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Identification of fish CMPK2 as an interferon stimulated gene against SVCV infection

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ABSTRACT

Cytidine/uridine monophosphate kinase 2 (CMPK2) is known as a nucleoside monophosphate kinase in mitochondria to maintains intracellular UTP/CTP, and could be induced by immunostimulants LPS and Poly (I:C) in mammals, suggesting its potential antiviral and antibacterial role. In this study, CMPK2 was cloned and characterized in Fathead minnow (FHM) cells. *In vivo* analysis of tissue distribution revealed that CMPK2 transcript was detected in all the tissues of zebrafish (*Danio rerio*) examined in this study, particularly abundant in liver, spleen and kidney. In addition, indirect immunofluorescence showed that CMPK2 was localized in the cytoplasm of FHM cells. Expression of CMPK2 mRNA was significantly up-regulated following challenge with Spring viraemia of carp virus (SVCV), poly(I:C), or zebrafish IFN1 and IFN3 both *in vitro* and *in vivo*. Furthermore, overexpression and RNA interference of CMPK2 in SVCV-infected FHM cells showed significantly antiviral effect. In summary, this study for the first time shows the presence and distribution of CMPK2 in different tissues of zebrafish, but also demonstrates its antiviral potential against SVCV infection *in vivo*. These new findings could contribute to explain the molecular mechanism of the CMPK2 mediated antiviral function.

1. Introduction

Innate immunity is the immune defense function of host animals in the process of development and evolution, which constitutes the first line of defense against infectious diseases and the basis of activating adaptive immunity. Previous researches showed that innate immunity is associated with the process of infection resistance to pathogenic microorganisms, anti-tumor, self-tolerance maintenance, and specific immunity [1–4].

Aquatic viral infection, as one of the most serious diseases endangering aquatic animals, is a major obstacle to the development of world aquaculture. Spring viraemia of carp virus (SVCV), which belongs to Rhabdoviridae family and subfamily of Vesiculovirus, is a single negative-stranded RNA virus that is able to cause large-scale spring viraemia of carp (SVC) in common carp in spring [5]. Thus, it is necessary to study the role of host innate immunity in combating SVCV infection in order to control SVC effectively.

In response to virus infection, a cell-autonomous antiviral program is created and immune-related cells are activated in fish. This antiviral program consists of type I interferon (IFN) production followed by the response to IFN signaling [6]. That is, the IFNs produced consecutively can induce the expression of hundreds of interferon stimulated genes (ISGs) to establish host antiviral systemin in mammals [7,8]. Similarly, a conserved fish IFN antiviral response is evoked following infection [9]. These fish IFNs can set up host antiviral state via inducing the expression of many fish IFN-stimulated genes (ISGs) such as PKR, ISG15, Mx and some novel ISGs including Gig1, PKZ and Gig2 [10–15].

In mammals, cytidine/uridine monophosphate kinase 2 (CMPK2), also known as TYKi/TMPK2, is a nucleoside monophosphate kinase. This kinase gene is located in mitochondria implicating in maintaining intracellular UTP/CTP, which is an ISG with the potential role to restrain HIV replication [16–19]. A recent study has indicated that CMPK2 primarily functions to ensure adequate CTP for viperin-mediated antiviral effect [20] and also plays a crucial role in NLRP3 activation and IL-1 β production [21]. CMPK2 has been cloned from Atlantic salmon and could be induced by immunostimulants LPS and

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Table 1
Primer and siRNA sequences used in this study.

Primer name	Sequence (5'–3')	Application
pcDNA4-CMPK2-F	CGGAATTCATGTTGACATCAAACCAAGTTG	Eukaryotic expression
pcDNA4-CMPK2-R	CCGCTCGAGCCAGTCCAGTTTCATATC	
pEGFP-DrIFN1-F	CCGCTCGAGGCCACCATGTGGACCTATATTTTTGTGATAT	Eukaryotic expression
pEGFP-DrIFN1-R	CCCAAGCTTAGGATTGACCCTTGCGTTGCTT	
pEGFP-DrIFN3-F	CCGCTCGAGGCCACCATGGACCTTCACCGTGTGGCCTGGC	Eukaryotic expression
pEGFP-DrIFN3-R	CCCAAGCTTTAACATGATGTCCGAGTTGTTT	
TBP-F	TTACCCACCAGCAGTTTAG	qPCR
TBP-R	ACCTTGGCACCTGTGAGTA	
40s-F	CCGTGGGTGACATCGTTACA	qPCR
40s-R	TCAGGACATTGAACCTCACTGTCT	
qSVCV-G-F	CGACCTGGATTAGACTTG	qPCR
qSVCV-G-R	AATGTTCCGTTTCTCACT	
qSVCV-*N*-F	GCGGTTTTCTGTATGTGTCTC	qPCR
qSVCV-*N*-R	CTCTGCCAAATCACCATACTC	
qCMPK2-FHM-F	ACAGCAGCAGAGCATCACC	qPCR
qCMPK2-FHM-R	GCTTGTCTCCAACCGTTCATC	
qCMPK2-dr-F	GGCTCCTTTCAGACAACG	qPCR
qCMPK2-dr-R	TTCAACCCTACCACCAAC	
promCMPK2-F	GGGGTACCTTACCTGCTTCTTTCAGCAG	promoter cloning
promCMPK2-R	CCCTCGAGCAGCTGATCAGCTATTTA	
siRNA1-F	GCUGUGGUCACCUGUCAAUTT	siRNA knockdown
siRNA1-R	AUUGACAGGUGACCACAGCTT	siRNA knockdown
siRNA2-F	GGAGACAAGCACUGGUGAUTT	siRNA knockdown
siRNA2-R	AUCACCAGUGCUUGUCUCCTT	siRNA knockdown
siRNA3-F	GCUUCUCACUGUCAAUCCATT	siRNA knockdown
siRNA3-R	UGGAUUGACAGUGAGAAGCTT	siRNA knockdown
si–NC–F	UUCUCCGAACGUGUCACGUTT	siRNA knockdown
si–NC–R	ACGUGACACGUUCGGAGAATT	siRNA knockdown

