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miRNAs and genes expression in MARC-145 cell in response to PRRSV infection

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ABSTRACT

The regulation of viral replication is under control of miRNAs and their target genes. Several articles report the cross-talk between host and virus. The drastic effects of Porcine reproductive and respiratory syndrome virus (PRRSV) pressed us to investigate the expression profiling of miRNAs and immunity related genes during PRRSV infection. This was performed by qPCR in MARC145 cells during PRRSV infection. It was observed that miRNAs and genes show different expression patterns at different time points during PRRSV infection. The early infected stage was accompanied with increased expression of some miRNAs including miR-204, miR-21, miR-181a, miR-29 while a decrease was observed for the same in late infection stage. The opposite condition also existed in parallel. An interesting observation was seen when miR-145 was strongly induced by PRRSV infection, whereas miR-127 expression was significantly reduced in all infection points. Taken together, our studies have revealed that the expressions of miRNAs and immune-related genes were regulated in PRRSV infected MARC-145 cells and had important roles in the immune response, providing a basis for further investigations.

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), a single stranded RNA virus, causes acute and persistent respiratory infection in piglets and reproductive failure in pregnant sows (Stadijek et al., 2002; Grebennikova et al., 2004), which leads to serious economic losses to the swine industry worldwide. The emergence of highly pathogenic PRRSV (HP-PRRSV) strain in China during the year 2006, resulted in high fever, morbidity and mortality in pigs of all ages (Tian et al., 2007). Numerous researches have been made to reveal the pathogenesis of PRRSV, such as receptor-mediated infection, but the factors that influence viral infection and host innate immune response are very scarce.

Viral infection induces immediate and robust gene expression changes in host cells, which are not only involved in innate and adaptive immune response (Bowen and Walker, 2005; Saito et al., 2008; Takeuchi and Akira, 2009) but also related to apoptosis and anti-apoptosis genes (Tran et al., 2013; El Maadidi et al., 2014), these processes are multifactorial and highly regulated. microRNAs (miRNAs), as a post-transcriptional regulator of gene expression in a sequence specific manner, are highly conserved, 19–22 nt in length, endogenous non-coding RNA (Lee et al., 2004; Zhao and Srivastava, 2007). Substantial numbers of research articles demonstrate that miRNAs play a pivotal role in regulating a wide range of crucial physiologic and pathological processes, including innate and adaptive immunity and apoptosis (Wienholds and Plasterk, 2005; Taganov et al., 2006). Although there are some miRNAs associated with virus infection, host immune-related miRNAs expression affected by PRRSV infection has not been investigated.

In the current study, to explore the important miRNAs regulation in PRRSV infection, we screened numerous highly conserved miRNAs and their target genes related to immunity and apoptosis. We identified that different miRNAs and genes have different expression patterns in different time during PRRSV infection. The data suggested that host miRNAs might have significant roles in modulating PRRSV and provide new therapies for prevention and treatment against porcine reproductive and respiratory syndrome (PRRS).

2. Materials and methods

2.1. Cell and virus

MARC-145 cell which is a clone of the African green monkey kidney cell line MA-104, were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 100 U/ml

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penicillin, and 100 g/ml streptomycin, and then held at 37 °C in a humidified 5% CO_2 incubator.

PRRSV strain WUH3, a highly pathogenic North American type PRRSV 97, which was either cultivated on porcine alveolar macrophages or adapted to Marc-145 cells at a MOI of 0.5. Virus stocks of PRRSV were prepared in MARC-145 cells and infectious virus titers were analyzed in MARC-145 cells using the Reed and Muench method. For virus infection, cells were initially adsorbed with virus at the indicated MOI for 1 h at 37 °C. After 1 h of adsorption, cells were gently washed with medium.

2.2. RNA isolation

At 0, 6, 12, 24, 36 and 48 h post-infection (*p.i.*), cells were washed three times with PBS, and 4 ml TRIZOL reagent (Invitrogen) was added to each flask in both groups. The homogenized samples were incubated for 5 min at room temperature, and total RNA was isolated according to the manufacturer's protocols.

2.3. miRNA expression profiles using quantitative real time PCR (qRT-PCR)

miRNAs were prepared with an All-in-One™ miRNA qRT-PCR Detection Kit (GeneCopoeia, Rockville, MD) according to the manufacturer's instructions. Briefly, the extracted RNA was reverse-transcribed in the presence of a Poly-A polymerase with an oligo-dT adaptor. Quantitative PCR was then carried out with SYBR green detection with a forward primer for the mature miRNA sequence and a universal adaptor reverse primer. All standards and samples were run in triplicate on 96-well reaction plates. The cycle conditions were set as follows: start with 5 min template denaturation 95 °C, 30 cycles of denaturation at 95 °C for 10 s, and elongation at 60 °C for 20 s. This cycle was followed by a melting curve analysis, baseline and cycle threshold values (*Ct* values) were automatically determined for all plates using Roche LightCycler 480 software. miRNA expression levels in each sample were normalized to the expression of the housekeeping gene of U6. The real-time PCR results were analyzed and expressed as relative expression of CT (threshold cycle) value using the $2^{-\Delta\Delta Ct}$ method (Table 1).

