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Impact of VHSV on CIITA-mediated MHCII expression and antigen presentation in largemouth bass

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ABSTRACT

Viral hemorrhagic septicemia virus (VHSV) infection poses a significant threat to fish immunity by evading antigen presentation. Our previous study indicated that VHSV inhibited the transcriptional activation of MHC class II (MHCII) to impair antigen presentation in vitro, but the underlying mechanisms remain unclear. Here, we cloned and characterized the class II major histocompatibility complex transactivator (CIITA) in largemouth bass (*Micropterus salmoides*), a key regulator of MHCII expression, demonstrating that it activates MHCII transcription and possesses antiviral properties against VHSV. Following VHSV infection, CIITA and its downstream target MHCII were significantly downregulated in vivo. Further analysis revealed that the VHSV N protein degrades CIITA, primarily via its H3 and FCH domains, thereby impairing MHCII expression and antigen presentation. This study uncovers a critical mechanism through which VHSV inhibits adaptive immunity in fish and provides a theoretical basis for developing live-attenuated VHSV vaccines.

1. Introduction

In vertebrates, the major histocompatibility complex II (MHCII) is essential for orchestrating immune responses by presenting foreign antigen peptides to $CD4^+$ T lymphocytes, which is a vital step in the activation and differentiation of these T cells [1]. Typically, MHCII molecules are constitutively expressed in specialized antigen-presenting cells (APCs), which include macrophages, dendritic cells (DCs) and B cells. Interestingly, some non-professional APCs, such as fibroblasts, endothelial cells and epithelial cells, could also be stimulated to express MHCII in response to signals such as interferon-gamma (IFN-γ). This induction expands the repertoire of cells capable of presenting antigens to T cells, thereby enhancing the immune response in various tissues [2]. Importantly, both constitutive and inducible expression of MHCII molecules is fundamentally dependent on CIITA, a key transactivator that facilitates their transcription [3]. CIITA assembles enhanceosomes, recruits chromatin remodeling complexes, and facilitates the transcription of MHCII genes. In mammals, deficiencies in CIITA expression, as observed in bare lymphocyte syndrome (BLS), result in severely compromised immune function, highlighting its indispensable role in maintaining immune homeostasis [4].

Although CIITA plays a pivotal role in modulating MHCII expression for effective immune responses, it does not directly bind to the MHCII promoter due to the absence of the DNA-binding domain. Instead, CIITA facilitates the recruitment of other transcription factors, including nuclear transcription factor Y (NF-Y), the regulatory factor X (RFX), the cAMP-responsive element binding protein (CREB), forming the enhanceosome and then binding to the MHCII promoter, thereby activating MHCII transcription [5]. In addition, CIITA also serves as a platform that recruits some transcriptional co-activators, containing histone methyltransferases, histone deacetylases, histone acetyltransferases, and chromatin remodeling factors. This recruitment modulates enhanceosome activity and alters chromatin accessibility, thereby further influencing the transcriptional regulation of MHCII [6].

In addition to its regulatory role in antigen presentation, CIITA has been recognized for its antiviral properties. In mammals, CIITA is recognized as a restriction factor that counteracts viral infections. For instance, it has been reported that CIITA suppresses the multiplication of human immunodeficiency virus by competing with Cyclin T1, an essential host factor that is recruited by the viral transactivator Tat to

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facilitate transcript elongation in human T cells [7]. By disrupting the interaction between Tax-1 and key factors promoting transactivation of Tax-1-mediated LTR, CIITA could significantly restrict human T-cell lymphotropic virus replication [8]. Moreover, CIITA also promotes resistance by upregulating the expression of the p41 isoform of the invariant chain CD74, thereby preventing viral entry through the blockade of cathepsin-mediated processing of the Ebola virus glycoprotein [9]. These studies indicate that CIITA exerts antiviral function through various molecular mechanisms. In bony fish, CIITA has been identified in channel catfish [10], Chinese tongue sole [11], and its function in regulating MHCII expression is also analyzed in zebrafish [12] and grass carp [13]. However, the function of CIITA in modulating immune responses in fish, particularly in the context of viral infections, remains underexplored.

The largemouth bass has significant economic value in aquaculture due to its popularity among consumers. However, farms cultivating largemouth bass suffer from significant losses due to disease outbreaks associated with various viral pathogens. Recently, our laboratory successfully isolated a strain of viral hemorrhagic septicemia virus (VHSV) from diseased largemouth bass at a fish farm in Guangdong, China [14]. VHSV is a highly infectious disease capable of affecting numerous freshwater and marine fish species, causing latent infections or hemorrhagic symptoms, organ damage, and mortality, which in turn leads to significant economic losses in the global aquaculture industry [15]. Therefore, investigating the infection dynamics and pathogenesis of VHSV, along with its effects on fish immune system, is essential for developing effective strategies to prevent VHSV infection. Previous studies have shown that the VHSV N protein interacts with STAT1 and degrades it via the proteasome pathway, resulting in the downregulation of MHCII expression [16]. However, the broader impact of VHSV on CIITA-mediated MHCII regulation remains poorly understood. Furthermore, the viral factors and molecular mechanisms responsible for this suppression, particularly in vivo, have not been elucidated.