Poly (I:C), suggesting its potential antiviral and antibacterial function [22]. Since little is currently known about the role of CMPK2 in SVCV infection, this study is aimed to investigate the potential role of CMPK2 in SVCV infection and understand the related molecular mechanism of immune response against SVCV in fish.

2. Materials and methods

2.1. Cells and viruses

Fathead minnow (FHM) cell line (ATCC® CCL-42™) was maintained in Medium 199/EBSS (M199, Hyclone, USA) supplemented with 10% fetal bovine serum (Natocor, Córdoba, Argentina) containing streptomycin (100 μg/mL) and penicillin (100 U/ml) at 28 °C in a 5% CO_2 atmosphere. SVCV (ATCC: VR-1390) was prepared in FHM cells at 25 °C and viral titers were determined by plaque assay. The viral titer of SVCV was 2×10^7 PFU/mL.

2.2. Sequence analysis

The sequence of CMPK2 was confirmed by NCBI BLAST analysis [23].The multiple sequence alignment was performed with CLUSTALW program. And the phylogenetic tree was constructed basing on the amino acid sequence of CMPK2 according to Neighbor-joining (NJ) algorithm using MEGA7.

2.3. Zebrafish

Zebrafish were purchased from China Zebrafish Resource Centre (CZRC) and the care, breeding, feeding and challenging were described previously [24].

2.4. Plasmids and transfections

The FHM CMPK2 amplification primers were designed by reference zebrafish CMPK2 sequence (GenBank: XM_693963.8) and FHM RefSeq sequence (GenBank: JNCD00000000.1). The full length CDS of CMPK2 was amplified using primers of CMPK2-F and CMPK2-R, *Eco*R I and *Xho*I were added at the primers, and resulted sequence was subcloned into the eukaryotic expression vector pcDNA4 (Invitrogen, USA) to generate the plasmid pcDNA4-CMPK2-His. The pEGFP-DrIFN1 and pEGFP-DrIFN3 plasmids were generated by inserting zebrafish IFN1 and IFN3 CDS into the pEGFP-N1 vector, respectively. All plasmids were verified by sequence analysis. The primers used for plasmids construction were listed in Table 1.

As for transient transfection, FHM cells were seeded in 6-well plates or 10 cm^2 cell culture dishes with 70–90% monolayer. Approximately 24 h later, transfection was performed with FuGENE HD Transfection Reagent (Promega) according to the manufacturer's instructions.

2.5. Promoter cloning and sequencing

The promoter sequence of the fathead minnow CMPK2 was cloned from FHM cells genomic DNA which was purified by HiPure Bacterial DNA Kit (Magen, China). The promoter primers were designed by reference FHM RefSeq sequence (GenBank: JNCD00000000.1). The primers used for promoter cloning were listed in Table 1.

2.6. siRNA knockdown

To test the inhibitory effect of CMPK2 siRNA on the expression of CMPK2, the siRNA was transfected according to the established standard protocols [25]. CMPK2 siRNA was purchased from GenePharma (Shanghai, China). In the transfection experiment, 100 pmol CMPK2-specific siRNA duplex or the negative control was mixed with 5 μL of TransIntro EL Transfection Reagent (TransGen Biotech, China), then transfected into FHM cells according to the manufacturer's instructions, the sequences of siRNA were listed in Table 1.

2.7. Quantitative real-time PCR (qRT-PCR)

Total RNAs were extracted with TRIzol reagent (Invitrogen) according to manufacturer's instructions. The reverse transcription was conducted using PrimeScript™ RT reagent Kit with gDNA Eraser

