-N domain, an acetyltransf 1 domain and a bromo domain. (C) Multiple sequences alignment of KAT2A. The amino acid sequences of KAT2A are from *C. quadricarinatus* and other species, including *P. vannamei* (XP_027221844.1), *H. sapiens* (NP_066564.2), *M. musculus* (NP_064388.2), *D. rerio* (XP_009297593.1), and *D. persimilis* (XP_026845620.1).



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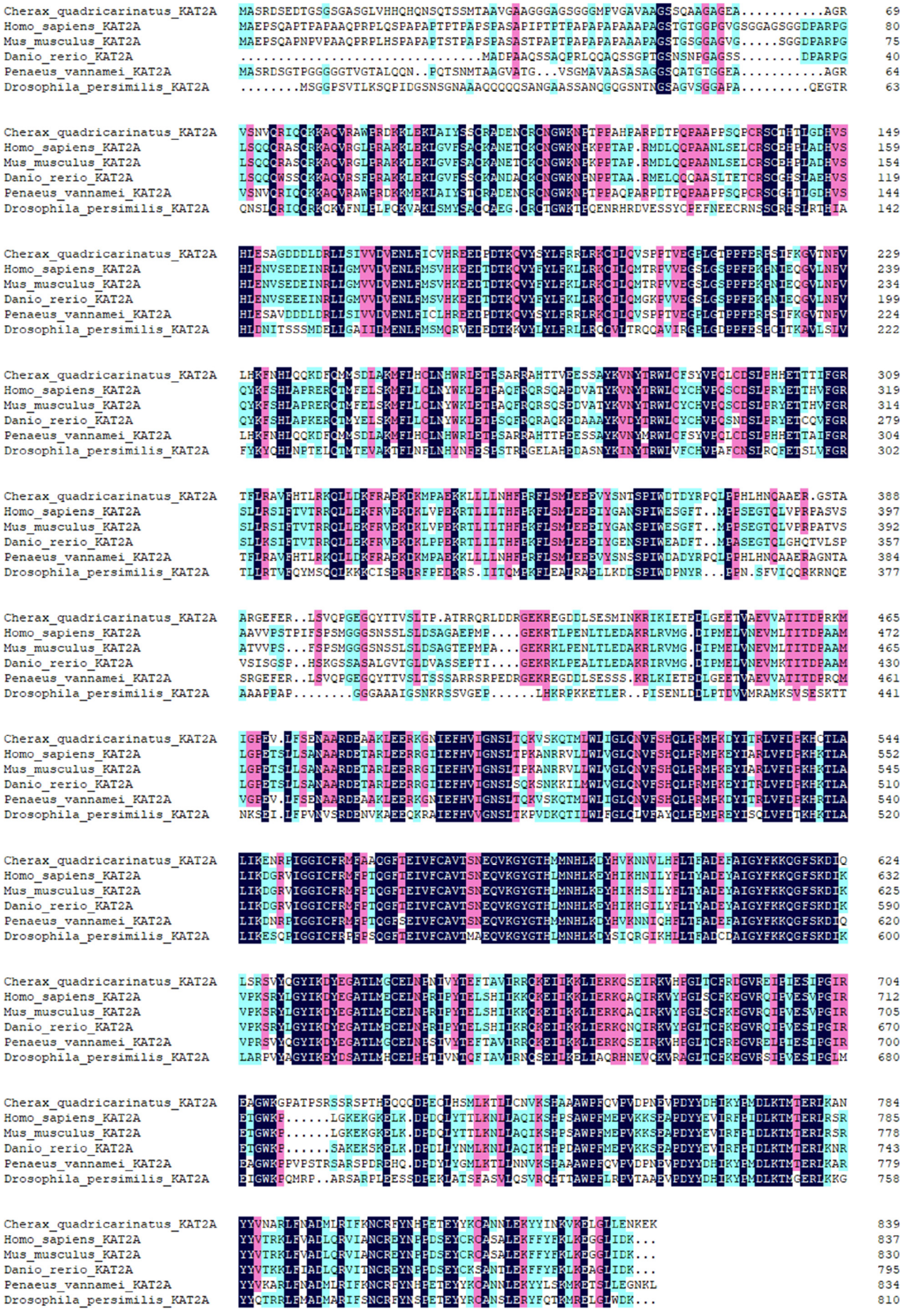

