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Role of the dimerization domain of black carp STING during the antiviral innate immunity

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ABSTRACT

Stimulator of interferon gene (STING) functions importantly as antiviral adaptor protein during the innate immune activation. The role of the dimerization domain (DD) of STING remains obscure although other domains of this molecule have been studied extensively. To clarify the mechanism of black carp STING (bcSTING) in the innate immunity, bcSTING-ΔDD (bcSTING without DD) and bcSTING-ΔCTT (bcSTING without CTT domain) were constructed and analyzed in this manuscript. The reporter assays revealed that the induced transcription of IFN promoters mediated by bcSTING-ΔDD were much higher than that of the wild type bcSTING; however, bcSTING-ΔCTT almost lost the activities to trigger the interferon (IFN) promoters transcription. The mRNA transcriptional levels of IFN and interferon stimulated genes (ISGs) in EPC cells expressing bcSTING-ΔDD were obviously higher than those of EPC cells expressing wild type bcSTING; however, the transcriptional levels of the above cytokines of EPC cells expressing bcSTING-ΔCTT were basically the same as those of control cells. EPC cells overexpressing bcSTING-DD showed stronger antiviral activity than those overexpressing wild-type bcSTING. Furthermore, the co-immunoprecipitation assay identified the self-interaction between bcSTING-ΔDD molecules. And it was interesting that the affinity between bcSTING-ΔDD and bcTBK1 was obviously stronger than that between bcTBK1 and wild-type bcSTING. Thus, our data suggests that DD of black carp STING plays a negative regulatory role in STING-mediated antiviral immunity, which provides a new perspective for further researching the function of fish STING in the innate immunity.

1. Introduction

Innate immunity is a natural defense mechanism gradually established in the long-term evolution process, which is the first line of defense against infectious microorganisms, such as viruses and bacteria [1]. Stimulator of interferon genes (STING, also known as MITA and MPYS, TMEM173), as an essential antiviral adaptor protein, has been shown to play a pivotal part in response to both non-self-cytosolic RNA and dsDNA [2,3]. Many DNA viruses, such as adenovirus, cowpox virus and papillomavirus, activate cGAS-STING signaling, one of the main signaling pathways against DNA viruses, leading to the activation of antiviral innate immunity [4]. STING significantly also facilitated innate immune responses to resist negative-stranded RNA viruses including vesicular stomatitis virus (VSV) and sendai virus (SEV) [5]. Recent studies have shown that STING-deficient mice were highly sensitive to VSV infection [6]. Accumulating evidences have showed that STING could interact with retinoic acidinducible gene I (RIG-I), mitochondrial antiviral signaling protein (MAVS) and interferon regulatory factor 3 (IRF3) respectively, and recruiting the kinase TANK-binding kinase 1 (TBK1), activating IRF3 and inducing interferon (IFN) production [3,5,6]. These results reveal that STING is imperative for RLRs-mediated type I IFN production.

The positive role of STING in type I IFN production in the antiviral innate immunity has been clarified, however, some specific mechanisms behind this are still remained obscure. STING, as an endoplasmic reticulum (ER)-associated transmembrane protein, was originally identified to contain two parts: the N-terminus and the C-terminus [7]. The N-terminus consists of approximately 130 amino acids and contains four transmembrane regions (TM), anchoring STING to the endoplasmic reticulum. The C-terminus consists of 250 amino acids and contains a

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spherical C-terminal domain (CTD), which faces the cytoplasm and is involved in STING-mediated signaling [6]. Subsequently, crystal structure analysis of the CTD of human STING identified that CTD contains three parts [7]: the dimerization domain (DD) of about 153-173aa, the C-terminal tail (CTT) domains of 343-379aa and the c-di-GMP binding domains (CBD) between DD and CTT. Recent studies have shown that CTT domain is indispensable for activating TBK1 and IRF3 [8], which is Co-localized with TBK1 and IRF3 [9,10]. The activation of human STING can induce not only type I IFN response, but also nuclear factor (NF)-κB-mediated response [11]. In zebrafish (*Danio rerio*), the CTT domain contains an additional motif that is more prone to eliciting NF-κB response [12]. Compared with CTT domain, there are less studies on DD. The previous studies have discovered that DD of STING is the most conserved region in all species by sequence alignment of full-length STING, so it is speculated that this region may exert a vital role in the function of STING [7]. It has been reported that STING dimerization could lead to high expression of IFN, which may be related to the DD [2]; however, the role of DD of STING is still unknown. STING ortholog have been extensively investigated in teleost fish. For example, zebrafish, common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), fathead minnow (*Pimephales promelas*), grouper (*Epinephelus coioides*), Japanese flounder (*Paralichthys olivaceus*) and black carp (*Mylopharyngodon piceus*) [13–19]. All these researches have shown that STING plays a key part in antiviral immunity in fish, which is consistent with mammalian STING.

In present study, we have found that black carp STING (bcSTING) can enhance host cell antiviral ability against SVCV/GCRV, and its C-terminal two serine residues are critical for STING activation [19]. In this paper, our data showed that the DD-deleted mutant of bcSTING (bcSTING-ΔDD) significantly improved the transcription activity of IFN promoter and antiviral activity compared with wild-type bcSTING. Furthermore, the deletion of the DD did not impact the self-interaction of bcSTING and increased its interaction with TBK1. Thus, our data implies that the DD of bcSTING might be a negative regulatory domain, which may be unrelated to the dimerization of bcSTING. The paper provides a new perspective for further researching the function of fish STING in the antiviral innate immunity.

2. Materials and methods

2.1. Cell lines and recombinant plasmids

All the cells, including Human embryonic kidney (HEK293T) cells, *Epithelioma Papulosum Cyprinid* (EPC) cells were kept in the lab, and these cell were cultured as previously described [20]. pcDNA5/FRTTO, pcDNA5/FRTTO-Flag/HA-bcSTING, pcDNA5/FRTTO-Flag-bcTBK1, pEGFP-N1, pRL-TK, Luci-bcIFNa, Luci-epcIFN and Luci-DrIFNφ1/3 pro were kept in the lab.