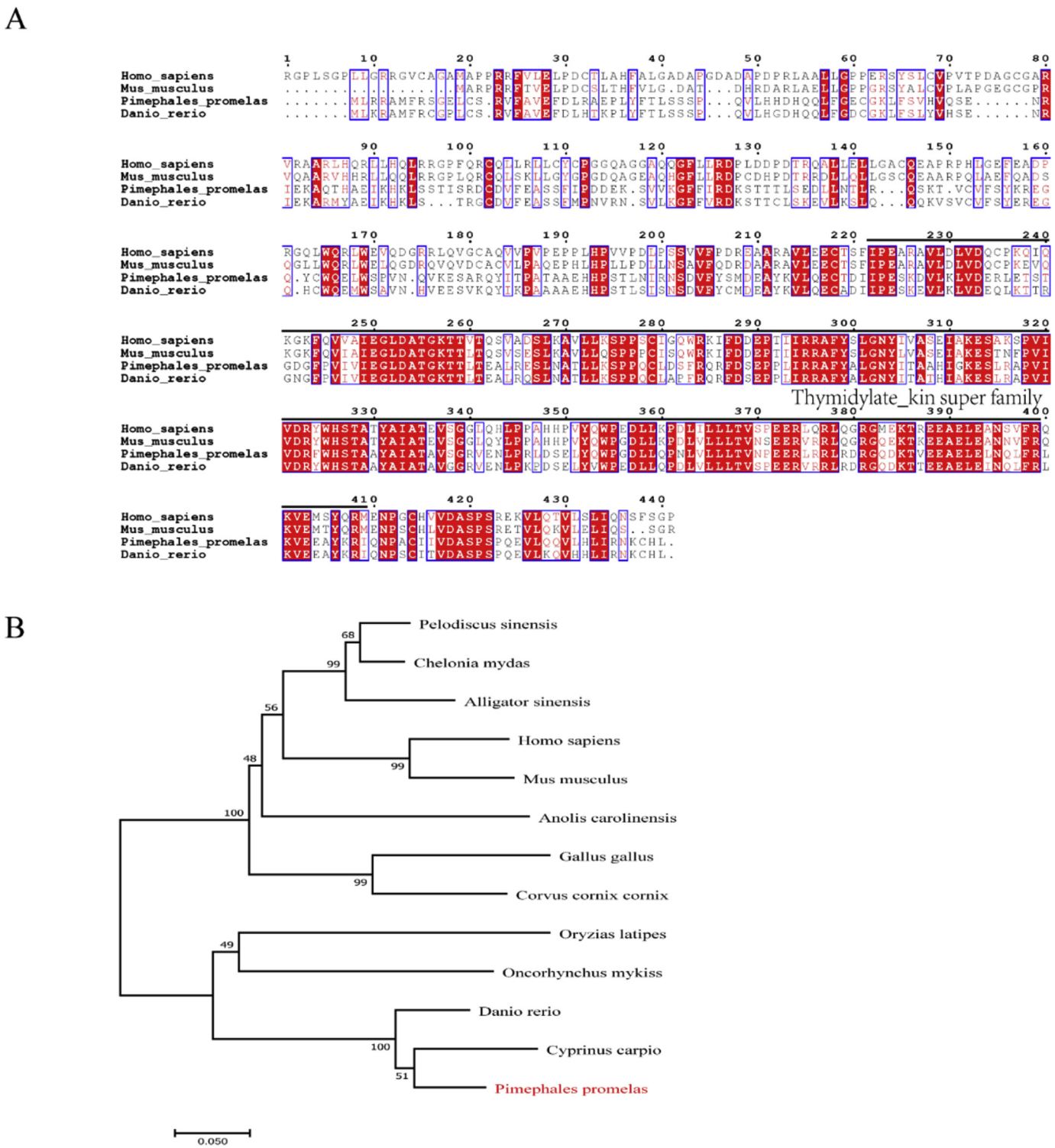

Fig. 1. Sequence analysis of CMPK2. (A) Alignment of the amino acid sequences of CMPK2 homologues. The functional domains of CMPK2 across different species were marked with a solid bold line. The GenBank accession numbers of the aligned sequences are as follows: *Homo sapiens*, AAH89425.1; *Mus musculus*, AAH57565.1; XP_699055.5. (B) The phylogenetic tree was constructed with the neighbor-joining algorithm using the Mega 7.0 program based on the multiple sequence alignment. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) is shown next to the branches.

(Perfect Real Time) (TAKARA, China). The reaction volume and conditions were reported previously [26]. In particular, SYBR Green qPCR SuperMix was purchased from TaKaRa (Dalian, China), and amplification and detection were performed on LightCycler® 480II Real Time Detection System (Roche, German).

2.8. Western blot

Western blot assay was used to detect the relative level of CMPK2 protein in FHM cells in the following steps: a) the extracted proteins were transferred into polyvinylidene fluoride membrane (Bio-Rad, USA). b) after blocking for 2 h with 2% BSA in tris-buffered saline with

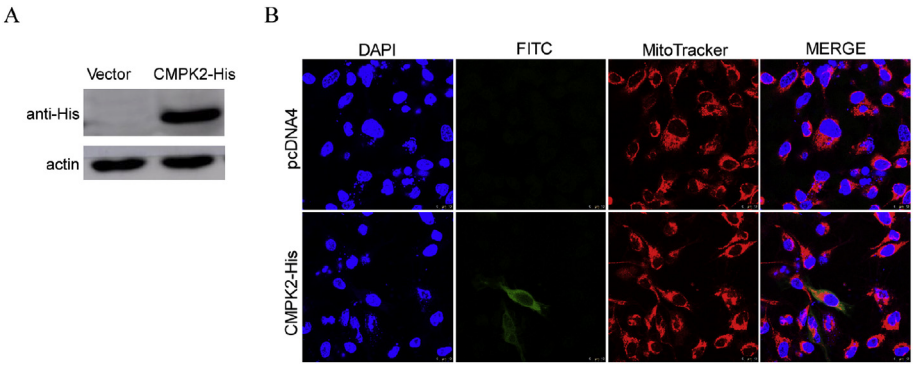

Fig. 2. The subcellular localization of CMPK2. (A) Western blot assay used to detect the overexpression of CMPK2; pcDNA4-CMPK2-His recombinant plasmids were transfected into FHM cell line, pcDNA4 empty plasmid was used as a control. (B) Subcellular localization of CMPK2 in FHM cells. Cell nucleus ware stained with DAPI, samples were observed under confocal laser scanning microscopy.

