The Capsid Protein of Hepatitis E Virus Inhibits Interferon Induction via its N-terminal Domain

S. Lin, Y. Yang, Y. Nan, Z. Ma, L. Yang, and Y. Zhang

Department of Veterinary Medicine, University of Maryland, College Park, MD

ABSTRACT

Hepatitis E virus (HEV) is the causative agent for a liver inflammation that mainly presents as an acute, self-limiting liver disease. In pregnant women, the fulminant liver disease can be exacerbated and result in up to 30% case fatality. Chronic HEV infection with rapid progression in immunocompromised patients has been a challenge in industrialized countries in recent years. The transmission of HEV is mainly through contaminated drinking water or foods. HEV is a positive-sense single-stranded RNA virus of the family *Hepeviridae*. The HEV genome contains three open reading frames: ORF1 encodes the non-structural polyprotein involved in viral RNA replication; ORF2 encodes the capsid protein; ORF3 encodes a small multifunctional protein. HEV ORF1 product is known to inhibit the induction of type I interferons (IFNs), but the expression of ORF1 product in HEV infected patients and cells is relatively low. In this study, we discovered that the capsid protein, the most abundantly expressed viral protein, may play a major role in the HEV antagonizing IFN induction. Mechanistically, the capsid protein blocked the phosphorylation of IRF3 via interaction with the MAVS-TBK1-IRF3 complex. The N-terminal domain of the capsid protein appears to be responsible for the inhibition of IRF3 activation. Further study showed that the first 50 amino acids in the N-terminal domain are mainly responsible for the blocking of IRF3 phosphorylation. Our data provides further insight of HEV interference with the IFN signaling.

INTRODUCTION

- HEV is a positive-sense single-stranded RNA virus of the family *Hepeviridae.* There are two genera: *Orthohepevirus* and *Piscihepevirus* in the family.
- The genus *Orthohepevirus* contains the previously known genotype 1-4 and the newly recognized genotype 5-8.
- The genotype 1 and 2 are restricted to humans, whereas the genotype 3 and 4 strains cause zoonotic infections.
- HEV genome encodes three open-reading frames (ORFs): ORF1, ORF2 and ORF3. An additional ORF, ORF4, is found in genotype 1.
- HEV ORF2 is predicted to encode a 72 kDa protein.
- The capsid protein contains an N terminal domain (aa1-111), a VLP (aa112-608) and a C terminal domain (aa608-660).
- RIG-I-like receptors (RLR) pathway plays a vital role in antiviral response. The activation of RIG-I pathway leads to the activation of IRF3 and IFN synthesis.
- HEV infection induces high level expression of RIG-I and MDA5, while the IFN production is very low in cultured cells.
- The HEV non-structural protein (NSP) X and PCP downregulate the IFN production, while their expression level is below detection. In contrast, the capsid protein is the most abundant protein in HEV infected cells.
- The objective of this study was to determine the role of the capsid protein in the IFN induction.

RESULTS

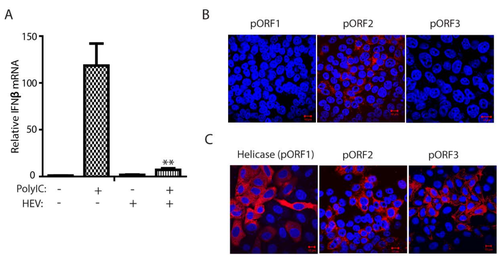

Figure 1. HEV infection downregulates IFN induction. A. The inhibition of polyI:C-induced IFN-β expression in HepG2/C3A cells by infection of HEV Kernow-P6 strain. Significant difference between the polyI:C-treated samples is denoted with ** for P < 0.01. B. Indirect immunofluorescence assay (IFA) of the HepG2/C3A cells infected with Kernow-p6. Antibodies against products of helicase (ORF1), ORF2 and ORF3 were used. Red indicates HEV-positive staining and blue is DAPI staining of nuclear DNA. C. IFA was done with corresponding antibodies as described in "B" above. IFA of HeLa cells transiently transfected with plasmids of helicase, ORF2, and ORF3.

