Virology 444 (2013) 233–240

Contents lists available at ScienceDirect

Virology

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

Involvement of unfolded protein response, p53 and Akt in modulation of porcine reproductive and respiratory syndrome virus-mediated JNK activation

Yazhen Huo [a], Lihong Fan [b,*], Shutao Yin [a], Yinhui Dong [a], Xiao Guo [a], Hanchun Yang [b], Hongbo Hu [a,**]

[a] Division of Nutrition and Health, College of Food Science and Nutritional Engineering, China Agricultural University, no. 17 Qinghua East Road, Haidian District, Beijing 100083, China
[b] College of Veterinary Medicine, China Agricultural University, no. 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

ARTICLE INFO

Article history:
Received 5 March 2013
Returned to author for revisions
5 April 2013
Accepted 10 June 2013
Available online 11 July 2013

Keywords:
PRRSV
JNK
Unfolded protein response (UPR)
Akt
p53
Apoptosis

ABSTRACT

Our previous study has shown that activation of JNK plays a critical role in Porcine reproductive and respiratory syndrome virus (PRRSV)-mediated apoptosis. In this follow-up study, we further investigated the mechanisms involved in modulation of PRRSV-mediated JNK activation and apoptosis. We found that unfolded protein response (UPR) was induced in response to PRRSV infection which in turn triggered JNK activation and apoptosis. We also found that p53 and Akt were activated at the early stage of infection and functioned as negative regulator of JNK activation to counteract the PRRSV-mediated apoptosis. Furthermore, induction of UPR, p53 and Akt was not only involved in modulation of PRRSV-mediated apoptosis, but also contributed to the virus replication. Our findings indicated that multiple signaling pathways were involved in modulation of PRRSV-mediated apoptosis of the host cells via regulating JNK signaling pathway and provided novel insights into understanding the mechanisms of pathogenesis of PRRSV infection.

Introduction

The endoplasmic reticulum (ER) is a multifunctional signaling organelle that controls a variety of biological processes. One major function of ER is to serve as the protein-folding factory. The status of protein-folding in ER is tightly monitored and regulated by a primitive, evolutionary conserved signaling pathways, collectively termed the unfolded protein response (UPR) or ER stress response (Walter and Ron, 2011). Three ER resident transmembrane proteins ATF6 (activating transcription factor 6), IRE1 (inositol requiring enzyme 1) and PERK (double-stranded RNA-activated protein kinase (PKR)-like ER kinase) are the stress sensors of ER. Activation of these sensors results in increase of ER protein-folding capacity and decrease of ER protein load. The final outcome of UPR is mitigation of ER stress. However, prolonged activity of the UPR could lead to cell death (Tabas and Ron, 2011). Growing evidence shows that UPR signaling pathways play a critical role in modulation of apoptosis of host cells in response to virus infection (Mahoney et al., 2011; Rodrigues et al., 2012; Wang et al., 2012). The mechanisms involved in UPR modulation of cell death include activating c-Jun N-terminal kinases (JNK)-mediated apoptotic signaling pathways (Ye et al., 2008), activation of ER-resident caspase-12 (Roberson et al., 2012), and modulating functions of Bcl-2 family proteins such as Noxa and Mcl-1 (Rosebeck et al., 2011; Huang et al., 2011).

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome (PRRS) that causes great economic losses worldwide each year (Lunney et al., 2010). Apoptosis induction of the host cells plays a critical role in pathogenesis of PRRSV infection (Suarez, 2000; Karniychuk et al., 2011). It has been demonstrated in our previous study that activation of JNK signaling pathway is a key event in apoptosis induction of the host cells in response to PRRSV infection (Yin et al., 2012). The purpose of the present study was to further decipher the mechanisms involved in modulation of PRRSV-induced JNK activation and apoptosis induction. We found that UPR was induced in response to PRRSV infection, which in turn triggered JNK activation and apoptosis. In addition, p53 and Akt were activated at the early stage of infection and functioned as negative regulator of JNK activation to counteract the PRRSV-mediated apoptosis. Our findings indicated that multiple signaling pathways were involved in modulation of PRRSV-mediated apoptosis of the host cells via regulating JNK signaling pathway.

* Corresponding author. Fax: +86 10 6273 3837.
** Corresponding author. Fax: 86 10 6273 8653.
E-mail addresses: flh@cau.edu.cn (L. Fan), hongbo@cau.edu.cn (H. Hu).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.06.015

Results

Activation of UPR signaling pathways contributed to JNK-mediated apoptosis in response to PRRSV infection

Marc-145 cells were infected with PRRSV at MOI=1. The Whole cell lysates from both adherent and floating cells were then prepared at 12 or 24 h after infection. Western blotting was used to analyze changes of ER stress markers in response to PRRSV infection. As shown in Fig. 1A (left), PRRSV infection significantly increased phosphorylation of the ER resident kinase PERK and IRE1, but did not affect expression of ER chaperone BIP. In line with PERK and IRE1 induction, phosphorylation of eIF2α and expressions of CHOP and XBP1s were significantly induced in response to the virus infection. The data suggest that PERK and IRE1, the two arms of the UPR are activated in response to PRRSV infection in Marc-145 cells. To investigate if UPR was also activated by PRRSV in porcine alveolar macrophages (PAM), the in vivo target cells of the virus, we analyzed the changes of ER stress-related proteins using western blotting. As shown in Fig. 1A (right), similar changes of the ER stress-related proteins were also observed in PAM, suggesting the general applicability of UPR activation by PRRSV in the host cells.