In this study, we identified and characterized the CIITA gene in largemouth bass, focusing on its role in MHCII transcriptional regulation and antiviral defense. We investigated the impact of VHSV infection on CIITA and MHCII expression in vivo, revealing that VHSV suppresses CIITA transcription and promotes its degradation via the N protein. Functional domain analyses of the N protein identified specific regions critical for this degradation, providing novel insights into how VHSV disrupts antigen presentation. These findings not only uncover a key mechanism through which VHSV evades adaptive immunity but also lay the groundwork for the development of attenuated vaccines and other control measures for VHSV.

2. Materials and method

2.1. Fish treatment and ethical considerations

Largemouth bass fry, approximately 5–8 cm in body length, were purchased from a fish farm in Zhuhai (Guangdong, China) and maintained at our lab for more than 14 days before tissue sampling or viral challenge experiments. All experimental procedures in largemouth bass individuals were in accordance with the ethical guidelines of the Committee on the Ethics of Animal Experiments of Sun Yat-sen University.

2.2. Cells, strain and virus

Largemouth bass spleen (LMBS) cells were established in our labs and cultured at 28 °C in DMEM medium (Invitrogen). Human embryonic kidney (HEK) 293T cells were maintained at 37 °C in 5 % CO_2 in DMEM. Fathead minnow (FHM) cells were grown at 28 °C in Medium 199 (Invitrogen). All the aforementioned cell culture media contained 10 % fetal bovine serum. For plasmid amplification, *Escherichia coli* Top10 was employed. VHSV was propagated in FHM cells and preserved at −80 °C in our lab.

2.3. Gene cloning and sequence analysis

Utilizing homologous sequences from *Siniperca chuatsi* and *Larimichthys crocea* as queries, the sequences of IRF1, CIITA and MHCII were retrieved from databases containing largemouth bass genomic and transcriptomic information [17]. According to the nucleotide sequences acquired by homologous alignment, specific primers were developed to amplify the cDNA sequences of IRF1 and CIITA. The online software (http://doua.prabi.fr/software/sim4) was employed to perform the alignment of the genomic sequence with the amplified cDNA sequence of CIITA. Phylogenetic analyses were conducted using MEGA version 8.0.

2.4. The construction of plasmids

The open reading frames (ORFs) of CIITA with a Flag-tag on its N terminal, and IRF1 (GenBank Accession No. PQ660446) were cloned into pcDNA3.1 vector (Invitrogen). The sequences of N protein and its truncated mutants were cloned into pCMV-Myc (Clontech), and the ORF of G protein was inserted into pEGFP-N3 (Clontech). To construct the promoters of MHCII-α and MHCII-β, the 5′ flanking regions of two genes, MHCII DA alpha chain (accession number XM_038731627.1) and beta chain (accession number XM_038731622.1), were analyzed using online PROMO program and Promoter 2.0. Subsequently, the predicted sequences, containing 1059 bp and 689 bp respectively, were subcloned into pGL3-basic luciferase reporter vector (Invitrogen). Validation of all constructed plasmids was performed through DNA sequencing at Tsingke Biotechnology Company, and primers used in this study were listed in Table S1.

2.5. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total extraction from collected tissue and treated cell samples was performed using the TRIzol reagent (Invitrogen), and reverse transcription was carried out with GoScript™ Reverse Transcription kit (Promega). qRT-PCR was conducted with SYBR Green master mix (Vazyme) on the LightCycler 480 II (Roche). The PCR conditions included an initial denaturation step at 95 °C for 2 min, followed by 42 cycles of amplification at 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s. Largemouth bass *beta-actin* (*β-actin*) was chosen as an internal reference gene for normalization. The relative fold changes were determined by comparing the samples to their respective controls through the comparative CT (2-ΔΔCt) method. Information regarding all the primers utilized in the qRT-PCR assays is available in Table S1.

2.6. Expression patterns of largemouth bass CIITA gene

Three healthy largemouth bass individuals were anesthetized with MS-222 (Merck), and different tissues, including brain, heart, gill, intestine, kidney, spleen, muscle, skin, and liver, were collected. Total RNA was extracted from above tissues, and the expression level of CIITA gene was further detected using specific primers listed in Table S1. The transcripts of CIITA in different tissues were assessed by the cycle threshold (Ct) method and normalized to *β-actin* via the $2^{-\Delta Ct}$ method. Subsequently, the expression levels were compared to the lowest expression level in the tissue, assigned a value of 1, using the $2^{-\Delta\Delta Ct}$ method.

2.7. Transient transfection and luciferase activity assay

HEK 293T or LMBS cells seeded in 6-well plates overnight were transfected indicated plasmids with Lipofectamine™ 6000 (Invitrogen) following manufacturer's instructions. FHM cells were cultured in 24-well plates overnight and then co-transfected with indicated firefly luciferase reporter plasmids (PGL3-MHCII-α-pro or PGL3-MHCII-β-pro) and other overexpression plasmids. The *Renilla* luciferase vector (pRL-TK) was used as an internal control. After 24 h transfection, the medium

was removed, the cells were washed with PBS, and then lysed for detection of luciferase activity through the Dual-Luciferase Reporter Assay System (Promega). The experiments were conducted independently at least three times, and the average results were utilized for statistical analysis.