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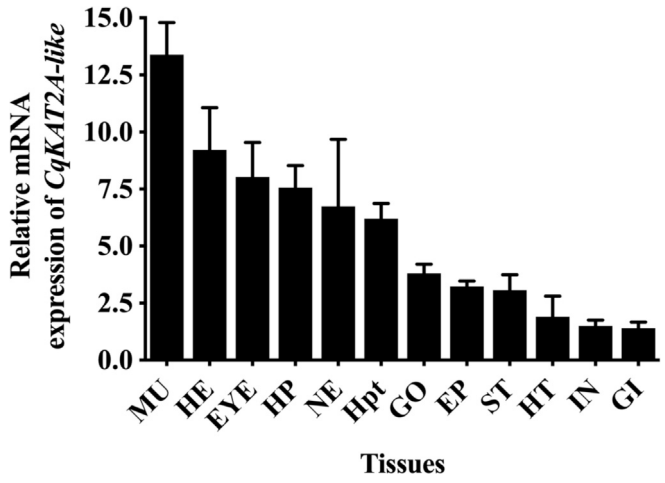
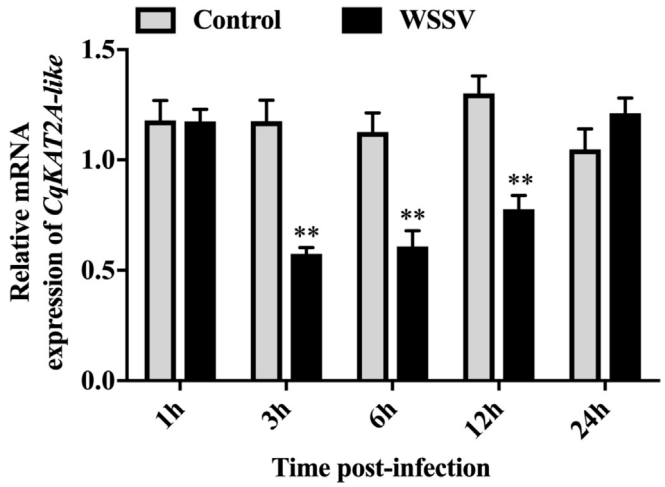


Fig. 2. Tissue distribution and expression profile of *CqKAT2A-like* gene post WSSV infection. (A)The mRNA expression profile of *CqKAT2A-like* gene in different tissues from *Cherax quadricarinatus*. HE: haemocyte; Hpt: haematopoietic tissue; IN: intestine; GI: gill; ST: stomach; GO: gonad; NE: nerve; EP: epithelial tissue; MU: muscle; HP: hepatopancreas; HT: heart; EYE: eyestalk. The relative transcript levels in HT was employed as the calibrator (value set as 1). (B) The mRNA expression profile of *CqKAT2A-like gene* in Hpt cells post WSSV infection. CFS treatment was used as control group. The relative transcript levels in control of 1 h were employed as the calibrator (value set as 1). The experiment was biologically repeated for three times. The asterisk indicated significant difference compared with those of controls (*$p < 0.05$, **$p < 0.01$).



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cell cycle progression and cell death (Kelly et al., 2009; Meritxell et al., 2014; Paolinelli et al., 2009; Xie et al., 2002), we speculated that the high expression of *CqKAT2A-like* gene in muscle might be related to the maintenance of normal physiological function. Importantly, haemocyte plays an important role in innate immune response against microbial infection in crustaceans, including microbial recognition, release of antimicrobial substances, phagocytosis and so on (Johansson et al., 2000). Therefore, the high expression of *CqKAT2A-like* gene in crayfish haemocyte suggests that *CqKAT2A-like* gene may participate in innate immune response to pathogenic infection such as WSSV, a highly infectious pathogen in crustaceans. To further study whether *CqKAT2A-like* gene was responsive to WSSV infection, the transcript of *CqKAT2A-like* was detected in Hpt cells cultured at different time points after WSSV infection. As shown in Fig. 2B, the expression of *CqKAT2A-like* gene was significantly down-regulated at 3, 6, 12 h in Hpt cells post WSSV infection, which was consistent with our previous finding in that