2.2. Virus produce and titration

Spring viremia of carp virus (SVCV) was kept in the lab (SVCV741). Virus were produced in EPC cells. Specifically, virus were harvested together with the supernatant media and frozen at −80 °C when the cytopathic effect (CPE) was up to 50%. After freezing and thawing for three times, the titers were tested through the plaque assay as reference [21].

2.3. Dual-luciferase reporter assay

EPC cells seeded in 24-well plate (3×10^5 cells/well) were co-transfected with pRL-TK, Luci-bcIFNa or Luci-epcIFN or Luci-DrIFNφ1/3 pro and plasmids expressing bcSTING or its mutants (bcSTING-ΔDD or bcSTING-ΔCTT). The cells lysed by PLB were used for reporter assay as previously [22].

2.4. Quantitative real-time PCR (q-PCR)

q-PCR was used to analyze the relative mRNA transcriptional levels of *IFN*, *MXI*, *ISG15* and *Viperin* genes of EPC cells expressing bcSTING or its mutants for 24 h and then infected with SVCV for 2 h. Specifically, Total RNA from EPC cells were extracted by the RNA rapid extraction kit, and the RNA were reverse-transcribed by reverse transcriptase (Takara); q-PCR was detected using the SYBR Green; The program was: 1 cycle of 50 °C/2min, 1 cycle of 95 °C/10min, 40 cycles of 95 °C/15s, 60 °C/1min, followed by dissociation curve analysis (60 °C–95 °C) to verify the amplification of a single product. The threshold cycle (CT) value was determined by using Applied Biosystems QuantStudio 5 Real-Time PCR Systems (Thermo Fish, USA). The data were analyzed by the 2-ΔΔCT method as previously described [23]. The primers were listed in Table 1.

2.5. Immunoblotting

HEK293T cells expressing bcSTING or its mutants in 6-well plate were collected with PBS buffer at 48 h post transfection, the cells were lysed with 1% NP40 supplemented with a protease inhibitor for immunoblot (IB) assay as previously described [24].

2.6. Co-immunoprecipitation (Co-IP)

Self-interaction of bcSTING or bcSTING-ΔDD and its interaction with the downstream factor bcTBK1 were analyzed using Co-IP assay. bcSTING or bcSTING-ΔDD was co-transfected with bcTBK1 or empty vector in HEK293T cells by PEI, Co-IP experiments were conducted as previously described [25]. In briefly, Whole-cell lysates were centrifuged at 12,000 g for 8 min and the resultant supernatant were collected. The protein A/G agarose beads were added to the supernatant for 1.5 h. Subsequently, the supernatant was incubated with anti-Flag (anti-HA) conjugated protein A/G agarose beads for 4 h. The beads were heat-denatured in 5 × SDS-sample buffer after 5 times washes with 1% NP-40 and used for immunoblotting as above.

3. Result

3.1. Sequence analysis of bcSTING and construction of its mutant

The previous studies in this lab have identified that bcSTING protein consists of 4 different domains, which contains the transmembrane domains (TD), dimerization domain (DD), c-di-GMP-binding domain and CTT domain [7]. To investigate the function of these domain, the sequences of STING from different species were compared, including *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *D. rerio* and *M. piceus*, which showed that the homologies of DD of STING were very high among the five species (more than 90%). However, the homologies of other domains of STING among these species were much lower, suggesting that the function of DD of bcSTING is basically the same as that of human (Fig. 1-A). Previous studies have found that the DD of human STING was associated with its dimerization, and the CTT domain was a key region for recruitment and activation of TBK1 and IRF3 [26]. In order to further explore the functions of DD and CTT domain of bcSTING, bcSTING-ΔDD (DD deleted) and bcSTING-ΔCTT (CTT domain deleted) were constructed respectively (Fig. 1-B). Besides, the expression of the bcSTING and its mutants were confirmed in HEK293T cells by IB assay (Fig. 1-C), in which the protein level of bcSTING-ΔDD was obviously lower than that of bcSTING or bcSTING-ΔCTT.

3.2. IFN induction ability of bcSTING mutants

Recent studies have shown that STING without CTT domain is unable to recruit the downstream factor TBK1 to induce IFN production [26]. Nevertheless, the functional mechanism of the DD of STING was

Table 1
Primers used in the study.

Primer name	Sequence (5′-3′)	Primer information
Mutants		
bcSTING-N-F	ACTGACGGTACCATGTGTGGTGTGATCGGA	HA-bcSTING
bcSTING-N-R	ACTGACCTCGAGCTACTTGTAATAATCA	
bcSTING-ΔDD-N-F	ACTGACGGTACCGTGAAGTGAAGAAGGATTTACAGAACAAGA	HA-bcSTING-ΔDD
bcSTING-ΔDD-N-R	ACTGACCTCGAGTTGTTCTGTAAATCCTTCTTCACTTCACAG	
bcSTING-ΔCTT-N-R	ACTGACCTCGAGTTAAACTGGATGGATTTC	HA-bcSTING-ΔCTT
Q-PCR		
Q-EPC actin-F	AAGGAGAAGCTCTGCTATGTGGCT	ex vivo q-PCR
Q-EPC actin-R	AAGGTGGTCTCATGGATACCGCAA	
Q-EPC viperin-F	GCAAAGCGAGGGTTACGAC	
Q-EPC viperin-R	CTGCCATTACTAACGATGCTGAC	
Q-EPC Mx1-F	TGGAGGAACCTGCCTTAAATAC	
Q-EPC Mx1-R	GTCTTTGCTGTTGTCAGAAGATTAG	
Q-EPC ISG15-F	TGATGCAAATGAGACCGTAGAT	
Q-EPC ISG15-R	CAGTTGTCTGCCGTTGTAAATC	
Q-SVCV-G-F	GATGACTGGGAGTTAGATGGC	
Q-SVCV-G-R	ATGAGGGATAATATCGGCTTG	
Q-SVCV-P-F	GAGAAAGTAGCAGCATC	
Q-SVCV-P-R	ACTATCCCAGGTCCAA	
Q-SVCV-M-F	GCCAAATGCCTCCTT	
Q-SVCV-M-R	AGCCCGACCTCCTCTA	
Q-SVCV-N-F	TCTTCTTGCTGGGTCT	
Q-SVCV-N-R	TTGTGAGTTGCCGTTA	