2.8. Western blot

Western blot was performed as described in a previous study [18]. In brief, the boiled cell lysates were separated using 10 % or 15 % SDS-PAGE, followed by transfer to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). After blocking for 2 h in tris-buffered saline (TBST) buffer (25 mM Tris-HCl, 150 mM NaCl, 0.1 % Tween 20, pH 7.5) with 5 % skimmed milk powder, the membrane was incubated with diluted primary antibodies (Abs) overnight at 4 °C, washed three times with TBST buffer, and then incubated with diluted secondary Abs for 1 h at room temperature. The Minichemi system (Sagecreation) image analyzer was used to capture photographs of the blots. Abs used in this study were diluted as follows: anti-Flag/Myc(Abmart) at 1:4000, anti--β-actin (Abmart) at 1:3000, and HRP-conjugated anti-mouse IgG (Beyotime) at 1:1000.

2.9. Statistics analysis

The data from qRT-PCR and luciferase assays were analyzed through SPSS software (Version 19.0, IBM-SPSS Inc., Chicago, Illinois, USA) and presented as the mean ± SEM. The p values were determined using the Student's t-test, with a p value of less than 0.05 being regarded as statistically significant.

3. Results

3.1. Molecular characterization of largemouth bass CIITA

The cDNA sequence of largemouth bass CIITA was cloned and subsequently deposited into GenBank under accession numbers (PQ660445). Genomic structure analysis revealed that CIITA gene contained 21 exons and 20 introns (Fig. 1A). The ORF of CIITA consisted of 3684 bp encoding 1227 amino acids. The prediction of functional domain displayed that largemouth bass putative CIITA protein contained three conserved domains belonging to the Atrophin-1, P-loop_NTPase, and PPP1R42 superfamilies (Fig. 1B). The amino acid alignment of CIITAs showed substantial variability in the N-terminal region across different species, while all sequences contained conserved GTP-binding motif and nuclear localization sequence (NLS) (Fig. S1). Phylogenetic analysis found that all CIITA molecules in teleost fish were clustered together in a same clade, which was then clustered with another clade from other lineages of higher vertebrates (Fig. S2).

3.2. Tissue-specific expression of CIITA in largemouth bass

Among nine examined tissues, CIITA expression was highest in the spleen, a key immune organ, showing an 8.65-fold increase compared to the brain, which exhibited the lowest expression (Fig. 2).

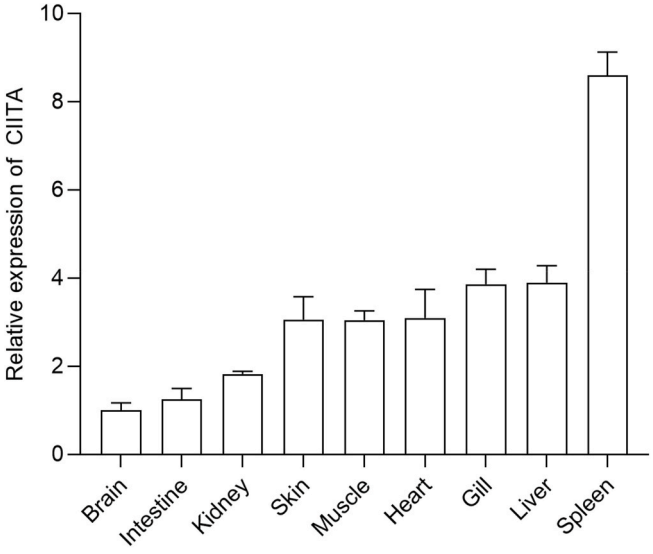

Fig. 2. Tissue expression of CIITA in largemouth bass. Nine tissues, containing the gill, intestine, kidney, spleen, liver, skin, heart, brain, and muscle, were collected from three healthy largemouth bass. The expression levels of CIITA in various tissues were measured using qRT-PCR. The relative expression of CIITA was determined by normalizing the expression levels in different tissues against that of the brain, which was designated as a reference value of 1, utilizing the $2^{-\Delta\Delta Ct}$ method.

