Virology 509 (2017) 222–231

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Foot-and-mouth disease virus induces lysosomal degradation of host protein kinase PKR by 3C proteinase to facilitate virus replication

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ARTICLE INFO

Keywords:
Foot-and-mouth disease virus
PKR
$3C^{pro}$
Lysosomes

ABSTRACT

The interferon-induced double-strand RNA activated protein kinase (PKR) plays important roles in host defense against viral infection. Here we demonstrate the significant antiviral role of PKR against foot-and-mouth disease virus (FMDV) and report that FMDV infection inhibits PKR expression and activation in porcine kidney (PK-15) cells. The viral nonstructural protein 3 C proteinase ($3C^{pro}$) is identified to be responsible for this inhibition. However, it is independent of the well-known proteinase activity of $3C^{pro}$ or $3C^{pro}$-induced shutoff of host protein synthesis. We show that $3C^{pro}$ induces PKR degradation by lysosomal pathway and no interaction is determined between $3C^{pro}$ and PKR. Together, our results indicate that PKR acts an important antiviral factor during FMDV infection, and FMDV has evolved a strategy to overcome PKR-mediated antiviral role by downregulation of PKR protein.

1. Introduction

Foot-and-mouth disease virus (FMDV) is an RNA virus belonging to the genus *Aphthovirus* of family *Picornaviridae*. FMDV is the causative agent of foot-and-mouth disease (FMD) that causes highly contagious viral disease of cloven-hoofed animals (Grubman and Baxt, 2004). The outbreak of FMD often results in severe economic consequences due to its impact on trade and the slaughtering of large amounts of animals (Jamal and Belsham, 2013). The genome of FMDV is a positive-strand RNA of ~8.5 kb encoding a large polyprotein. The viral leader proteinase (L^{pro}), 2 A protein, and 3 C proteinase ($3C^{pro}$) subsequently cleaves the polyprotein to yield mature structural and nonstructural proteins (Steinberger et al., 2014). L^{pro} and $3C^{ro}$ are multifunctional proteins. In addition to the vital roles in processing of the polyprotein precursor, it is well-known that L^{pro} and $3C^{ro}$ are significantly involved in the viral antagonistic activity against host antiviral responses (Lawrence et al., 2012; Liu et al., 2015; Stenfeldt et al., 2016).

L^{pro} is widely known to promote virus propagation by disrupting the interferon (IFN) signaling pathway to suppress host innate immune responses (Liu et al., 2015; Steinberger and Skern, 2014). $3C^{pro}$ belongs to the family of chymotrypsin-like cysteine proteases, and plays an important role in FMDV pathogenesis (Curry et al., 2007). $3C^{pro}$ can suppress host antiviral responses by different mechanisms, such as, inducing cleavage of eukaryotic translation initiation factor 4G (eIF4G) to shut off host protein synthesis; cleaving host antiviral proteins and inhibition of IFN-α/β production (Du et al., 2014a; Lawrence et al., 2012; Liu et al., 2015). The significant roles of $3C^{pro}$ in viral pathogenesis make $3C^{pro}$ as an attractive target for the design of antiviral agents (Sweeney et al., 2007). The mechanisms about the $3C^{pro}$-mediated antagonistic effects are further exploited to provide insights for uncovering the viral pathogenesis.

The interferon-induced double-strand RNA activated protein kinase (PKR) plays important roles in host defense against viral infection. PKR is a serine-threonine kinase that constitutively expresses in mammalian cells (Habjan et al., 2009; Nallagatla et al., 2011). As an antiviral factor, PKR can be induced by IFN treatment and is activated by binding of double-stranded RNA (dsRNA) or the PKR activator (PACT) protein. Activation results in PKR phosphorylation and subsequently phosphorylates the α subunit of the eIF2 translation initiation factor (eIF2α), blocks cellular and viral protein synthesis, thereby interfering with viral transcription or translation in infected cells (Nallagatla et al., 2011). To disrupt or antagonize antiviral effects of PKR, various viruses have evolved different strategies to inhibit PKR activation. Influenza A virus non-structural (NS1) protein interacts with PKR

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http://dx.doi.org/10.1016/j.virol.2017.06.023
Received 16 June 2017; Accepted 20 June 2017
Available online 26 June 2017
0042-6822/

or directly binds the double-stranded RNA to block PKR activation and counteract PKR-mediated inhibition of viral replication (Bergmann et al., 2000; Li et al., 2006; Lu et al., 1995). Hepatitis C virus (HCV) internal ribosome entry site binds to PKR in competition with PKR ligands and prevents the phosphorylation and activation of PKR (Vyas et al., 2003). Our previous study found that FMDV infection leads to a significant decrease of PKR protein as infection progresses (Li et al., 2016b). It implies that PKR may suppress FMDV infection and the virus decreases PKR protein expression to block PKR activation and antagonize the antiviral response.

In the present study, we examined the FMDV modulation of PKR singling in porcine cells. Here we report that overexpression of PKR could significantly suppress FMDV replication. FMDV infection led to decreased levels of PKR protein in comparison with that in the mock-infected cells. The viral $3C^{pro}$ was determined to be responsible for this decrease of PKR. $3C^{pro}$-induced PKR reduction was independent of its proteinase activity and the ability to cleave eIF4G.

