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Fish SAMHD1 performs as an activator for IFN expression

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ABSTRACT

As a host limiting factor, Sterile Alpha Motif and Histidine-Aspartate Domain 1 protein (SAMHD1) is associated with IRF3-mediated antiviral and apoptotic responses in mammals. However, the antiviral mechanism of SAMHD1 remains indistinct in fish. In this study, we found the expression of *Ctenopharyngodon idella* SAMHD1 (MF326081) was up-regulated after transfection with poly I:C (dsRNA analog), B-DNA or Z-DNA into *C. idella* kidney cells (CIKs), but these expression profiles had no obvious change when the cells were incubated with these nucleic acids. These data may indicate that *Ci*SAMHD1 participates in the intracellular PRR-mediated signaling pathway rather than extracellular PRR-mediated signaling pathway. Subcellular localization assay suggested that a part of over-expressed *Ci*SAMHD1 were translocated from nuclear to cytoplasm when *C. idella* ovary cells (COs) were transfected with poly I:C, B-DNA or Z-DNA. Nucleic acid pulldown assays were performed to investigate the reason for nuclear-cytoplasm translocation of *Ci*SAMHD1. The results showed that *Ci*SAMHD1 had a high affinity with B-DNA, Z-DNA and ISD-PS (dsRNA analog). In addition, co-IP assays revealed the interaction of *Ci*SAMHD1 with *Ci*STING (KF494194). Taken together, all these results suggest that grass carp SAMHD1 performs as an activator for innate immune response through STING-mediated signaling pathway.

1. Introduction

Virus infection is controlled by kinds of mechanisms at different levels in host cells. In this process, innate immune system and cell intrinsic restriction system are induced and make the host cells go into an antiviral state. How these different ways of antiviral response operate effectively is important for successful immunity. Pattern-recognition receptor (PRR) which monitors virus infection has been well documented (Zhong et al., 2008; Takaoka et al., 2007; Bürckstümmer et al., 2009; Unterholzner et al., 2010).

A variety of host restriction factors, such as APOBECs, TRIM5α and Tetherin/BST-2 which restrict and interfere with viral replication, have been discovered and analyzed (Rehwinkel, 2014; Simon et al., 2015; Harris et al., 2012). Sterile α motif and histidine-aspartic acid (HD) domain containing protein 1 (SAMHD1) is HIV-1 restriction factor (Pilakkakanthikeel et al., 2015). Many studies have indicated the role of SAMHD1 in the evading mechanism of HIV-1 infection. SAMHD1, a deoxynucleoside triphospate (dNTP) triphosphohydrolase, depletes the intracellular pool of dNTPs and prevents HIV-1 reverse transcription in myeloid cells and resting $CD4^+$ T cells (Ayinde et al., 2012; Baldauf et al., 2012; Berger et al., 2011; Descours et al., 2012). Besides HIV-1, SAMHD1 reveals a relatively broad-spectrum anti-virus capability against numerous types of virus (Kim et al., 2013; Hollenbaugh et al., 2013). SAMHD1 functions to restrict the replication of HSV-1 DNA genome in differentiated macrophage cell lines (Kim et al., 2013). In the same way, SAMHD1 also prevents HP-PRRSV replication (Yang et al., 2014). Recently, another mechanism of SAMHD1 inhibiting virus infectivity through degradation and/or binding to viral nucleic acids has been illuminated (Ballana and Esté, 2015; Choi et al., 2015). In addition, SAMHD1 promotes viral intermediators complex with the innate immune sensor STING and initiates IRF3-Bax-directed apoptosis under the infection of HTLV-1 (Sze et al., 2013). Hence, SAMHD1 may undertake some complicated functions in response to virus infections.

In the past few years, significant progress has been made in fish antiviral signaling pathway. Zebrafish SAMHD1 is associated with Aicardi-Goutières syndrome (AGS) (Kasher et al., 2015). However, it still remains elusive about the function of fish SAMHD1 in innate immunity. In present study, we found that grass carp SAMHD1 (*Ci*SAMHD1) was up-regulated by the transfection of B-DNA, Z-DNA

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or poly I:C, and then some of them were translocated to cytoplasm from nucleus. Furthermore, the repositioned *Ci*SAMHD1 interacted with the nucleic acids and *Ci*STING. The interaction can facilitate the transcription of *IFN*. These results elucidated *Ci*SAMHD1 can respond to the nucleic acids, and then activate innate immune response through STING-mediated signaling pathway.

2. Materials and methods

2.1. Cells, DNA and RNA analogues, vectors

Grass carp (*C. idella*) kidney cells (CIKs) and grass carp (*C. idella*) ovary cells (COs) were cultured at 28 °C in medium 199 supplemented with 10% FCS. Z-DNA, B-DNA and ISD-PS were synthesized by Sangon Biotech (Shanghai, China). Their sequences were presented in Table 1. Poly I:C was purchased from Sigma (USA). pCMV-FLAG and pcDNA3.1(+) were both purchased from Invitrogen (USA). pET32a vector, pEGFP-C1, PGL3, *E. coli* strains DH5α and BL21 were all bought from Promega (USA).

2.2. Prokaryotic expressed proteins and antibodies

Grass carp SAMHD1 coding sequence (135-819 bp) was digested with *EcoR* I/*Xho* I and subcloned into pET32a expression vector. The recombinant plasmid pET32a/*Ci*SAMHD1-ORF was sequenced and transformed into competent *E. coli* BL21 cells, the cells were cultured in incubator shaker at 37 °C until the OD_{600} reaching 0.6–0.8 and then added 1 mM IPTG for 4 h. After that the cells were harvested, centrifuged and completely resuspended in binding buffer (20 mM Trise HCl, 500 mM NaCl, 5 mM imidazole, pH 7.9). The suspensions were broken by sonication and centrifuged for 15 min (4 °C, 12,000 rpm). Then the supernatant was purified by affinity chromatography with Ni-NTA resin (Novagen). The purified recombinant proteins were electrophoresed on a 12% SDS-PAGE gel.