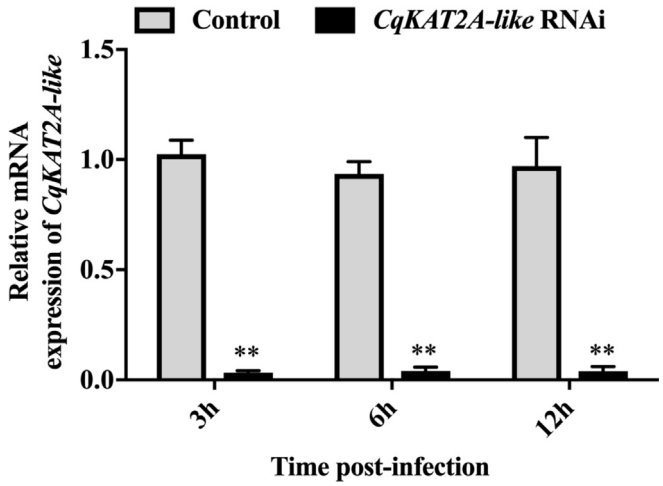


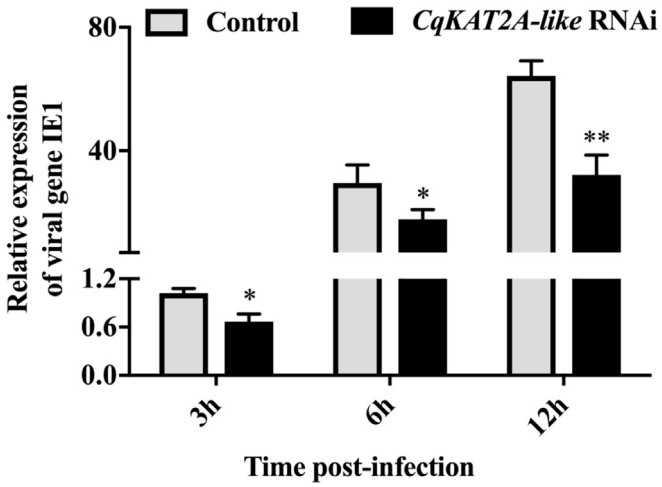
Fig. 3. WSSV replication is inhibited by gene silencing of *CqKAT2A-like* gene in Hpt cells. (A) The mRNA expression of *CqKAT2A-like* gene is significantly decreased after gene silencing of *CqKAT2A-like*. The mRNA expression of *CqKAT2A-like* gene during WSSV infection was determined by qRT-PCR. GFP dsRNA treatment was used as the control groups. (B) The viral gene expression of immediate early gene IE1 is decreased after gene silencing of *CqKAT2A-like*. The expression of IE1 was determined by qRT-PCR in *CqKAT2A-like* gene silenced Hpt cells post WSSV infection at 3, 6 and 12 hpi, respectively. The relative transcript levels in control of 3 h were employed as the calibrator (value set as 1). (C) The viral gene expression of late gene VP28 is decreased after gene silencing of *CqKAT2A-like*. The expression of VP28 was determined by qRT-PCR in *CqKAT2A-like* gene silenced Hpt cells post WSSV infection at 3, 6 and 12 hpi, respectively. The relative transcript levels in control of 3 h were employed as the calibrator (value set as 1). This experiment was repeated for three times. The asterisk indicated significant difference compared with those of controls (*$p < 0.05$, **$p < 0.01$).

CqKAT2A-like gene was significantly down-regulated in transcription library from Hpt cells at 12 h after WSSV infection (unpublished data). Hence, this finding clearly demonstrates that *CqKAT2A-like* gene was likely to be involved in the WSSV infection in red claw crayfish.

3.3. The WSSV replication is inhibited by gene silencing of CqKAT2A-like in crayfish Hpt cells

To verify whether *CqKAT2A-like* gene could affect WSSV infection in host cells, the *CqKAT2A-like* gene was silenced by using RNAi assay in Hpt cells followed by WSSV infection. As shown in Fig. 3A, the gene silencing efficiency of *CqKAT2A-like* was more than 90% in comparison to that of control cells. Importantly, the transcripts of viral immediate early genes IE1, and the late viral gene VP28 of WSSV exhibited significant decrease than those of control groups at 3, 6 and 12 hpi, respectively, in the Hpt cells after gene silencing of *CqKAT2A-like* (Fig. 3B and C). This result clearly implies that the presence of *CqKAT2A-like* gene is necessary for WSSV replication in crayfish Hpt cells.

