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Small interfering RNA targeting nonstructural protein1 α (nsp1α) of porcine reproductive and respiratory syndrome virus (PRRSV) can reduce the replication of PRRSV in MARC-145 cells

Xibao Shi a,b,c,1, Yongzhe Chang b,c,1, Xiaozhuan Zhang a,d, Li Wang c, Chunxi Li a, Kai Jiang d, Jing Chen c, Chao Wang c, Ruiguang Deng c, Jianming Fan e, Gaiping Zhang b,c,*

* Corresponding author. College of Animal Science and Veterinary Medicine, Henan Agricultural University, No. 63 Nongye Road, Zhengzhou 450002, People’s Republic of China. Tel.: +86 371 63550369; fax: +86 371 63558998.
E-mail address: zhanggaiping2003@163.com (G. Zhang).

1 These authors contributed equally to this study and share first authorship.

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PRRSV, a positive-stranded RNA virus, is a member of family Arteriviridae (Snijder et al., 2013). PRRSV is one of the most economically important diseases of swine which is characterized by severe reproductive failure in sows and respiratory distress in piglets and growing pigs (Rossow, 1998). Infection with PRRSV also predisposes pigs to secondary infection by bacterial and viral pathogens (Mateu and Diaz, 2008). However, to date, there is no efficient antiviral agent or method available against PRRSV, and unfortunately, both traditional-control strategies and conventional vaccines are insufficient to provide sustainable control of PRRSV (Darwich et al., 2010), so it is very important and urgent to develop therapeutic strategies to control PRRSV effectively.

RNA interference (RNAi) is sequence-specific gene silencing which is mediated by 21- to 25-nt RNA duplexes (siRNAs) and can be as an exciting method to silence viral genes, especially for the single strand RNA genomes (Meister and Tuschl, 2004). To date, RNAi has been used against several viruses including hepatitis B virus and dengue virus (Idrees and Ashfaq, 2013; Kahana et al., 2004). The PRRSV genome has ten open reading frames (ORFs) and could produce 16 nonstructural proteins (termed nsp1α, nsp1β, etc.) (Snijder et al., 2013). Previous studies have indicated that the nsp1α was essential for the synthesis of PRRSV subgenomic mRNA and it may play an important role in the virulence of PRRSV (Kroese et al., 2008; Nedialkova et al., 2010). However, whether the PRRSV nsp1α facilitates the replication
The effect of over-expression of nsp1α on the replication of PRRSV. (A) 293T cells were transfected with the plasmid pcDNA 3.1-GFP-nsp1α or pcDNA 3.1-GFP, and 48 h later, the cells were prepared for the western blot. (B) MARC-145 cells grown in 24-well plates were transfected in triplicate with the nsp1α plasmid pcDNA3.1-GFP-nsp1α (800 ng/well) or pcDNA 3.1-GFP. Six hours later, the cells were infected with PRRSV at a MOI of 0.1 or mock infected, and 24 hours later, the cells were lysed, and then viral titers were measured by TCID50. The results shown were from one of three independent experiments with similar observations. Error bars represented the standard deviations. *P < 0.05 compared with the results in control.
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Fig. 2. The effect of siRNAs targeting nsp1α on the replication of PRRSV. (A) siRNAs targeting nsp1α could inhibit the expression of GFP-nsp1α and did not influence the expression of GFP in MARC-145 cells. MARC-145 cells grown in 24-well plates were co-transfected with pcDNA 3.1-GFP-nsp1α (800 ng/well) or pcDNA 3.1-GFP (800 ng/well) and nsp1α siRNA 1 (100 nM), nsp1α siRNA 2 (100 nM), nsp1α siRNA 3 (100 nM) or control siRNA (100 nM). Twenty-four hours later, the cells in five random fields were analyzed by fluorescence microscopy (50×) and only one of them was shown. (B) MARC-145 cells grown in 24-well plates were transfected with nsp1α siRNA 1 (100 nM), nsp1α siRNA 2 (100 nM) or control siRNA (100 nM). Six hours later, the cells were infected with PRRSV at MOI of 0.1 or mock infected, and 24 hours later, the cells were lysed and then viral titers were measured by TCID50. The results shown were from one of three independent experiments with similar observations. Error bars represented the standard deviations. *P < 0.05 compared with the results in control.