Virology 450-451 (2014) 182–195

Contents lists available at ScienceDirect

Virology

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

MicroRNA-23 inhibits PRRSV replication by directly targeting PRRSV RNA and possibly by upregulating type I interferons

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ARTICLE INFO

Article history:
Received 18 September 2013
Returned to author for revisions
19 October 2013
Accepted 17 December 2013
Available online 4 January 2014

Keywords:
PRRSV
microRNAs
miR-23
miR-378
miR-505
IFNs
IRF

ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and play critical roles in intricate networks of host–pathogen interactions and innate immunity. Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases affecting swine industry worldwide. Here, we demonstrated that miR-23, miR-378, and miR-505 were antiviral host factors against PRRS virus (PRRSV). Over-expression of the three miRNAs inhibited PRRSV infection in a dose-dependent manner, respectively. Blockage of the three endogenously expressed miRNAs significantly enhanced PRRSV replication. Different type 2 PRRSV strains harbored conserved miR-23, miR-378, and miR-505 target sites (TSs) that were sufficient to confer miRNA-mediated repression of PRRSV replication. Interestingly, miR-23 was capable of inducing type I interferon expression during PRRSV infection through IRF3/IRF7 activation, which might further lead to the inhibition of virus infection. These results suggest that miR-23, miR-378, and miR-505, especially miR-23, may have the potential to be used as antiviral therapy against PRRSV infection.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in swine industry worldwide, and has been causing significant economic losses since it was first reported in the United States and Canada in 1987 (Wensvoort et al., 1992). Porcine reproductive and respiratory syndrome virus (PRRSV), the etiological agent of the disease, is an enveloped, positive-sense, and single-stranded RNA virus belonging to the *Arteriviridae* family within the order *Nidovirales* (Cavanagh, 1997). PRRSV includes two major genotypes, the European type (type 1) and the North American type (type 2) (Allende et al., 1999). The PRRSV genome is about 15.4 kb in length, containing at least two large replication-related genes (ORF1a and ORF1b), and eight structural protein genes (GP2a, GP2b, GP3, GP4, GP5a, GP5b, the matrix protein M, and the nucleocapsid N protein) (Firth et al., 2011; Johnson et al., 2011).

MicroRNAs (miRNAs) are small (~23 nt) non-coding RNAs that regulate gene expression post-transcriptionally through degradation of mRNAs or inhibition of translation (Bartel, 2009). The RNA-induced silencing complex (Rani et al., 2011) is the core miRNA-mediated silencing machinery, where miRNA serves as a guide RNA to target mRNAs bearing the complementary sequence. The complementarity between the miRNA 'seed region' (positions 2–8 from the 5′ end) and the sequence of the mRNA target is considered to be critical for the specificity of miRNA-target mRNA interaction (Carthew and Sontheimer, 2009). Yet specificity of the miRNA is also influenced by other factors such as the presence and cooperation between multiple target sites (TSs), the spacing between TSs, proximity to the stop codon, position within the mRNA, AU composition, and the target mRNA secondary structure (Bartel, 2009; Shukla et al., 2011).

Mounting evidence suggests a complicated interplay between viruses and miRNAs. On the one hand, a virus can exploit the miRNA system to facilitate its replication (Gottwein et al., 2007). On the other hand, multiple miRNAs in hosts have been identified as inhibitors of viral replications by directly targeting viral genomes (Huang et al., 2007; Otsuka et al., 2007; Song et al., 2010; Zheng et al., 2013) or inhibiting factors necessary for viral life cycles (Ouda et al., 2011; Triboulet et al., 2007). Additionally, host

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0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.12.020

Table 1
Target sites prediction of 12 candidate miRNAs and conservation analysis on 168 PRRSV strains of target sites.

Candidate miRNAs					
	Predicted seed-matched target sites in the viral RNA (miRNA seed sequences and the corresponding target sites are underlined and bolded)	Predicted seed-matched target sites in the viral RNA	Seed match	Conservation **(identity)** in type 2	Conservation **(identity)** in type 1
miR-23	ORF 3: 5'-TATTTGGGATAGGG**AATGTGA**G-3' miR-23: 3'-CCUUUAGGGACCG**UUACACU**A-5'	ORF3	7mer-m8	98.13%	86.62%
miR-505	ORF 3: 5'-ATGTGAGTCAAGTTTA**TGTTGACA**-3' miR-505: 3' -UCCUUUGGUCGUUC**ACAACUG**C -5' ORF 5: 5'- TGTCATCTTCCCCG**TGTTGAC**T -3' miR-505: 3' -UCCUUUGGUCGUUC**ACAACUG**C -5'	ORF3; ORF5	8mer; 7mer-m8	95.00%; 81.25%	0.00%
miR-378	ORF 7: 5'-GCCCAACAAAACC**AGTCCAGA** -3' miR-378: 3' -GGAAGACUGAGGU**UCAGGUC**A- 5'	ORF7	8mer	96.84%	0.00%