As the key domain of an important histone acetyltransferase KAT2A, the bromo domain regulates site specificity of lysine acetylation on histone H3 (Cieniewicz et al., 2014). By analyzing the previous experimental results, we speculate that *CqKAT2A-like* gene promotes the replication of WSSV in Hpt cells, probably depending on acetylating histone H3 via bromo domain, which is worthy of further exploration. Furthermore, KAT2A is also associated with replication of some other viruses. For example, KAT2A inhibits peroxisome proliferator-activated receptor-γ coactivator 1α-induced enhancement of Hepatitis B virus transcription and replication through its acetyltransferase activity (Tian et al., 2013). Besides, KAT2A participates in the integration step of HIV-1 replication cycle (Terreni et al., 2010). Therefore, the genome replication of WSSV likely benefits from the presence of *CqKAT2A-like* gene in Hpt cells, probably via regulating the histone acetylation



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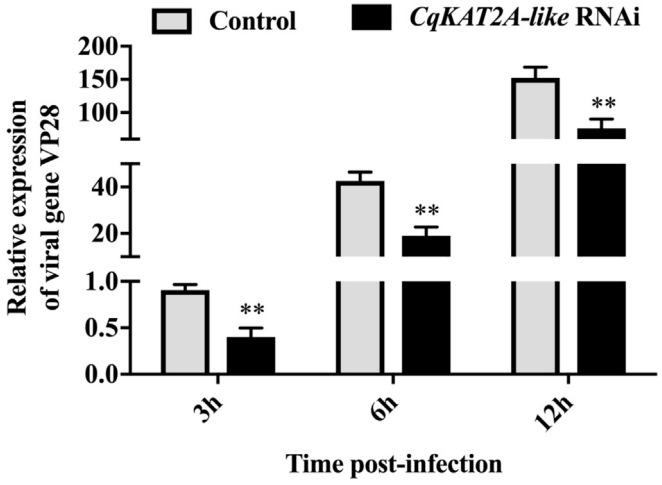

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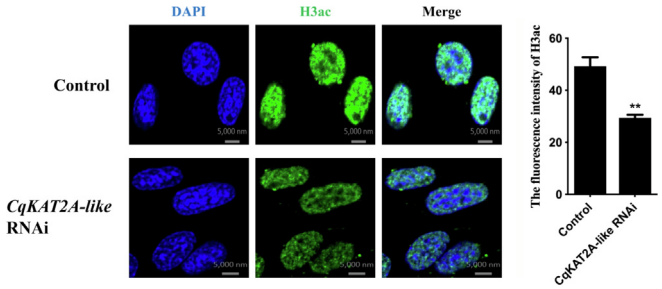
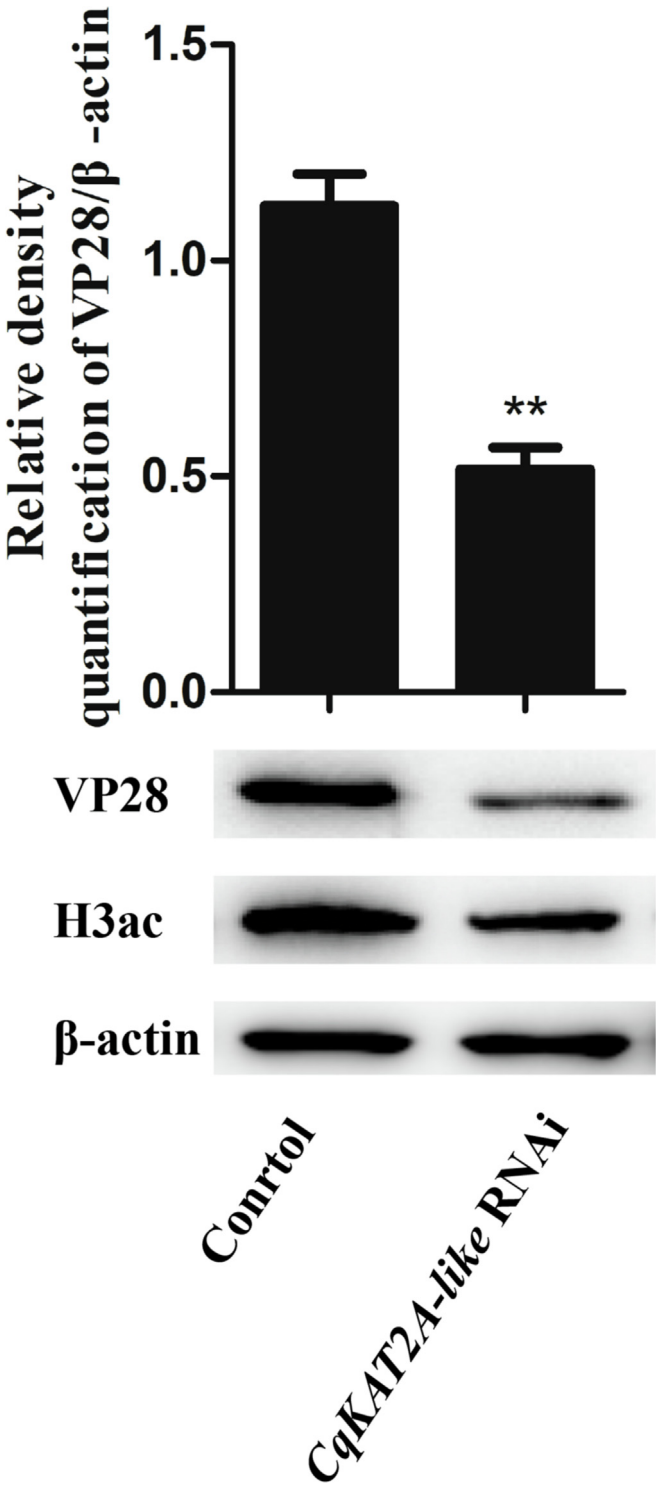


