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Infectious bursal disease virus protein VP4 suppresses type I interferon expression via inhibiting K48-linked ubiquitylation of glucocorticoid-induced leucine zipper (GILZ)

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ABSTRACT

Viruses have developed a variety of methods to evade host immune response. Our previous study showed that infectious bursal disease virus (IBDV) inhibited type I interferon production via interaction of VP4 with cellular glucocorticoid-induced leucine zipper (GILZ) protein. However, the exact underlying molecular mechanism is still unclear. In this study, we found that IBDV VP4 suppressed GILZ degradation by inhibiting K48-linked ubiquitylation of GILZ. Furthermore, mutation of VP4 (R41G) abolished the inhibitory effect of VP4 on IFN-β expression and GILZ ubiquitylation, indicating that the amino acid 41R of VP4 was required for the suppression of IFN-β expression and GILZ ubiquitylation. Moreover, IBDV infection or VP4 expression markedly inhibited endogenous GILZ ubiquitylation. Thus, IBDV VP4 suppresses type I interferon expression by inhibiting K48-linked ubiquitylation of GILZ, revealing a new mechanism employed by IBDV to suppress host response.

1. Introduction

Infectious bursal disease (IBD), also called Gumboro disease, is a highly contagious and acute disease among young chickens (Pitcovski et al., 2003). Infectious bursal disease virus (IBDV), the causative agent for IBD, can cause severe damage in its target organs of birds, especially the bursa of Fabricius (Liu and Vakharia, 2004; Muller et al., 2003; Sharma et al., 2000). Besides, chickens infected with IBDV suffer from severe immunosuppression, leading to an enhanced susceptibility to various avian diseases (Sharma et al., 2000; Stricker et al., 2010).

IBDV, an Avibirnavirus belonging to Birnaviridae, has two segments of double-stranded RNAs (segment A and B) (Azad et al., 1985a; Fahey et al., 1989). The short RNA, segment B (2.8 kb), encodes VP1, a RNA-dependentRNA polymerase (RdRp) (Escaffre et al., 2013; Wang et al., 2009), mainly taking part in viral replication and virulence (Pan et al., 2009), forming complexes with the capsid protein VP3, and leading to efficient encapsidation into virus-like particles (Lombardo et al., 1999; Tacken et al., 2000); while the large molecule, segment A (3.17 kb), contains two partially overlapping open reading frames (ORFs). ORF1 encodes the nonstructural viral protein VP5. Recent reports demonstrate that the nonstructural viral protein VP5 plays different roles in IBDV-induced apoptosis during IBDV infection. VP5 inhibits apoptosis early during IBDV infection (Liu and Vakharia, 2006; Wei et al., 2011), but induces apoptosis at a later stage of infection (Li et al., 2012; Mundt et al., 1997). While, ORF2 encodes the polyprotein pVP2-VP4-VP3 (110 kDa) that autoproteolytically cleaved into viral proteins pVP2, VP3 and VP4, and then pVP2 is further processed into the mature capsid protein VP2 (Azad et al., 1985b; Casanas et al., 2008; Delgui et al., 2009; Kibenge et al., 1991). VP2 and VP3 are the major structural proteins of virus, constituting 51% and 40% of the viron, respectively (Dobos et al., 1979), and they are involved in antigenicity, cell tropism, pathogenic phenotype, and apoptotic regulation (Brandt et al., 2001). VP4, a viral protease, is able to cleave *in trans* and is responsible for the interdomain proteolytic autoprocessing of the pVP2-VP4-VP3 polyprotein into pVP2 precursor (48 kDa) and VP4 (28 kDa) as well as VP3 (32 kDa) (Birghan et al., 2000; Stricker et al., 2010). Our previous report showed that viral protease VP4 suppressed type I interferon (IFN) expression in cells with IBDV infection (Li et al., 2013).