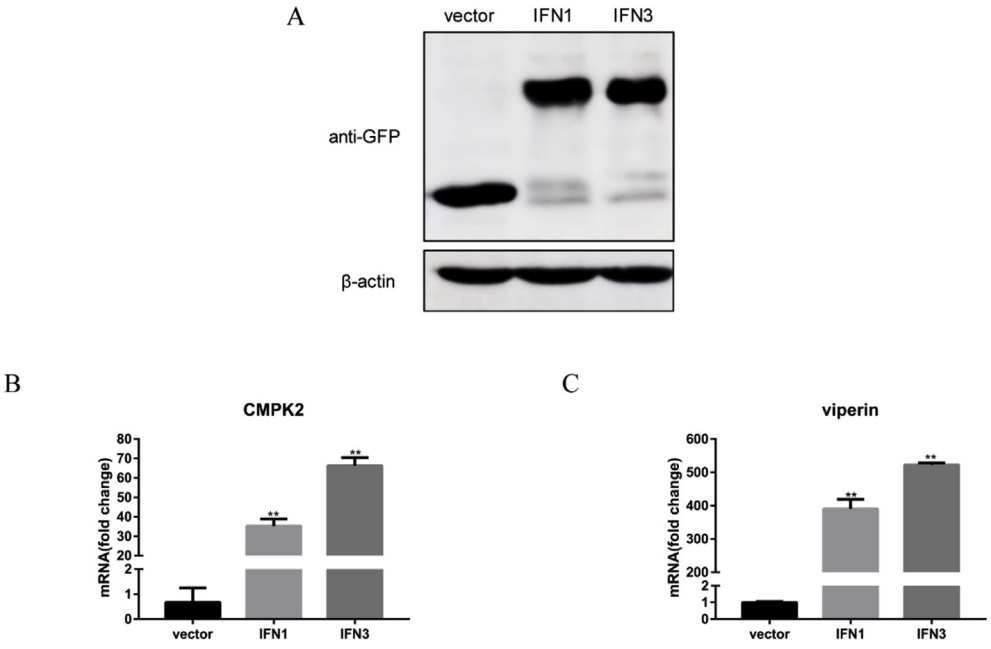

Fig. 3. Induction of CMPK2 by fish IFNs. FHM cells seeded in 6-well plates were transfected with DrIFN1, DrIFN3 and empty vector (2 μg each), respectively. Cells were collected at 24 h post transfection. (A) Western blot was used to verify overexpression of DrIFN1 and DrIFN3. (B, C) qRT-PCR was performed to detect the mRNA of CMPK2 and viperin after transfection.

tween 20 (TBST) at room temperature, the membranes were incubated with the primary antibody [anti-His-tag and *anti*-β-actin (ABclonal, 1:2000 dilution), SVCV-G [27]] for 2 h at room temperature. c) the membranes were washed thrice with TBST, and again incubated with corresponding HRP conjugated secondary antibodies (ABclonal, 1:5000 dilution) for 1 h at room temperature. d) the signal intensity was then determined using Amersham Imager 600 (GE, USA).

2.9. Indirect immunofluorescence assay

An indirect immunofluorescence assay was carried out to determine the subcellular localization of CMPK2 via detected the expression of CMPK2-His fusion proteins in FHM cells. The mitochondria were stained with the MitoTracker-Red (Invitrogen, China). The FHM cells were seeded on the 10 cm^2 dishes and then washed with PBS (pH 7.4) after 24 h long transfection. Then they were fixed with methanol for 30 min, and incubated with mouse anti-His antibodies (ABclonal, 1:500 dilution) for 1 h and then with FITC-conjugated goat anti-mouse antibodies (ABclonal, 1:2000 dilution) for 45 min. Next, the cells were stained with 4,6-diamidino-2-phenylindole (DAPI) (1 μg/mL) for 15 min. Finally, the cells were washed with PBS, fixed with 50% glycerol, and observed with a confocal laser scanning microscopy (Leica TCS SP8, Japan).

2.10. Plaque assay

Plaque assay was implemented with FHM cells to determine virus titer. Briefly, while FHM cells were seeded in 12-well plates, viruses were 10-fold diluted and inoculated into cell monolayers. After 1 h adsorption at 25 °C, the cells were washed with serum-free M199 and covered with an overlay medium containing 3% FBS and 1.5% carboxymethyl cellulose (CMC, Sigma-Aldrich). At 72 h post-infection, infected cells were fixed with 10% paraformaldehyde and stained with 0.5% crystal violet. After vigorous washing with tap water, visible plaques were counted and viral titers were calculated. All data were expressed as the mean of triplicated samples.

2.11. Statistics analysis

All related experiments were performed at least three times with similar results. The data were shown as mean ± standard deviation (S.D.). Two-way ANOVA tests were used to compare the difference between two groups. All statistical analyses and calculations were performed using GraphPad Prism 7.0. Significant differences were indicated with p-value. * represents p-value < 0.05 while ** represents p-value < 0.01.