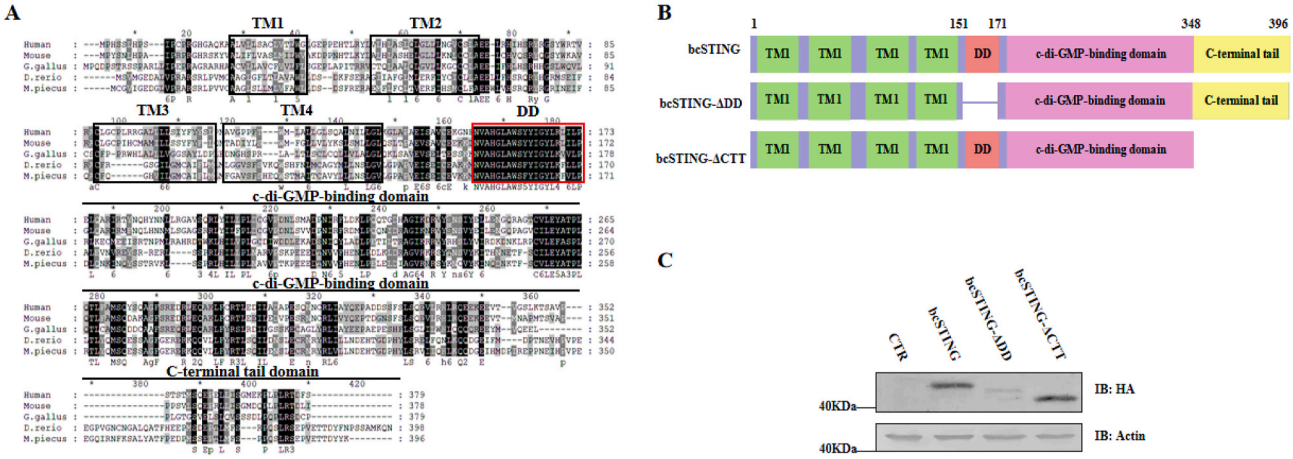

Fig. 1. Sequence analysis of bcSTING and construction of its mutant.
(A). Multiple alignments of STING from *M. piceus* (AQY10109.1), *D. rerio* (NP_001265766.1), *H. sapiens* (AVQ94753.1), *M. musculus* (AVQ94754.1), *G. gallus* (NP_001384384.1). **(B).** structure domain of bcSTING mutants. **(C).** HEK293T cells were transfected with HA-bcSTING or HA-bcSTING-ΔDD or HA-bcSTING-ΔCTT, by PEI and the expression of bcSTING and its mutants were detected by immunoblotting (IB). HA-bcSTING: pcDNA5/FRTTO/HA-bcSTING; HA-bcSTING-ΔDD: pcDNA5/FRTTO/HA-bcSTING-ΔDD; HA-bcSTING-ΔCTT: pcDNA5/FRTTO/HA-bcSTING-ΔCTT; CTR: pcDNA5/FRTTO/HA.

indistinct. To further reveal the part of bcSTING mutants in IFN signaling, EPC cells expressing bcSTING or its mutants were used for reporter assay. The data revealed that the induced transcription of bcIFNa promoter by bcSTING-ΔDD was about 15-fold higher than that by wild type bcSTING (Fig. 2-A), and the induced transcription of *epcIFN* promoter, *DrIFNφ1* promoter and *DrIFNφ3* promoter by bcSTING-ΔDD was 6-fold, 3-fold and 8-fold of that by wild type bcSTING, respectively (Fig. 2-B, C, D), which indicated that DD deletion offered bcSTING much improved IFN-inducing ability. However, the induced transcription of above IFN promoters by bcSTING-ΔCTT were similar to that of control, demonstrating that CTT domain deletion resulted in the loss of ability of bcSTING to induce IFN, which were consistent with the data in mammals.

3.3. The antiviral activity of bcSTING mutants

To explore whether the transcriptional level of IFN/ISG was influenced by bcSTING mutants, EPC cells were transfected with bcSTING and its mutants, and the vector separately, then used for q-PCR assay. The relative transcription levels of *epcIFN*, *epcViperin*, *epcMX1*, *epcISG15* (Fig. 3-A, B, C, D) were holistically higher in the cells expressing bcSTING-ΔDD than those in the cells expressing bcSTING, and the transcriptional levels of these genes of the cells expressing bcSTING-ΔCTT were basically the same as those of the control, which matched the results of reporter assay. Thus, these results indicated that bcSTING lacking the DD was more capable of inducing IFNs and ISGs.