Fig. 4. Gene silencing of *CqKAT2A-like* results in reduced H3ac and WSSV replication in Hpt cells. (A) The activity of H3ac is decreased after gene silencing of *CqKAT2A-like*. The H3ac was determined by immunofluorescence assay in *CqKAT2A-like* gene silenced Hpt cells (left panel). Analysis of the relative fluorescence intensity (righ panel). GFP dsRNA treatment was used as the control groups. (B) The H3ac and viral envelope proteins VP28 are decreased after gene silencing of *CqKAT2A-like.* The main viral envelope proteins VP28 and H3ac were immunoblotted post WSSV infection at 6 h. The results are representative of three independent experiments. The asterisk indicates significant difference compared with those of controls ($*p < 0.05$, $**p < 0.01$).



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modification.

3.4. H3ac was reduced by gene silencing of CqKAT2A-like in crayfish Hpt cells

Generally, histone acetyltransferases (HATs) can promote histone acetylation levels in eukaryotes (Yang and Seto, 2007), and the enhancement of H3ac represents the “open chromatin” conformation and transcriptional activation (Görisch et al., 2005). The bromo domain of KAT2A regulates site specificity of lysine acetylation on histone

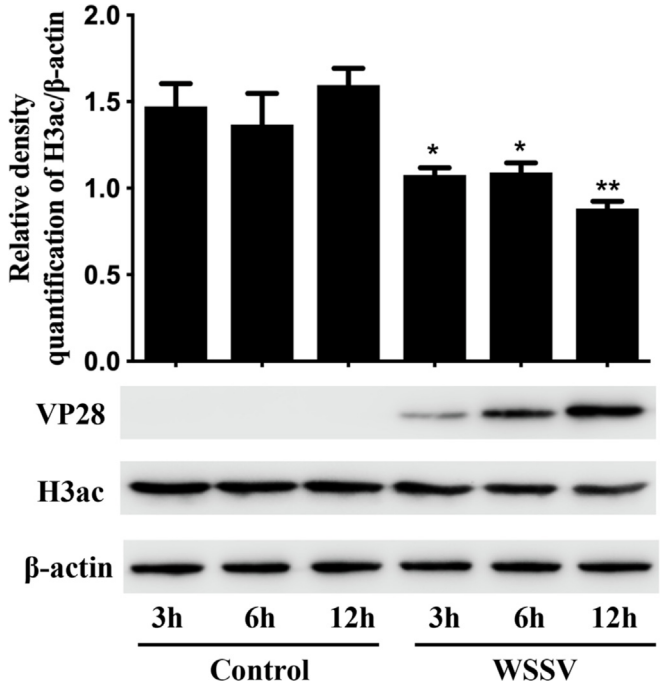
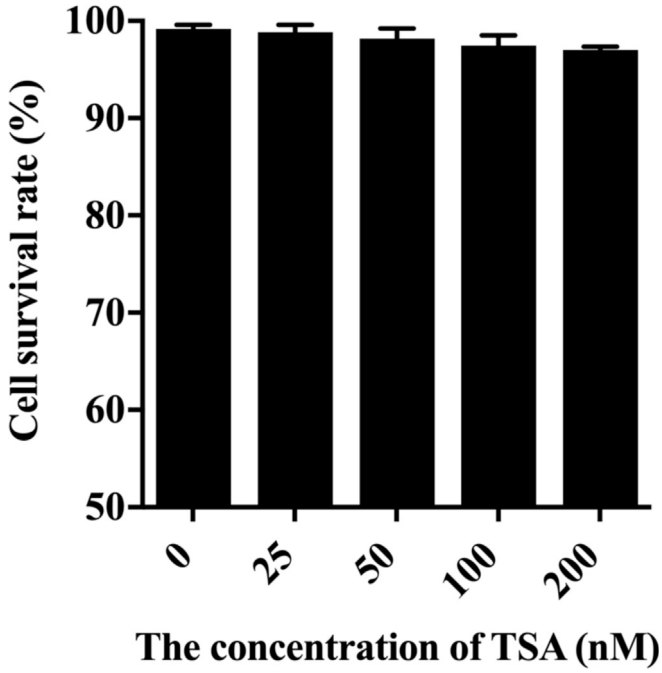


