Virology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Virology

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

Intercellular transfer of mitochondria rescues virus-induced cell death but facilitates cell-to-cell spreading of porcine reproductive and respiratory syndrome virus

Rui Guo[a], Duane Davis[b], Ying Fang[a,*]

[a] Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS 66506, United States
[b] Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, United States

ARTICLE INFO

Keywords:
Porcine reproductive and respiratory syndrome virus (PRRSV)
Intercellular nanotube
Mitochondria
Cell death

ABSTRACT

Our recent study showed that intercellular tunneling nanotubes (TNTs) serve as an alternative pathway for cell-to-cell spreading of infectious materials of porcine reproductive and respiratory syndrome virus (PRRSV). In this study, we found that PRRSV infection could induce the formation of TNTs between infected and uninfected cells. Co-culturing PRRSV-infected cells with uninfected cells, including porcine umbilical cord stem cells, rescued PRRSV-induced cell death. Mitochondrion, an important regulator of cell survival/death, was observed transferring from uninfected to PRRSV-infected cells. Importantly, impaired formation of nanotube or defective mitochondrion was unable to rescue infected cells from apoptosis/necrosis. Certain PRRSV proteins were detected to associate with mitochondria and transport from infected to uninfected cells through TNTs. Our results suggest that TNTs-transfer of functional mitochondria rescued PRRSV-infected cells from apoptosis/necrosis in the early stage of infection. On the other hand, mitochondria could be utilized as a vehicle to transport viral materials for spreading the infection.

1. Introduction

Porcine reproductive and respiratory syndrome has caused tremendous economic losses to swine industry worldwide. The etiologic agent, porcine reproductive and respiratory syndrome virus (PRRSV) belongs to the family of *Arteriviridae*, order Nidovirales (Fang and Snijder, 2010; Snijder and Meulenberg, 1998). PRRSV is an enveloped positive-stranded RNA virus. The viral genome is packed by nucleocapsid (N) proteins, and surface glycoproteins and membrane proteins are inserted into the lipid-bilayered envelope, which surrounds the nucleocapsid to form virion particles. The PRRSV genomic RNA molecule is about 15 kb in length and contains eleven known open reading frames. The replicase gene consists of the large ORFs 1a and 1b, which are situated in the 5′-proximal three quarters of the polycistronic genome. They encode two long nonstructural polyproteins, pp1a and pp1ab, with expression of the latter depending on a −1 ribosomal frameshift signal in the ORF1a/ORF1b overlap region. Following their synthesis from the genomic mRNA template, the pp1a and pp1ab replicase polyproteins are processed into at least 14 nonstructural proteins (nsps), including nsp1α/β, nsp2-nsp12 (Fang and Snijder, 2010). In our recent studies (Fang et al., 2012; Li et al., 2014b), a novel ORF (TF) was identified, which is translated via an efficient −2 ribosomal frameshift mechanism, resulting in the expression of a transframe protein, nsp2TF. At the same frameshifting site, −1 ribosomal frameshift also occurs, yielding a truncated nsp2 protein (nsp2N). The 3′ end of the genome encodes four membrane-associated glycoproteins (GP2a, GP3, GP4 and GP5), three unglycosylated membrane proteins (E, ORF5a and M) and a nucleocapsid protein (N) (Snijder et al., 2013).

In infected pigs, PRRSV mainly infects subsets of swine macrophages that are present in lungs and lymphoid organs (Duan et al., 1997; Labarque et al., 2000). In cultured cells, PRRSV grows in primary porcine alveolar macrophages, monocyte-derived macrophages or monocyte-derived dendritic cells (Chitko-McKown et al., 2013; Duan et al., 1998; Loving et al., 2007; Oleksiewicz and Nielsen, 1999; Singleton et al., 2016; Wang et al., 2007). In addition to primary cell cultures, PRRSV replicates in a certain African green monkey kidney cell line (MA-104) and its derivatives, such as MARC-145 cells (Kim et al., 1993). Previous studies investigated the significance of apoptosis/necrosis in the pathogenesis of PRRSV infection (Costers et al., 2008; Kim et al., 2002; Lee and Kleiboeker, 2007; Miller and Fox, 2004). Necrosis and apoptosis are two major types of cell death, which differ both morphologically and biochemically. Necrosis is a passive

* Corresponding author.
E-mail address: yfang@vet.k-state.edu (Y. Fang).

https://doi.org/10.1016/j.virol.2017.12.018
Received 29 September 2017; Received in revised form 14 December 2017; Accepted 15 December 2017