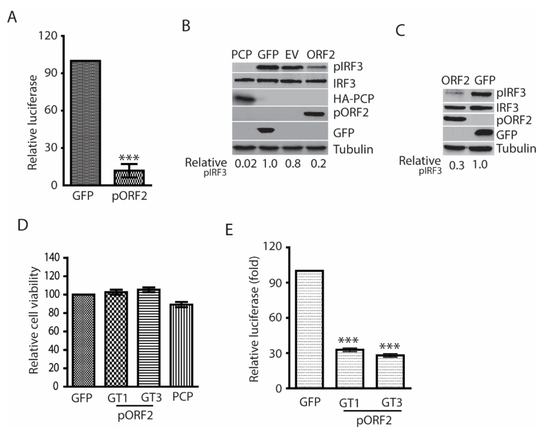

Figure 2. The capsid proteins of both genotype 1 and 3 HEV inhibit polyI:C-induced IFN production. A. The capsid protein of Kernow (genotype 3, GT3) inhibits IFN-β reporter expression in 293T cells. The cells were transfected with plasmids of GT3 ORF2 and IFN-β reporter. At 36 hpt, the cells were transfected with polyI:C at 1 μg/ml. The Luciferase activity was detected after 24 h treatment. "***" denotes P < 0.001. B. The capsid protein of HEV Kernow inhibits polyI:C-induced IRF3 phosphorylation. The cells were harvested for Western blotting (WB) after 8 h treatment. The PCP of HEV ORF1, GFP and empty vector (EV) were included as controls. C. HEV Sar55 (GT1) ORF2 blocks IRF3 phosphorylation induced by polyI:C. D. Relative cell viability of 293T cells after transfection. The cells were transfected with plasmids of GFP, HEV ORF2 and PCP for 48h before the cell viability assay. E. HEV pORF2 downregulates Sendai virus-induced IFN-β expression in 293T cells.

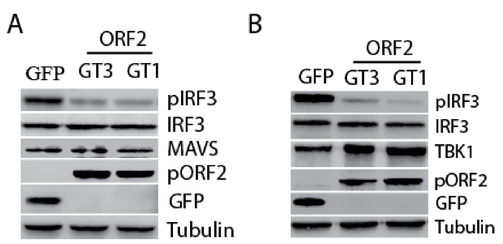

Figure 3. The capsid protein inhibits IFN induction by blocking TBK1-mediated IRF3 phosphorylation. A. The capsid protein of HEV GT1 and GT3 blocks MAVS-induced IRF3 activation. The 293T cells were co-transfected with ORF2, IRF3 and MAVS plasmids. The cells were harvested 24 h later for WB. B. The capsid protein blocks TBK1-induced IRF3 activation. Transfection and WB were conducted as described in "A" above.

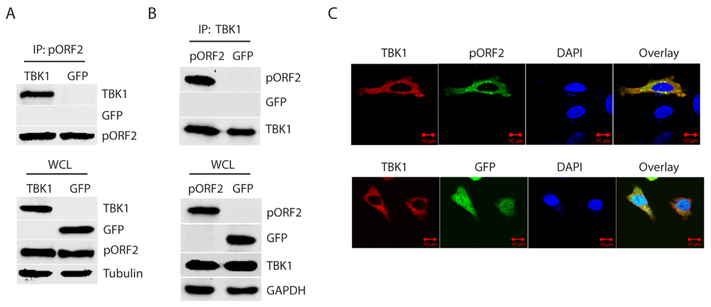

Figure 4. The capsid protein interacts with TBK1. A. TBK1 presents in the Co-IP complex of pORF2. HEK293-IRF3 stable cells were transfected with pORF2 and TBK1 plasmids. B. pORF2 presents in the Co-IP complex of TBK1 from 293-IRF3 stable cells. C. The capsid protein co-localizes with TBK1. HeLa cells were transfected with pORF2 and TBK1 plasmids. IFA and confocal microscopy were done at 36hpt. Green stands for ORF2, red indicates TBK1, and blue is DAPI staining. The bar in the lower right of the images denotes 10 μm.

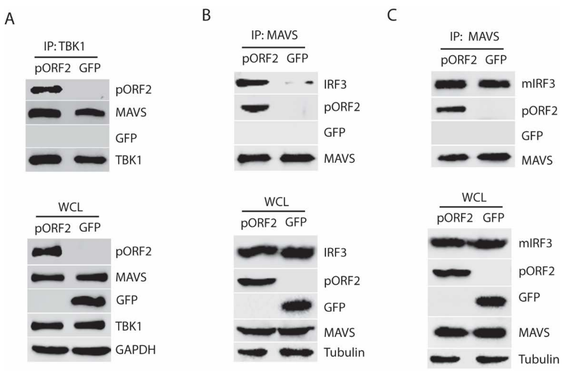

Figure 5. The capsid protein inhibits dissociation of IRF3 from MAVS-TBK1 complex. A. TBK1 interacts with pORF2 in the presence of MAVS. B. MAVS IP pulls down IRF3 and pORF2, whereas much less IRF3 in the control of GFP. The 293T cells were transfected with Myc-MAVS, IRF3 and pORF2. Co-IP was done with Myc-MAVS antibody, and the product was detected by WB with antibodies against MAVS, IRF3 and pORF2. WB of WCL is shown in the lower panel. C. Presence of ORF2 does not affect mutant IRF3 interaction with MAVS. The 293T cells were transfected with Myc-MAVS, mutant IRF3 (S385A/S386A) and pORF2. The Co-IP detection was done as panel B.