Since our data have showed that bcSTING-ΔDD heighten the transcription of IFN and the antiviral proteins, we further investigated whether bcSTING lacking the DD could improve antiviral ability. bcSTING and its mutants were transfected with into EPC cells separately, and then the cells were infected with SVCV. Compared with wild-type bcSTING, the antiviral results showed that both cytopathic effect (CPE) ratio and viral titer were markedly decreased in the EPC cells expressing bcSTING-ΔDD. Moreover, the antiviral capacity in EPC cells expressing bcSTING-ΔCTT were basically the same as those of control cells (Fig. 4-A). At the same time, the infected cells were harvested and

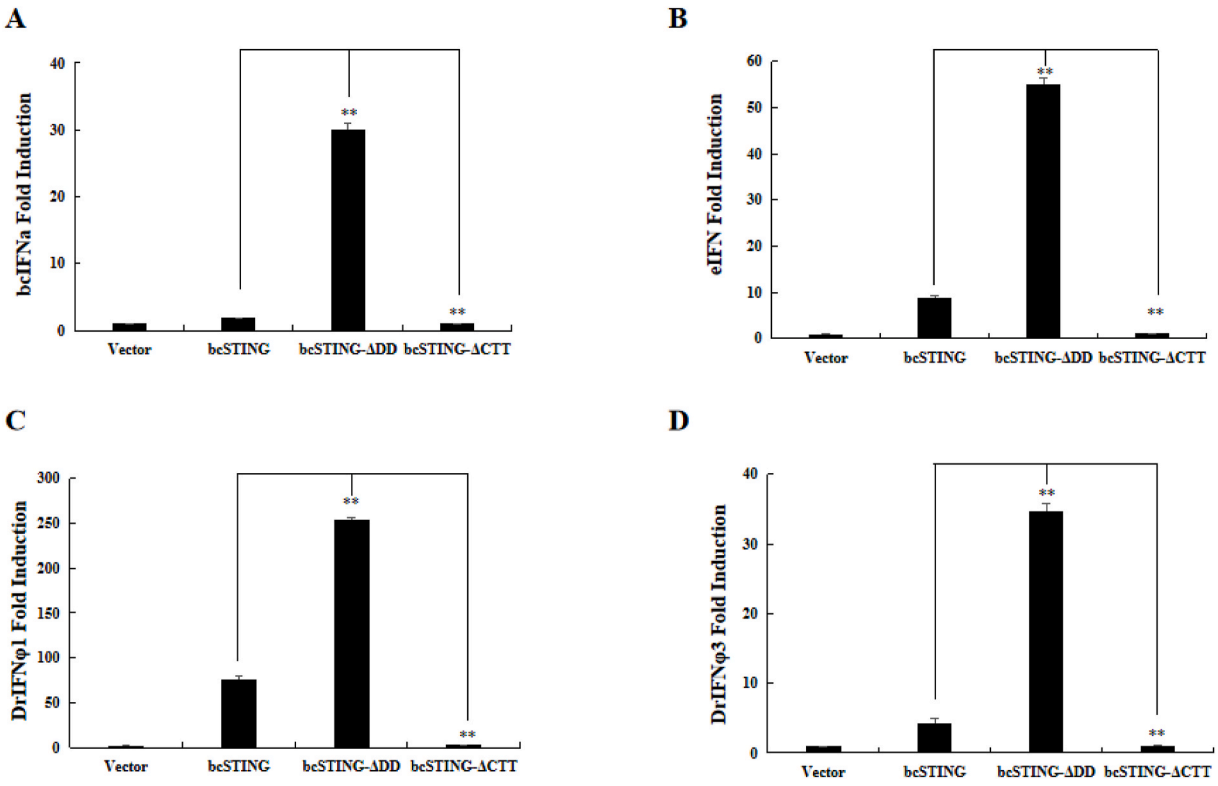

Fig. 2. The induced IFN promoter transcription by bcSTING or its mutants.
EPC cells in 24-well plates were transfected with the reporter plasmids pRL-TK (25 ng), Luci-bcIFNa (200 ng) **(A)**, or Luci-epcIFN (200 ng) **(B)**, or Luci-DrIFNφ1 (200 ng) **(C)**, or Luci-DrIFNφ3 (200 ng) **(D)**, along with bcSTING (200 ng), or bcSTING-ΔDD (200 ng), or bcSTING-ΔCTT (200 ng). At 24 h post transfection, the induced transcription of IFN promoters were examined through luciferase reporter assay. bcSTING: pcDNA5/FRTTO/HA-bcSTING; bcSTING-ΔDD: pcDNA5/FRTTO/HA-bcSTING-ΔDD; bcSTING-ΔCTT: pcDNA5/FRTTO/HA-bcSTING-ΔCTT.

tested by q-PCR assay, and the data also showed that the transcriptional levels of SVCV proteins, including *SVCV-G*, *SVCV-P*, *SVCV-N* and *SVCV-M,* in EPC cells expressing bcSTING-ΔDD were remarkably decreased than those of bcSTING-expressing, bcSTING-ΔCTT-expressing and control cells (Fig. 4-B, C, D, E), which demonstrated that bcSTING-ΔDD possessed a stronger antiviral ability against SVCV.

3.4. Self-interaction of bcSTING-ΔDD

In human and mammals, the DD of STING was associated with its dimerization [7]. Therefore, in order to explore whether the DD of teleost STING is related to its dimerization, HEK293T cells co-expressing bcSTING-ΔDD/bcSTING were used for Co-IP experiment. As was expected, Co-IP data revealed that HA-bcSTING was pulled down by Flag-bcSTING-precipitated proteins (Fig. 5-A), which was correlated with the data of its mammalian counterpart. To our surprise, HA-bcSTING-ΔDD (red arrow indicated) was tested in Flag-bcSTING-ΔDD precipitated proteins (Fig. 5-B), which indicated that the deletion of DD did not affect the self-interaction of bcSTING. Thus, our data implied that the DD of black carp STING might not be related to its dimerization, which was different with that of its mammalian counterpart.

