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The down-regulation of casein kinase 1 alpha as a host defense response against infectious bursal disease virus infection

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ABSTRACT

Infectious bursal disease virus (IBDV) is an important immunosuppressive virus of chickens. Although the gene functions of IBDV have been well characterized, the host responses during IBDV infection remain much poor. In the present study, casein kinase 1 alpha (CK1α), a novel VP2-associated protein, was down-regulated during IBDV replication in DF1 cells. Further experiments showed that siRNA-mediated knockdown of CK1α inhibited IBDV replication, while overexpression of CK1α promoted IBDV growth. Finally, we revealed that the effects of CK1α expression level on IBDV replication were involved in the negative regulation of CK1α on type I interferon receptor (IFNAR1), because ubiquitination assay analyses demonstrated that CK1α could promote the ubiquitination of IFNAR1, thereby affecting the stability of this receptor. In conclusion, down-regulation of CK1α during IBDV infection as a host defense response increased abundance of IFNAR1, which in turn enhanced an inhibitory effect on IBDV replication.

1. Introduction

Infectious bursal disease (IBD, also called Gumboro disease) is an acute, highly contagious immunosuppressive disease in young chickens (Cosgrove, 1962; Kibenge et al., 1988). The etiological agent, infectious bursal disease virus (IBDV), destroys the central immune organ (bursa of Fabricius) of chickens, resulting in immunosuppression and reduced responses to vaccines, as well as increased susceptibility to other pathogens (Nagarajan and Kibenge, 1997). Indeed, IBD is considered as a major threat to the global commercial poultry industry (Lasher and Shane, 1994; Müller et al., 2003; Sharma et al., 2000).

IBDV is an *Avibirnavirus* of family *Birnaviridae* (Dobos et al., 1979; Müller et al., 1979), and contains a bi-segmented (segment A and B) double-stranded RNA (dsRNA) genome (Nagarajan and Kibenge, 1997). Segment A (approximately 3.2 kb) encodes two partially overlapping open reading frames (ORFs). Of these, the larger ORF encodes a 110 kDa polyprotein precursor, NH_2-pVP2VP4VP3-COOH, which is cotranslationally processed into pVP2 (48 kDa), VP3 (32 kDa), and VP4 (28 kDa) by the viral protease VP4 (Hudson et al., 1986; Jagadish et al., 1988; Sánchez and Rodriguez, 1999). During viral assembly, pVP2 is further processed into mature VP2 (~ 40 kDa) via several C-terminal proteolytic cleavage reactions (Chevalier et al., 2002; Da Costa et al., 2002). The small ORF of segment A encodes VP5, a 17 kDa non-structural protein that is dispensable for viral replication (Mundt et al., 1995, 1997), whereas segment B (approximately 2.8 kb) encodes VP1 (91 kDa), an RNA-dependent RNA polymerase.

Viruses as the obligate parasites must rely on their host to live and replicate, therefore virus-host interactions including host immune responses and viral immune evasions, will constantly happen through the entire life cycle of the viruses once the viral infection is initiated. Following the better understanding of IBDV gene functions, researchers have begun to focus on revealing the mystery of the interaction between IBDV and the host. For example, several papers has shown that IBDV infection would activate the host antiviral response, such as up-regulation of interferon-related genes (Lee et al., 2014; Raj et al., 2011; Smith et al., 2015), but meanwhile, researchers have also found the viral protein VP3 and VP4 played a role in suppressing interferon expressions via blockage of dsRNA

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recognition by MDA5 (Ye et al., 2014b) or binding to glucocorticoid-induced leucine zipper protein (GILZ) (Li et al., 2013b). And ribonucleoprotein complexes of IBDV cloud interact with voltage-dependent anion channel 1 to enhance viral polymerase activity (Han et al., 2017). In contrast, the interaction of VP4 with Cyclophilin A are not beneficial for IBDV replication (Wang et al., 2015) and eukaryotic translational initiation factor 4AII would inhibit viral polymerase activity through binding to VP1 (Gao et al., 2017). Also, researchers recently revealed that the role of VP5 in IBDV-induced apoptosis was mediated by the interaction of VP5 with voltage-dependent anion channel 2 (VDAC2) (Li et al., 2012) and receptor of activated protein kinase C1 (RACK1) (Lin et al., 2015). Additionally, as we known, VP2 as the capsid protein of IBDV is responsible for attachment and entry to the host (Delgui et al., 2009; Lin et al., 2007), however, beyond the entry step, there is much poor information about the detailed interactions with host and the resulting responses of host.

In this study, we demonstrate that casein kinase 1 alpha (CK1α), a member of the casein kinase l family, is a novel VP2-associated protein, and down-regulation of CK1α plays an important role in suppression of IBDV replication by promoting type I interferon (IFN-I) signaling.

2. Materials and methods

2.1. Plasmids

The eukaryotic expression vectors pCAGGS-HA (pCAH) and pCAGGS-FLAG (pCAF), containing an N-terminal HA-tag and a C-terminal FLAG-tag, respectively, were reconstructed from pCAGGS (Niwa et al., 1991). pCAH-GtVP2, which contains the VP2 gene of IBDV Gt, was constructed previously (Zhang et al., 2015). *Gallus* CK1α cDNA (GenBank no. AF042862.1) was cloned into vectors pCAH and pCAF, forming pCAH-CK1α and pCAF-CK1α, respectively. pCAF-IFNAR1, containing the *Gallus* type I IFN receptor subunit 1 (IFNAR1) coding gene, was constructed by cloning the full-length IFNAR1 gene (GenBank no. AY744159.1) from DF1 cells into pCAF. Then the plasmid pCAF-IFNAR1^{S545A} containing substitution of the amino acid Ser545 was also constructed by oligonucleotide-directed mutagenesis as described previously (Qi et al., 2007), with specific primer pairs (IFNAR1-S545A-U: AAACAGAGCAGTCGAGATGCA GGGAATTATTCTTAC and IFNAR1-S545A-L: GTAAGAATAATTC CCTGCATCTCGACTGCTCTGTTT). And, the *Gallus* ubiquitin B (GenBank no. NM_001293174.1) coding sequence was cloned into pCAH, generating pCAH-UBB. Lastly, to express the extracellular domain (100aa-200aa) of chicken IFNAR1 as the immunogen for antibody preparation, the coding region of this domain was cloned into pET32a to construct the expression vector pET32a-IFNAR1^{truncated}