Fig. 5. Histone H3 acetylation is involved in WSSV infection. (A) The modification level of H3ac was decreased after WSSV infection. The H3ac was examined by Western blotting in Hpt cells after infection by WSSV for 3, 6 and 12 h, respectively, with a MOI of 1. (B) The H3ac is promoted by TSA treatment in Hpt cells. The H3ac was examined by Western blotting in Hpt cells treated by different concentrations of TSA (0, 25, 50, 100 and 200 nM) for 12 h. (C) TSA treatment does not affect the Hpt cell survival rate. The survival rate of Hpt cells was determined by MTT assay with different concentrations of TSA (0, 50, 100 and 200 nM) for 12 h. (D) The viral gene expression of immediate early gene IE1 is increased by promoted H3ac. The H3ac activity is promoted by TSA (25 nM) treatment in Hpt cells. The expression of IE1 was determined by qRT-PCR in Hpt cells treated with TSA followed by infection with WSSV at 3, 6 and 12 h, respectively. The relative transcript levels in control of 3 h were employed as the calibrator (value set as 1). (E) The viral gene expression of late gene VP28 is increased by promoted H3ac. The H3ac activity is promoted by TSA (25 nM) treatment in Hpt cells. The expression of VP28 was determined by qRT-PCR in Hpt cells treated with TSA followed by infection with WSSV at 3, 6 and 12 hpi, respectively. The relative transcript levels in control of 3 h were employed as the calibrator (value set as 1). (F) The expression of WSSV protein is increased by promoted H3ac activity. The H3ac and presence of viral protein VP28 were determined by Western blotting at 0 and 6 hpi, respectively, after WSSV infection in Hpt cells pretreated by TSA. The results were representative of three independent experiments. The asterisk indicates significant difference compared with those of controls (*$p < 0.05$, **$p < 0.01$).

(Cieniewicz et al., 2014). To prove whether *CqKAT2A-like* gene promoted lysine acetylation on histone H3, we detected the change of H3ac modification after gene silencing of *CqKAT2A-like*. As shown in Fig. 4A, gene silencing of *CqKAT2A-like* resulted in the clear decrease of H3ac fluorescence intensity by immunofluorescence analysis when compared with those of control groups. This result implies that the activity of *CqKAT2A-like* gene was positively correlated with H3ac. Similarly, the H3ac was decreased by the lacking of *CqKAT2A-like* gene activity, accompanied with the decreased amount of VP28 protein level, by Western blotting analysis (Fig. 4B). These data together prove that *CqKAT2A-like* gene can enhance the H3ac modification, accompanied with the increase in WSSV replication. As KAT2A is a highly conserved HAT that has been shown to acetylate multiple histone lysines in vitro, primarily lysine of histone H3, histones H4 and H2B (Cieniewicz et al., 2014), we thus speculate that WSSV can hijack *CqKAT2A-like* gene to regulate histone acetylation levels. However, it was not clear whether histone acetylation has a direct effect on the replication of WSSV, which needs to be further investigated.