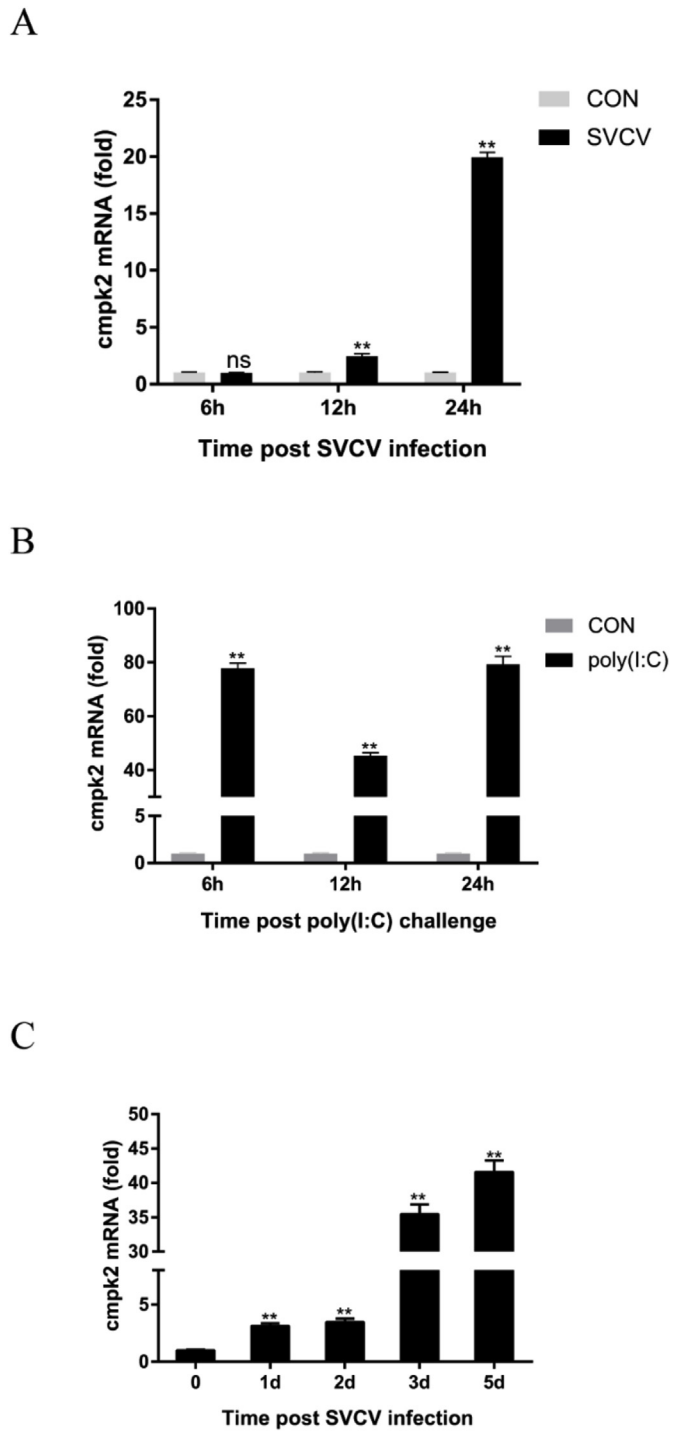

Fig. 4. The expression profile of CMPK2 after SVCV infection and poly(I:C) stimulation. (A) The mRNA expression changes of CMPK2 in FHM cells after SVCV infection, the transcript levels were normalized to TBP. (B) The mRNA expression changes of CMPK2 in FHM cells after poly (I:C) challenge, 40s was used as the internal control. (C) The relative expression of CMPK2 in liver was calculated in SVCV infected zebrafish as the folds relative to that in PBS injected control at the same time point.

3. Results

3.1. Sequence analysis of CMPK2

The full-length of CMPK2 coding DNA sequence (CDS) region was generated from FHM cells. Sequence analysis showed that the CMPK2 CDS region was 1251 bp in length and encoded a putative protein of 416 amino acids containing a highly conserved Thymidylate_kin superfamily domain (AA221-AA410). Multiple alignment showed that the Thymidylate_kin superfamily domain of *Pimephales promelas* CMPK2 shared a high sequence similarity with other reported CMPK2 genes, the conserved domain amino acids shared 61%, 67% and 88% identity to *Homo sapiens*, *Mus musculus* and *Danio rerio*, respectively (Fig. 1A). A phylogenetic tree of CMPK2 was also constructed across different species, and the phylogenic analysis showed that *Pimephales promelas* CMPK2 was closest to the *Cyprinus carpio* CMPK2 (Fig. 1B).

3.2. Subcellular localization

To detect the subcellular localization of CMPK2, plasmid pcDNA4-CMPK2-His was constructed and then transfected into FHM cells, the high expression of CMPK2 in transfected FHM cells was verified using Western blot assay (Fig. 2A), and the fluorescence was observed under a confocal microscopy. As shown in (Fig. 2B), the green fluorescence in CMPK2-His fusion protein was distributed mainly in the cytoplasm of FHM cells. According to the reports from mammal species, CMPK2 was localized in the mitochondria of HeLa cells [28]. In this study, the mitochondria were stained by the MitoTracker dye, CMPK2 distributed in the whole cytoplasm, and not just localized in the mitochondria of FHM cells.

