Fish and Shellfish Immunology 88 (2019) 606–618

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Identification of the SAMHD1 gene in grass carp and its roles in inducing apoptosis and inhibiting GCRV proliferation

Xiaowen Xu[a], Meifeng Li[a], Dongming Li[b], Zeyin Jiang[a], Changxin Liu[a], Xiao Shi[a], Chuxin Wu[c], Xingxing Chen[a], Gang Lin[a], Chengyu Hu[a,*]

[a] College of Life Science, Nanchang University, Nanchang, 330031, China
[b] Fuzhou Medical College, Nanchang University, Fuzhou 344000, China
[c] Yuzhang Normal University, Nanchang 330031, China

ARTICLE INFO

Keywords:
SAMHD1
IRF3 and IRF9
Transcriptional regulation
Apoptosis
GCRV

ABSTRACT

SAMHD1 is an innate immunity restriction factor that inhibits virus infection through IRF3-mediated antiviral and apoptotic responses. Fish SAMHD1 shares some similar properties with those in mammals. In this study, a *SAMHD1* orthologue from grass carp (*Ctenopharyngodon idellus*) was cloned and characterized. The full-length cDNA of *CiSAMHD1* is 2792 bp with an ORF of 1884 bp encoding a polypeptide of 627 amino acids. Multiple alignments showed that SAMHD1 is highly conserved among different species. Phylogenetic tree analysis revealed that *CiSAMHD1* shared a high degree of homology with *Sinocyclocheilus rhinocerous SAMHD1*. Expression analysis indicated that *CiSAMHD1* was widely expressed in all tissues tested including the brain, eyes, spleen, gill, intestine, liver, heart and kidney. It was significantly up-regulated in spleen, liver and intestines after treatment with poly I:C. Also, *CiSAMHD1* can be induced following stimulation with recombinant IFN in CIK cells. The promoter sequence of *CiSAMHD1* was identified to explore the mechanism underlying the transcriptional regulation of *CiSAMHD1*. The promoter sequence of *CiSAMHD1* (1370 bp) consists of IRF1, IRF3, IRF9 and p65 binding elements. Gel mobility shift assay also showed that IRF1, IRF3, IRF9 and p65 prokaryotic proteins can separately interact with *CiSAMHD1* promoter. Dual luciferase assay and q-PCR suggested that the promoter of *CiSAMHD1* can be activated by the overexpression of *Ci*IRF3 and *Ci*IRF9, but cannot be triggered by *Ci*IRF1 and *Ci*p65. In contrast, knockdown of *Ci*IRF3 or *Ci*IRF9 inhibits the transcription of *CiSAMHD1*. Intriguingly, CCK assay suggested that *Ci*SAMHD1 decreased cell viability. TUNEL apoptosis assay and Hoechst 33258 staining assay indicated that apoptosis is induced by the overexpression of *Ci*SAMHD1. Crystal violet staining, detection of two GCRV genes (vp3 and vp5) and viral titration showed that *Ci*SAMHD1 can suppress the proliferation of grass carp reovirus (GCRV) in CIK cells.

1. Introduction

Host restriction factors such as APOBEC3G, TRIM5α, Tetherin and Mx2 inhibit viral replication by blocking nucleotide duplication, viral uncoating and the release of viral particles [1–4]. Another host restriction factor is sterile alpha motif (SAM) and histidine-aspartic (HD) domain-containing protein 1 (SAMHD1). It is a nuclear protein associated with Aicardi–Goutières syndrome (AGS) [5–8]. It is known that the function of SAMHD1 is entirely mediated by its HD domain [9].

Recent studies identify that SAMHD1 plays an antiviral function. In response to human T-lymphotropic virus type 1 (HTLV-1) infection, SAMHD1 inhibits the production of reverse transcription intermediates (RTI) and induces IRF3-mediated apoptosis [10]. SAMHD1 is proposed to inhibit the replication of DNA and RNA viruses. It can restrict the replication activity of HSV-1 (DNA virus) and HP-PRRSV (RNA virus) [11,12]. However, the functional mechanism of SAMHD1 is not very clear. Tetramerization of SAMHD1 is required for its biological activity [13]. SAMHD1 may act as a deoxynucleoside triphosphate (dNTP) triphosphohydrolase that depletes the intracellular pool of dNTPs, and thereby prevents HIV-1 reverse transcription in some cell types [14]. SAMHD1 also can directly bind and hydrolyze viral nucleic acids [15].

During the past decades, tremendous advances have been made in fish antiviral response. The mechanism of innate immunity and apoptosis is relatively conserved in fish as compared to their mammalian counterparts [16]. The dimer of STING and ZDHHC1 can activate IRF3, then initiates IFN expression [17]. In response to poly I:C stimulation,

* Corresponding author.
E-mail addresses: lgang@ncu.edu.cn (G. Lin), hucy2008@163.com (C. Hu).

https://doi.org/10.1016/j.fsi.2019.03.028
Received 27 December 2018; Received in revised form 10 March 2019; Accepted 12 March 2019
Available online 15 March 2019

Table 1
Sequences and applications of the primers used in this study.

Primer name	Primer sequence(5′→3′)	Application
SAMHD1-5′RACE1	TCAAACATCTGGACTGAGGCA	5′RACE cloning
SAMHD1-5′RACE2	GCTTCCATTTCTCACCAGGTCGA	
SAMHD1-3′RACE1	CGCAAGAATCAGGTGTCCAAA	3′RACE cloning
SAMHD1-3′RACE2	AGTACTTTGTTCAGTGGTGCATGG	
Long	CTAATACGACTCACTATAGGGCAAAGCAGTGGTATCAACGCAGAGT	RACE PCR
Short	CTAATACGACTCACTATAGGGC	
NUP	AAGCAGTGGTATCAACGCAGAGT	
SAMHD1-SP1	TGCAAACAGACTACACAACACAC	Promoter cloning
SAMHD1-SP2	TGTTCTGTGGCCTCTTGTATTTC	
SAMHD1-SP3	GACTGTGCCTTTAGATCACCTGTT	
APP	CGGGATCCCGGAATTCCCCCICCCCICCCCIC	
SAMHD1-RT-F	GGGCTATTTGGCAGGATGTC	Q-PCR
SAMHD1-RT-R	AAGACAGAGTTATCCAGGGGC	
IRF1-RT-F	CGTGTGGGTCAACAAGGAG	
IRF1-RT-R	GTTCATGGCACAGCGGAAA	
IRF3-RT-F	TCCAGGCCAAGCATACGAA	
IRF3-RT-R	CCATTTGCAACAGCCATCAT	
IRF9-RT-F	GTAAAATGGAGAGAGGAAGCGA	
IRF9-RT-R	ATTGCAAATGGGTCTCTGAGG	
p65-RT-F	AACCAAGAACCAGCCATACAAG	
p65-RT-R	CGCTTCAGGAATATTAAAGGGG	
Bax-RT-F	GCTTTGGGTTCTGCACTTCT	
Bax-RT-R	CGGTTGAAGAGCAGAGTCAT	
BCL2-RT-F	GACTCCTCTCCAAACTCTGAC	
BCL2-RT-R	TCCTTTCTATCTCGTCTCCAG	
Vp3-RT-F	GCTCAAGTTCGATGCTACCAG	
Vp3-RT-R	TTCATCGGTCCATTAGGGTCA	
Vp5-RT-F	CCGACTGACGTGCAAAAATTC	
Vp5-RT-R	TTATCCGGGCTACGATGCAT	
ISG15-RT-F	GGTGAAAGTTGATGCCACAGTTG	
ISG15-RT-R	TTGGAAAGGGGGGTTCGTG	
β-actin-F	CACTGTGCCCATCTACGA	
β -actin-R	CCATCTCCTGCTCGAAGTC	
SAMHD1-ORF-F	ACGTATTTAATGATGGATAAGCGGA	Prokaryotic/eukaryotic expression vector construction
SAMHD1-ORF-R	TTAGAGGTTGTTTTGTCCCTGA	
IRF1-ORF-F	CGGGATCCAAACCATGCCTGTGTCCAGAATGC	
IRF1-ORF-R	CGGAATTC CTGATGCTCTCAGAGAGGACAC	
IRF3-ORF-F	CGGAATTCATGACCCATCCAAAACCG	
IRF3-ORF-R	CGGCGGCCGCTCACTTGGTGTCACACAACTC	
IRF9-ORF-F	CGGGATCCATGGCATCTGGAAGGATTCGT	
IRF9-ORF-R	CGCTCGAGGGGCTTTAATGTCAAGAATGCAGC	
p65-ORF-F	CGGAATTCATGGACGGACTGTTTCAC	
p65-ORF-R	CGCTCGAGTTAGGTCGGGTGTCCAGACAG	
SAMHD1-pro-F	ATTGAGTGATGGGTCTTTGGC	Construction of promoter vector
SAMHD1-pro-R	ACTCTCCATCACAAACCTGCT	

Table 2
The sequences of *siRNA* assay.

Sequence name	Sequence (5′→3′)
siRNA-*CiIRF1*-175	UAUGUAGGCUCAUAAGUGCTT
siRNA-*CiIRF1*-712	AAGCAACUGCAAUAAAUGCTT
siRNA-*CiIRF1*-2143	AAAGAUGAAGAGAACACGCTT
siRNA-*CiIRF9*-106	GGUGAACAGUGGGAAGUAUTT
siRNA-*CiIRF9*-697	GCUGAACUCUUUCCACAUATT
siRNA-*CiIRF9*-1065	CCACCCAGCUGUUCAACAUTT
siRNA-*CiIRF3*	GCAGGAAAGUCCCUUACAATT
siRNA-*Cip65*	UUAACGAUACUUCGUUUGGTT
Negative control (N.C)	UUCUCCGAACGUGUCACGUTT

fish PKR, PKZ and PERK can activate eIF2α and induce apoptosis [18–20]. IRF3-dependent and ISGF3-dependent pathways are also conserved in fish [21,22]. These conserved proteins and pathways are essential for the efficient antiviral responses in fish.