Virus can be sensed by cellular pattern recognition receptors (PRRs), causing type I interferon (IFN) and inflammatory cytokine responses (Cunha, 2012; Ward, 2010). However, aberrant PRRs activation might cause acute or chronic inflammation and tissue damage (Kato and

Abbreviations: GILZ, glucocorticoid-induced leucine zipper; CHX, cycloheximide; MG132, Z-Leu-Leu-Leu-al; Ub, ubiquitylation; IBDV, , infectious bursal disease virus

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Fujita, 2014; Smith and Jefferies, 2014). To prevent these inflammatory disorders, host post-translational modifications (PTMs), including phosphorylation, ubiquitylation and acetylation might play important roles in the modulation of PRRs and their downstream signaling. Among them, ubiquitylation, an important process of post-translational modifications (PTMs), is involved in the covalent bonding of about 8.3 kDa protein ubiquitin to the target proteins (Chernorudskiy and Gainullin, 2013; Pickart and Eddins, 2004). Viruses have developed a variety of strategies to counteract host immune response and facilitate their replication by manipulating the state of host cells. Among them, subverting the ubiquitin or ubiquitin-like modification system is an important part (Heaton et al., 2016).

Our previous study established that IBDV VP4 suppressed type I interferon expression via interacting with the glucocorticoid-induced leucine zipper (GILZ) (Li et al., 2013). In this study, we provide further evidence to show that VP4 inhibited IFN-β production through impairing the K48-linked ubiquitylation of GILZ. These data suggest that IBDV VP4 suppresses type I interferon expression by inhibiting GILZ ubiquitylation, unveiling a new mechanism employed by IBDV to suppress host response.

2. Materials and methods

2.1. Cell lines, E.Coli cells and virus

Human Embryonic Kidney 293T (HEK-293T) and immortal chicken embryo fibroblast (DF-1) cells were purchased from ATCC and these cells were maintained in Dulbecco modified Eagle medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37 ˚C in 5% CO_2 incubator. Lx, a IBDV strain propagated in DF-1 cells, was kindly afforded by Dr. Jue Liu (Beijing Academy of Agriculture and Forestry, Beijing, China). Rosetta (DE3) *E.Coli* cells and DH_{10}Bac *E.Coli* cells were purchased from Transgen company (Beijing).

2.2. Reagents and antibodies

All the restriction enzymes and PrimeScript RT reagent Kit were purchased from TaKaRa (Japan). pRK5-flag, pRK5-HA, pCMV-Myc, pCMV-HA, pEGFP-N1 vectors were obtained from Clontech (USA). Anti-c-Myc (sc-40), anti-green fluorescent protein (anti-GFP, sc-9996), Rabbit anti-HA polyclonal antibodies (sc-805), anti-β-actin (sc-1616-R) antibodies were obtained from Santa Cruz Biotechnology (USA). GILZ monoclonal antibody was established by our laboratory (He and Wang, 2017) and VP4 monoclonal antibodies were purchased from CAEU Biological Company (Beijing). Anti-GAPDH monoclonal antibody, Fastagen RNA extraction Kit and protease inhibitor cocktail C were obtained from GBC company (Beijing). Anti-K48-Ub and anti-K63-Ub were purchased from cell signaling technology (USA). Poly(I:C), MG132 (proteasome inhibitor), NH_4Cl (lysosome inhibitor), anti-FLAG monoclonal antibody (F1804) were purchased from Merck-Sigma-Aldrich Corporation. Horseradish peroxidase (HRP)-conjugated goat anti-mouse and anti-rabbit IgG antibodies were purchased from DingGuo (China). An enhanced chemiluminescence (ECL) kit was purchased from Merck Millipore (Germany). OPTI-MEM I was purchased from Invitrogen, Jetprime transfection reagent from Polyplus company, protein A/G plus-agrose from GE company (Sweden). Quantitative reverse transcription-PCR performed with Roche LC480 system (Roche life science, USA).