3.3. Induction of CMPK2 by fish IFNs

In mammals, CMPK2 was characterized as one of IFN-inducible antiviral effectors. To determine whether fish IFNs could also induce CMPK2 expression, two type I zebrafish IFN plasmids (DrIFN1, DrIFN3) were constructed, and high expression of IFNs in transfected cells was verified using Western blot assay (Fig. 3A). Similar to mammalian type I IFN, endogenous CMPK2 mRNA can be induced when the fish IFN1 and IFN3 are overexpressed in FHM cells (Fig. 3B), simultaneously consistent results were obtained for detection of endogenous viperin mRNA (Fig. 3C). In mammals, the promoters of IFN-inducible genes (ISGs) have characteristic sequences of ISRE (IFN-stimulated response element). In order to confirm whether the ISRE sequence exists in the fish cmpk2 promoter, the flanking sequence of the fathead minnow CMPK2 was cloned and analyzed, and one highly conserved ISRE sequences were identified, typically found in promoters of type I IFN-stimulated genes, at position −829 to −841 (Supplementary materials Fig. 1). Indicating that fish CMPK2 is a new ISG that can be induced by zebrafish type I IFNs.

3.4. Expression patterns of CMPK2 after SVCV and poly(I:C) challenge

In mammals, CMPK2 was upregulated in response to LPS, poly(I:C) and several viruses, such as Hepatitis E genotype 4 Virus (HEV) and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection [29,30]. In this study, qRT-PCR assay was performed to examine the mRNA expression of CMPK2 *in vitro* and *in vivo*. The transcription level of CMPK2 after challenging with SVCV and poly(I:C) was examined in FHM cells, and the expression levels of CMPK2 were significantly up-regulated following the stimulations (Fig. 4A. B). The expression of CMPK2 mRNA in the SVCV infected liver tissue was also detected at series time points (0 d, 1 d, 2 d, 3 d and 5 d post infection). The results showed that compared with control group (which was injected with PBS), the relative transcripts of CMPK2 were significantly up-regulated and reached to peak at 5 d post-infection (Fig. 4C).

3.5. Effects of overexpression CMPK2 on SVCV replication

In order to further determine the role of CMPK2 in SVCV infection, plasmid pcDNA4-CMPK2-His or pcDNA4 (control group) was transfected into FHM cells and then infected with SVCV. Overexpression of

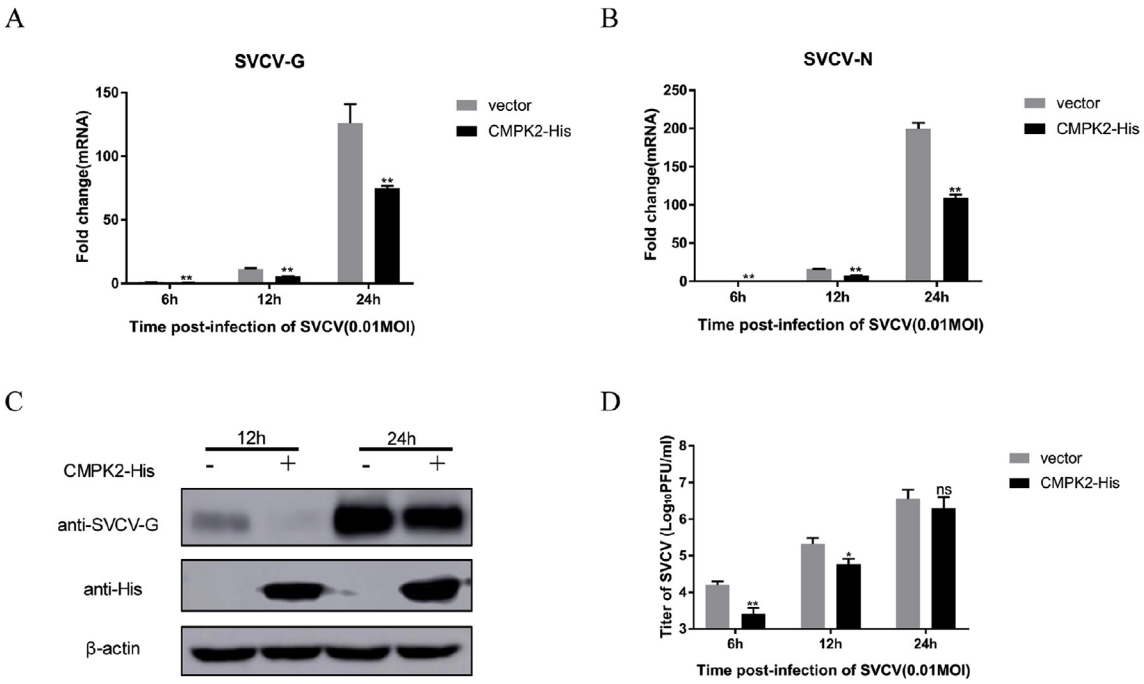

Fig. 5. The effect of CMPK2 over-expression on the transcription and translation of viral genes and SVCV production. FHM cells were transfected with pcDNA4-CMPK2-His plasmid and mock vector, respectively, followed by SVCV infection at 0.1MOI. The cells were harvested at various time points. (A, B) SVCV G and N mRNA were quantified using qRT-PCR. TBP was used as the internal control. (C) SVCV G Protein and CMPK2-His were detected by Western blot. β-Actin was used as the internal control. (D) The SVCV titers in the supernatants were measured using Plaque assay. All the results are representative of at least two independent assays, with each determination performed in triplicate (mean ± SD). (*P < 0.05; **P < 0.01).