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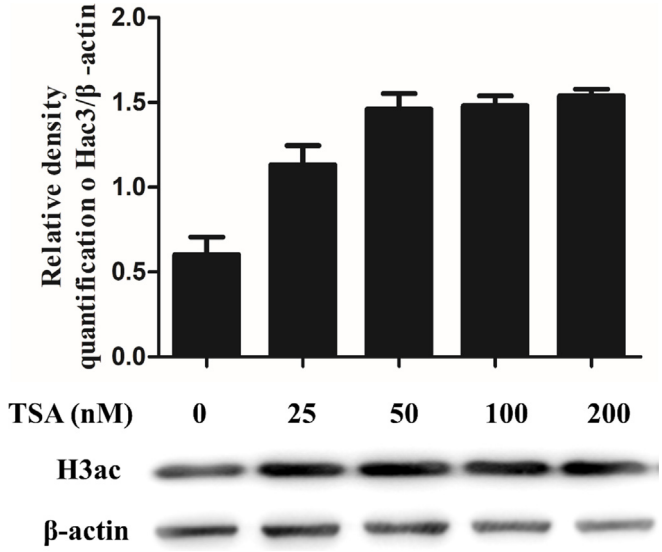

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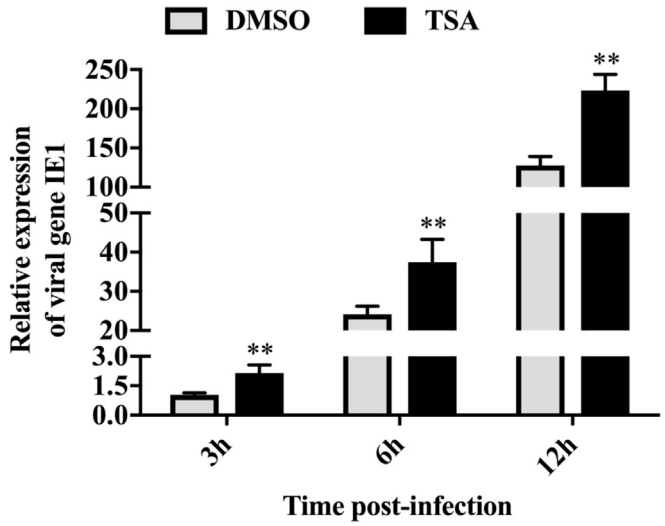
3.5. Histone H3 acetylation promotes WSSV replication in crayfish Hpt cells

Based on the previous results, we speculated that *CqKAT2A-like* gene promoted WSSV replication via acetylating histone. However, it is not clear whether H3ac activity was indeed associated with WSSV infection in red claw crayfish. To answer this question, we determined the modification level of H3ac in Hpt cells at 3, 6 and 12 hpi, respectively, post WSSV infection. The results showed that the modification level of H3ac was continuously decreased (Fig. 5A), indicating the involvement of H3ac on WSSV infection in Hpt cells. Furthermore, the H3ac activity was induced by using a histone deacetyltransferase inhibitor TSA in Hpt cells. As shown in Fig. 5B, TSA could effectively enhance the H3ac at concentrations of 25, 50, 100 and 200 nM, in which the cell survival rate could reach more than 95% at 25, 50, 100 and 200 nM of TSA accordingly (Fig. 5C), suggesting the non-toxic effect caused by TSA



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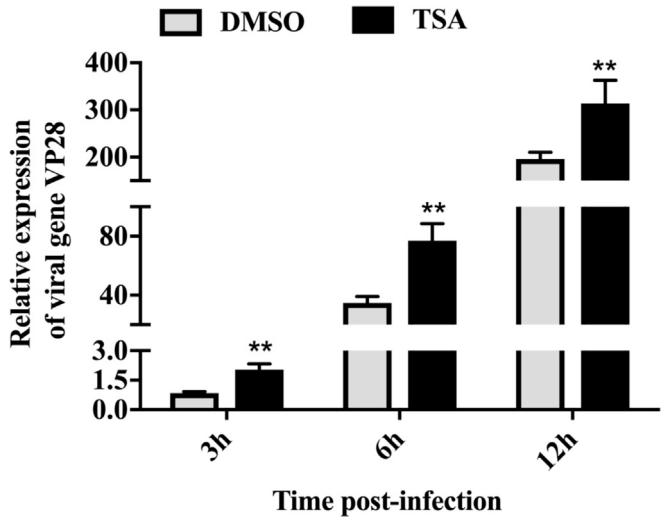