Rabbit polyclonal anti-grass carp SAMHD1 was made by immunizing rabbits with the prokaryotic-expressed protein SAMHD1 (21-248 aa). The rabbit serum was directly used as antibody. Rabbit anti-*Ci*GAPDH, anti-*Ci*IFN antibodies were previously prepared in our lab (Xu et al., 2017). Histone H3 Ab was purchased from Affinity (USA). FLAG and GFP Ab were purchased from Sigma (USA) and Abmart (USA), respectively. The goat anti-rabbit and anti-mouse antibodies were bought from Sangon Biotech (Shanghai, China).

2.3. Plasmids construction

The open reading frame (ORF) for *CiSAMHD1* and *CiSTING* were inserted into pcDNA3.1 (+) vector to construct the expression plasmids and used for over-expression experiment. The promoter of *CiIFN* was inserted into pGL3 for luciferase assays. The ORF of *CiSAMHD1, CiTBK1 and CiSTING* were cloned into pEGFP-C1 and pCMV-FLAG for subcellular localization and co-IP assays. DNA sequencing confirmed all constructs. The primers used for plasmid construction are shown in Table 1.

2.4. Subcellular localization and nucleic acid pull-down assay

CO cells were plated on microscopic petri dishes for 12 h. Transfection systems were as follow: 2 μg pEGFP-SAMHD1 plasmid and 6 μl FuGENE®6. After 24 h of transfection, 2 μg Poly I:C, B-DNA and Z-DNA were severally transfected into CO cells. Then the cells were washed with PBS for three times and fixed with 4% (v/v) paraformaldehyde for 15 min at room temperature. These cells were dyed with DAPI (0.1 mg/ml) and examined under a confocal microscope (Olympus).

Nucleic acid pull-down assays were performed as described previously (Karayel et al., 2009). In brief, the synthetic ISD-PS replaced poly I:C because ISD-PS can conjugate with biotin label (White et al., 2017). ISD-PS, B-DNA and Z-DNA were synthesized with a 5′-biotin tag by Sangon Biotech (China), then 5 μg biotin-labelled ISD-PS, B-DNA and Z-DNA were dissolved in 100 μl 1 × BW buffer (5 mM Tris-Hcl pH 7.5, 0.5 mM EDTA, 1 M NaCl), then the dissolved ISD-PS, B-DNA and Z-DNA were incubated with M-280 streptavidin-coupled Dynabeads (Invitrogen) for 5 h. Finally, the unbound biotin-labelled nucleic acids were washed with 1 × BW buffer for five times. Cells were lysed using cell total protein lysis buffer (TransGen) and lysates were cleared by centrifugation.

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

Primer name	Primer sequence(5′-3′)	Application
SAMHD1-RT-F	GGGCTATTTGGCAGGATGTC	Real-time PCR
SAMHD1-RT-R	AAGACAGAGTTATCCAGGGGC	
IFN-RT-F	CATTGCCAACAGACGATA	
IFN-RT-R	ATTAGCTTGCTTGATCAGATT	
SAMHD1-ORF-pcDNA3.1-EcoRI-F	CGGAATTCCATGGATAAGCGGATTAAACGGCC	Eukaryotic vector construction
SAMHD1-ORF-pcDNA3.1-kpnI-R	CGGGTACCTTAGAGGTTGTTTTGTCCCTGAGC	
SAMHD1-ORF-pEGFP-EcoRI-F	CGGAATTCCATGGATAAGCGGATTAAACGGCC	
SAMHD1-ORF-pEGFP-kpnI-R	CGGGTACCTTAGAGGTTGTTTTGTCCCTGAGC	
STING-0RF-pEGFP-EcoRI-F	CGGAATTCAATGTGTGGTGTGATCGGAG	
STING-ORF-pEGFP-Sall-R	CGGTCGACAAATAATGACTGAAAAACCAAGAAATCATG	
SAMHD1-ORF-FLAG-EcoR1-R	CGGAATTCCATGGATAAGCGGATTAAACGGCC	
SAMHD1-ORF-FLAG-KpnI-F	CGGGTACCTTAGAGGTTGTTTTGTCCCTGAGC	
STING-GFP-F	CGGAATTCAATGTGTGGTGTGATCGGAG	
STING-GFP-R	CGGTCGACAAATAATGACTGAAAAACCAAGAAATCATG	
TBK1-FLAG-F	CGGAATTCAATGCAGAGTACGGCGAACT	
TBK1-FLAG-R	CGGGATCCAGAGGTTCGGCACGCTGA	
IFN-pro-F	CGGGTACCTGGTTGGTTTTAAAGTAGGCCTAATTG	IFN promoter vector
IFN-pro-R	CGACGCGTCGTTTCCAAACTAGAAGAGATGCG	
SAMHD1-antibody-F	CGGAATTCGTTTGTGATGGAGAGTTTTGG	Construction of antibody
SAMHD1-antibody-R	CGGCGGCCGCTCAAATAAGGTCATCAGGAAGTCTCA	
$(GC)_{10}$	CTGATACTACATTGAATTCGCGCGCGCGCGCGCGCGCGCGAATTCAATGTAGTATCAGA	Z-DNA
$(TA)_{10}$	CTGATACTACATTGAATTCTATATATATATATATATATATAGAATTCAATGTAGTATCAGA	B-DNA
ISD-PS-F	TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA	ISD-PS
ISD-PS-R	TGTAGATCATGTACAGATCAGTCATAGATCACTAGTAGATCTGTA	