CMPK2 was verified by Western blot assay (Fig. 5C). Compared to the control group, overexpression of CMPK2 in transfected cells decreased the expressions of SVCV G and N protein mRNA (Fig. 5A and B). The production of SVCV G protein was also down-regulated significantly in pcDNA4-CMPK2-His transfected cells (Fig. 5C). In addition, the viral titers were also reduced by CMPK2 overexpress as detected at both 6 and 12 h post-infection times (Fig. 5D and supplementary materials Fig. 2). These results demonstrated the crucial roles of CMPK2 during SVCV infection.

3.6. Effects of knockdown CMPK2 on SVCV replication

To investigate the effect of CMPK2 in antiviral immunity, CMPK2 expression was knocked down by siRNA in FHM cells. The decrease expression level of CMPK2 was confirmed by qRT-PCR and the mRNA level of CMPK2 was decreased by approximately 73.76%, 49.31%, and 53.86% by siRNA-1, siRNA-2, and siRNA-3, respectively, as compared to the expression in the si-NC cells (negative control) (Fig. 6A). Then, the transcription, translation and production of SVCV was evaluated after CMPK2 silencing by siRNA-2 in FHM cells. At 12 and 24 h post-infection times, the cells were collected in order to detect SVCV mRNA protein and titers by qRT-PCR, Western blot and plaque assay, respectively. As shown in (Fig. 6B and C), the mRNA expressions of SVCV G and N were significantly increased at 12 and 24 h post-infection. However, SVCV G protein was under detection at this period of times. The G protein expression was increased significantly in the cells transfected with siRNA-2 at 24 h (Fig. 6D). The SVCV titers in supernatants were significantly increased at 12 and 24 h post-infection (Fig. 6E and supplementary materials Fig. 3).

4. Discussion

In mammals, CMPK2 has been identified plays an important role in host innate immunity [19]. However, the function of CMPK2 on virus infection has not yet been revealed in fish. In the present study, fish CMPK2 was characterized and its potential role involved in SVCV infection was assessed.

As reported in mammal, CMPK2 is located in mitochondrial of HeLa cells [17,28]. However, in this study, CMPK2 was identified as a cytoplasmic protein but not completely localized to mitochondria. There may be two possible explanations for these different observations: one is the species difference, the *N*-terminus of fish CMPK2 cannot be found in mammals through sequence alignment (Fig. 1A), thereby the positioning is different; the other reason is that CMPK2 is localized differently from cells to cells, such as the FADD protein which is localized to the nucleus in HeLa cells [31] but to the nucleus and cytoplasm in FHM and GS cells [32].

Understanding the expression of genes in different tissues and their regulatory responses to different conditions the basic approaches to examine the specific function of genes [33]. In the present study, the mRNA expression of CMPK2 was distributed in all the ten tissues examined, significantly high level in the liver, spleen, kidney and skin, suggesting the possibility of miscellaneous functions in fish (Supplementary Materials Fig. 4). In particular, head kidney, liver, gill and hindgut of fish are generally considered as immune organs and central to the immune responses [34], our findings indicate that CMPK2 may exert immune effect by tissue-specific mechanisms.

According to the previous exploration in mammal, CMPK2 was reported as a IFN stimulated gene [17]. However, the role of fish CMPK2 has not been investigated. In fishes, IFNs possess IFN-I and IFN-II, and IFN-I is further categorized as group I and II. In zebrafish, group I contains IFN1 and IFN4, and group II contains IFN2 and IFN3 [35,36]. Group II IFNs would control the viral replication during the early stages of infection, whereas group I IFNs would display a more powerful but delayed antiviral action [37]. For this reason, endogenous CMPK2 was evaluated after stimulated with zebrafish IFN1 and IFN3. Based on our results, the fish CMPK2 expression showed markedly upregulation when treated with IFN. Viperin is a well-known ISG [38] and upregulated after IFN stimulation as well in this experiment. Our results indicated that CMPK2 was a new ISG in fish. In addition, previous