Danio rerio SAMHD1 is the first identified analogue in fish. Kasher et al. (2015) indicated that it is associated with AGS [23]. However, the biological function and transcriptional mechanism of fish SAMHD1 remain elusive. In this study, the cDNA and promoter sequences of grass carp *SAMHD1* were first cloned in *Ctenopharyngodon idellus* kidney cells (CIK cells). We studied the phylogeny, expression patterns and transcriptional mechanism of grass carp SAMHD1, and evaluated its biological functions in inducing apoptosis and antiviral property. These findings provided the essential perspective into the biological roles of fish SAMHD1.

2. Materials and methods

2.1. Fish, plasmids, cells, virus and strains

Grass carp (mean weight 20 g) were obtained from Nanchang Shenlong Fisheries Development Co., Ltd., Jiangxi, China. The fish were

allowed to acclimatize for at least 2 weeks in aerated freshwater tanks at room temperature prior to experiments. The pcDNA3.1, pET32a and pEASY-T1 were purchased from TransGene Biotech (China). Taq DNA Polymerase, LA Taq DNA polymerase and dNTP were bought from TaKaRa (Japan). The pRL-TK, pGL3-basic, *E.coli* strains DH5α and BL21 were bought from Promega (USA). CIK cells (derived from *Ctenopharyngodon idellus* kidney tissue) were preserved in our lab and maintained at 28 °C in M199 medium containing 10% inactivated fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. GCRV 097 were kindly provided by Dr. Jianguo Su (Huazhong Agricultural University, Wuhan, China) and propagated in CIK cells.

2.2. Identification of the full length cDNA of CiSAMHD1 and its promoter sequence

The full-length cDNA of *CiSAMHD1* (MF326081.1) was cloned by RACE-PCR method. Firstly, homologous fragment of *CiSAMHD1* was cloned according to *DrSAMHD1* (NM_001159933). Then, total RNA was extracted from CIK cells using RNA simple Total RNA Kit (Tiangen, China) and SMART cDNA was prepared using Super Script III reverse polymerase (Invitrogen, USA). Specific primers *SAMHD1-3RACE1*, *SAMHD1-3RACE2*, *SAMHD1-5RACE1* and *SAMHD1-5RACE2* were designed to obtain *CiSAMHD1-3′UTR* and *CiSAMHD1-5′UTR* sequences. The PCR cycling conditions were: 95 °C/3 min, 35 cycles of 95 °C/30 s, 56 °C/30 s, 72 °C/2 min, and 72 °C/10 min. PCR products were ligated into pEASY-T1 vector and sequenced as described previously. After the cDNA sequence of *CiSAMHD1* was verified, polypeptide was determined by online-software ORF Finder (http://www.ncbi.nlm.nih.Gov/gorf/gorf.html).

Genomic DNA was purified from whole blood of healthy grass carp using a Universal Genomic DNA Extraction Kit (TaKaRa, Japan). The promoter sequence of *CiSAMHD1* was cloned by a genomic walking

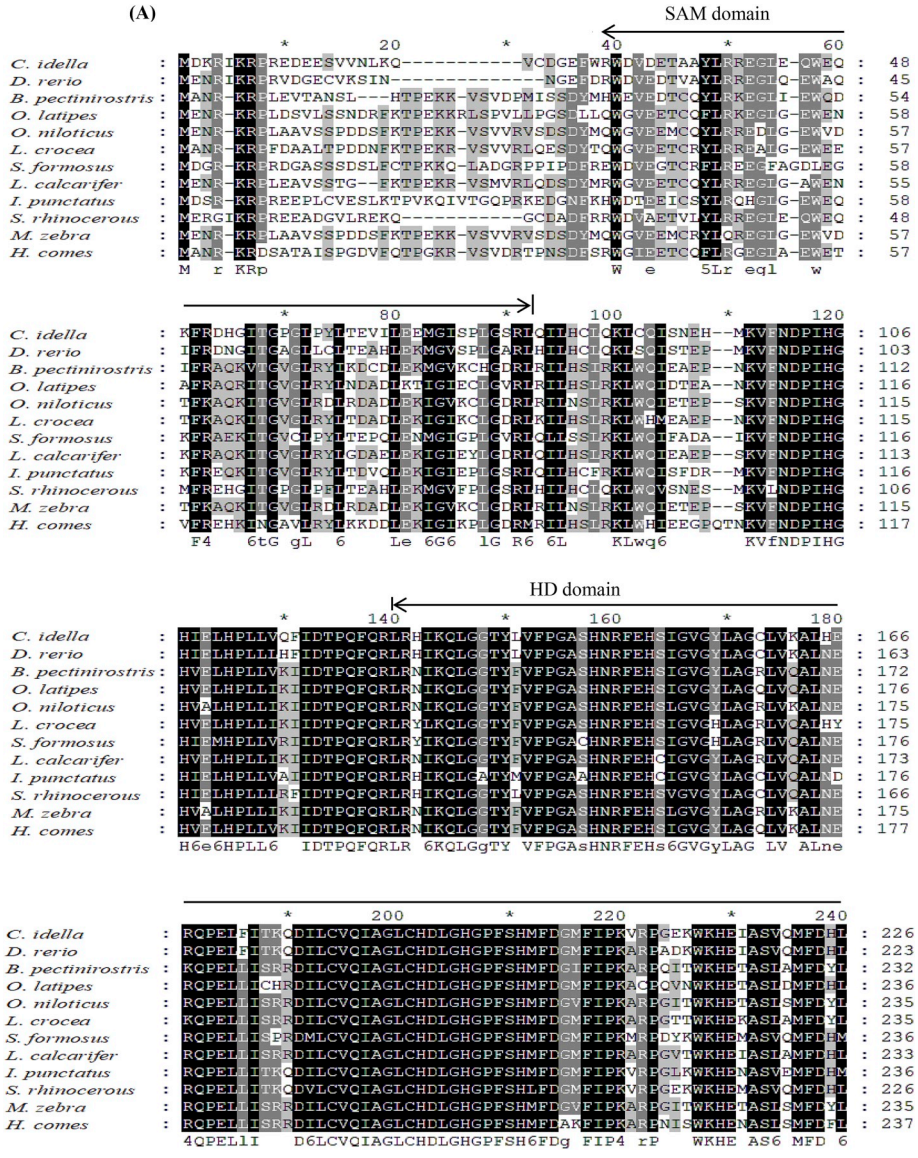

Fig. 1. Amino acid sequences and phylogenetic analysis of SAMHD1.

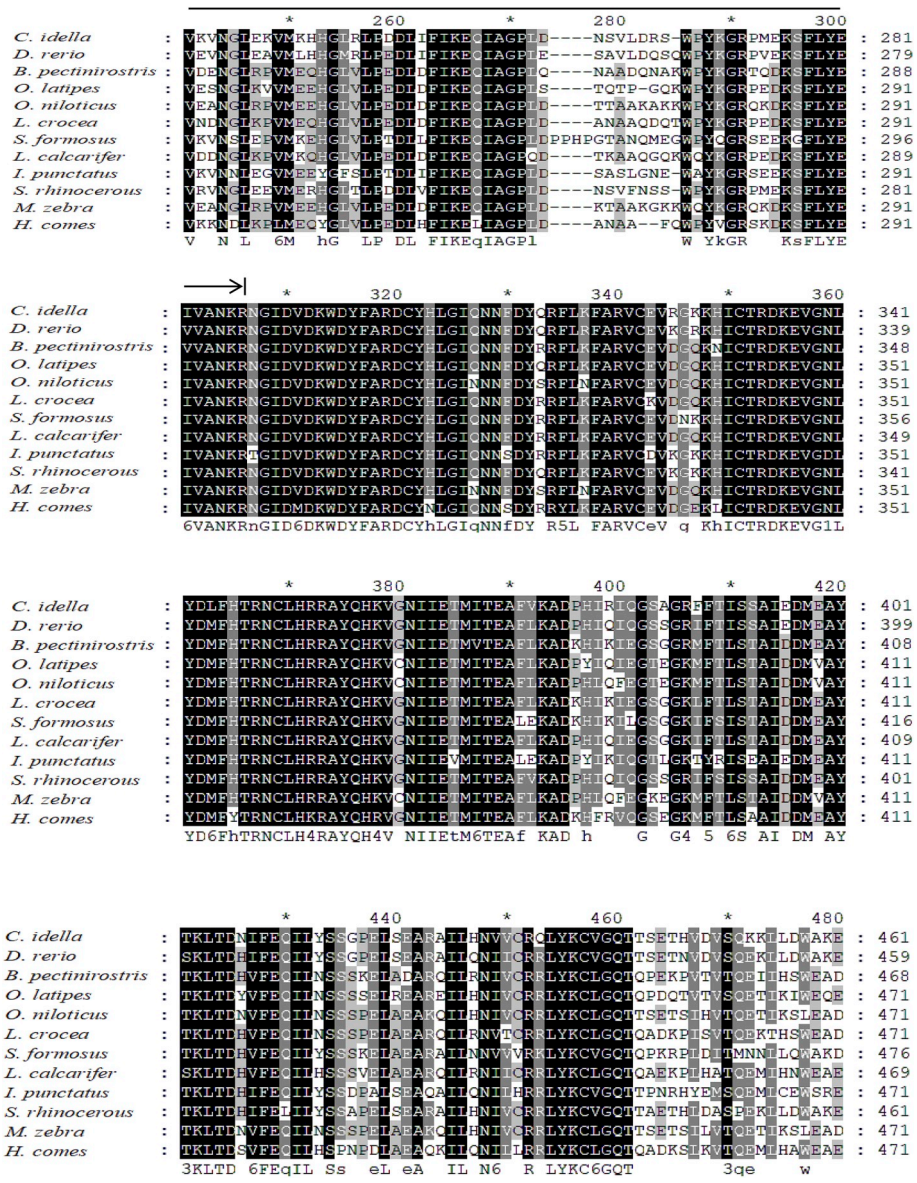

Fig. 1. (*continued*)