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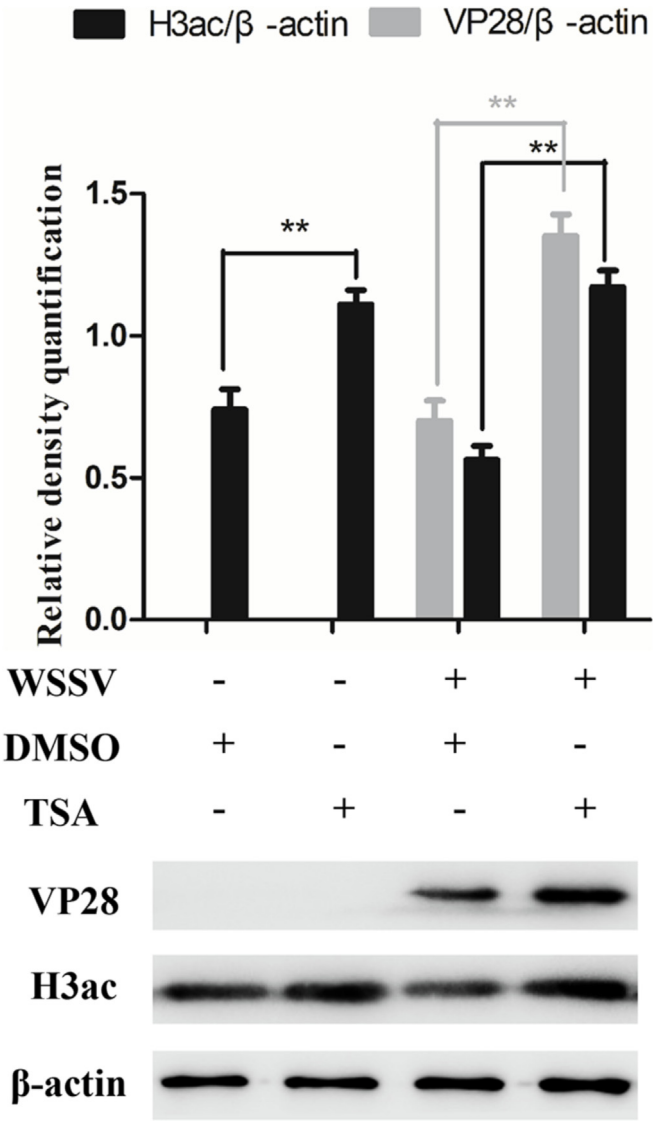

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treatment with these concentrations used. Interestingly, the transcript of both the viral gene IE1 and VP28 was significantly increased at 3, 6 and 12 hpi post WSSV infection in Hpt cells after promoting the H3ac activity with TSA treatment when compared to those of negative controls (Fig. 5D and E). The increased H3ac was also confirmed by Western blotting in Hpt cells, which was in correspondence to the increase of WSSV envelope proteins VP28 (Fig. 5F). These results suggested that the modification level of H3ac was positively correlated with the efficiency of WSSV replication. In other words, the increased H3ac could promote WSSV replication in Hpt cells.

As we know that the increased acetylation of histones is associated with activation of gene transcription, while low acetylation level is associated with inactivation of gene transcription (Görisch et al., 2005; Lo et al., 2003). WSSV can utilize different strategies to inhibit or subvert diverse intracellular signaling events. For example, the NF-κB related signaling pathways can be actively subverted to benefit WSSV infection, and a metabolic shift that resembles a Warburg effect is induced by WSSV to support its genome replication (Li et al., 2019). Hence, we speculate that WSSV might also utilize the activity of histone H3 acetylation to enhance the viral replication. The down-regulation of H3ac post WSSV infection in Hpt cells (Fig. 5A) may be caused by the reduction of *CqKAT2A-like* mRNA expression post WSSV infection (Fig. 2B). Compared with other viruses, some DNA viruses have a similar phenomenon, such as herpes simplex virus (HSV-1) and bovine herpesvirus 1 (BoHV-1). The viral proteins ICP0 and VP16 are required to enhance histone acetylation on the viral genome to achieve efficient gene expression of HSV-1 (Cliffe and Knipe, 2008; Hancock et al., 2010). Meanwhile, histone H3 acetylation (H3K9ac and H3K18ac) was significantly decreased in MDBK cells, and the BoHV-1 replication was positively correlated with histone acetyltransferase activity (Zhu et al., 2018). We speculate that the H3ac may be directly involved in the transcriptional regulation of WSSV genome, but its specific regulatory mechanism needs further investigation.

4. Conclusion

A histone acetyltransferase *CqKAT2A-like* gene was identified from a crustacean red claw crayfish, and the replication of WSSV gene and H3ac were both significantly decreased by gene silencing of *CqKAT2A-like* in Hpt cells. Meanwhile, histone H3 acetylation was shown to regulate the WSSV replication. Importantly, we demonstrate that *CqKAT2A-like* gene could promote the replication of WSSV in host cells by enhancing H3ac, which provides a possible antiviral target for the control of WSSV diseases in aquaculture.

Acknowledgements

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