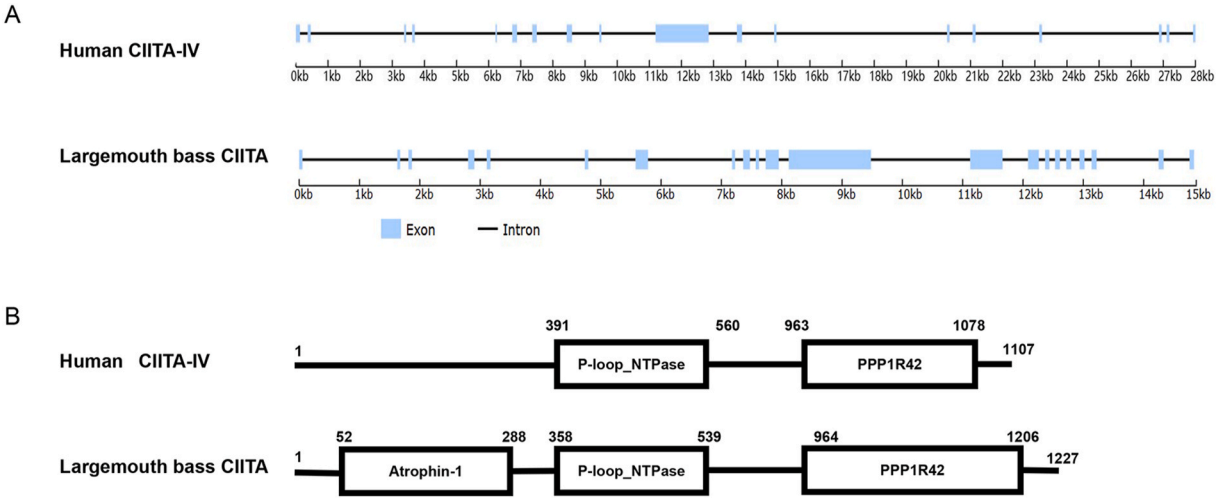

Fig. 1. Schematic representations of CIITA gene and the prediction of conserved domains in human and largemouth bass. (A)The sequences of human type IV CIITA were sourced from the UCSC Genome Browser, and largemouth bass CIITA sequence was obtained through a local BLAST search using genomic data downloaded from the National Center for Biotechnology Information (NCBI). (B) The conserved functional domains of largemouth bass and human CIITA proteins were predicted with Conserved Domain search in NCBI.

3.3. Regulation of MHCII expression by CIITA and IRF1

To investigate the role of CIITA in MHCII transcription, the regulatory sequences in the promoters of MHCII-α and MHCII-β were predicted, which contained RFX-1, NFY, AP1, CREB and ISRE (Fig. S3). Then the promoters of MHCII-α or MHCII-β and pcDNA3.1 or Flag-CIITA were co-transferred into FHM cells. Luciferase assays indicated that the promoter activities of both MHCII-α and MHCII-β were significantly activated in cells overexpressed CIITA (Fig. 3A and B). In zebrafish, it has been reported that CIITA cooperates with IRF1 to induce MHCII-DAB expression [12]. To identify whether this mechanism is conserved in different fish, Flag-CIITA, pc-IRF1 and MHCII-α or MHCII-β promoters, were co-transfected into FHM cells. It was found that largemouth bass CIITA also collaborated with IRF1 to promote the activation of MHCII-α and MHCII-β promoter (Fig. 3A and B).

3.4. Antiviral role of CIITA against VHSV

To explore the antiviral properties of CIITA, LMBS cells were transfected with Flag-CIITA and subsequently infected with VHSV. qRT-PCR analysis showed a significant reduction in VHSV N and G transcripts in CIITA-overexpressing cells (Fig. 4), indicating that CIITA inhibits VHSV replication.

3.5. Suppression of CIITA and MHCII expression following VHSV infection

In previous studies, we found that VHSV infection could inhibit the transcriptional activation of MHCII in IFN-γ stimulated cells [16], but it is uncleared that whether VHSV infection affects MHCII expression in vivo. To explore the expression pattern of CIITA after VHSV infection, the expression dynamics of CIITA and MHCII following VHSV infection were investigated. qRT-PCR analysis of spleen tissues revealed significant downregulation of CIITA mRNA levels within 12 h post-infection (Fig. 5A). Correspondingly, the expression of both MHCII-α and MHCII-β was also markedly suppressed (Fig. 5B and C), indicating that VHSV infection affects CIITA expression at the transcriptional level, thereby inhibiting the expression of MHCII.

3.6. VHSV impairs MHCII transcription mediated by CIITA

To determine whether VHSV directly affects CIITA-mediated MHCII transcription, FHM cells were co-transfected with Flag-CIITA and MHCII promoters, followed by VHSV infection. Luciferase assays revealed that VHSV significantly suppressed the activity of both MHCII-α and MHCII-β promoters (Fig. 6A and B). Western blot analysis further demonstrated a reduction in CIITA protein abundance in VHSV-infected cells (Fig. 6C), indicating that VHSV disrupts CIITA function at both transcriptional and post-transcriptional.

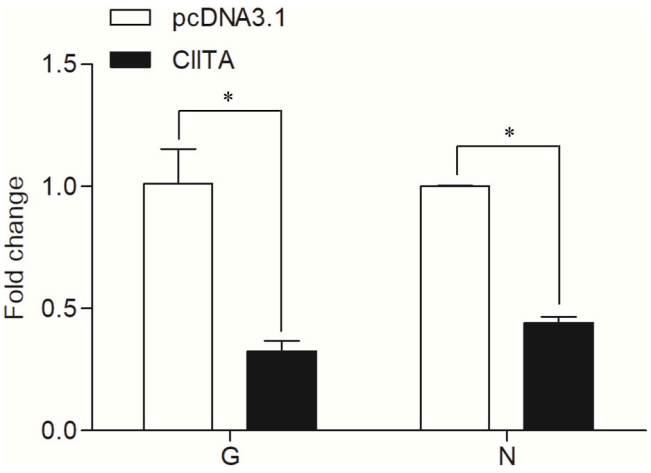

Fig. 4. CIITA suppresses VHSV replication in LMBS cells. LMBS cells were seeded in 6-well plates for 24 h and then transfected with 2.0 μg of pcDNA3.1 or Flag-CIITA. After 24 h transfection, the cells were infected with VHSV (MOI = 0.2). Cell samples were harvested at 24 h after VHSV incubation, and the transcripts of G and N genes were assessed using qRT-PCR. Asterisks (*) indicates statistically significant differences compared to the control group ($p < 0.05$).