approach [24]. The single primer linear PCR was initially performed with primer *SAMHD1-SP1*, and then followed by adding dGTP and terminal transferase (TdT) (TaKaRa, Japan) to the purified PCR product to produce a Poly (G) tail. Two cycles of nested PCR were performed, using the adapter primer AAP and two specific primers *SAMHD1-SP2* and *SAMHD1-SP3* in turn. The PCR product was cloned and sequenced as described above. Transcription start sites of *CiSAMHD1* were identified by using 5′-Full RACE Kit with Tap (TaKaRa, Japan). All of the PCR products were sequenced by Sangon Biotech (Shanghai, China) to confirm DNA sequences.

2.3. Homologous amino acid sequences alignment and phylogenetic tree construction

The homologous protein sequences were obtained from NCBI blast server. The domain structures were predicted using SMART program (http://smart.emblheidelberg.de/smart/save_user_preferences.pl). The similarity analysis of amino acid sequences were displayed by Gene doc. The phylogenetic tree was created using Neighbour-Joining algorithm from MEGA X program.

2.4. Plasmids construction

Each of the open reading frames (ORF) of *CiSAMHD1*, *CiIRF1*, *CiIRF3*, *CiIRF9* and *Cip65* were separately inserted into pcDNA3.1 vector to construct expression plasmids, and these recombinant plasmids were used to conduct overexpression experiments. *CiIRF1, CiIRF1-nDBD, CiIRF3, CiIRF3-nDBD, CiIRF9, CiIRF9-nDBD, Cip65, Cip65-nDBD* and *CiIFN* were separately inserted into pET32a vector, and these modified vectors were transformed to competent *E. coli* BL21 cells to

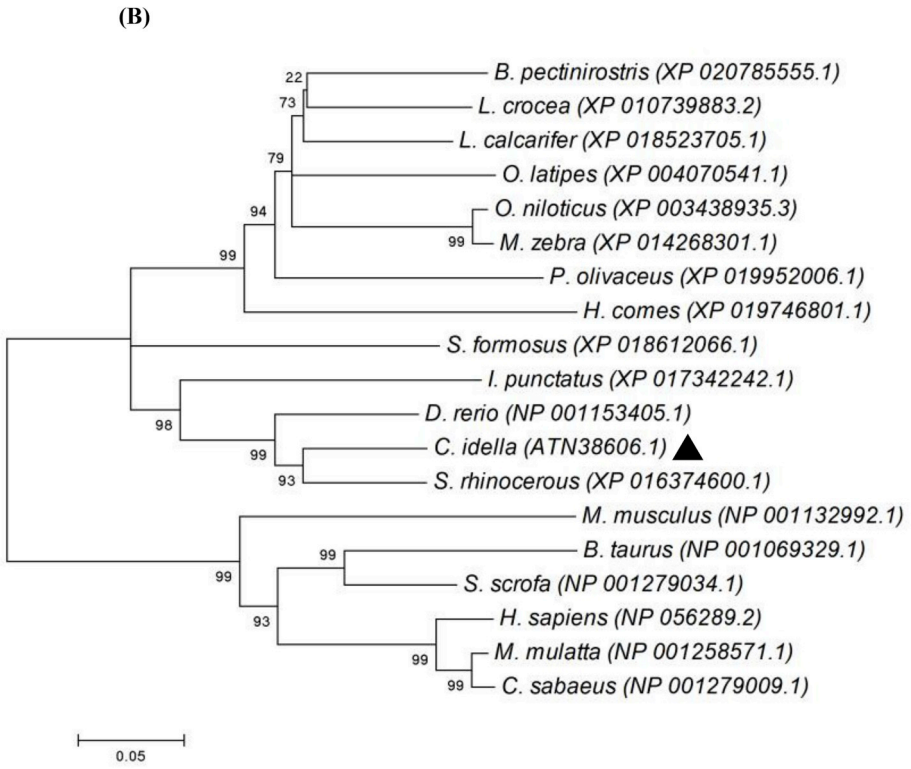

Fig. 1. (*continued*)

express recombinant protein. The promoter of *CiSAMHD1* was inserted into pGL3 and used in luciferase assays. The primers used for plasmids construction were shown in Table 1.

2.5. Q-PCR analysis of CiSAMHD1 mRNA level in grass carp tissues and CIK cells

The healthy grass carps (mean weight 20 g) were injected intraperitoneally (ip) with 150 μl (1 mg/ml) poly I:C (Sigma, USA). Total RNA samples were extracted from the eyes, gill, liver, spleen, kidney, intestine, brain and heart of three individuals at 6 h, 12 h, 24 h, 48 h and 72 h post-stimulation by RNA simple Total RNA Kit (Tiangen, China). CIK cells were seeded in 6-well plates to obtain 80% confluence, then directly stimulated with 10 ng recombinant IFN protein. The Quantscript RT Kit (TaKaRa, Japan) was used to clear genomic DNA and synthesized first strand cDNA. Quantitative real-time (q-PCR) was used to detect the mRNA levels of *SAMHD1* in CFX Connect™ Real-Time System (BIO-RAD, USA) with SYBR Green Real-Time PCR Master Mix (TaKaRa, Japan). The expression values were normalized to *β-actin*. *β-actin* is popular used as normalizer gene [24,25]. Its stability was also confirmed in supplementary Fig. 1. *CiSAMHD1* mRNA was analyzed by the comparative CT method ($2^{-\Delta\Delta CT}$ method). The primers for q-PCR were listed in Table 1.

2.6. Gel mobility shift assays

The recombinant plasmids (pET32a-*IRF1*, pET32a-*IRF1-nDBD*, pET32a-*IRF3*, pET32a-*IRF3-nDBD*, pET32a-*IRF9*, pET32a-*IRF9-nDBD*, pET32a-*p65*, pET32a-*p65-nDBD* and pET32a-*IFN*) were separately transformed into *E. coli* BL21 cells. Bacteria were cultured in incubator shaker (ZHWY-200H). When cells were grown to an OD_{600} of 0.6–0.8, they were added 1 mM IPTG for 4 h. Then the cells were harvested, centrifuged, resuspended through binding buffer (20 mM Trise HCl, 500 mM NaCl, 5 mM imidazole, pH 7.9). Recombinant proteins were purified by NI-NTA resign (Novagen, USA) and dialyzed by dialysis bag (Biotopped, China). The purified recombinant proteins were quantified and electrophoresed on a 12% SDS-PAGE gel.

Gel mobility shift assays were used as a method for studying the affinity of *CiSAMHD1* promoter with PBS, *Ci*IFN (negative control), *Ci*IRF1, *Ci*IRF1-nDBD, *Ci*IRF3, *Ci*IRF3-nDBD, *Ci*IRF9, *Ci*IRF9-nDBD, *Ci*p65 and *Ci*p65-nDBD prokaryotic proteins. The concrete reaction system was as follow: 20 ng of *CiSAMHD1* promoter sequence mixed with 1 μg of purified prokaryotic protein in a 10 μl reaction system, and incubated in ice for 1 h, then was analyzed by a 1% agarose gel. The gels were stained in ethidium bromide (EtBr, 0.5 mg/ml) and photographed.

2.7. Luciferase activity assays and transfection

CIK cells seeded in 24-well plates were grown to 70% confluence. Transfection systems were as follow: 0.25 μg of each eukaryotic expression vector (pcDNA3.1-*IRF1*, pcDNA3.1-*IRF3*, pcDNA3.1-*IRF9* and pcDNA3.1-*p65*), 0.25 μg of reporter gene vectors (pGL3-*SAMHD1-pro*), 0.025 μg of pRL-TK renilla luciferase vector and 2 μl of FuGENE®6 were