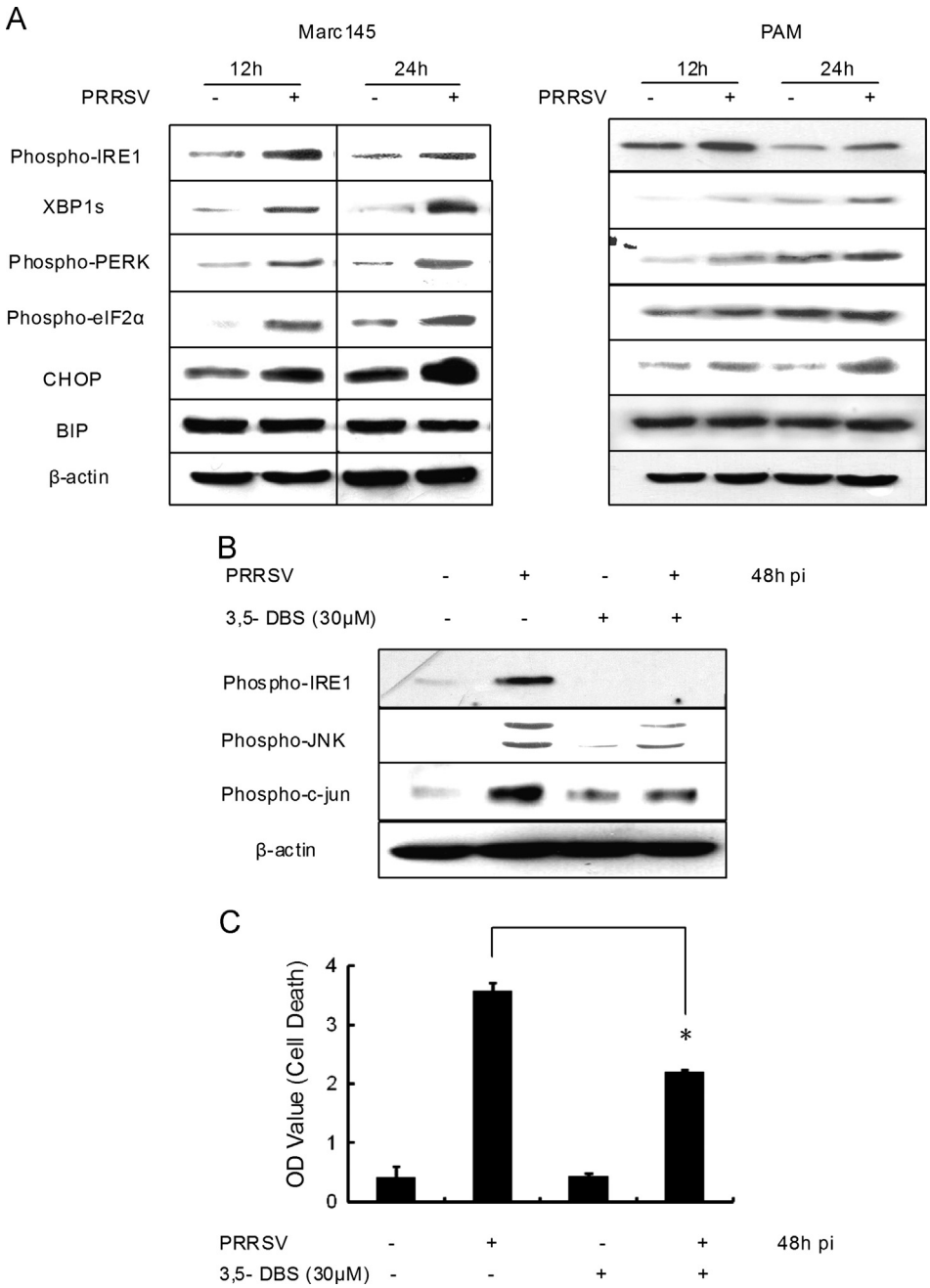

Fig. 1. Activation of UPR signaling pathways contributed to JNK-mediated apoptosis in response to PRRSV infection. (A) PRRSV induced activation of UPR in Marc-145 cells (left) and porcine alveolar macrophages (PAM) (right). The cells were infected with PRRSV at MOI=1. At 12 and 24 h post-infection, the cells were harvested for western blot analysis of ER stress markers. (B) Effect of IRE1 inhibition by its inhibitor 3-Ethoxy-5,6-dibromosalicylaldehyde (DBS) on PRRSV-induced JNK activation. The cells were infected with PRRSV in the presence or absence of DBS for 24 h and phosphorylation of JNK and its a substrate c-jun was examined by western blotting; and (C) Effect of IRE1 inhibition on PRRSV-induced apoptosis. The cells were infected with PRRSV in the presence or absence of DBS for 48 h and cell death was measured by Cell Death ELISA Kit ($n=3$, $p<0.05$).