

Expression of miR-29a and IFN- γ in pigs vaccinated with modified live PRRS vaccine

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the important RNA viruses that affects swine production worldwide. To better improve vaccine efficacy, understanding the molecular mechanisms and viral-host interaction is important to increase the knowledge of vaccine response. The objective of this study was to investigate the expression levels of miR-29a and IFN- γ in pigs that received modified live PRRSV vaccine. The study revealed that both miR-29a and IFN- γ transcript levels increased in vivo early, at day 3 post vaccination, whereas levels of miR-29a and IFN- γ mRNA were not different at day 7 post vaccination. Our study is the first attempt to demonstrate the level of this miR-29a in response to PRRSV vaccination. However, the molecular mechanisms of miR-29a and its functional role during PRRSV infection require further investigation.

Keywords: miR-29a, PRRSV, IFN- γ , vaccine

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important virus that affects swine production worldwide. It is an RNA enveloped virus that infects the respiratory and reproductive systems, causing abortion, stillborn piglets, mummified fetuses and premature farrowing (Ladinig et al., 2014). It has been shown that PRRSV could be detected in semen, stillborn and mummified fetuses/piglets between 63.3 to 74.1% in pigs in Thailand (Olanratmanee et al., 2015). In nursery and grower pigs, PRRSV causes respiratory problems commonly found with secondary bacterial and other viral infections (Park et al., 2014; Yu et al., 2012). PRRSV has been characterized into genotypes I and II based on genomic sequence. Upon infection, PRRSV enters pigs through inhalation or venereal transmission and then rapidly spreads to the lymphoid organs and lung of infected animals. Currently, the virus has been reported among pig population throughout Thailand and Asia. In Thailand, PRRSV has been found in swine serum since 1989 (Thanawongnuwech et al., 2004). Although vaccines are available for PRRSV control, the genetic diversity and immunosuppressive mechanism of the virus have limited vaccine efficacy and PRRSV control (Mateu and Diaz, 2008). Accordingly, the interplay between host innate and adaptive immunity and PRRSV has been extensively evaluated (Wang et al., 2011).

To combat PRRSV, both humoral and cellular immunity is critical for PRRSV control and elimination of PRRS virus in infected pigs (Amadori and Razzuoli, 2014). For appropriate immune responses, multiple regulatory processes including the newly emerging roles of microRNAs have been examined. MicroRNAs are small non-coding RNAs that regulate post-transcriptional gene expression. More than thousand microRNAs have been identified to date from plants, insects and mammals. Among these, groups of microRNAs have been shown to contribute in host immunity, pathogen clearance and response to vaccine (Bela-ong et al., 2015; Tsitsiou and Lindsay, 2009). For example, the role of miR-29 family against IFN- γ production was demonstrated in mice during intracellular bacterial infection including *Mycobacterium bovis* and *Listeria monocytogenes* (Ma et al., 2011). Given the important roles of cell-mediated immune response and IFN- γ production during PRRSV infection, it is interesting to investigate the expression level of miR-29a and IFN- γ in pigs that received PRRSV vaccine.

In this study, the expression level of miR-29a and IFN- γ in pigs vaccinated with modified live virus PRRS was examined. The qRT-PCR was used to quantify expression levels of miR-29a and IFN- γ in vaccinated animals. Results of this study may lead to better understanding of host-virus interaction and development of more effective PRRSV vaccine.

Materials and Methods

Animals and vaccination protocols: Twenty pigs free of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), PseudoRabies virus, Swine Fever Virus

(SFV), and Foot and Mouth Disease Virus (FMDV) were maintained at the animal research facility, Faculty of Veterinary Medicine, Kasetsart University. The pigs were divided into two groups, 10 pigs in each, as the control and vaccinated groups. Six pigs were randomly selected from each group for blood collection. Genotype II (US strain) modified live virus PRRS vaccine (Fostera™ PRRS, Zoetis) was intramuscularly administered at 2 mL/pig. Three and seven days post vaccination, blood samples were collected for gene expression analysis.

