Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

MOV10 inhibits replication of porcine reproductive and respiratory syndrome virus by retaining viral nucleocapsid protein in the cytoplasm of Marc-145 cells

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ARTICLE INFO

Article history:
Received 13 August 2018
Accepted 25 August 2018
Available online xxx

Keywords:
Porcine reproductive and respiratory syndrome virus
Moloney leukemia virus 10-like protein
Nucleocapsid protein
Viral replication

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) has been a major threat to global industrial pig farming ever since its emergence in the late 1980s. Identification of sustainable and effective control measures against PRRSV transmission is a pressing problem. The nucleocapsid (N) protein of PRRSV is specifically localized in the cytoplasm and nucleus of virus-infected cells which is important for PRRSV replication. In the current study, a new host restricted factor, Moloney leukemia virus 10-like protein (MOV10), was identified as an inhibitor of PRRSV replication. N protein levels and viral replication were significantly reduced in Marc-145 cells stably overexpressing MOV10 compared with those in wild-type Marc-145 cells. Adsorption experiments revealed that MOV10 did not affect the attachment and internalization of PRRSV. Co-immunoprecipitation and immunofluorescence co-localization analyses showed that MOV10 interacted and co-localized with the PRRSV N protein in the cytoplasm. Notably, MOV10 affected the distribution of N protein in the cytoplasm and nucleus, leading to the retention of N protein in the former. Taken together, these findings demonstrate for the first time that MOV10 inhibits PRRSV replication by restricting the nuclear import of N protein. These observations have great implications for the development of anti-PRRSV drugs and provide new insight into the role of N protein in PRRSV biology.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most problematic infectious diseases for the swine industry worldwide. Since 2006, a highly-pathogenic form of PRRS virus (HP-PRRSV) has been circulating in China resulting in considerable economic loss [1].

PRRSV, the causative agent of PRRS, is the family Arteriviridae of the order Nidovirales [2]. PRRSV genome is approximately 15.4-kb and encodes at least 10 open reading frames (ORFs) [3]. ORF7 encodes the nucleocapsid (N) protein. The N protein is a small basic protein, of 123 or 128 amino acids in the North American and European genotypes, respectively [4]. During infection, the N protein is the most abundant viral protein, accounting for approximately 40% of the virion protein content [5]. It plays essential roles in the virus life cycle, including encapsidation of the viral RNA [6]. The entire life cycle of PRRSV occurs in the cytoplasm of infected cells, but the N protein is found both in the cytoplasm and nucleus of the host cell [7]. A stretch of basic amino acids of the PRRSV N protein comprises a functional nuclear localization signal (NLS). Entry of the N protein into the nucleus plays an important role in viral replication. However, it is currently unclear if the host impacts the cellular distribution of N protein.

The innate immune system represents the first line of defense against viral infection. It is activated by various pathogen

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https://doi.org/10.1016/j.bbrc.2018.08.148
0006-291X/

Abbreviations

Co-IP	co-immunoprecipitation
DAPI	4′,6′-diamidino-2-phenylindole
DMEM	Dulbecco's modified Eagle's medium
FBS	fetal bovine serum
hpi	hours post infection
hpt	hours post transfection
IFN	interferon
MAb	monoclonal antibody
MOI	multiplicity of infection
MOV10	Moloney leukemia virus 10-like protein
N protein	nucleocapsid protein
NLS	nuclear localization signal
ORF	open reading frame
pAb	polyclonal antibody
PBS	phosphate-buffered saline
PRRS	pathogenic porcine reproductive and respiratory syndrome
PRRSV	PRRS virus
qRT-PCR	quantitative reverse-transcription polymerase chain reaction
RIG	retinoic acid-inducible gene
WB	western blotting

components via specific host pattern recognition receptors [8]. Many host restriction factors that result in PRRSV resistance have been identified [9,10]. Moloney leukemia virus 10-like protein (MOV10) is one such factor, induced by IFN [11]. It is a putative member of the helicase superfamily 1 [12] and is involved in RNA interference [13]. Further, MOV10 gene is an IFN-stimulating gene that inhibits various viruses, including retrovirus, influenza A virus, and vesicular stomatitis virus [11,14,15]. However, it is not known whether MOV10 also regulates PRRSV replication.

The aim of the current study was to examine the putative anti-PRRSV effect of MOV10. The investigation revealed the anti-PRRSV activity of MOV10, which is mediated through interaction with the PRRSV N protein, interfering with the cellular distribution of N protein in the host cell.