3.7. N protein blocks MHCII expression mediated by CIITA

To identify the viral factor inhibiting MHCII transcription mediated by CIITA, the N and G proteins of VHSV were co-transfected with CIITA and MHCII promoters. The N protein significantly inhibited MHCII promoter activity and reduced CIITA protein levels, whereas the G protein showed no such effects (Fig. 7A-C). To identify the potential domains of N protein affecting CIITA function, five mutants of the N protein were constructed (Fig. 7D). Then FHM cells were co-transfected

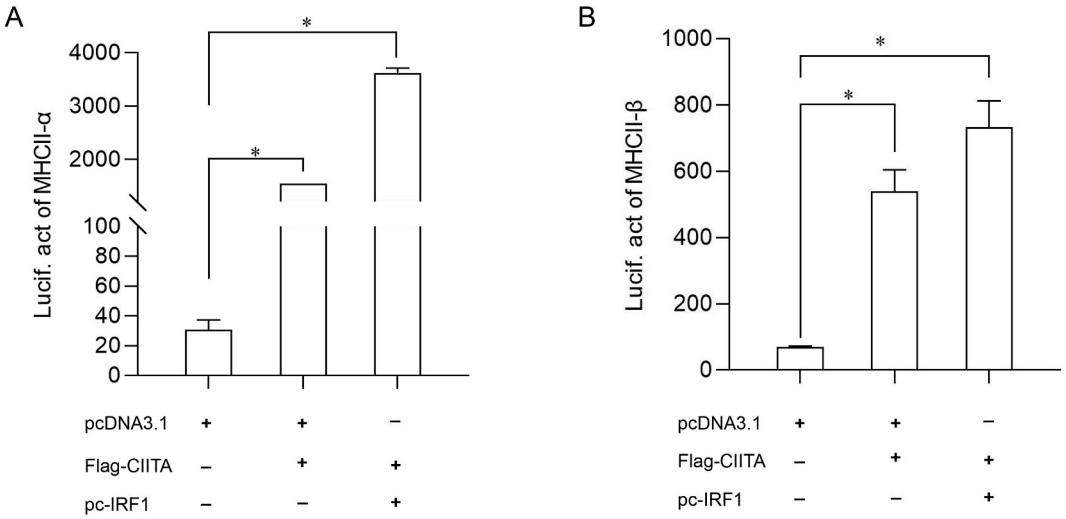

Fig. 3. CIITA activates MHCII transcription and collaborates with IRF1. FHM cells were cultured in 24-well plates overnight, 250 ng of Flag-CIITA was co-transfected with the 250 ng of pcDNA3.1 or pc-IRF1, 250 ng of MHCII-α or MHCII-β promoters, and 25 ng of pRL-TK. Promoter activity was assessed after 24 h transfection. Asterisks (*) indicates statistically significant differences ($p < 0.05$).

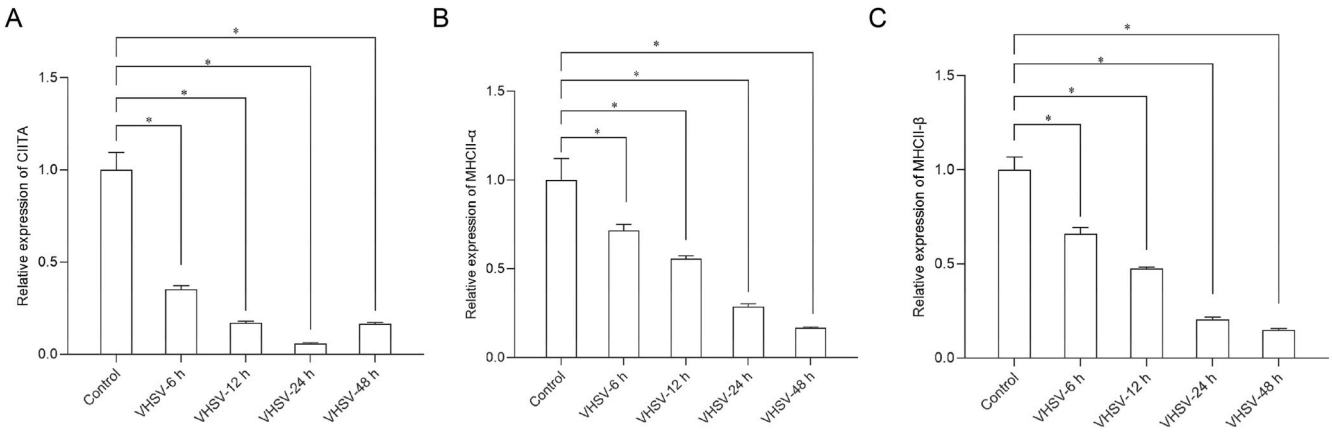

Fig. 5. VHSV infection suppresses CIITA and MHCII expression in vivo. Largemouth bass individuals were inoculated by intraperitoneal injection with 20 μl of VHSV (MOI = 0.2), and the spleen was harvested at 0 h, 6 h, 12 h, 24 h and 48 h after VHSV infection, respectively. Total RNAs were isolated to assess the transcriptional levels of CIITA (A), MHCII-α (B) and MHCII-β (C). Asterisks (*) indicates statistically significant differences from the control group ($p < 0.05$).