3.5. Interaction between bcSTING-ΔDD and bcTBK1

Previous studies in human and mammals have identified that STING could interact with and activate TBK1 during DNA virus invasion, activated TBK1 in turn phosphorylates and triggers the nuclear translocation of IRF3, resulting in the transcription of IFN and antiviral immune activation finally [4]. Therefore, to further whether DD affects the interaction between STING and TBK1 in teleost, HEK293T cells co-expressing bcSTING/bcSTING-ΔDD and bcTBK1 were used for Co-IP experiment. The specific bands of bcTBK1 (~72 KDa) were tested in the proteins precipitated by bcSTING-ΔDD and wild type bcSTING (red arrow indicated in Fig. 6). It is worth mentioning that, according to the gray analysis, the amount of bcTBK1 protein precipitated by bcSTING-ΔDD was approximately 1.49-fold of bcTBK1 protein precipitated by wild-type bcSTING, which indicated that the affinity between bcSTING-ΔDD and bcTBK1 was much stronger than that between wild type bcSTING and bcTBK1.

4. Discussion

STING is a transmembrane protein located on the membrane of endoplasmic reticulum [3]. STING activation induces IFN production through the kinase TBK1 and the transcription factor IRF3 [6]. In mammals, the domains of STING have been extensively studied. There were several additional residues in the transmembrane domain that are required for IFN production but not for ligand binding, suggesting that the transmembrane domain plays a key part in STING activation. In addition, recently studies have shown that the CBD (c-di-GMP domain) and CTT (C-terminal tail) domains of STING are related to the recognition of c-di-GMP and the activation of downstream factors, among which the CTT domain is more important for the activation of TBK1 and IRF3-mediated IFN responses. However, the DD of STING may be involved in its dimerization, which needs further investigation [7]. The study found that the DD of STING is the most conserved in many species, so it is speculated that this domain may play a crucial role in innate immunity. The study in zebrafish has shown that CTT domain of STING could recruit TRFA6 to activate NF-κB signaling pathway, which was inconsistent with the data of its mammalian counterpart [12]. Although the DD is suggested to be related to the self-interaction of STING molecules, the mechanism behind this has never been elucidated. And,

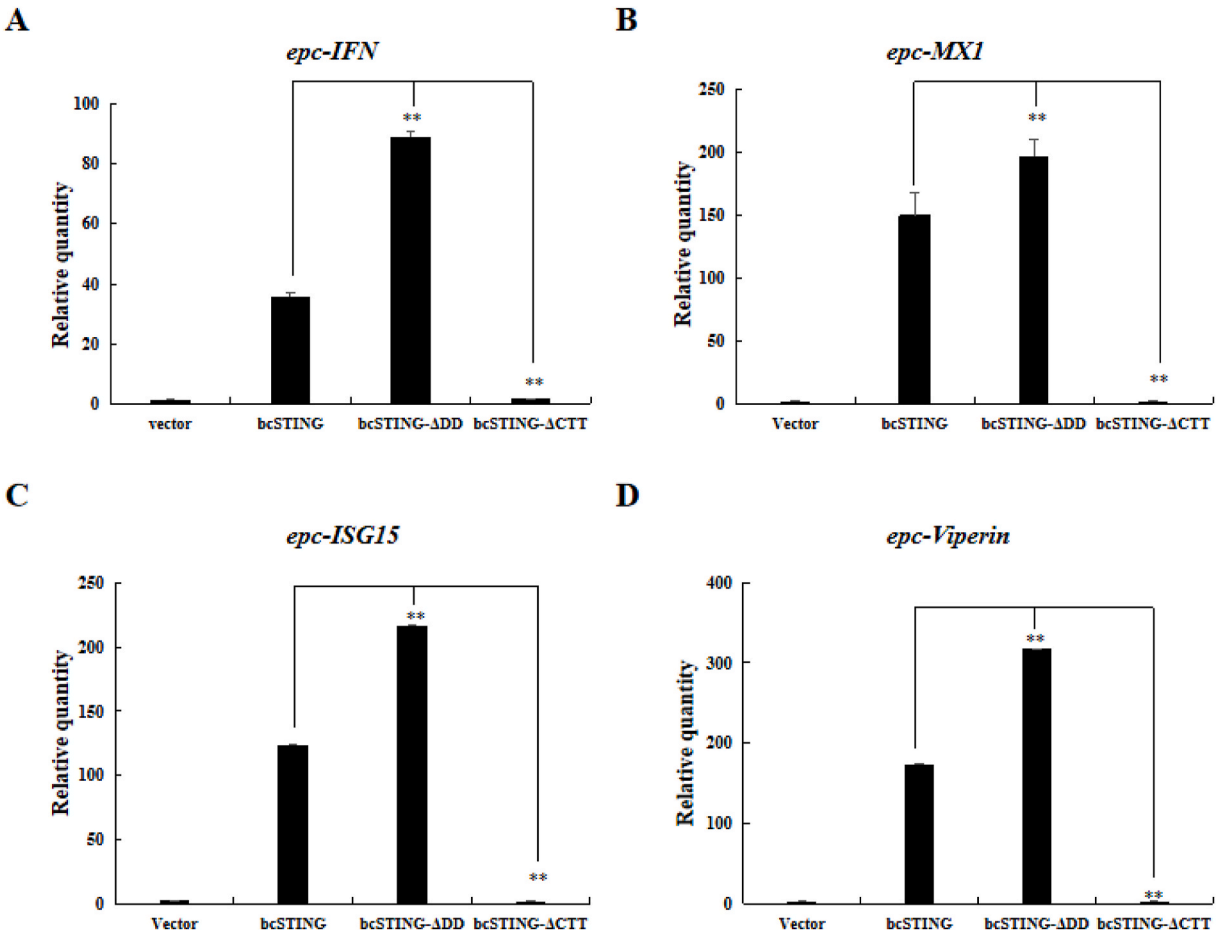

Fig. 3. The expression of antiviral genes in EPC cells expressing bcSTING or its mutants.
EPC cells in 6-well plate were transfected with bcSTING, bcSTING-ΔDD, bcSTING-ΔCTT, or the empty vector (3 μg/well) separately, and the cells were harvested at 48 h post transfection and used for RNA isolation. The relative mRNA transcriptional levels of (A) *epcIFN*, (B) *epcMX1*, (C) *epcISG15*, (D) *epcViperin*, were examined by q-PCR. bcSTING: pcDNA5/FRTTO/HA-bcSTING; bcSTING-ΔDD: pcDNA5/FRTTO/HA-bcSTING-ΔDD; bcSTING-ΔCTT: pcDNA5/FRTTO/HA-bcSTING-ΔCTT.