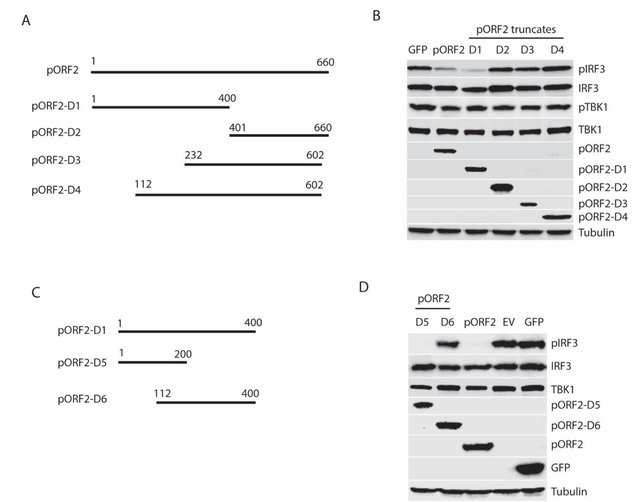

Figure 6. The first 111 aa of the capsid protein is responsible for inhibition of IRF3 activation. A. Schematic illustration of ORF2 truncation mutants. B. Domain screening of ORF2 truncation mutants in the inhibition of TBK1-mediated IRF3 activation. 293-IRF3 stable cell line was co-transfected with ORF2 truncations and TBK1 plasmids, and at 36hpt, the samples were subjected to WB for p-IRF3 detection. C. Schematic illustration of ORF2 truncation mutants D5 and D6. D. The pORF2-D5 inhibits IRF3 activation. ORF2 D5 and D6 were co-transfected to 293-IRF3 cells and at 36hpt, the samples were subjected to WB for p-IRF3 detection.

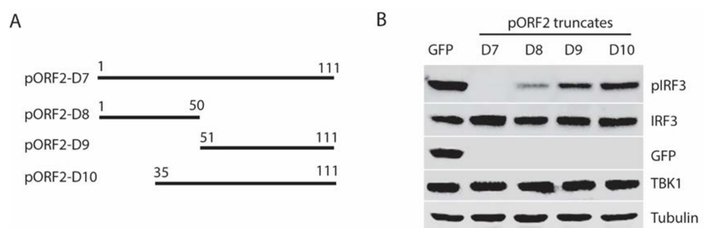

Figure 7. Further mapping of N-terminal domain of the capsid protein for inhibition of IRF3 phosphorylation. A. Schematic illustration of truncation mutants of the first 111 aa of the capsid protein. B. Domain screening of ORF2 truncations in the inhibition of TBK1-mediated IFN induction. 293-IRF3 stable cell line was co-transfected with ORF2 N-terminal truncations and TBK1 plasmids for 36 h, and the samples were harvest for WB.

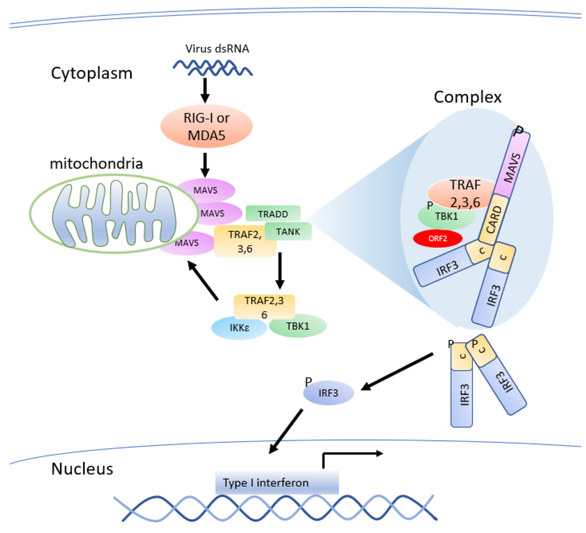

Figure 8. The proposed model for HEV ORF2 inhibition of IFN signaling.

Summary

- The HEV capsid protein inhibits type I IFN production via blocking IRF3 activation
- The capsid proteins of both genotype 1 and 3 inhibit IFN induction
- The capsid protein interacts with TBK1 and inhibits the dissociation of IRF3 from the MAVS-TBK1 complex.
- The N terminal 111 aa of the capsid protein is essential to the inhibition of IRF3 phosphorylation