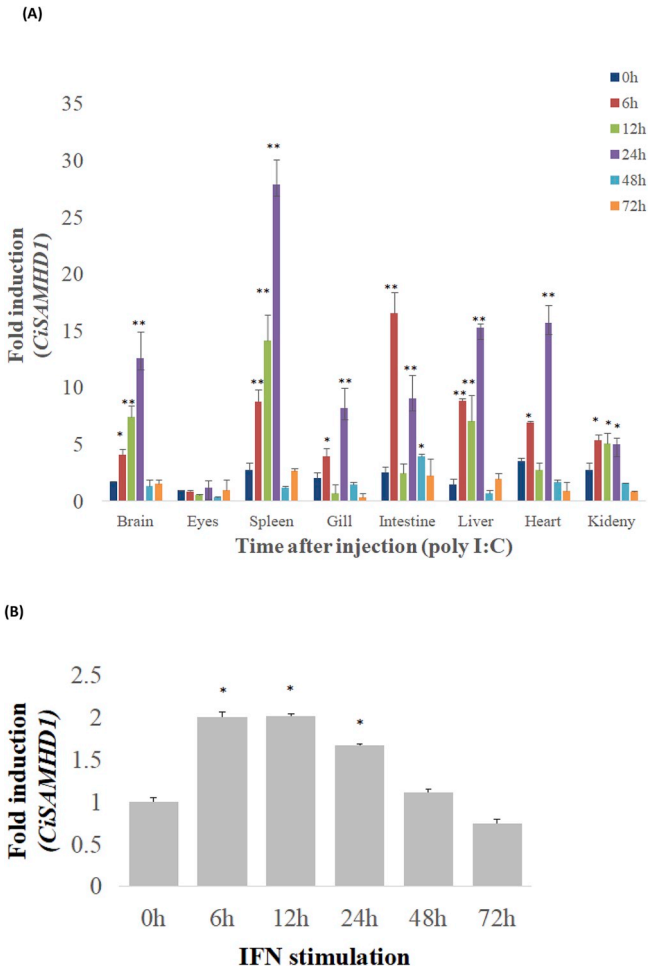

Fig. 2. Expressional characteristic of *CiSAMHD1* in tissues and CIK cells. **(A)** *CiSAMHD1* mRNAs in grass carp tissues (Eyes, Gill, Liver, Spleen, Kidney, Intestine, Brain and Heart) were evaluated at different time points (0 h, 6 h, 12 h, 24 h, 48 h and 72 h) of poly I:C stimulation. The expression in eyes (treatment for 0 h) was used as the calibrator. **(B)** *CiSAMHD1* mRNAs in CIK cells were evaluated at different time points of recombinant IFN (10 ng/well) stimulation. Data represent mean ± SD (n = 3) of three experiments were tested for statistical significance using unpaired two-tailed *t*-test. $*p < 0.05$, $**p < 0.01$.

completely mixed and incubated with medium for 15 min. The mixture was added to three parallel wells (each with 25 ml mixture) and grown for 24 h. The lysate was obtained using 100 μl 1 × passive lysis buffers at room temperature for 10 min and transferred to a plate. Luciferase activities of all samples were measured using the Luciferase Assay System (Promega, USA) according to the manufacturer's instructions.

CIK cells were grown to 70% confluence and then transfected with 2 μg of pcDNA3.1-basic or 2 μg of pcDNA3.1-*SAMHD1*.

2.8. RNA interference-mediated gene-knockdown assays

In the siRNA-mediated gene-knockdown assay, the specific small interfering RNA (siRNA) sequences against *CiIRF1, CiIRF9* and the negative control RNA oligonucleotides (*N.C*) were designed and synthesized by Shanghai GenePharma (Table 2). In this assay , we used the same siRNA sequences against *CiIRF3* and *Cip65* as our previous studies [26,27]. The protocol of transfection was followed in the same manner as the previous description [19]. Cells were separately transfected with 2 μg of *siRNA-IRF1*, 2 μg of *siRNA- IRF3*, 2 μg of *siRNA-IRF9*, 2 μg of *siRNA-p65* and 2 μg of *N.C*. *CiSAMHD1* mRNA was detected by q-PCR.

2.9. Cell viability and apoptosis assays

*Ci*SAMHD1-induced cell viability was evaluated by cell counting kit (CCK). Approximately 2×10^3 CIK cells were seeded and cultured in each well of a 96-well plate at 28 °C for 12 h, and then transfected with 2 μg of pCDNA3.1-basic or 2 μg of pCDNA3.1-*SAMHD*1. Twenty-four hours later, 10 μl of Trans Detect® Cell Counting Kit (CCK) (Transgene, China) was added to each well, and then continued to incubate for 2 h. The plates were measured spectrophotometrically on Microplate Manager 6 (Bio-Rad) at a wavelength of 450 nm. Cell viability (%) = $(OD_{pcDNA3.1\text{-}SAMHD1/pcDNA3.1\text{-}basic}\text{-}OD_{M199-/-})/(OD_{cell}\text{-}OD_{M199-/-}) \times 100\%$. $OD_{pcDNA3.1\text{-}SAMHD1/pcDNA3.1\text{-}basic}$: the absorbance of well with cells, CCK solution and transfected recombinant plasmid pCDNA3.1-*SAMHD1* or pCDNA3.1-basic; $OD_{M199-/-}$: the absorbance of well with the M199 medium and CCK solution; OD_{cell}: the absorbance of well with cells and CCK solution.

Cell apoptosis was analyzed by One Step TUNEL Apoptosis Assay Kit and Hoechst 33258 (Beyotime, China). CIK cells were plated on microscopic petri dishes for 12 h, and then transfected with 2 μg of pcDNA3.1-basic or 2 μg of pcDNA3.1-*SAMHD1* for 24 h. The protocol of kit was followed by manufacturer's instructions.

2.10. Antiviral activity test

To evaluate the antiviral activities of *Ci*SAMHD1, CIK cells were seeded in 35 cm^2 petri dishes and transfected with 2 μg of pcDNA3.1-*SAMHD1* or 2 μg of pcDNA3.1-basic for 12 h. Each well of cells were infected with GCRV at a 1000 $TCID_{50}$ (standard of 50% tissue culture infective dose) for 48 h. The culture medium was collected as titer samples. The viral titers of GCRV were measured by $TCID_{50}$ method, according to the Reed-Muench method [28]. The infected cells were washed with PBS, fixed by 30% formaldehyde for 30 min, stained by 1% (w/v) crystal violet 30 min, and observed for cytopathic effect (CPE). The two viral genes of GCRV, vp3 (Genbank ID: JQ782741) and vp5 (Genbank ID: JQ782742), were detected by q-PCR.

2.11. Statistical analysis

Statistical analysis and graphs of q-PCR, dual-luciferase assay and cell viability assay were performed and produced by Microsoft excel. Confocal images were analyzed and performed by image J. Data from q-PCR, dual-luciferase assay, cell viability assay and viral titration were presented as mean and ± SD (n = 3). Each data from EMSA assay and TUNEL apoptosis assay was representative of at least three independent experiments. Significant differences were determined using a two-tailed Student's *t*-test ($*p < 0.05$, $**p < 0.01$).

Fig. 3. Characterization of *CiSAMHD1* promoter. Promoter sequence was shown in small letter. Transcription start site is in box and marked +1. IRF1, IRF3, ISGF3 and NF-κB binding elements are in box.

3. Results

3.1. Amino acid sequences and phylogenetic analysis of SAMHD1

In this study, the full-length cDNA sequence of *CiSAMHD1* (MF326081.1) was generated by RACE PCR cloning. *CiSAMHD1* is 2792 bp in length and comprises 1884 bp open reading frame that translates into 627 aa. *Ci*SAMHD1 protein contains sterile α motif (SAM) domain and a histidine aspartic (HD) domain. To understand amino acid sequences similarity of SAMHD1 between grass carp and other species, amino acid sequence multiple alignments and phylogenetic three were constructed. The sequences of SAMHD1 protein showed a high degree of homology between different fish species, especially in their histidine aspartic (HD) domain (Fig. 1A). The phylogenetic tree of *SAMHD1* indicated that *C. idella SAMHD1* shares high homology (> 90% bootstrap value) with other fish *SAMHD1*, especially with *S. rhinocerous SAMHD1* (Fig. 1B).

3.2. Expressional characteristic of CiSAMHD1 in tissues and CIK cells

Q-PCR was employed to analyze the transcription of *CiSAMHD1* in different tissues using *β-actin* as a control gene. *CiSAMHD1* was ubiquitously expressed at low level in almost all tested tissues. After injection with poly I:C, the mRNA levels of *CiSAMHD1* were significantly up-regulated in almost test tissues, especially in the brain, spleen, intestine and liver, but there was no increase in the eyes. In detail, the expression of *CiSAMHD1* was heavily up-regulated at 6 h post-induction and peaked at 12 h post-induction. Then the expression gradually recovered to the normal level at 48 h post-induction (Fig. 2A).