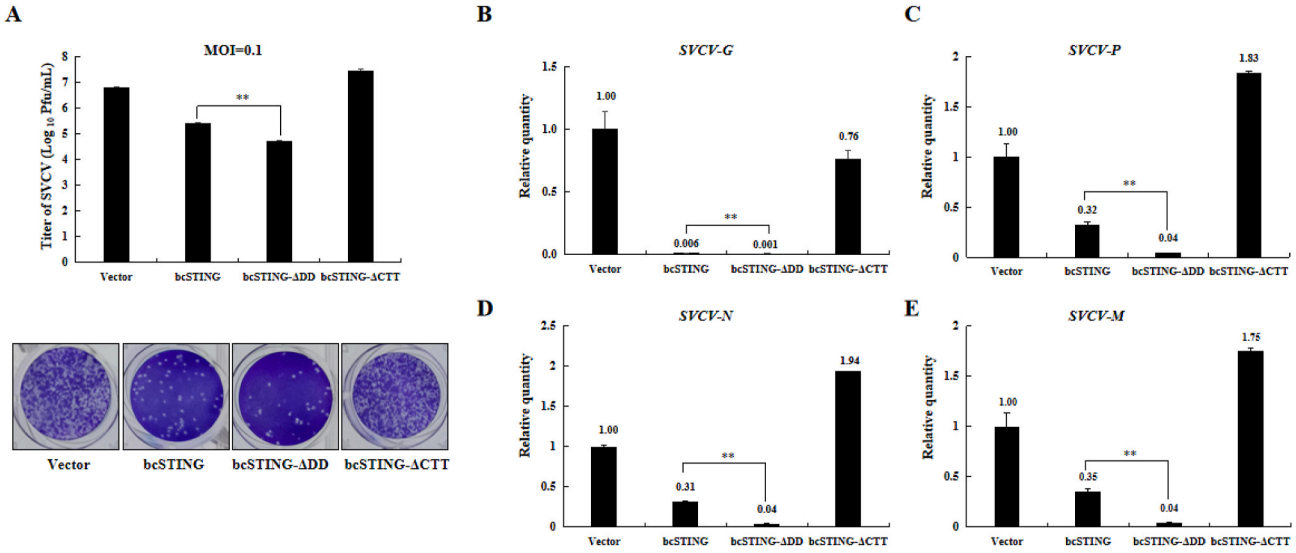

Fig. 4. The antiviral activity of bcSTING or its mutants.
EPC cells in 24-well plates were transfected with 250 ng of bcSTING, bcSTING-ΔDD, bcSTING-ΔCTT, or the empty vector separately and infected with SVCV (MOI = 0.1) at 24 h post transfection. The virus titers were examined by plaque assay at 48 h post-transfection (A, the upper one) and the cells used for virus titer assay were stained with crystal violet (A, the lower one). EPC cells in 6-well plate were transfected with bcSTING, bcSTING-ΔDD, bcSTING-ΔCTT, or the empty vector (3 μg/well) separately and infected with SVCV (MOI = 0.1) at 24 h post transfection. The cells were harvested at 48 h post transfection and used for RNA isolation. The relative mRNA transcriptional levels of SVCV proteins, *SVCV-G* (B), *SVCV-P* (C), *SVCV-N* (D) and *SVCV-M* (E), were examined by q-PCR.

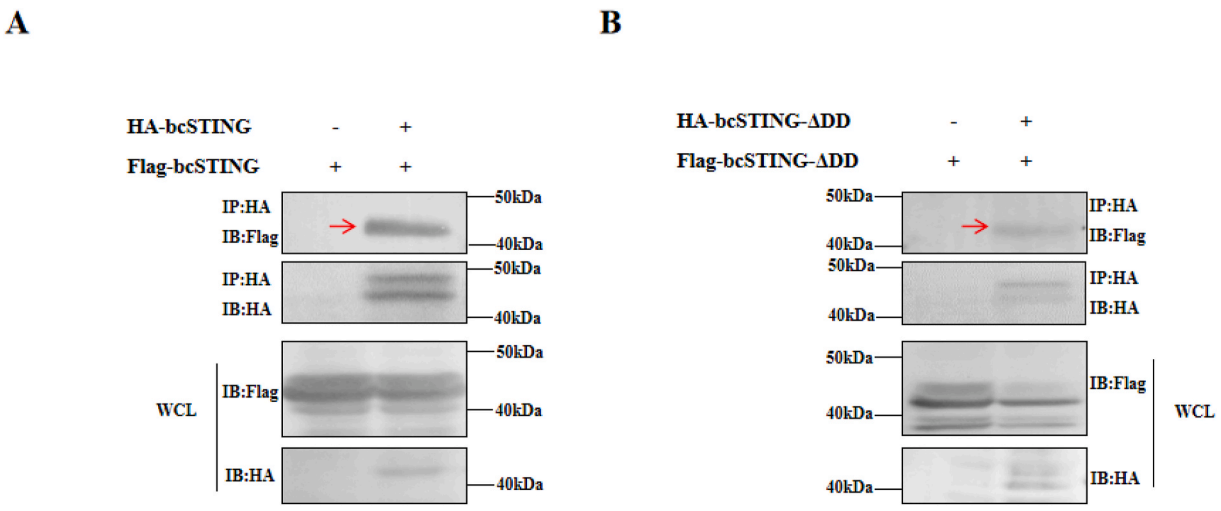

Fig. 5. Self-interaction of bcSTING or bcSTING-ΔDD.
HEK293T cells in 10 cm dishes were co-transfected with bcSTING (7.5 μg) **(A)**, or bcSTING-ΔDD (7.5 μg) **(B)**. The cells were harvested 48 h post transfection and used for co-immunoprecipitation assay. IP: immunoprecipitation; IB: immunoblot; WCL: whole cell lysate; HA-bcSTING: pcDNA5/FRTTO/HA-bcSTING; HA-bcSTING-ΔDD: pcDNA5/FRTTO/HA-bcSTING-ΔDD; Flag-bcSTING: pcDNA5/FRTTO/Flag-bcSTING; Flag-bcSTING-ΔDD: pcDNA5/FRTTO/Flag-bcSTING-ΔDD.