2.3. Plasmid construction

pCMV-HA-*ub* plasmid and its mutants pCMV-HA-K48-*ub* (all lysines except that at position 48 were mutated to arginines), pCMV-HA-K63-*ub* (all lysines except that at position 63 were mutated to arginines) were kindly provided by Professor Jun Tang (China Agricultural University). IBDV *vp4* was cloned from IBDV strain *Lx* using the following specific primers: sense, 5′-AGGATAGCTGTGCCGGTGGTCTC CACAT-3′; antisense, 5′-TTTGATGAACGTTGCCCAGTT-3′ (GenBank accession no.6539893). Chicken *gilz* was cloned from DF-1 cells using the specific primers 5′-ATGAGCACCGGCGTGTACCA-3′ (sense) and 5′-TTACACAGCAGAACCACCA-3′ (antisense) with reference to the sequence in GenBank (accession no. NM_001077234). All the primers were synthesized by Sangon Company (China), and the constructs were made by standard biological procedures.

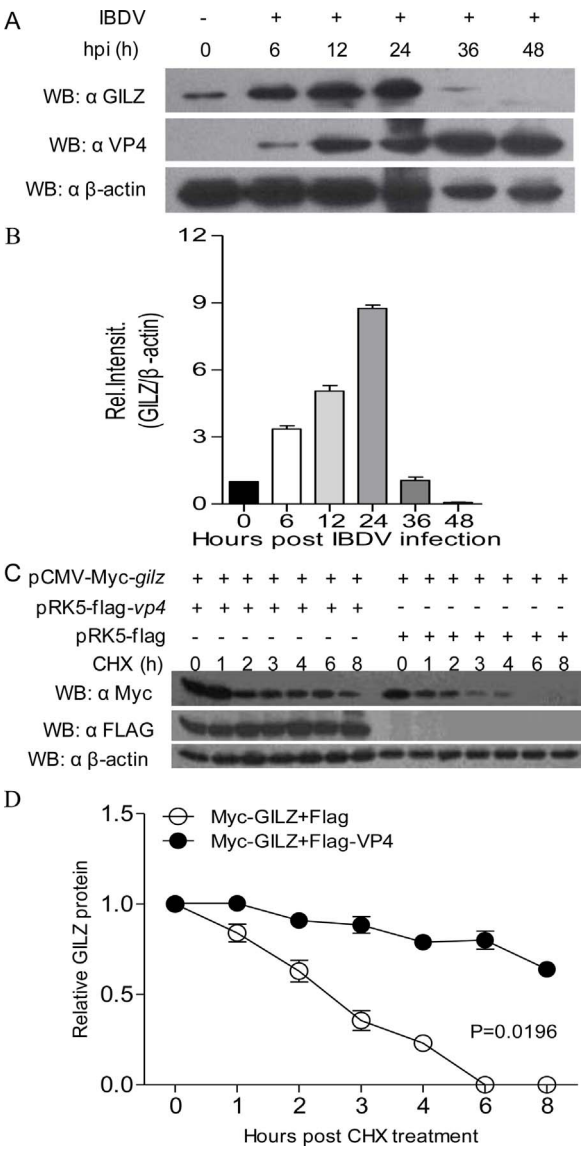

Fig. 1. IBDV infection or IBDV VP4 expression inhibits GILZ degradation. (A&B) Effect of IBDV on the expression of endogenous GILZ in DF-1 cells. (A) DF-1 cells were mock infected or infected with IBDV at an MOI of 1. Cells were harvested at the indicated time points (6, 12, 24, 36, 48 hpi) and examined by Western Blot using anti-VP4, anti-GILZ and anti-β-actin antibodies. (B) The histogram represented the change of protein level of GILZ. The protein level of GILZ was normalized to β-actin. (C and D) Effect of IBDV VP4 on the half-life of GILZ in HEK-293T cells. HEK-293T cells were cotransfected with 1 μg of pCMV-Myc-*gilz* and pRK5-flag-*vp4* or control empty vector. Cells were treated with cycloheximide (CHX) at certain times (0, 1, 2, 3, 4, 6, 8 h) before they were harvested. Twenty-four hours after transfection, cell lysates were prepared and examined with Western Blot using indicated antibodies. The relative levels of GILZ were calculated as follows: band density of GILZ/band density of β-actin. The relative level of GILZ without CHX treatment was set as 1. Results are representative of three independent experiments with similar results. Datas are shown as means ± SD; n = 3. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.