The expression analysis of *CiSAMHD1* was also performed in CIK

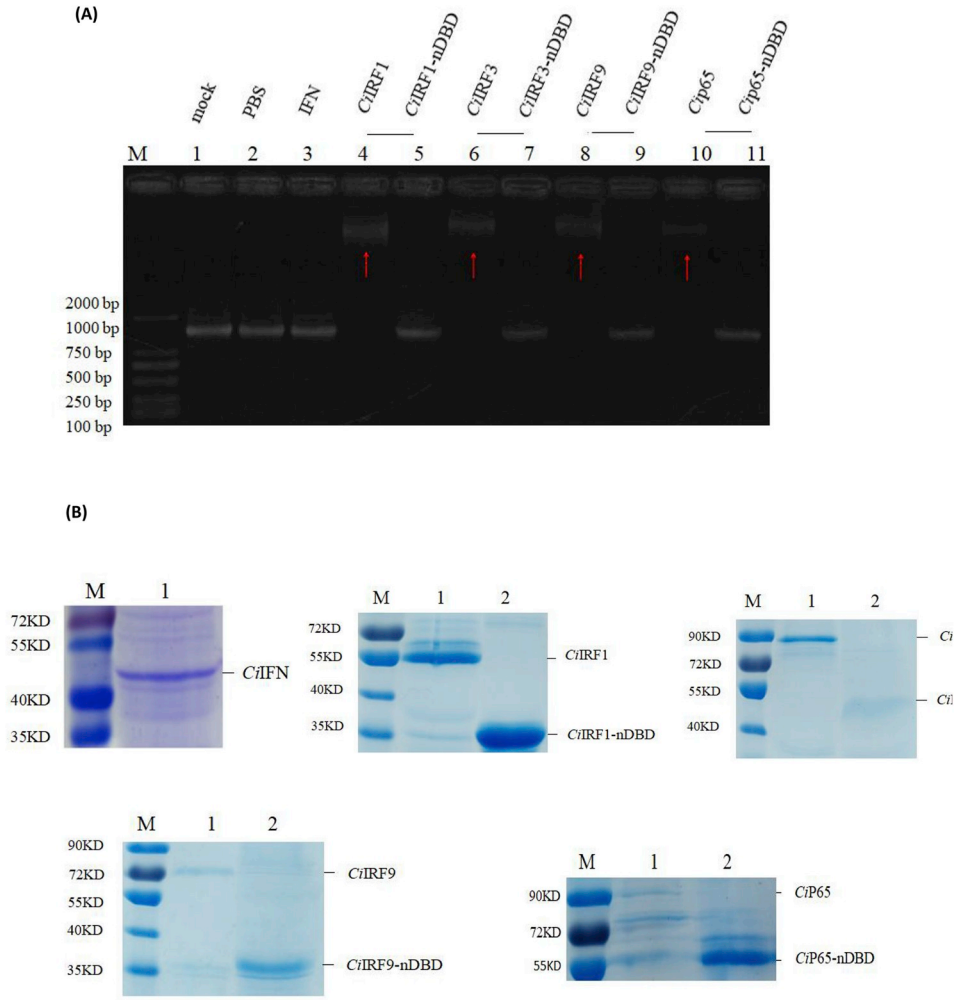

Fig. 4. Affinity of *CiSAMHD1* promoter with regulatory factors. 20 ng *CiSAMHD1* promoter was incubated with 1 μg of fusion peptides (*Ci*IRF1, *Ci*IRF1-nDBD, *Ci*IRF3, *Ci*IRF3-nDBD, *Ci*IRF9, *Ci*IRF9-nDBD, *Ci*p65, *Ci*p65-nDBD, PBS, *Ci*IFN, respectively) in a 10 μl reaction system, and incubated in ice for 1 h, then analyzed by a 1% agarose gel **(A)**. Non-proteins were performed as a mock; non-DNA binding domain (nDBD) of regulatory factors (*Ci*IRF1, *Ci*IRF3, *Ci*IRF9, *Ci*p65), PBS and *Ci*IFN protein were performed as negative controls. M: DNA molecular length marker (TaKaRa, Japan). The purified recombinant proteins (*Ci*IRF1, *Ci*IRF1-nDBD, *Ci*IRF3, *Ci*IRF3-nDBD, *Ci*IRF9, *Ci*IRF9-nDBD, *Ci*p65, *Ci*p65-nDBD, PBS, *Ci*IFN) were analyzed by 12% SDS-PAGE **(B)**. The sizes of recombinant proteins *Ci*IFN, *Ci*IRF1, *Ci*IRF1-nDBD, *Ci*IRF3, *Ci*IRF3-nDBD, *Ci*IRF9, *Ci*IRF9-nDBD, *Ci*p65 and *Ci*p65-nDBD were 43 kDa, 55 kDa, 35 kDa, 86 kDa, 50 kDa, 73 kDa, 38 kDa, 90 kDa and 55 kDa, respectively. M: Page Ruler™ Prestained Protein Ladder (Thermo, USA). These experiments were representative of at least three independent experiments.

cells. The cells were stimulated with 10 ng/well of grass carp recombinant IFN protein. Q-PCR results indicated that *CiSAMHD1* transcription was dramatically up-regulated at 6 h post-stimulation with rIFN (Fig. 2B).

3.3. Characterization of CiSAMHD1 promoter region

The promoter region of *CiSAMHD1* was obtained from grass carp database and analyzed in ALGGEN-PROMO program (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi? dirDB¼TF_8.3). The IRF1, IRF3, ISGF3 and NF-κB elements were found in *CiSAMHD1* promoter region (Fig. 3). The relevant transcriptional regulators were chosen in the subsequent experiments.

3.4. Affinity of CiSAMHD1 promoter with recombinant proteins

*Ci*IRF1 and *Ci*IRF1-nDBD, *Ci*IRF3 and *Ci*IRF3-nDBD, *Ci*IRF9 and *Ci*IRF9-nDBD, *Ci*p65 and *Ci*p65-nDBD prokaryotic proteins were all used in gel mobility shift assays. The shifting of *CiSAMHD1* promoter on agarose gel was all blocked by *Ci*IRF1, *Ci*IRF3, *Ci*IRF9 and *Ci*p65; however, non-DNA binding domain (nDBD) of *Ci*IRF1, *Ci*IRF3, *Ci*IRF9 and *Ci*p65 proteins had no blockage effects on *CiSAMHD1* promoter shift. Mock represented non-protein-added group. PBS and IFN performed as negative controls (Fig. 4A). The prokaryotic proteins were further analyzed by 12% SDS-PAGE (Fig. 4B). The results indicated that *Ci*IRF1, *Ci*IRF3, *Ci*IRF9 and *Ci*p65 proteins can interact with *CiSAMHD1* promoter *in vitro*.

3.5. IRF3 and IRF9 regulate CiSAMHD1 transcription

To understand the mechanism of transcriptional regulation of *CiSAMHD1*, dual luciferase assay and q-PCR were used to analyze the promoter activity and mRNA level of *CiSAMHD1*, respectively. In dual luciferase assay, *Ci*IRF3 and *Ci*IRF9 up-regulated the promoter activity of *CiSAMHD1*; however, *Ci*IRF1 and *Ci*p65 cannot induce the promoter activity of *CiSAMHD1* (Fig. 5A). These results were consistent with the results from q-PCR (Fig. 5B). The efficiency of overexpressing *Ci*IRF1, *Ci*IRF3, *Ci*IRF9 and *Ci*p65 were shown in supplementary Figure 2.

Subsequently, RNAi assay was performed to further confirm whether *Ci*IRF3 and *Ci*IRF9 are the regulators of *CiSAMHD1* or not. The

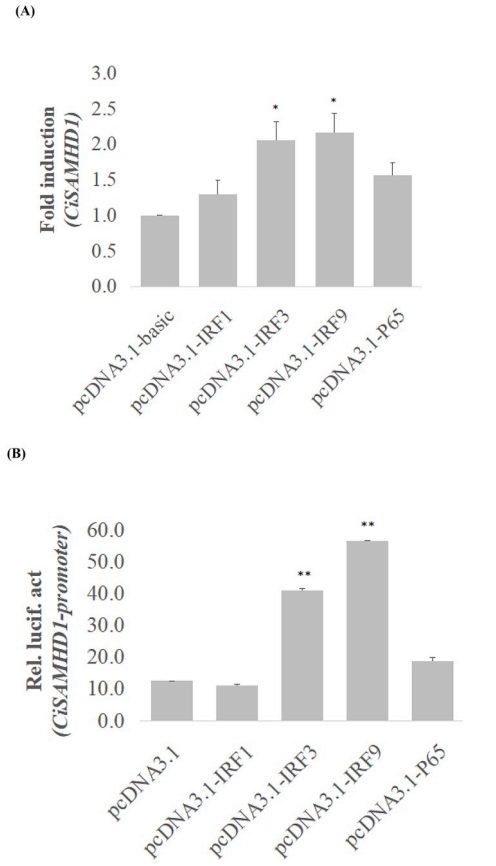

Fig. 5. *Ci*IRF3 and *Ci*IRF9 up-regulated the transcriptional level of *CiSAMHD1*. CIK cells were transfected with 2 μg of pcDNA3.1-*IRF1* or 2 μg of pcDNA3.1-*IRF3* or 2 μg of pcDNA3.1-*IRF9* or 2 μg of pcDNA3.1-*p65* respectively using 6 μl of FuGENE® in 6-well plate. 24 h later, total RNA was extracted. Q-PCR was used to detect the transcription of *CiSAMHD1* **(A)**. CIK cells were transfected wih 0.25 μg of pcDNA3.1-*IRF1* or 0.25 of μg pcDNA3.1-*IRF3* or 0.25 μg of pcDNA3.1-*IRF9* or 0.25 μg of pcDNA3.1-*p65*, or 0.25 μg of pcDNA3.1-basic separately with 0.25 μg of pGL3-*SAMHD1-pro* and 0.025 μg of pRL-TK plasmids. pRL-TK was used to standardize the expression level. 24 h later, cells were harvested and luciferase activities were measured by Dual Luciferase Reporter Assay System **(B)**. The data obtained from mean of three replicates is shown with ± S.D (n = 3). The level of statistical significance between control group and experiment group were determined by *t*-test (*$p < 0.05$; **$p < 0.01$).

efficiency of *siRNA-IRF1* and *siRNA-IRF9* were shown in Fig. 6A and Fig. 6B, *siRNA-IRF1-175* and *siRNA-IRF9-106* were used in subsequent experiment. Knockdown of *Ci*IRF3 and *Ci*IRF9 inhibited the activity of *CiSAMHD1* promoter, but knockdown of *Ci*IRF1 or *Ci*p65 had no influence on *CiSAMHD1* transcription (Fig. 6C). These data suggested that *CiSAMHD1* transcription were specifically regulated by *Ci*IRF3 and *Ci*IRF9.

3.6. CiSAMHD1 decreased cell viability and induced apoptosis

To further delineate the biological function of *Ci*SAMHD1, cell viability was examined in overexpressed *Ci*SAMHD1 CIK cells. In comparison with the control, *Ci*SAMHD1 dramatically decreased cell viability (~45%) (Fig. 7A).