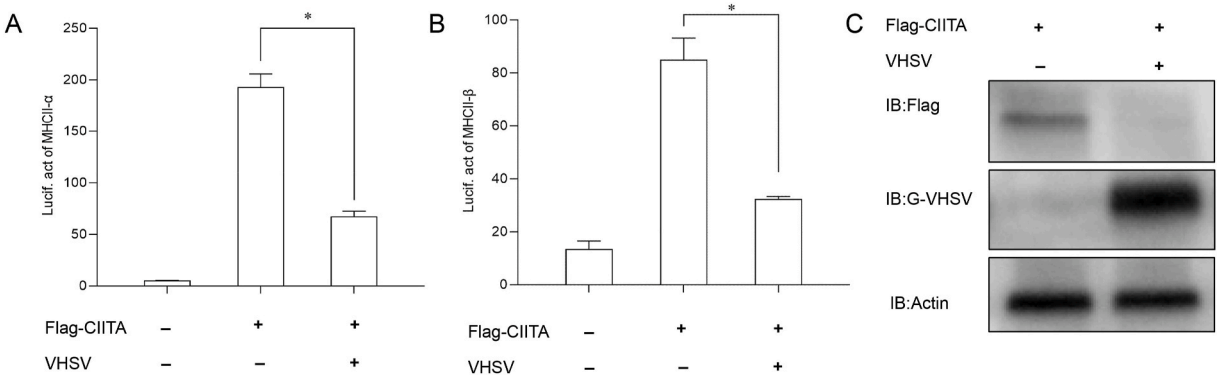

Fig. 6. VHSV blocks MHCII expression mediated by CIITA. (A and B) FHM cells seeded in 24-well plates were co-transfected with 250 ng of Flag-CIITA, 250 ng of MHCII-α or MHCII-β promoters and 25 ng of pRL-TK. After 24 h transfection, cell was infected with VHSV (MOI = 0.2). After 24 h infection, the cells were harvested and then lysed for luciferase assay. (C) FHM cells cultured in 6-well plates were transfected with 2.0 μg of Flag-CIITA and then incubated with VHSV (MOI = 0.2). After 24 h infection, the protein levels of CIITA were detected by immunoblotting (IB) with indicated Abs. Asterisks (*) indicates statistically significant differences from the control group ($p < 0.05$).

with Flag-CIITA, Myc-N or its five mutants and the promoters of MHCII-α or MHCII-β. The results indicated that the third and fourth mutants of N protein have lost partial ability of blocking activation of MHCII-α and MHCII-β promoters mediated by CIITA (Fig. 7E and F). Western blot also demonstrated that the deletion of the third and fourth domains in N protein largely restored the abundance of CIITA (Fig. 7G), suggesting that H3 and FCH domains of the N protein play important role in influencing CIITA function.

4. Discussion

As a key transcription factor that regulates the adaptive immune response, CIITA is essential for modulating both cellular and humoral immunity by precisely activating the transcriptional expression of MHCII. In mammals, the genetic characteristics and functions of CIITA have been explored. Although CIITA have been identified in several fish species, but its antiviral function and the impact of viral infection on its expression in vivo have not been reported in bony fish. In this study, we initially cloned CIITA from the largemouth bass and then analyzed its gene structure and functional domains. Comparing the gene structures of CIITA between largemouth bass and human, it was found that largemouth bass CIITA gene consists of 20 introns and 21 exons, while the type IV CIITA in human contains 17 introns and 18 exons, which might be due to the loss of intron during the evolution. Studies in grass carp had shown that there are 20 exons and 19 introns in CIITA [13], indicating that the number of exons and introns in CIITA varies among different fish species. Despite differences in gene structure, both the lower vertebrate and higher vertebrate have two conserved functional domains in CIITA, belonging to the P-loop_NTPase and PPP1R42 superfamilies. Interestingly, a novel Atrophin-1domain was identified in largemouth bass CIITA, and predicted to interact with the short glutamine repeat in the transcriptional coactivator CREB binding protein and involve in development or neuronal process.