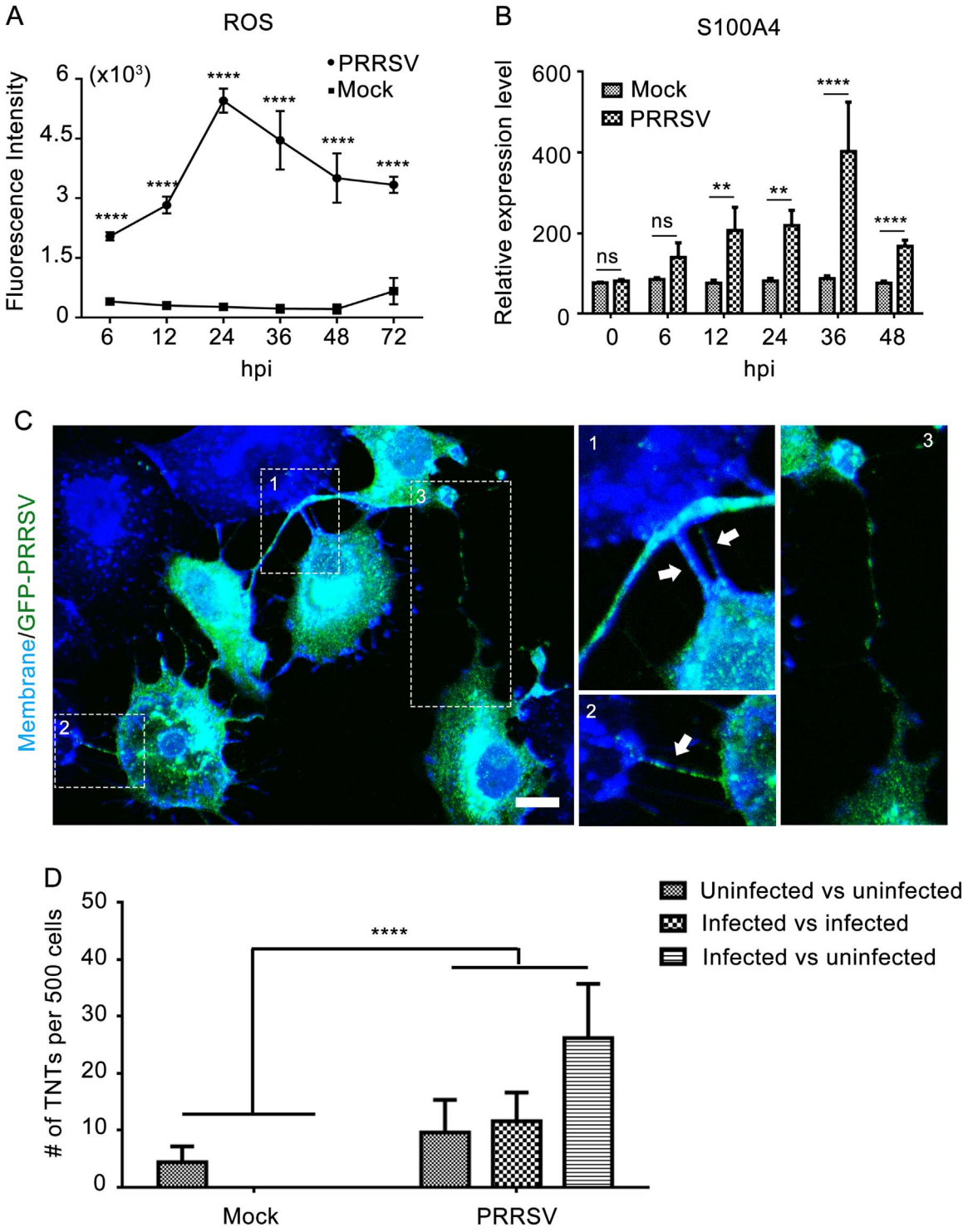

Fig. 1. *PRRSV-infected cells release 'call-for-help' signals (ROS, S100A4) and increased formation of nanotubes.* (A) Intracellular ROS levels detected in PRRSV-infected or uninfected MARC-145 cells. MARC-145 cells were infected with PRRSV strain SD95-21 or mock-infected with cell culture medium. Cells collected from indicated time points were subject to cellular assay for detecting ROS expression. The fluorescence signals were recorded by FLUOstar Omega microplate reader (BMG Lab Tech) at 485 nm for excitation and 535 nm for emission. (B) Relative expression level of S100A4 in PRRSV-infected or uninfected MARC-145 cells. MARC-145 cells were infected with PRRSV or mock infected with cell culture medium. Cells collected from indicated time points were subjected to cellular total RNA isolation and qRT-PCR analysis. The amount of S100A4 was normalized to the expression level of endogenous GAPDH mRNA. (C-D) Formation of intercellular nanotube connections between infected and uninfected cells during early stage of PRRSV infection. MARC-145 cells were infected with GFP-PRRSV or mock-infected with cell culture medium. At indicated time points, cell membrane was stained by blue fluorescent dye, WGA-AF350 (Invitrogen), and fixed with 4% paraformaldehyde. (C) Nanotube formation was visualized by confocal microscopy with 40× oil immersion objective lens. Arrows indicate nanotubes between GFP-PRRSV-infected and uninfected cells. Images were taken by a confocal microscope (LSM 880, Zeiss). Scale bar, 10 μm. (D) Nanotubes connecting between uninfected and uninfected cells; infected and uninfected cells; and infected and infected cells were counted at 12 h post infection.