Apoptosis were analyzed by TUNEL and Hoechst 33258 staining assays. In the TUNEL assay, obvious green fluorescence were detected in the cells which were transfected with pcDNA3.1-*SAMHD1* plasmids (Fig. 7B), as compared to cells transfected with pcDNA3.1-basic plasmids or mock group. The cells transfected with pcDNA3.1-*SAMHD1* were induced apoptosis using Hoechst 33258 staining assay (Fig. 7C). The transcripts of *Bax* (a gene inducing apoptosis) were up-regulated when CIK cells were transfected with pcDNA3.1-*SAMHD1*. However, the cells transfected with pcDNA3.1-*SAMHD1* had less impact on the transcription level of *BCL2* (Fig. 7D). These results suggested that *Ci*SAMHD1 can decrease cell viability and induces apoptosis.

3.7. CiSAMHD1 inhibits the proliferation of GCRV and increases the transcription of ISG15

To explore the antiviral effect of *Ci*SAMHD1, we monitored GCRV infection in CIK cells. CIK cells were transfected with pcDNA3.1-*SAMHD1* or pcDNA3.1-basic, followed by GCRV infection. Crystal violet staining showed a markedly reduced number of cytopathic effects (CPEs) in *Ci*SAMHD1 overexpressed cells as compared with control cells (Fig. 8A). The detection of virus titer revealed that overexpression of *Ci*SAMHD1 inhibited viral replication (Fig. 8B). The genes of *vp3* and *vp5* are part of GCRV genome [29]. Consistently, the transcription levels of *vp3* and *vp5* were decreased in the cells overexpressed *Ci*SAMHD1 (Fig. 8C). Surprisingly, overexpression of *Ci*SAMHD1 up-regulated the transcriptional level of *ISG15* (Fig. 8D).

4. Discussion

Restriction factors and innate immune signaling pathways are significant for cells to fight against pathogens invasion [30]. SAMHD1 plays a pivotal role in resisting various viruses in different ways [14,31,32]. Up to now, some homologs of *SAMHD1* have been found in fish. In this study, grass carp SAMHD1 is first identified. Similarly, *Ci*SAMHD1 protein also contains a sterile α motif domain (SAM) and a histidine-aspartate domain (HD) (Fig. 1A). Phylogenetic analysis also demonstrated that SAMHD1 is highly conserved in vertebrates, and *Ci*SAMHD1 shares the highest level of homology with *Sinocyclocheilus rhinocerous* SAMHD1 (Fig. 1B). Therefore, it provides the structural basis for the similar function between *Ci*SAMHD1 and mammalian SAMHD1.

Human SAMHD1 is induced by IL-12/IL-18 and TNF-α in monocyte-derived macrophages and lung fibroblasts, respectively. Its expression is regulated by promoter methylation [33,34]. Porcine SAMHD1 can be induced by HP-PRRSV (highly pathogenic porcine reproductive and respiratory syndrome virus) and regulated by IRF3 [12,35]. In fish, *CiSAMHD1* constitutively expressed in tested tissues, and was up-regulated by poly I:C stimulation in the tissues of the brain, spleen, gill, intestine, liver, heart and kideny. Interestingly, the expression of *CiSAMHD1* in eyes had no obvious changes under poly I:C stimulation (Fig. 2A), this pattern may suggest functional diversity of *Ci*SAMHD1 in different grass carp tissues. Moreover, recombinant IFN can induce the transcription level of *CiSAMHD1* in CIK cells (Fig. 2B). These results indicated that the expression of *Ci*SAMHD1 is regulated by poly I:C-mediated and IFN-mediated pathway.

To investigate the regulatory mechanism of *Ci*SAMHD1 gene, the promoter of *CiSAMHD1* is determined. ALGGEN-PROMO program

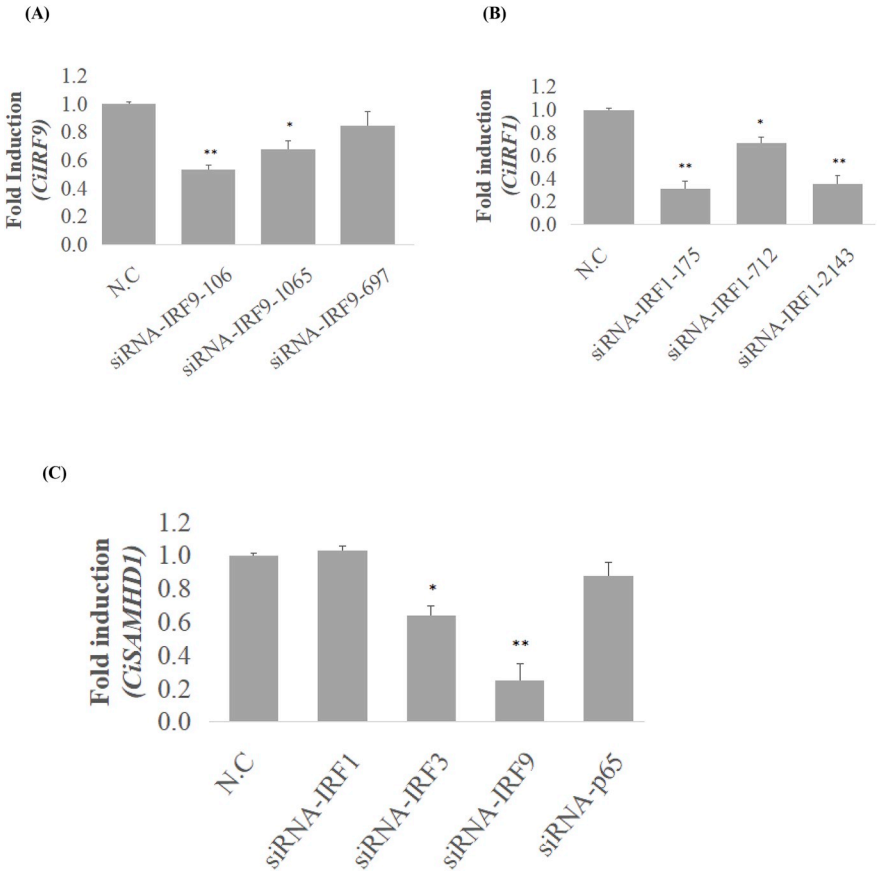

Fig. 6. Knockdown of *Ci*IRF3 and *Ci*IRF9 down-regulated the transcription level of *CiSAMHD1*. CIK cells were transfected with (2 μg of *siRNA-IRF1-175/712/2143* or 2 μg of *siRNA-IRF9-106/1065/697* or 2 μg of *N.C*) using 6 μl of Hiperfect® (QIAGEN, Germany) in 6-well plates respectively. N.C acted as a control. 24 h later, total RNA was extracted. Q-PCR detected mRNA levels of *CiIRF9* **(A)** and *CiIRF1* **(B)** respectively. CIK cells were transfected with (2 μg of *siRNA-IRF1-175* or 2 μg of *siRNA-IRF3* or 2 μg of *siRNA-IRF9-106* or 2 μg of *siRNA-p65* or 2 μg of N.C). The mRNA of *CiSAMHD1* was detected **(C)**. The data of q-PCR obtained from mean of three replicates is shown with ± SD. The level of statistical significance between control group and experiment group were determined by *t*-test (*$p < 0.05$; **$p < 0.01$).

showed that the IRF1, IRF3, IRF9 and p65 binding elements are found in *CiSAMHD1* promoter (Fig. 3). Actually, prokaryotic *Ci*IRF1, *Ci*IRF3, *Ci*IRF9 and *Ci*p65 proteins interact with *CiSAMHD1* promoter *in vitro* (Fig. 4). As we all know, IRF1 [23], IRF3 [36], IRF9 [21] and p65 [37] are all positive regulators of innate immunity and inflammatory response genes in many fish. However, intracellular experiment demonstrated that *Ci*IRF3 and *Ci*IRF9 rather than *Ci*IRF1 and *Ci*p65 up-regulated the promoter activity of *CiSAMHD1* in CIK cells (Fig. 5A and B). On the contrary, knockdown of *Ci*IRF3 and *Ci*IRF9 in cells inhibited *CiSAMHD1* transcription (Fig. 6C). These results indicated that the transcription of *CiSAMHD1* was specifically controlled by *Ci*IRF3 and *Ci*IRF9. It is well-known that IRF3 and IRF9 are downstream regulator for poly I:C-TLRs and IFN-JAK-STAT mediated pathway respectively [20,38]. Therefore, the transcription of *CiSAMHD1* is regulated by TLRs and JAK-STAT mediated pathway.

HTLV-1 (Human T cell leukemia virus type 1) infection induces SAMHD1-mediated apoptosis in monocytic cells. SAMHD1 promotes HTLV-1 reverse transcription intermediates (RTI) sensed by STING and initiates IRF3-Bax-driven apoptosis (10). In this paper, overexpression of *Ci*SAMHD1 induced cell apoptosis and increased the transcription level of *Bax* in CIK cells (Fig. 7).

Overexpression of porcine SAMHD1 blocks the proliferation of HuN4 in MARC-145 cells and increases the transcription of *ISG15* (12). *Ci*SAMHD1 inhibited the proliferation of GCRV and increased the transcriptional levels of *CiISG15* in the absence of external IFN stimulation (Fig. 8). These results showed that grass carp SAMHD1 shares similar functions with those of mammals.

In summary, the full-length cDNA sequence of grass carp *SAMHD1* was firstly identified and characterized in this study. *CiSAMHD1* transcription was up-regulated by poly I:C and recombinant IFN stimulation. *Ci*IRF3 and *Ci*IRF9 were specifical regulators for *CiSAMHD1* transcription. The overexpression of *Ci*SAMHD1 can induce apoptosis and inhibit the proliferation of GCRV in CIK cells.