Tissue expression profile analysis revealed that CIITA exhibits the highest expression in spleen of largemouth bass. In grass carp, CIITA is most highly expressed in the kidney [13], and the highest levels of CIITA expression are observed in the muscle of zebrafish and in the gill of Chinese tongue sole [11], indicating that the distribution of CIITA among different species are inconsistent. The cis-acting elements for RFX, NF-Y and CREB proteins, which form an enhanceosome with CIITA, as well as the ISRE sites bound by IRF1, were predicted on both MHCII-α and MHCII-β promoters. Function analysis also showed that CIITA not only induces the activation of MHCII, but also collaborates with IRF1 to enhance MHCII expression, which is consistent with that has been observed in zebrafish [12], suggesting that this mechanism might be conserved across different fish species. In grass carp, overexpression of CIITA in GCO cells enhanced the expression of MHCII-α and MHCII-β [13]. The suppression of MHCII expression was observed through RNA interference targeting CIITA in Chinese tongue sole [11]. All of these studies indicated that fish CIITA plays a crucial role in

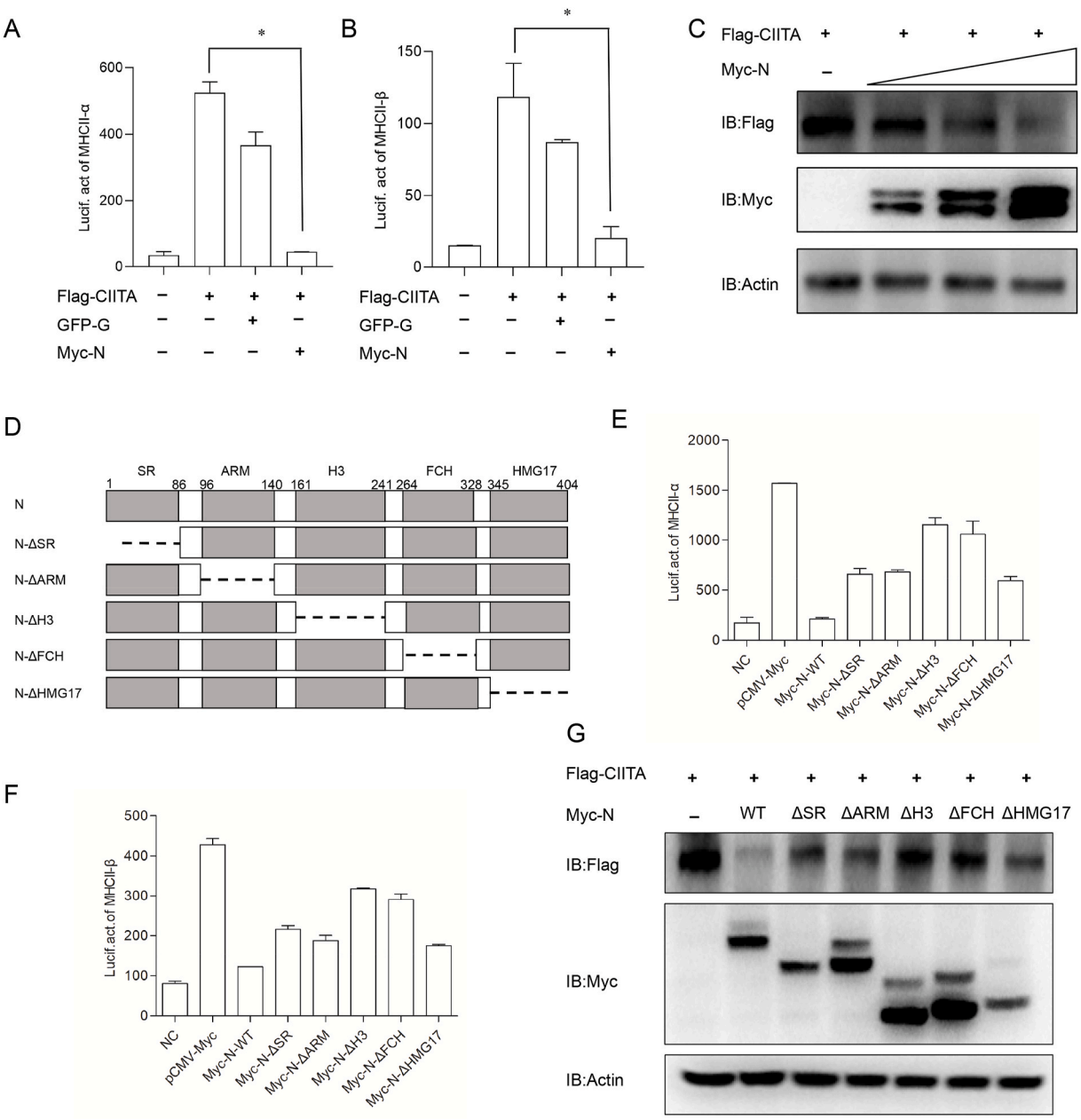

Fig. 7. N protein of VHSV inhibits CIITA function and MHCII transcription. (A and B) FHM cells were seeded in 24-well plates overnight and then transfected with 250 ng of Flag-CIITA, 250 ng of GFP-G or Myc-N, 250 ng of MHCII-α or MHCII-β promoters, and 25 ng of pRL-TK. The cells were lysed for monitoring luciferase activity after 24 h transfection. (C) HEK 293T cells were cultured in six-well plates overnight and then transfected with 2.0 μg of Flag-CIITA and 2.0 μg of empty vector or Myc-N (0.5, 1.0 and 2.0 μg, respectively). The cell samples were collected after 24 h transfection, and detected by IB with indicated Abs. (D) Schematic diagram of the wild-type N protein and its mutants. (E and F) FHM cells were cotransfected with 250 ng of Flag-CIITA, 250 ng of Myc-N or its mutants, 250 ng of MHCII-α or MHCII-β promoter, and 25 ng of pRL-TK. The promoter activities were measured after 24 h transfection. (G) HEK 293T cells seeded in 6-well plates overnight were cotransfected with 2.0 μg of Flag-CIITA and 2.0 μg of Myc-N or its mutants. After 24 h transfection, the cell samples were collected and the lysates were detected by IB with indicated Abs. Asterisks (*) indicates statistically significant differences from the control group ($p < 0.05$).

promoting MHCII transcription.

In mammals, CIITA not only regulates antigen presentation but also possesses antiviral function. Studies have shown that CIITA inhibits the transcription of hepatitis B virus (HBV) through ERK1/2-mediated down-regulation of the expression of HNF1α and HNF4α, which are two important factors for HBV replication in hepatocytes [19]. CIITA also enhances cell resistance by promoting the expression of the p41 isoform of the invariant chain CD74, which in turn blocks viral entry by preventing the cathepsin-mediated processing of the Ebola virus glycoprotein [9]. These results indicated that the antiviral molecular mechanisms of CIITA vary depending on the different viruses. In largemouth bass, we also found that CIITA suppressed VHSV reproduction, but the specific molecular mechanisms of CIITA inhibiting VHSV replication need to be explored in future.

Given the importance of CIITA in antiviral defense and antigen presentation, pathogens may evade host immune recognition by influencing its function through various molecular mechanisms. Previous studies found that the X protein of HBV interferes with anti-HBV activity of CIITA via its amino acid sequence 51–154 binding to CIITA, and thus impairing CIITA function [19]. The direct interaction of LANA encoding by KSHV with IRF4 dramatically blocks the DNA-binding ability of IRF4 on both pIII and pIV promoters of CIITA, which could suppress MHCII