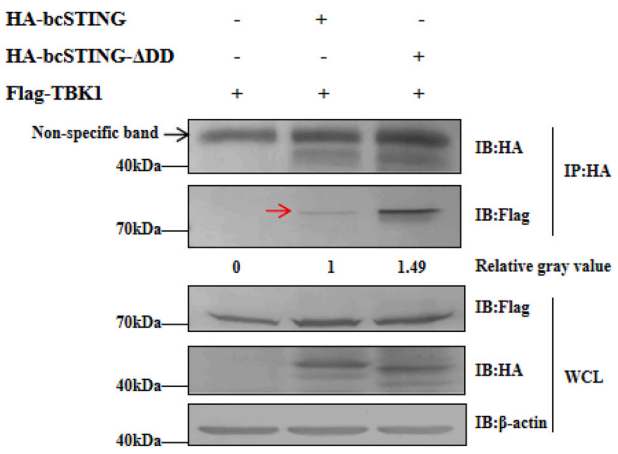

Fig. 6. The interaction between bcTBK1 and bcSTING.
HEK293T cells in 10 cm dishes were co-transfected with bcTBK1 (7.5 μg) and bcSTING (7.5 μg) or bcSTING-ΔDD (7.5 μg). The cells were harvested at 48 h post transfection and used for co-immunoprecipitation assay. IP: immunoprecipitation; IB: immunoblot; WCL: whole cell lysates; bcTBK1: pcDNA5/FRTTO/Flag-bcTBK1; bcSTING: pcDNA5/FRTTO/HA-bcSTING; bcSTING-ΔDD: pcDNA5/FRTTO/HA-bcSTING-ΔDD.

before this study, there was no report about the role of the DD of STING in fish. In this paper, bcSTING-ΔDD showed stronger IFN-inducing and antiviral capacity while bcSTING-ΔCTT basically had no function. Thus, CTT domain of black carp STING, like its mammalian counterpart, functions crucially in bcSTING-mediated signaling. However, DD of bcSTING is suggested to negatively regulate in bcSTING/IFN signaling, and is not related to the self-interaction of bcSTING molecules.

In recent years, great progress has been made in the study of the multiple immune pathways involved in STING, and the cGAS-STING signaling pathway has become a major pathway for recognizing cytoplasmic DNA. But the mechanism of STING involved in the regulation of RNA virus infection still needs to be further investigated. STING is closely related to autoimmune diseases, overactivation of STING signaling triggers inflammatory responses in autoimmune and inflammatory diseases. For instance, STING-related infantile vascular disease (SAVI) is caused by mutation of STING gene and the mutated residues are located at the dimerization interface of STING, which triggers excessive activation of STING and production of IFN, leading to severe autoimmune diseases [27]. Therefore, the strict regulation of antiviral innate immune signaling is very significant for the host to maintain the balance of immune environment. In this manuscript, the Co-IP results identified that DD was not indispensable for self-interaction of bcSTING (Fig. 5-B), but DD deletion strengthened the interaction between bcSTING and bcTBK1 (Fig. 6). Thus, DD of STING functions negatively in STING/IFN signaling in black carp, which is different to that of its mammalian counterpart. However, the mechanism behind the negative role DD of teleost STING needs to be further explored.

Author contributions

H. Feng contributes to conceptualization, formal analysis, project administration and writing. J. Yan contributes to data curation, formal analysis and writing. G. Qiao, H. Chang and H. Wu play the roles of investigation and original draft writing. J. Tu and H. Wu play the roles of investigation and formal analysis. M. Liu and Y. Zhang play the role of investigation and data curation. All authors reviewed and approved the submitted manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] X. Zhang, X.C. Bai, Z.J. Chen, Structures and mechanisms in the cGAS-STING innate immunity pathway, Immunity 53 (1) (2020) 43–53.