Blood collection and PBMC isolation: Ten mL of blood samples were collected from the jugular vein and then placed into a heparinized container. To separate PBMCs, three mL of blood samples were mixed with Ficoll-Hypaque gradient (Sigma, USA), followed by centrifugation separation as previously described (Nantakhrua et al., 2013). The cell suspension was further centrifuged at 800 g for 20 min at 4°C. The cell pellets were resuspended in RPMI medium (RPMI 1640 culture medium) supplemented with antibiotic and 5% fetal bovine serum. The cells were plated at a density of 6×10^6 cells per well in a 24-well plate.

RNA isolation and cDNA synthesis : RNAs were isolated from the whole blood samples using Trizol extraction reagent (Invitrogen, USA) following the manufacturer's instructions. Briefly, 50 μ L of PBMCs were mixed with 1 mL Trizol and then processed for phenol and chloroform extraction. RNA quality and quantity were assessed using NanoDrop spectrophotometer. The extracted RNA was transcribed into cDNA using cDNA synthesis kit (Invitrogen, USA).

Analysis of gene expression: The forward and reverse primers for miR-29a, IFN- γ and beta-actin were previously described (Kametani et al., 2012; Sun et al., 2012; Surachetpong et al., 2014). For standard RT-PCR, the samples were mixed with PCR mastermix and run in T100 mastercycler (Biorad, USA). The PCR condition included denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 53°C for 30 sec, 37°C for 30 sec, with final extension at 72°C for 7 min. For qRT-PCR, 2 μ L of RNA samples were mixed with SYBR mastermix (Biorad, USA) and analyzed in CFX96 (BioRad, USA) real-time thermocycler. The PCR condition was 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec.

Statistical analysis: The $2^{-\Delta\Delta C_T}$ difference between the groups was determined by paired Student's t-test using Prism software version 5.0.2 (GrapPad, San Diego, California). A p value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

MicroRNAs are small non-coding RNAs that regulate post-transcriptional gene expression in eukaryote cells. The pathological and physiological roles of microRNAs have been revealed during host-pathogen interaction and response to vaccination. For

viral infection, microRNAs may directly bind to complementary sequences on viral RNA, resulting in viral RNA degradation or inhibition of protein synthesis (Roberts et al., 2011). A recent study suggested that miR-29a promoted PRRSV replication at early stage of virus infection in porcine alveolar macrophage (Zhou et al., 2016). Additionally, our previous study demonstrated that pigs inoculated with PRRSV showed higher expression of miR-29a both in cultured PBMCs and in PRRS-infected pigs (Surachetpong et al., 2014). In the present study, RT-PCR and real-time RT-PCR were used to compare the expression level of miR-29a in modified live PRRS vaccinated and non-vaccinated pigs. The RNA samples were collected from isolated PBMCs of each pig and subsequently reverse transcribed to cDNA for PCR analysis. Gel electrophoresis of miR-29a showed a clear

and specific band product only in the modified live virus (MLV) vaccinated pigs at three days post vaccination, while no specific band was observed in the non-vaccinated pigs (Fig. 1a). The beta actin gene was used as a loading control for all samples. To compare the expression level of miR-29a from both groups, the band intensity of each control and vaccinated pigs was analyzed. Figure 1B shows the significant upregulation in the level of miR-29a in the vaccinated pigs with 7.62 ± 2.08 fold difference compared to that of the non-vaccinated pigs. However, there was no difference in the expression level of miR-29a between both groups at seven days post vaccination (data not shown). The present study revealed the upregulation of both miR-29a and IFN- γ levels in vaccinated pigs at 3 days post vaccination.

