Transcription elements AREB6 and miR-34a affect apoptosis of PAMs by regulating the expression of SS2-related gene PPP1R11

Guisheng Liu\textsuperscript{a,b}, Junjing Wu\textsuperscript{a,b}, Mu Qiao\textsuperscript{a,b}, Jiawei Zhou\textsuperscript{a,b}, Huayu Wu\textsuperscript{a,b}, Xianwen Peng\textsuperscript{a,b}, Supamit Mekchay\textsuperscript{c} and Shuqi Mei\textsuperscript{a,b}

\textsuperscript{a}Institute of Animal Science and Veterinary Medicine, Hubei Academy of Agricultural Sciences, Wuhan, China; \textsuperscript{b}Hubei Key Lab for Animal Embryo Engineering and Molecular Breeding, Wuhan, China; \textsuperscript{c}Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT
In our previous work, gene PPP1R11 (protein phosphatase 1 regulatory subunit 11) was significantly expressed in pigs after \textit{Streptococcus suis 2} (SS2) challenged. This study firstly confirmed that SS2 induced significant expression of PPP1R11 gene in porcine alveolar macrophage (PAM) cells, and apoptosis of PAM cells were observed. After that, the core promoter of porcine PPP1R11 was identified and its transcription factor AREB6 which significantly regulated PPP1R11. We also characterized that the PPP1R11 gene is a target of miR-34a. Further, we found that PPP1R11 helped to inhibit apoptosis of PAM cells under SS2 infecting, through transcription factor AREB6 was negatively correlated with apoptosis whereas miR-34a was positively correlated. Those findings provide a functional connection among the transcription factor AREB6, miR-34a, PPP1R11 gene and apoptosis of PAM cells in the pathogenesis of the SS2 infection.

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Introduction

\textit{Streptococcus suis 2} (\textit{S. suis} 2 or SS2) is an important swine pathogen and emerging zoonotic agent, causing arthritis, pneumonia, septicemia, meningitis and even acute death, and has broken out several times worldwide, and been isolated from a wide range of mammalian species [1,2]. In our previous work, PPP1R11 (protein phosphatase 1 regulatory subunit 11) gene was significantly expressed in pigs after SS2 challenged[3]. The PPP1R11 gene is a member of the PPP (protein phosphatases) superfamily, which encodes protein inhibitor 3 (Inh3) to inhibit PP1, which regulates diverse cellular processes, such as cell cycle, transcription, protein synthesis and metabolism [4–7]. The role of PPP1R11 in immune response has been paid more attention than in other functions [8,9]. Recently, PPP1R11 was found as an uncharacterized RING finger E3 ligase, which attenuates TLR (Toll-like receptor) 2 signaling in response to \textit{Staphylococcus aureus} infection by targeting TLR2 for proteasomal degradation[9].

AREB6 [\textit{Atp1a1} (Na, K-ATPase a1 subunit) regulatory element binding factor 6] is a zinc-finger-homeodomain transcription factor (is also known as Zinc finger E-box binding homeobox 1, ZEB1) that induce epithelial–mesenchymal transition (EMT), which plays important roles in both normal physiological and pathological processes [10–12]. For example, AREB6 was identified as a repressor on the immunoglobulin heavy-chain enhancer[13]; and as a negative transcription factor for gene interleukin 2 (IL-2) to turn off the IL-2 gene transcription just after T-cell activation [14,15].

MiRNAs (microRNAs) are noncoding small RNA molecules capable of regulating gene expression at the post-transcriptional level[16]. Some host miRNAs have been reported to be involved in the host-bacteria crosstalk [17,18], and miRNAs play important roles in modulating macrophages response toward pathogens, e.g. dynamic regulators of macrophage polarization and plasticity [19,20]. Macrophages are the first defense cells in the body which are exposed to pathogenic microorganisms[21], like SS2[22]. In literatures, alveolar macrophage (AM) cells were employed to study the function of genes, especially in pathogen infection [22–27].
Apoptosis is considered as a major defense mechanism of the body. Multiple pathogens induce macrophage apoptosis as a mode of immune evasion [28]. The resistance or susceptibility to viruses or bacteria was strongly linked to apoptosis [29,30]. In the case of S. suis, reports about apoptotic effects are limited [31–33]. Zeng & Lu (2003) demonstrated the muramidase released protein (MRP) which is a virulence factor of SS2 induced HEp-2 cell apoptosis. [31] Tenenbaum, et al. (2006) indicated that S. suis causes cell death in PCPEC (porcine choroid plexus epithelial cell) predominant by necrosis, although apoptosis may be involved in the process of PCPEC cell death[33]. By comparing S. suis wild strain with suilysin-deficient mutant in porcine respiratory epithelial cells under ALI (air-liquid interface cultures) conditions, Meng et al. (2016) demonstrated that suilysin contributes to apoptosis, and the cytolytic activity of suilysin is crucial for this effect [34].

The aim of this study focused on the PPP1R11 gene, try to explain the mechanism underlying its differential expressions in pigs after SS2 infection. The results demonstrated that transcription factor AREB6 binds to and activates PPP1R11 promoter, and the PPP1R11 is a target of miR-34a. AREB6 and miR-34a both supported to regulate porcine AM (PAM) cell apoptosis through PPP1R11.