[2] W. Sun, Y. Li, L. Chen, H. Chen, F. You, X. Zhou, Y. Zhou, Z. Zhai, D. Chen, Z. Jiang, ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization, Proc. Natl. Acad. Sci. U. S. A 106 (21) (2009) 8653–8658.
[3] B. Zhong, Y. Yang, S. Li, Y.Y. Wang, Y. Li, F. Diao, C. Lei, X. He, L. Zhang, P. Tien, H.B. Shu, The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation, Immunity 29 (4) (2008) 538–550.
[4] H. Ishikawa, Z. Ma, G.N. Barber, STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity, Nature 461 (7265) (2009) 788–792.
[5] K.M. Franz, W.J. Neidermyer, Y.J. Tan, S. Whelan, J.C. Kagan, STING-dependent translation inhibition restricts RNA virus replication, Proc. Natl. Acad. Sci. U. S. A 115 (9) (2018) E2058–E2067.
[6] J.W. Schoggins, D.A. MacDuff, N. Imanaka, M.D. Gainey, B. Shrestha, J.L. Eitson, K.B. Mar, R.B. Richardson, A.V. Ratushny, V. Litvak, R. Dabelic, B. Manicassamy, J. D. Aitchison, A. Aderem, R.M. Elliott, A. García-Sastre, V. Racaniello, E.J. Snijder, W.M. Yokoyama, M.S. Diamond, C.M. Rice, Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity, Nature 505 (7485) (2014) 691–695.
[7] S. Ouyang, X. Song, Y. Wang, H. Ru, N. Shaw, Y. Jiang, F. Niu, Y. Zhu, W. Qiu, K. Parvatiyar, Y. Li, R. Zhang, G. Cheng, Z.J. Liu, Structural analysis of the STING adaptor protein reveals a hydrophobic dimer interface and mode of cyclic di-GMP binding, Immunity 36 (6) (2012) 1073–1086.
[8] C. Zhang, G. Shang, X. Gui, X. Zhang, X.C. Bai, Z.J. Chen, Structural basis of STING binding with and phosphorylation by TBK1, Nature 567 (7748) (2019) 394–398.
[9] B. Zhao, F. Du, P. Xu, C. Shu, B. Sankaran, S.L. Bell, M. Liu, Y. Lei, X. Gao, X. Fu, F. Zhu, Y. Liu, A. Laganowsky, X. Zheng, J.Y. Ji, A.P. West, R.O. Watson, P. Li, A conserved PLPLRT/SD motif of STING mediates the recruitment and activation of TBK1, Nature 569 (7758) (2019) 718–722.
[10] S.L. Ergun, L. Li, Structural insights into STING signaling, Trends Cell Biol. 30 (5) (2020) 399–407.
[11] T. Abe, G.N. Barber, Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1, J. Virol. 88 (10) (2014) 5328–5341.
[12] C.C. de Oliveira Mann, M.H. Orzalli, D.S. King, J.C. Kagan, A. Lee, P.J. Kranzusch, Modular architecture of the STING C-terminal tail allows interferon and NF-κB signaling adaptation, Cell Rep. 27 (4) (2019) 1165–1175.e5.
[13] F. Sun, Y.B. Zhang, T.K. Liu, J. Shi, B. Wang, J.F. Gui, Fish MITA serves as a mediator for distinct fish IFN gene activation dependent on IRF3 or IRF7, J. Immunol. 187 (5) (2011) 2531–2539. Baltimore, Md. : 1950.
[14] S. Biacchesi, E. Mérour, A. Lamoureux, J. Bernard, M. Brémont, Both STING and MAVS fish orthologs contribute to the induction of interferon mediated by RIG-I, PLoS One 7 (10) (2012), e47737.
[15] X. Feng, C. Yang, Y. Zhang, L. Peng, X. Chen, Y. Rao, T. Gu, J. Su, Identification, characterization and immunological response analysis of stimulator of interferon gene (STING) from grass carp *Ctenopharyngodon idella*, Dev. Comp. Immunol. 45 (1) (2014) 163–176.
[16] J.I. Ma, S. Kang, H.B. Jeong, J. Lee, Isolation and expression analysis of stimulator of interferon gene from olive flounder, paralichthys olivaceus, Fish. Aquatic Sci. 21 (1) (2018) 5.
[17] Y. Huang, Z. Ouyang, W. Wang, Y. Yu, P. Li, S. Zhou, S. Wei, J. Wei, X. Huang, Q. Qin, Antiviral role of grouper STING against iridovirus infection, Fish Shellfish Immunol. 47 (1) (2015) 157–167.
[18] X.L. Cao, J.J. Chen, Y. Cao, G.X. Nie, J.G. Su, Identification and expression analysis of the sting gene, a sensor of viral DNA, in common carp *Cyprinus carpio*, J. Fish. Biol. 88 (5) (2016) 1949–1964.
[19] L. Lu, X. Wang, S. Wu, X. Song, Z. Zou, X. Xie, J. Xiao, S. Chen, H. Feng, Black carp STING functions importantly in innate immune defense against RNA virus, Fish Shellfish Immunol. 70 (2017) 13–24.
[20] J. Li, Y. Tian, J. Liu, C. Wang, C. Feng, H. Wu, H. Feng, Lysine 39 of IKKε of black carp is crucial for its regulation on IRF7-mediated antiviral signaling, Fish Shellfish Immunol. 77 (2018) 410–418.
[21] J. Li, C. Yan, J. Liu, J. Yan, H. Feng, SIKE of black carp is a substrate of TBK1 and suppresses TBK1-mediated antiviral signaling, Dev. Comp. Immunol. 90 (2019) 157–164.
[22] Z. Chen, Y. Cao, J. Huang, Y. Tan, J. Wei, J. Xiao, J. Zou, H. Feng, NLK suppresses MAVS-mediated signaling in black carp antiviral innate immunity, Dev. Comp. Immunol. 122 (2021), 104105.
[23] X. Lu, J. Liu, J. Yan, H. Wu, H. Feng, Identification and characterization of IRF9 from black carp *Mylopharyngodon piceus*, Dev. Comp. Immunol. 103 (2020), 103528.
[24] X. Song, W. Li, X. Xie, Z. Zou, J. Wei, H. Wu, H. Feng, NLRX1 of black carp suppresses MAVS-mediated antiviral signaling through its NACHT domain, Dev. Comp. Immunol. 96 (2019) 68–77.
[25] Y. Cao, Z. Chen, J. Huang, H. Wu, J. Zou, H. Feng, Black carp TUFM collaborates with NLRX1 to inhibit MAVS-mediated antiviral signaling pathway, Dev. Comp. Immunol. 122 (2021), 104134.
[26] Q. Yin, Y. Tian, V. Kabaleeswaran, X. Jiang, D. Tu, M.J. Eck, Z.J. Chen, H. Wu, Cyclic di-GMP sensing via the innate immune signaling protein STING, Mol. Cell 46 (6) (2012) 735–745.
[27] J. Ahn, G.N. Barber, Self-DNA, STING-dependent signaling and the origins of autoinflammatory disease, Curr. Opin. Immunol. 31 (2014) 121–126.