transcription and provide a unique strategy for KSHV to escape from immune surveillance by cytotoxic T cells [20]. By binding to the ZRE in the CIITA promoter, Zta of Epstein-Barr virus negatively regulates CIITA transcription and inhibited the expression of MHCII [21]. In bony fish, the studies have shown that grass carp MHCI was significantly upregulated, but MHCII was down-regulated after grass carp reovirus (GCRV) infection, indicating that GCRV infection may suppress MHCII transcription through an unknown mechanism [22]. Our previous study also found that N protein of VHSV degraded sea perch STAT1 and thus suppressed CIITA and MHCII expression induced by IFN-γ in vitro [16]. In this study, the expression of CIITA and MHCII in largemouth bass were significantly inhibited after VHSV infection in vivo, and considering the highly conserved amino acid sequence of the molecules involved in MHCII regulation in sea perch and largemouth bass, we speculated that the impacts of VHSV infection on antigen presentation might be consistent in vitro and in vivo. In addition to influencing the transcription of CIITA, VHSV infection also led to the degradation of CIITA and disturbed the promoter activities of MHCII-α and MHCII-β, indicating that VHSV interfered with CIITA function at the protein level, thereby impairing the overall antigen presentation in bony fish. Further analysis revealed that the deletion of the H3 or FCH domain from the N protein largely diminished its impact on the activities of the MHCII-α and MHCII-β promoters induced by CIITA, which suggests that these two domains of the N protein might be critical for modulating CIITA function. In other fish rhabdoviruses, it has also been demonstrated that the N protein suppress innate immune responses through various mechanisms. For example, the N proteins of spring viremia of carp virus and infectious hematopoietic necrosis virus degrade MAVS and MITA, respectively, thereby blocking IFN expression [23,24]. Our recent studies have further revealed that the N protein of VHSV interacted with MDA5 in sea perch, inhibiting the activation of IFNh (data not shown). Thus, as a major inhibitory factor modulating both innate and adaptive immunity, the N protein of VHSV could serve as a promising target for vaccine development. Currently, we have successfully generated recombinant VHSV using reverse genetics, but the deletion of the N protein from the VHSV genome hinders the generation of recombinant viruses. Further investigation is needed to determine whether the absence of the F3 or FCH domains in VHSV genome affects its recombination or virulence.

In summary, the present study has identified CIITA in largemouth bass and analyzed its gene structure and functional domains. Overexpression of CIITA was found to significantly inhibit VHSV infection and activate MHCII expression. Following VHSV infection, it not only impacts the expression of CIITA at the transcriptional level but also leads to the degradation of CIITA via its N protein, thereby inhibiting the transcription of MHCII. These findings enhance our understanding of the molecular mechanisms through which VHSV evades the adaptive immune response in bony fish, and also provide the theoretical foundation for designing attenuated vaccines against VHSV by reverse genetics.

Data availability

Experimental data supporting the findings of this study are available on request.

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.

Acknowledgments

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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.2025.110336.

Data availability

Data will be made available on request.

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