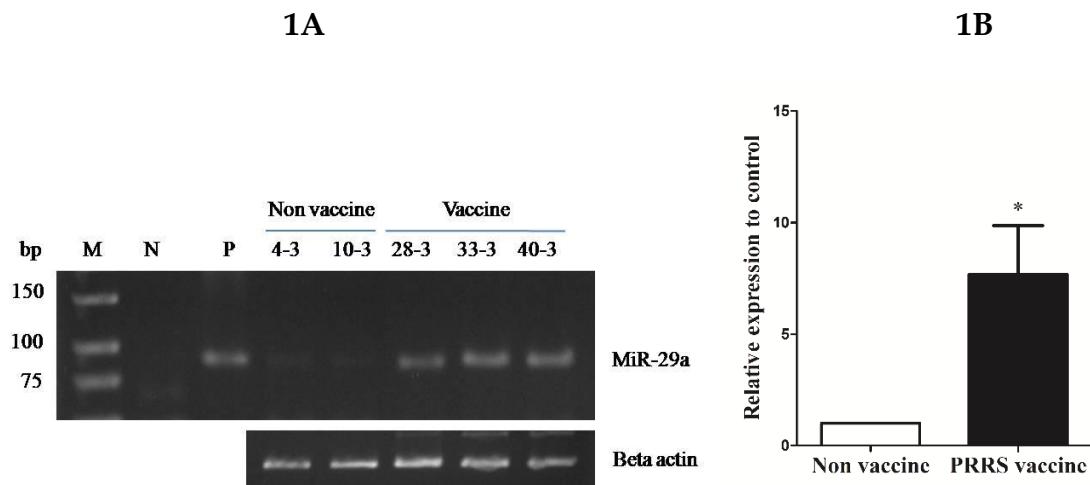


Figure 1 Elevated expression of miR-29a in pigs vaccinated with MLV PRRS vaccine

Figure 1A: Pigs were vaccinated with modified live virus (MLV) porcine reproductive and respiratory syndrome virus (pigs number 28-3, 33-3, and 40-3). Non-vaccinated pigs (pigs number 4-3 and 10-3) were used as controls. A beta-actin gene was amplified as an internal control. P = plasmid containing miR-29a gene, N = no template control, M = 10 bp marker.

Figure 1B: Band densities from each sample were quantified and calculated as mean. Data were collected from 5 individual pigs (two from control group and three from vaccinated group) * = $P < 0.05$.

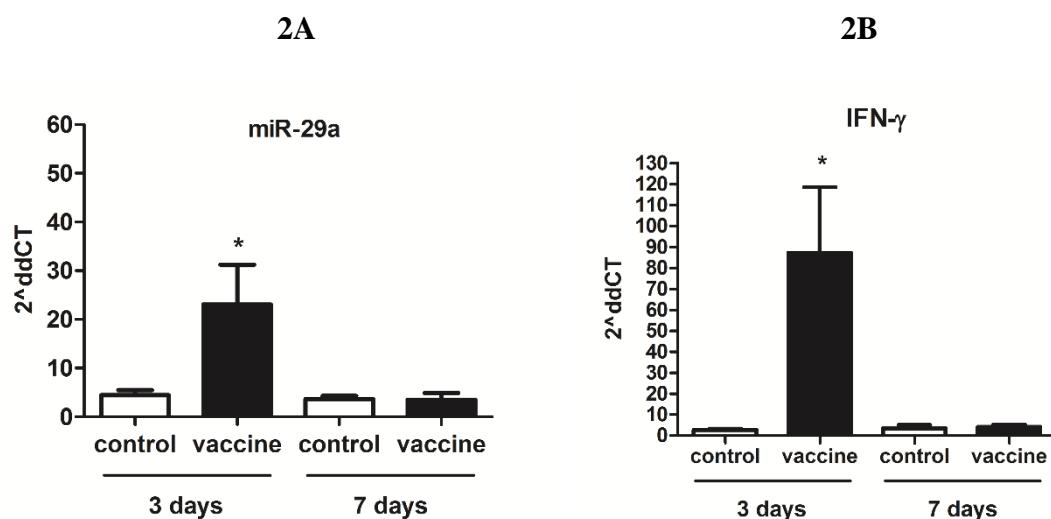


Figure 2 Elevated expression of miR-29a and IFN- γ in MLV PRRSV vaccinated pigs. Peripheral blood mononuclear cells were collected at 3 and 7 days post vaccination. PBMCs were isolated from each individual pigs ($n=6$) and subjected for real-time RT-PCR analysis. Levels of miR-29a and IFN- γ were normalized to beta-actin and shown as $2^{-\Delta\Delta CT}$. * = $P < 0.05$.