2. Materials and methods

2.1. Cells and virus

African green monkey kidney (Marc-145) cells and human embryonic kidney (HEK293T) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C and under 5% CO_2. The HP-PRRSV strain HuN4 (GenBank accession no. EF635006) was used in all experiments.

2.2. Construction of plasmids

Porcine *MOV10* gene (GenBank accession no. XM_021090739.1) was amplified and cloned into pLOV.CMV (modified version of pLOV.CMV.GFP) or pCAGGS to generate pLOV.CMV-MOV10 or pCAGGS-MOV10-Flag, respectively. ORF7 of PRRSV HuN4 was cloned into pCAGGS to generate pCAGGS-N-HA. All plasmids were constructed by homologous recombination with the NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs; Ipswich, MA) according to the manufacturer's instructions. The primers used for gene amplification are listed in Table S1.

2.3. Establishment of MOV10-overexpressing Marc-145 cells

HEK293T cells were seeded into a 10-cm dish and transfected with 21 μg of pLOV-CMV-MOV10 or pLOV-CMV-EGFP (control), together with 14 μg of psPAX2 and 7 μg of pMD2.G using X-treme GENE HP DNA reagent (Roche Applied Science, Penzberg, Germany). The packaging and transduction processes of lentivirus are based on previous studies [16]. Negative control was conducted in parallel with the transduction process. Marc-145 cells stably overexpressing MOV10 (Marc-145-Mov10) were analyzed by western blotting (WB) using a rabbit anti-Flag MAb (1:6000) (cat. no. F7425; Sigma) and the negative control cells were observed under inverted fluorescence microscope (Leica Microsystems, Germany).

2.4. Virus challenge

To determine the effect of MOV10 on PRRSV replication, Marc-145 cells and Marc-145-Mov10 cells were infected with PRRSV HuN4 (multiplicity of infection, MOI = 0.5). The cells were harvested 12, 24, 36, and 48 h post infection (hpi), and lysed using RIPA lysis buffer (Thermo). They were then analyzed by WB using a mouse anti-N pAb (1:1000) produced in our lab. Multistep growth curve and RNA copy number determinations were also performed, as previously described [17].

2.5. Viral attachment and internalization assay

Marc-145 cells and Marc-145-Mov10 cells were pre-chilled at 4 °C for 1 h, inoculated with PRRSV HuN4 (MOI = 0.1), and placed at 4 °C for an additional 1 h. The viruses were removed by aspiration and the cells washed three times with PBS to remove unbound viruses. For Internalization assay, the cells infected with HuN4 were incubated at 37 °C for 1 h to allow virus internalization followed attachment. The cells were washed with PBS and treated with citric acid buffer (40 mM citric acid, 10 mM KCl, and 135 mM NaCl, pH 3.0) for 1 min to inactivate the bound but not internalized viruses. The viral copy number was assessed by quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) [18,19].

2.6. Co-immunoprecipitation (Co-IP)

HEK293T cells were transfected with the indicated plasmids using X-tremeGENE DNA transfection reagent (Roch); 24 hpt, cells were lysed with ice-cold IP lysis buffer (Thermo) containing 1 mM phenylmethanesulfonyl fluoride and 1 mg/ml protease inhibitor cocktail (Roche), at 4 °C for 15 min. Approximately 10% of the lysate supernatant was used as an input control and the remaining lysates were incubated with anti-HA agarose beads for 6 h at 4 °C. The beads were washed five times with the IP lysis buffer and then analyzed by WB using the indicated antibodies. To exclude the involvement of cellular DNA or RNA in the interaction between MOV10 and N proteins, cell lysates were also treated with 100 μg/ml RNase and 100 μg/ml DNase, and then subjected to Co-IP [20].

To detect the interaction between the endogenous (host) MOV10 and N protein during PRRSV infection, Marc-145 cells were infected with HuN4 (MOI = 0.1) for 36 h. The cells were lysed in 500 μL of IP lysis buffer, and the supernatants were pre-cleared using protein A/G-agarose beads (Sigma). The supernatant was incubated with anti-N pAb (1:1000) for 6 h; then, 20 μL of protein A/G-agarose beads was added, followed by additional 1-h incubation. Immunoprecipitated pellets were gently washed five times with IP lysis buffer and then analyzed by WB using the indicated antibodies.

Please cite this article in press as: K. Zhao, et al., MOV10 inhibits replication of porcine reproductive and respiratory syndrome virus by retaining viral nucleocapsid protein in the cytoplasm of Marc-145 cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.148