Table 1
Primers used to detect miRNA expression by qRT-PCR.

miRNA	Primer (5′–3′)
miR-125b	CTGGTCCCTGAGACCCTAAC
miR-15	CGTGGTAGCAGCACATAATGG
miR-16	CGTGGTAGCAGCACGTAAATA
miR-127	CTGGTCGGATCCGTCTGAGC
miR-21	CGCGGTAGCTTATACAGACTG
miR-215	GGCAGATGACCTATGAATTG
miR-204	CGTGGTTCCCTTTGTCATCCT
miR-145	CTGGGTCCAGTTTTCCCAGG
miR-29	CGCGGTAGCACCATCTGAAAT
miR-34a	CTGGTGGCAGTGTCTTAGCT
miR-23a	CTGGATCACATTGCCAGGGA
miR-122	CAGGATGGAGTGTGACAATG
miR-182	CAGGTTTGGCAATGGTAGA
miR-181a	CGTGGAACATTCAACGCTGTC

2.4. Gene expression profiles using qRT-PCR

To detect the relative level of immune genes expression, quantitative RT real-time PCR was performed. All PCR was performed under the following conditions: 94 °C for 4 min followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. SYBR Premix ExTaqTM kit (TaKaRa, Madison, WI) was used according to the manufacturer's instructions, and the real-time PCR was performed on an iQ5 Real-Time PCR system (Bio-rad). Gene expression levels in each sample were normalized to the expression of the housekeeping gene of β-actin. The real-time PCR results were analyzed and expressed as relative expression of CT (threshold cycle) value using the $2^{-\Delta\Delta Ct}$ method (Table 2).

2.5. Target prediction and functional analysis of differentially expressed miRNAs

Four miRNAs target prediction databases (TargetScan http://www.targetscan.org/, miRDB http://mirdb.org/miRDB/index.html, and microRNA.ORG http://www.microrna.org/microrna/home.do) were used to infer the targets of the differentially expressed miRNAs. The intersection of these data sets was considered reliable and important. Gene ontology (GO) classifications and Kyoto Encyclopedia Genes and Genomes (KEGG) pathways analysis were performed using DAVID with default parameters.

2.6. Statistical analyses

All experiments were performed independently three times, and results were the means ± SD of 3 separate experiments. Data were analyzed by one-way ANOVA followed by Tukey's Honesty Significant Difference (HSD) test with SPSS version 17.0 (SPSS, Chicago, IL, USA). P value < 0.05 was considered as statistically significant.

3. Result

3.1. Morphological changes and host cell response during HP-PRRSV infection in MARC-145 cells

To determine the morphological changes in MARC-145 cells during virus infection, strain WUH1, a highly pathogenic North American type PRRSV (HP-PRRSV), was used to infect MARC-145 cells. Corresponding morphological changes in the cells are shown in (Fig. 1). In contrast to mock-infected MARC-145 cells, Typically

Table 2
Primers for analysis of gene expression using qRT-PCR.

Gene symbol	Forward primer (5′–3′))	Reverse primer (5′–3′))	Amplicon
IFNβ	CATTACCTGAAGGCCAAGGA	CAATTGTCCAGTCCCAGAGG	148bp
IL28a	CAGTTCAAGTCCCTGTCTCCA	AAGCGACTCTTCTAAGGCATC	72bp
ISG15	GTGGACAAATGCGACGAACC	CCCGCTCACTTGCTGCTTCA	117
IKBKB	TGTGGGCGGGAGAACGAAGT	CCCTCAGTTCGCTGGTCTCG	85bp
IFNGR2	GCTACGAAACAATGGCAGAT	ATCTGTAATGGGATGCTTGG	90bp
TRAM1	GTCTTCCTGCTGGGGCTCAT	TCTTCTGTTGCTGGGAGGGT	91bp
IRAK2	TCATTCCTTGAGAAGCGACC	TGATTGAAGTCATCGGTTGC	90bp
MAP2K1	TGGGCTTCTATGGTGCGTTCT	AGGATGTTGGAGGGCTTGAC	94bp
BCL2L2	CTGGTGGCAGACTTTGTAGG	CATCGGAGACCTGGGTGAAG	86bp
LAMP2	ATGATACTTGTCTGCTGGCTAC	CTGCCTGTGGAGTGAGTTGT	88bp
β-Actin	AGCAAGCAGGAGTATGACGAGT	CAAGAAAGGGTGAACGCAACT	80bp

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