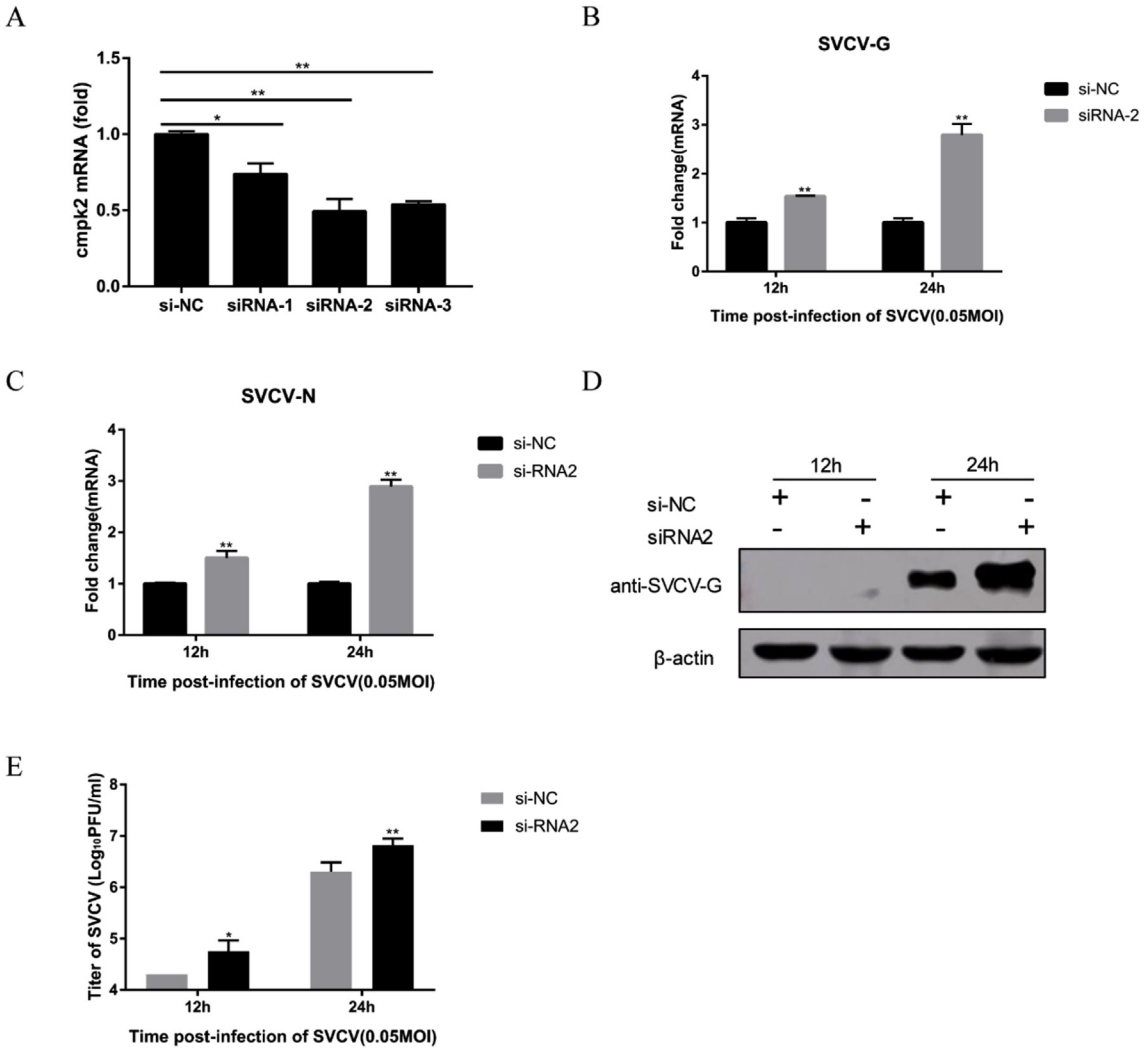

Fig. 6. Silence CMPK2 expression enhance SVCV replication. FHM cells were transfected with negative control (si-NC) and CMPK2 siRNA (siRNA-1, siRNA-2, siRNA-3), respectively, followed by SVCV infection at 0.05 MOI. The cells were harvested at 12 h and 24 h post-infection. (A) CMPK2 mRNA in FHM cells was measured using qRT-PCR. 40s was used as the internal control. (B, C) SVCV G and N protein mRNA was measured using qRT-PCR. TBP was used as the internal control and take the si-NC group as the standard. (D) SVCV G protein was detected by Western blot. β-Actin was used as the internal control. (E) The SVCV titers in the supernatants were measured using Plaque assay. All the results are representative of at least two independent assays, with each determination performed in triplicate (mean ± SD). (*P < 0.05; **P < 0.01).

research has shown that SVCV infection induced IFN production in EPC cells [39], all of these findings signify the possible involvement of CMPK2 in the host's antiviral immunity.

In order to investigate the role of CMPK2 in SVCV infection, we examined the mRNA expression of CMPK2 at different post infection times in liver of SVCV-infected zebrafish. It reveals that the CMPK2 mRNA was upregulated from 1d to 5d. it is in common with MX1 in liver of black carp after SVCV infection, but their upregulation were at different level [40]. *In vitro* assay reveals that CMPK2 was upregulated after challenging with SVCV and poly(I:C), and the latter is a kind of substance considered to be an RNA virus mimetic [41]. These results indicate that CMPK2 may play an important role in the host's response to the virus infection.

A recent report has indicated that human CMPK2 plays an important role in the inhibition of HIV replication [19]. Thus it will be also significant to understand the role of fish CMPK2 in viral infection because the antiviral activities of many proteins vary from species to species. In this study, we examined the effects of CMPK2 on SVCV infection, and found that CMPK2 overexpression inhibited the transcription and translation of the viral genes and production of SVCV in FHM cells, and siRNA silencing of CMPK2 promoted SVCV replication. Interestingly, the CMPK2 knock down resulted in the significant increase of SVCV titer in FHM cells at 12 and 24 h post-infection. However, CMPK2 overexpression only reduced SVCV titers at 6 h and 12 h post-infection times, but with no substantial difference at 24 h. One possible explanation for this observation is that the expression level of CMPK2 in control group has reached a high level as well at 24 h post-infection (Fig. 4A), therefore the SVCV titers were no significantly reduced by the CMPK2 overexpression in FHM cells at 24 h post-infection. All together, these results showed that CMPK2 is crucial in fish antiviral immunity.

In conclusion, CMPK2 was cloned and characterized in this study, and the response of CMPK2 to SVCV infection was investigated *in vivo* and *in vitro*. Intracellular localization was performed for CMPK2 *in vitro*. The results of overexpression and RNA interference of CMPK2 showed antiviral activities during SVCV infection. These results will be contributory to explaining of the molecular mechanism of the CMPK2 antiviral immune response in future.

Conflicts of interest

The authors declare that the research was conducted in the absence

of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledge

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.05.032.

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