Acknowledgement

This study was supported by Major projects of Natural Science Foundation of Jiangxi Province (20171ACB20004), the National Natural Science Foundation of China (31560594), the earmarked fund for Jiangxi Agriculture Research System (JXARS-04) and the Science & Technology Foundations of Education Department of Jiangxi (KJLD14002), the Science & Technology Foundations of Education Department of Jiangxi (GJJ161303) and the Science and Technology Project of Education Department of Jiangxi Province (GJJ180112).

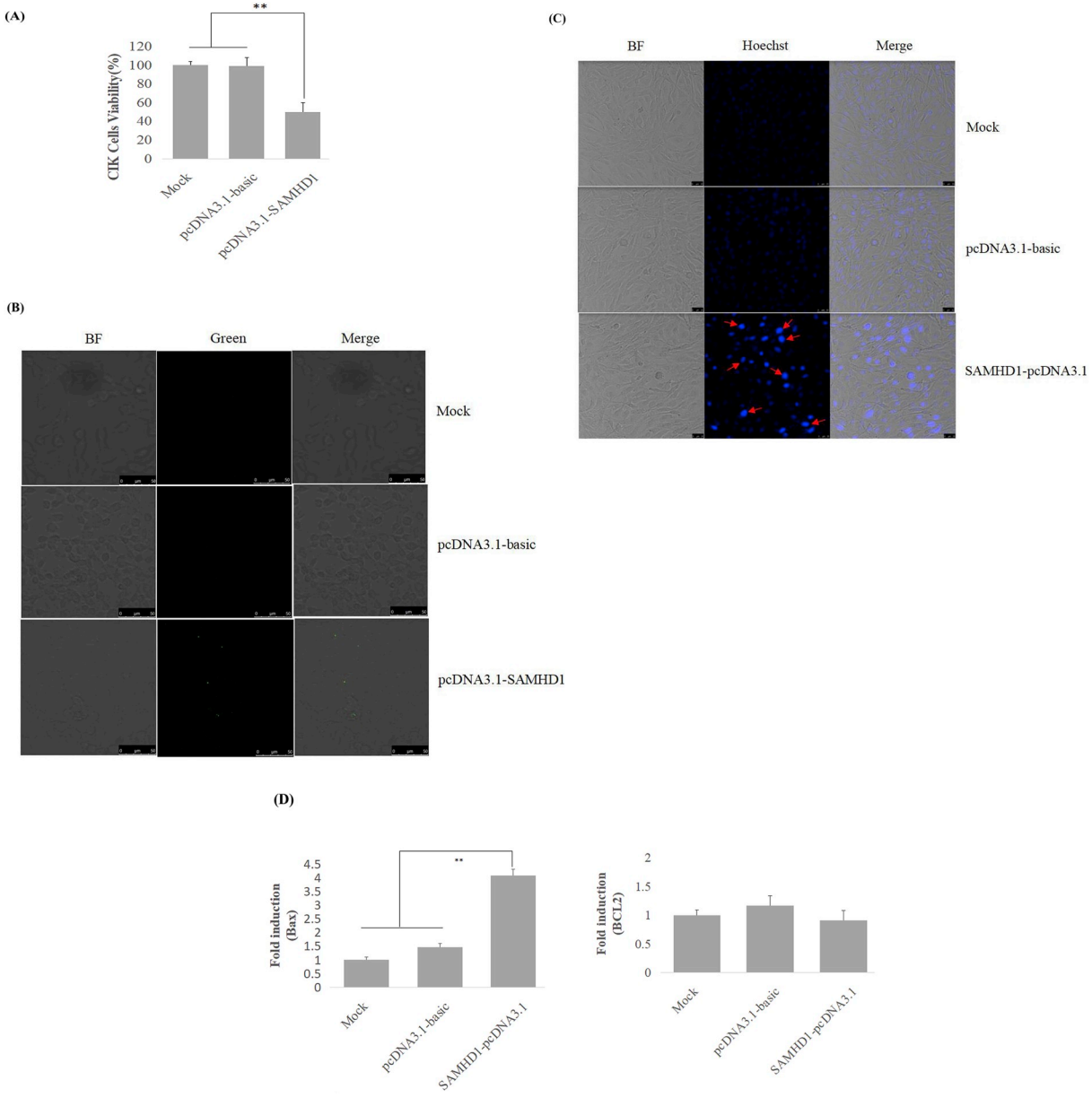

Fig. 7. *Ci*SAMHD1 decreased cell viability and induced apoptosis. CIK cells were seeded on 96-well plates and separately transfected with 2 μg of pcDNA3.1-*SAMHD1* and 2 μg of pcDNA3.1-basic. After 48 h of transfection, cell viability was evaluated by CCK assay **(A)**. Cell apoptosis was analyzed by TUNEL staining **(B)** and Hoechst 33258 staining **(C)**. The scale bar is 50 μm. NA is 40 × /0.60. Q-PCR detected mRNA levels of *Bax* and *BCL2* **(D)**. The data obtained from mean of three replicates were shown with ± SD. The level of statistical significance between control group and experiment group were determined by *t*-test (*$p < 0.05$; **$p < 0.01$).

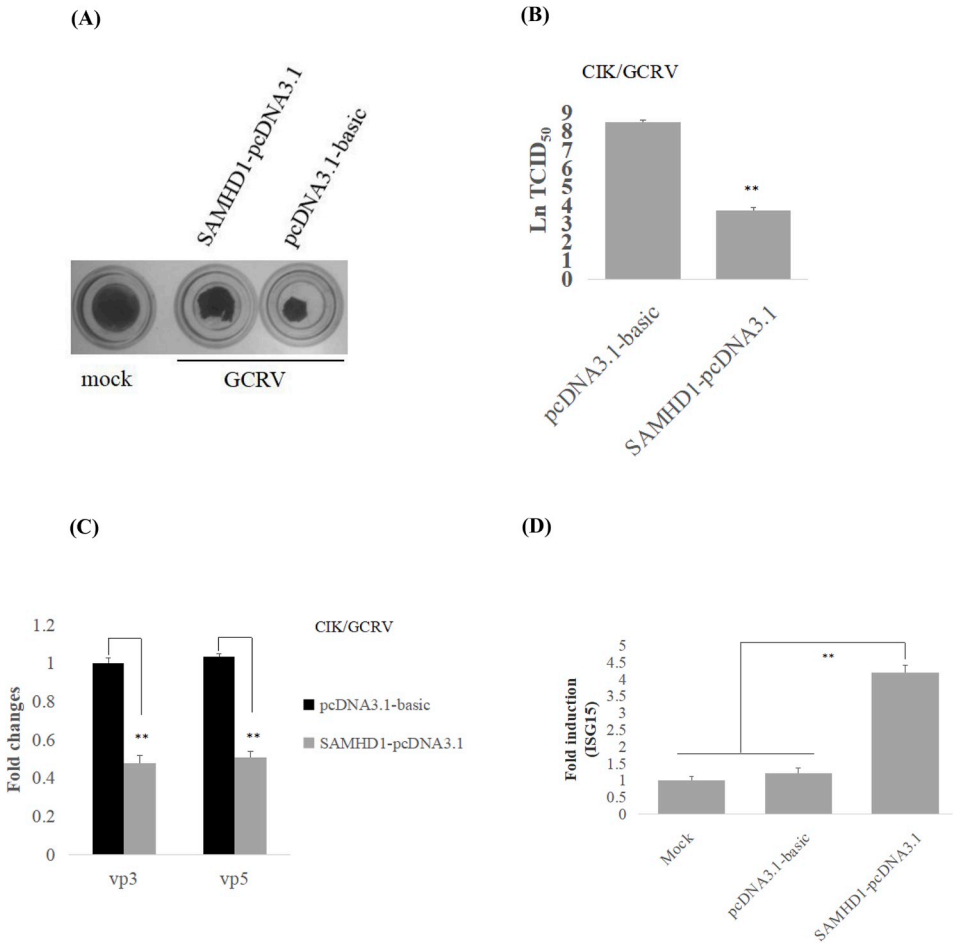

Fig. 8. *Ci*SAMHD1 inhibited proliferation of GCRV and increased the transcription of *ISG15*. CIK cells were seeded on 35 cm^2 petri dishes and separately transfected with 2 μg of pcDNA3.1-*SAMHD1*, 2 μg of pcDNA3.1-basic. After 12 h of transfection, cells were infected with GCRV (1000 $TCID_{50}$/ml). After another 48 h, cells were stained with crystal violet for CPEs **(A)**. The supernatants were harvested for $TCID_{50}$ assay to measure virus titers **(B)**. Q-PCR detected mRNA levels of *vp3, vp5* **(C)** and *ISG15* **(D)**. The data obtained from mean of three replicates is shown with ± SD. The level of statistical significance between control group and experiment group was determined by *t*-test (*$p < 0.05$; **$p < 0.01$).

Appendix A. Supplementary data

Supplementary data to this chapter can be found online at https://doi.org/10.1016/j.fsi.2019.03.028.

Identical (shaded in black) and similar (shaded in gray and light gray) residues identified by the Gene doc program are indicated. Both sterile α motif (SAM) domain and a histidine aspartic (HD) domain are indicated with arrows **(A)**. A neighbour-joining tree was constructed by MEGA X. All sequences used for analysis were derived from GenBank and their accession numbers were shown in parentheses. The bootstrap confidence values shown at the nodes of the tree were based on 1000 bootstrap replications **(B)**.