2.2. Cells, viruses, and antibodies

DF1 cells (immortalized chicken embryo fibroblasts) were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5%-CO_2 incubator. Chicken embryo fibroblast (CEF) cells prepared from 10-day-old specific-pathogen-free (SPF) chicken embryos were cultured in DMEM containing 5% FBS. IBDV strain Gt, a cell culture-adapted strain, was attenuated and identified in Harbin Veterinary Research Institute (HVRI), the Chinese Academy of Agricultural Sciences (CAAS), as described previously (Wang et al., 2004).

Anti-HA, anti-FLAG, and anti-β-actin antibodies, as well as FITC- and TRITC-conjugated goat anti-mouse IgG, and FITC-conjugated goat anti-Rabbit IgG were purchased from Sigma-Aldrich (St. Louis, MO, USA); polyclonal antibodies (pAb) specific to CK1α were purchased from Cell Signaling Technology (Danvers, MA, USA); monoclonal antibodies (mAb) specific to VP2 (6H7D) (Cheng et al., 2006) and VP5 (4B4) (Zhang et al., 2007), respectively, were prepared and preserved previously. To prepare IFANR1-specific antibodies, the extracellular domain (100aa-200aa) of chicken IFNAR1 was expressed by pET32a-IFNAR1^{truncated} in BL21 (DE3), then the purified extracellular domain as the immunogen was used to produce the IFNAR1 antibodies through immunizing the mice in the current study.

2.3. Viral infection and titration

DF1 cells were infected with IBDV Gt at a multiplicity of infection (MOI) of 1. After incubation for 1 h at 37 °C, the cells were washed three times with phosphate-buffered saline (PBS, pH 7.4) and then cultured in fresh medium. At the indicated times, cell supernatants were collected to detect extracellular viral titers as $TCID_{50}$ (tissue culture infectious dose; dose capable of infecting 50% of cells in a monolayer) per 100 μl, using the Reed and Muench (1938) formula. DF1 cell monolayers were subsequently washed and lysed using WB & IP lysis buffer (P0013, Beyotime Institute of Biotechnology, Shanghai, China, containing 20 mM Tris (pH7.5), 150 mM NaCl , 1% Triton X-100, sodium pyrophosphate, β-glycerophosphate, EDTA, Na_3VO_4, leupeptin, and 1 mM PMSF) at 36 h post transfection). The resulting lysates were then subjected to western blot analysis to evaluate the levels of intracellular VP5 expression of IBDV.

2.4. Coimmunoprecipitation/mass spectrometry (Co-IP/MS)

To identify host cell proteins that interact with VP2, 1×10^7 DF1 cells were infected with IBDV for 36 h and lysed by triplicate freeze-thaw treatments. Uninfected cells were used as a control. After centrifugation at 12,000×*g* for 10 min, supernatants were collected and cell debris were subjected to ultrasonication in 500 μl PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF). Cleared lysates were then pre-cleared with protein A/G beads at 4 °C for 2 h. After centrifugation at 4500×*g* for 2 min, the supernatants were incubated with the anti-VP2 mAb (Cheng et al., 2006) for 2 h at 4 °C, and then incubated with protein A/G beads overnight at 4 °C. Lastly, the bead complexes were washed three times with cold PBS, boiled in 5× loading buffer for 10 min, and subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The resulting gels were stained using a Fast Silver Stain Kit (Beyotime Institute of Biotechnology, China), and specific protein bands of interest were excised for MS analysis (Shanghai Institutes for Biological Sciences, China). For exogenous Co-IP, DF1 cells in 6-well plate were co-transfected with pCAF-CK1α (3 μg) and pCAH-VP2 (3 μg), and then cells were lysed in WB & IP lysis buffer (P0013, Beyotime Institute of Biotechnology, Shanghai, China) for Co-IP assay using FLAG antibody to precipitate CK1α-VP2 complex at 36 h post transfection.

2.5. Confocal laser scanning microscopy assays

To confirm the association between VP2 and CK1α, exogenous or endogenous Co-IP was performed in transfected or infected DF1 cells as showed above. In addition, the subcellular localization of VP2 and CK1α was evaluated by confocal laser scanning microscopy. Briefly, transfected or infected DF1 cells were fixed by incubation with absolute ethanol for 20 min at room temperature and blocked by incubation with 3% bovine serum albumin (BSA) for 1 h at 37 °C. Blocked cells were stained by incubation with mouse anti-HA antibodies (Sigma-Aldrich) or anti-VP2 mAb, followed by incubation with rabbit anti-FLAG antibodies (Sigma-Aldrich) or anti-CK1α pAb (Cell Signaling Technology) for 2 h. Stained cells were then incubated with the corresponding FITC- or TRITC-conjugated secondary antibodies (Sigma-Aldrich) for 1 h at room temperature. Finally, the cells were stained with 4, 6-diamidino-2-phenylindole (DAPI) for 10 min for visualization of nuclei, and examined using a Leica SP2 confocal system (Leica Microsystems, Wetzlar, Germany).