Based on the preliminary study of miR-29a expression in RT-PCR experiment, miR-29a and IFN- γ expression was investigated using the more sensitive gene expression method by real-time RT-PCR. The expression of miR-29a and IFN- γ was not different between the vaccinated and non-vaccinated pigs at 7 days post vaccination. The $2-\Delta\Delta CT$ of miR-29a was 3.64 ± 0.61 and 3.49 ± 1.37 , whereas that of IFN- γ was 3.56 ± 1.58 and 4.15 ± 1.17 in the vaccinated and non-vaccinated pigs, respectively. However, the upregulation of both miR-29a and IFN- γ was observed in the pigs receiving MLV PRRS at day 3 post vaccination. The $2-\Delta\Delta CT$ of miR-29a and IFN- γ was 23.03 ± 8.21 and 87.26 ± 31.29 versus 4.41 ± 1.07 and 2.74 ± 0.58 in the control group, respectively ($P<0.05$). In mice, a recent study suggested that miR-29 was the critical regulator of IFN- γ production during intracellular bacterial infection (Ma et al., 2011). IFN- γ is one of the important cytokines that determine the immunological outcome during PRRSV infection and vaccine response (Jeong et al., 2016; Piras et al., 2005). In addition to miR-29a, other microRNAs including miR-125b, miR-181 and miR-506 have been shown to regulate PRRSV replication by directly targeting PRRSV virus itself or indirectly modulating the host cellular responses (Gao et al., 2013; Wang et al., 2013; Wu et al., 2014). Moreover, miR-26a suppressed PRRSV replication through upregulated type I interferon (IFN) signaling pathway and higher expression of IFN-stimulated genes (Jia et al., 2015). In contrast, PRRSV could modulate host microRNAs to promote its replication by targeting heme oxygenase-1 (Xiao et al., 2016). miR-23, miR-378, and miR-505 suppress PRRSV replication by targeting viral RNA and modulating type I interferon responses (Zhang et al., 2014). miR-181 binds to PRRSV receptor CD163 in porcine alveolar macrophages and monocytes preventing viral entry into these cells (Gao et al., 2013). Such examples highlight the roles of microRNAs during viral infection including PRRSV in pigs. Nevertheless, the molecular mechanisms of miR-29a and its functional role during PRRSV infection require further investigation. Future studies of the role of microRNAs and their target genes will provide important insights into host-pathogen interaction and facilitate the design of better PRRSV vaccine.

Acknowledgements

This study was supported by the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand and from Kasetsart Veterinary Development Funds.

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บทคัดย่อ

ระดับ miR-29a และ IFN- γ ในสุกรที่ได้รับวัคซีน PRRS ชนิดเข็ือเป็น

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เข็ือไวรัสพืชาร์อาร์เอสจัดเป็นอาร์เอนเอไวรัสชนิดหนึ่งที่ก่อความเสียหายกับสุกรทั่วโลก ความเข้าใจเกี่ยวกับกลไกการก่อโรคและความรู้เกี่ยวกับการตอบสนองต่อไวรัสเป็นพื้นฐานสำคัญในการพัฒนาวัคซีนป้องกันโรคที่มีประสิทธิภาพ ในการศึกษาครั้นทั้งหมดจะได้ศึกษาระดับการแสดงออกของไมโครอาร์เอนเอ-29เอและอินเตอร์เฟอรอนแคมมาในสุกรที่ได้รับวัคซีนพืชาร์อาร์เอสชนิดเข็ือเป็น การศึกษาพบว่า ทั้งไมโครอาร์เอนเอ-29เอและอินเตอร์เฟอรอนแคมมามีระดับการแสดงออกที่เพิ่มสูงขึ้นในช่วงแรกในวันที่ 3 ภายหลังจากสุกรได้รับวัคซีน ในขณะที่ระดับของยืนทั้ง 2 ตัวมีการแสดงออกที่ไม่เปลี่ยนแปลงในวันที่ 7 ภายหลังจากได้รับวัคซีน ผลการศึกษานี้เป็นครั้งแรกที่แสดงให้เห็นถึงระดับของยืนทั้งสองตัวต่อการตอบสนองต่อวัคซีนพืชาร์อาร์เอสในสุกร อย่างไรก็ตาม กลไกและหน้าที่ของไมโครอาร์เอนเอในขณะที่มีการติดเชืื้อไวรัสพืชาร์อาร์เอสยังต้องการการศึกษาเพิ่มเติมต่อไป

คำสำคัญ: ไมโครอาร์เอนเอ-29เอ ไวรัสพืชาร์อาร์เอส อินเตอร์เฟอรอนแคมมา วัคซีน

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