References

[1] S.J. Neil, T. Zang, P.D. Bieniasz, Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu, Nature 451 (2008) 425–430.
[2] A.M. Sheehy, N.C. Gaddis, J.D. Choi, M.H. Malim, Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral VIF protein, Nature 418 (2002) 646–650.
[3] M. Stremlau, C.M. Owens, M.J. Perron, M. Kiessling, P. Autissier, J. Sodroski, The cytoplasmic body component TRIM5α restricts HIV-1 infection in old world monkeys, Nature 427 (2004) 848–853.
[4] V. Simon, N. Bloch, N.R. Landau, Intrinsic host restrictions to HIV-1 and mechanisms of viral escape, Nat. Immunol. 16 (2015) 546–553.
[5] A. Brandariz-Nuñez, J.C. Valle-Casuso, T.E. White, N. Laguette, M. Benkirane, J. Brojatsch, et al., Role of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac, Retrovirology 9 (2012) 49.
[6] V. Ramesh, B. Bernardi, A. Stafa, C. Garone, E. Franzoni, M. Abinun, et al., Intracerebral large artery disease in Aicardi-Goutières Syndrome implicates SAMHD1 in vascular homeostasis, Dev. Med. Child Neurol. 52 (2010) 725–732.
[7] D.C. Goldstone, V. Ennisadeniran, J.J. Hedden, H.C. Groom, G.I. Rice, E. Christodoulou, et al., HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase, Nature 480 (2011) 379–382.
[8] N. Laguette, B. Sobhian, N. Casartelli, M. Ringeard, C. Chablebessia, E. Ségéral, et al., SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx, Nature 474 (2011) 654–657.
[9] T.E. White, A. Brandariz-Nunez, J.C. Valle-Casuso, S. Amie, L. Nguyen, B. Kim, et al., Contribution of SAM and HD domains to retroviral restriction mediated by human SAMHD1, Virology 436 (2013) 81–90.
[10] A. Sze, S.M. Belgnaoui, D. Olagnier, R. Lin, J. Hiscott, G.J. Van, Host restriction factor SAMHD1 limits human T cell leukemia virus type I infection of monocytes via STING-mediated apoptosis, Cell Host Microbe 14 (2013) 422–434.
[11] E.T. Kim, T.E. White, A. Brandariznúñez, F. Diazgriffero, M.D. Weitzman, SAMHD1 restricts herpes simplex virus I in macrophages by limiting DNA replication, J. Virol. 87 (2013) 12949–12956.
[12] S. Yang, T. Shan, Y. Zhou, Y. Jiang, W. Tong, F. Liu, et al., Molecular cloning and characterizations of porcine SAMHD1 and its roles in replication of highly pathogenic porcine reproductive and respiratory syndrome virus, Dev. Comp. Immunol. 47 (2014) 234–246.
[13] S.D. de Silva, H. Hoy, T.S. Hake, H.K. Wong, P. Porcu, L. Wu, Promoter methylation regulates SAMHD1 gene expression in human CD4 T cells, J. Biol. Chem. 29 (2012) 9284–9292.
[14] D. Ayinde, N. Casartelli, O. Schwartz, Restricting HIV-1 the SAMHD1 way: through nucleotide starvation, Nat. Rev. Microbiol. 10 (2012) 675–680.
[15] E. Ballana, J.A. Esté, SAMHD1: at the crossroads of cell proliferation, immune responses, and virus restriction, Trends Microbiol. 23 (2015) 680–692.
[16] F.F. Yu, Y.B. Zhang, T.K. Liu, Y. Liu, F. Sun, J. Jiang, et al., Fish virus-induced interferon exerts antiviral function through Stat1 pathway, Mol. Immunol. 47 (2010) 2330–2341.
[17] T.K. Liu, Y.B. Zhang, Y. Liu, F. Sun, J.F. Gui, Cooperative roles of fish protein kinase containing Z-DNA binding domains and double-stranded RNA-dependent protein kinase in interferon-mediated antiviral response, J. Virol. 85 (2011) 12769–12780.
[18] C. Wu, Y. Hu, L. Fan, H. Wang, Z. Sun, S. Deng, et al., *Ctenopharyngodon idella* PKZ facilitates cell apoptosis through phosphorylating eIF2α, Mol. Immunol. 69 (2016) 13–23.
[19] T. Zhang, D. Li, L. Wan, X. Chen, X. Wang, B. Zhong, et al., *Ctenopharyngodon idella* PERK (EIF2AK3) decreases cell viability by phosphorylating eIF2α under ER stress, Fish Shellfish Immunol. 70 (2017) 568–574.
[20] F. Sun, Y.B. Zhang, T.K. Liu, L. Gan, F.F. Yu, Y. Liu, et al., Characterization of fish IRF3 as an IFN-Inducible protein reveals evolving regulation of IFN response in vertebrates, J. Immunol. 185 (2010) 7573–7582.
[21] Z. Wu, L. Wang, X. Xu, G. Lin, H. Mao, X. Ran, et al., Interaction of IRF9 and STAT2 synergistically up-regulates IFN and PKR transcription in *Ctenopharyngodon idella*,

Mol. Immunol. 85 (2017) 273–282.
[22] P.R. Kasher, E.M. Jenkinson, V. Briolat, D. Gent, C. Morrissey, L.A. Zeef, et al., Characterization of SAMHD1 morphant zebrafish recapitulates features of the human type I interferonopathy Aicardi-Goutieres Syndrome, J. Immunol. 194 (2015) 2819–2825.
[23] Q. Lai, G. Lin, M. Ma, S. Huang, W. Li, D. Li, et al., IRF-1 acts as a positive regulator in the transcription of grass carp (*Ctenopharyngodon idella*) IFN gene, Fish Shellfish Immunol. 34 (2013) 1432–1438.
[24] Q.M. Zhang, X. Zhao, Z. Li, M. Wu, J.F. Gui, Y.B. Zhang, Alternative splicing transcripts of zebrafish LGP2 gene differentially contribute to IFN antiviral response, J. Immunol. 200 (2017) 688–703.
[25] X.B. Yu, X.H. Chen, F. Ling, K. Hao, G.X. Wang, B. Zhu, Moroxydine hydrochloride inhibits grass carp reovirus replication and suppresses apoptosis in *Ctenopharyngodon idella* kidney cells, Antivir. Res. 131 (2016) 156–165.
[26] X. Xu, M. Li, Z. Wu, H. Wang, L. Wang, K. Huang, et al., Endoplasmic reticulum transmembrane proteins ZDHHC1 and STING both act as direct adaptors for IRF3 activation in teleost, J. Immunol. 199 (2017) 3623–3633.
[27] Q. Huang, D. Xie, H. Mao, H. Wang, Z. Wu, K. Huang, et al., *Ctenopharyngodon idella* p53 mediates between NF-κB and PKR at the transcriptional level, Fish Shellfish Immunol. 69 (2017) 258–264.
[28] L.J. Reed, H. Muench, A simple method of estimating fifty per centendpoints, Am. J. Hyg. 27 (1938) 493–497.
[29] Y. Rao, J. Su, Insights into the antiviral immunity against grass carp (*Ctenopharyngodon idella*) reovirus (GCRV) in grass carp, J Imuunol Res 2015 (2015) 670437.
[30] A.M. Kell, M. G Jr., RIG-I in RNA virus recognition, Virology 110 (2015) 479–480.
[31] C.T. Gelais, S.D. Silva, S.M. Amie, C.M. Coleman, H. Hoy, J.A. Hollenbaugh, et al., SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4+ T-lymphocytes cannot be upregulated by interferons, Retrovirology 11 (2012) 9–105.
[32] H.M. Baldauf, X.Y. Pan, E. Erikson, S. Schmidt, W. Daddacha, M. Burggraf, et al., SAMHD1 restricts HIV-1 infection in resting CD4+ T cells, Nat. Med. 18 (2012) 1682–1687.
[33] W. Liao, Z. Bao, C. Cheng, Y.K. Mok, W.S.F. Wong, Dendritic cell-derived interferon-γ-induced protein mediates tumor necrosis factor-α stimulation of human lung fibroblasts, Proteomics 8 (2008) 2640–2650.
[34] E. Pauls, E. Jimenez, A. Ruiz, M. Permanyer, E. Ballana, H. Costa, et al., Restriction of HIV-1 replication in primary macrophages by IL-12 and IL-18 through the up regulation of SAMHD1, J. Immunol. 190 (2013) 4736–4741.
[35] S. Yang, Y. Zhan, Y. Zhou, Y. Jiang, X. Zheng, L. Yu, et al., Interferon regulatory factor 3 is a key regulation factor for inducing the expression of SAMHD1 in antiviral innate immunity, Sci. Rep. 6 (2016) 29665.
[36] X. Xu, Q. Lai, M. Gu, D. Liu, Q. Hou, X. Liu, et al., Fish IRF3 up-regulates the transcriptional level of IRF1, IRF2, IRF3 and IRF7 in CIK cells, Fish Shellfish Immunol. 47 (2015) 978–985.
[37] H. Wang, Y. Zhu, X. Xu, X. Wang, Q. Hou, Q. Xu, et al., *Ctenopharyngodon idella* NF-kB subunit p65 modulates the transcription of IkBα in CIK cells, Fish Shellfish Immunol. 54 (2016) 564–572.
[38] J. Shi, Y.B. Zhang, T.K. Liu, F. Sun, J.F. Gui, Subcellular localization and functional characterization of a fish IRF9 from crucian carp *Carassius auratus*, Fish Shellfish Immunol. 32 (2012) 258–266.