2. Results

2.1. Dynamics of PKR expression in response to FMDV infection

The addition of 2-aminopurine (2-AP), an inhibitor of dsRNA inducible protein kinase (PKR), increases the yield of FMDV in PK-15 cells (Chinsangaram et al., 2001). This indicates the important role of PKR in inhibition of FMDV replication. It was therefore necessary to investigate the state of PKR during FMDV infection. We first investigated the kinetics of PKR to determine the state of PKR in FMDV-infected cells. PK-15 cells were infected by FMDV at an MOI of 0.5, and the dynamics of PKR were examined. It showed that the mRNA level of PKR was significantly upregulated starting at 6 h post-infection (hpi), and gradually increased as the infection progressed; no significant changes in PKR mRNA expression levels were observed in the mock-infected cells (Fig. 1A). To explore the correlation between PKR expression and FMDV replication, we also determined viral RNA levels. No viral RNA was detectable in mock-infected cells. In FMDV-infected cells, the viral RNA was detectable at 3 hpi, and gradually increased, similar to the increase of PKR (Fig. 1A). The state of PKR protein was also examined at different time points after FMDV infection, which showed that FMDV infection resulted in a significant loss of PKR protein. As shown in Fig. 1B, the amounts of PKR began to decrease at 6 hpi; and by 18 hpi, the amount of PKR almost could not be detected by western blotting. These results suggested that FMDV infection triggers PKR mRNA expression, while PKR protein levels are gradually reduced as infection progresses.

2.2. Upregulation of PKR inhibits FMDV replication

PKR plays an important role in inhibition of FMDV replication. FMDV infection reduced PKR expression. To explore whether upregulation of PKR suppresses FMDV replication, PK-15 cells were transfected with different amounts of empty vector plasmids or Myc-PKR expressing plasmids; at 12 h post-transfection (hpt), the cells were infected with 0.5 MOI of FMDV for 12 h. The viral RNA expression levels were determined and compared. The relative abundance of viral RNA was significantly reduced by overexpression of PKR, showing a dose-dependent manner (Fig. 2A). No remarkable inhibitive effect was observed in the vector-transfected cells. This observation was further confirmed by western blotting analysis. The presence of VP1 was used as an indicator of FMDV replication. As shown in Fig. 2B, a remarkable decrease of VP1 levels was detected in PKR overexpressing cells, and it also showed a clear dose-dependent manner. These results determined that overexpression of PKR significantly suppresses FMDV infection in the virus-infected cells, which indicated that the presence of PKR in FMDV-infected cells is essential for suppressing virus replication.

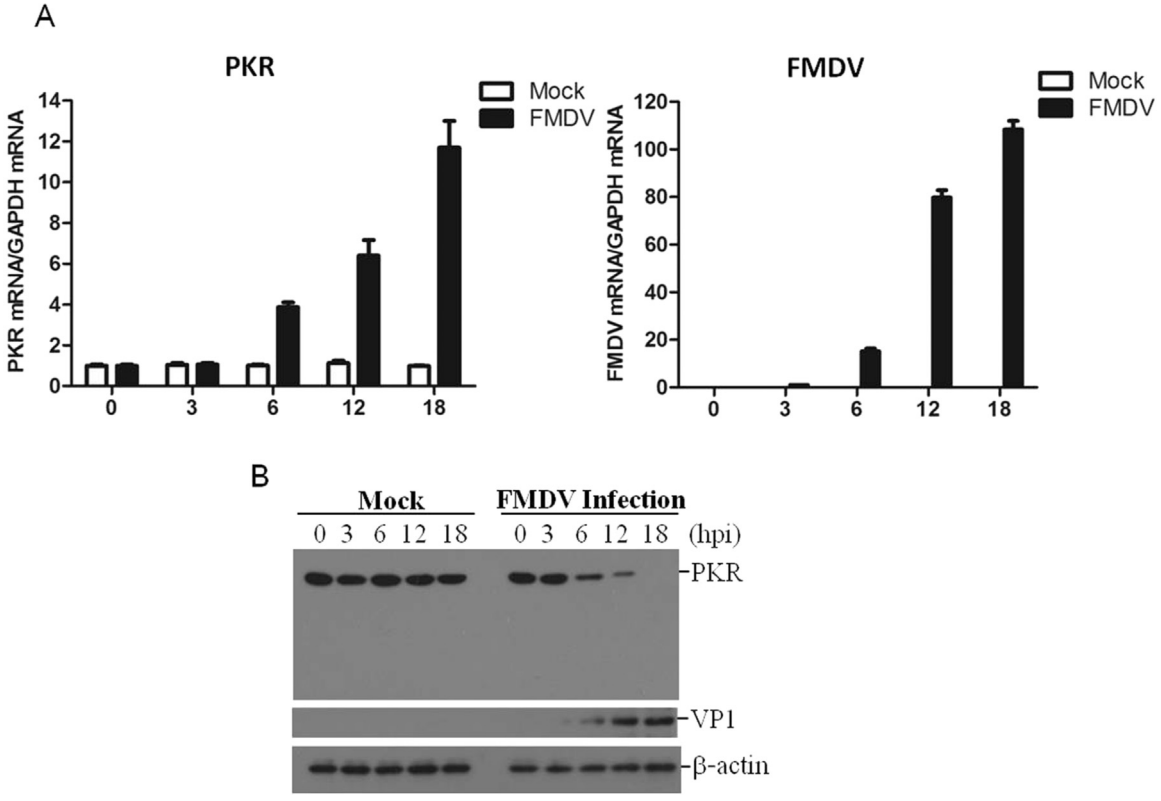

Fig. 1. FMDV infection inhibits PKR protein expression. (A) PK-15 cells were mock-infected or infected with FMDV for 0, 3, 6, 12 or 18 h, the transcripts of FMDV and PKR were detected by qPCR. (B) The expression levels of viral and PKR proteins in PK-15 cells were detected by western blotting at the indicated time points post-infection.