Materials and methods

Animal samples and bacteria SS2 and cells

The pig samples and bacteria SS2 were harvested according to previous study, [3] which experimental protocols were approved by the Laboratory Animal Monitoring Committee of Hubei Province in China (no. Y2017-0612) and was guided to perform. PAM (porcine alveolar macrophage) cells were isolated from pigs aged 30 ~ 40 days which were tested for being pathogen-free; i.e., free from porcine respiratory and reproductive syndrome virus, porcine circovirus type 2 (PCV2), PCV3, Pseudorabies virus and SS2. The isolation processing of PAMs were: the lung was washed 3 ~ 4 times with fetal bovine serum (PBS, pH 7.2) supplemented with 2% fetal bovine serum (Fisher Scientific, Waltham, MA, USA). The cell pellets were resuspended and mixed with prechilled Gibco RPMI-1640 medium (Fisher Scientific) containing 40% FBS (Hyclone Laboratories Inc., USA). The number of the prepared PAMs reached 10 [8],10 [9]/ml with >95% viability. Aliquots of PAMs were frozen and stored in liquid nitrogen before use. Porcine kidney 15 cells (PK cells) were obtained from the cell database of Wuhan University. Cells were cultured in F12 (Gibco, Waltham, USA) or DMEM (Gibco) supplied with 10% fetus bovine serum (Gibco) at 37°C in a humidified atmosphere of 5% CO₂. The cells were plated and grown until they were 70–80% confluent at the time of use.

Plasmid construction and dual-luciferase reporter assays

Deletion fragments from the potential promoter region of the porcine PPP1R11 gene were amplified and double-digested with KpnI and HindIII, then cloned into the pGL3-basic vector (Promega). In addition, the 3'-UTR of PPP1R11 was amplified, double-digested with PmeI and XhoI, and then cloned into the pmirGLO vector (Promega, USA). Mutated binding site was generated using a Fast Site-Directed Mutagenesis Kit (TIANGEN, China) and mutagenic primers (PPP1R11-mut-F1: CCTTTGTTCTCCTCGGTCTAGTCCTGGGTCCAGTTATCTGG; PPP1R11-mut-R1: GACAGGCAGTTACCGAGTGAGAACAAAGGGAAGGATTGGGACCAAGATGC; PPP1R11-mut-F2: CCAGCTTTCACTCGGTGGGTCCTAGTCAGATTCCAGGCCACCAAGATGC; PPP1R11-mut-R2: CGACTAGCAGTTACCGAGTGAGAACAAAGGGAAGGATTGGGACCAAGATGC).

The miRNA or siRNA was transfected into PAM cells and PK cells using Lipofectamine RNAi Max (Invitrogen, USA). Plasmids were transfected into the PAM cells using Lipofectamine 3000 (Invitrogen), while Lipofectamine 2000 (Invitrogen) was used when transfecting plasmids into the PK cells. The plasmid transfection amount was 2.5 μg, and the luciferase activities were measured with a PerkinElmer 2030 Multilabel Reader (PerkinElmer, USA).

qPCR analysis

Reverse transcription was performed using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Random hexamers, oligo(dT) or miRNA-specific stem-loop primers
were added to initiate cDNA synthesis. qPCR (quantitative PCR) was performed on a BioRad CFX384 system (Bio-Rad, USA) using the iTaq Universal SYBRGreen Supermix (Bio-Rad). The qPCR primers were as follows: 

| Primer   | Sequence of Primer (5′-3′)          | Size (bp) | Tm (°C) |
|----------|------------------------------------|-----------|----------|
| PPP1R11-D1-F | CTAGCTAGCTAGTCGGATTCTATTACACCAC  | 2000      | 61       |
| PPP1R11-D2-F | CTAGCTAGCTACATTAAACCATTCAATTTC    | 1500      | 61       |
| PPP1R11-D3-F | CTAGCTAGCTAGAAATGACATACATATT     | 1000      | 60       |
| PPP1R11-D4-F | CTAGCTAGCTAGTGTCCTGAGAAACA       | 700       | 59       |
| PPP1R11-D5-F | CTAGCTAGCTAGACGGGGTGTCCGCGATT  | 400       | 57       |
| PPP1R11-D6-F | CTAGCTAGCTAGGGAAAAAGGAAAAAGG    | 225       | 58       |
| PPP1R11-D7-F | CTAGCTAGCTAGGGAAAAAGGAAAAAGG    | 200       | 61       |
| PPP1R11-D-R   | CTAGCTAGCTAGGGAAAAAGGAAAAAGG    | 200       | 61       |

underline indicates the restriction enzyme sites, and italics indicate protective bases.

The targeted deletion was located upstream of the promoter at −2000 bp, −1500 bp, −1000 bp, −700 bp, −400 bp, −225 bp and −200 bp, respectively. Briefly, the work process as, the deleted core promoters were amplified and cloned into a vector. Subsequently, the successful plasmid bearing the deleted core promoter was transfected into PK cell. The luciferase activity was then determined upon transfection. Seven forward primers and one common reverse primer are shown in Table 1 for generating deletion fragments, and the restriction enzyme sites (KpnI and HindIII) and protective bases were added into primers.

### Western blot analysis

Cell protein lysates were generated using RIPA Lysis Buffer (Beyotime, China) and were added to PMSF (Beyotime) at a ratio of 100:1. Cellular proteins were extracted 48 h posttransfection, the number of cells was 10×10^6. Proteins were separated by SDS-PAGE and were transferred into polyvinylidene fluoride membranes (Millipore, USA). Primary antibodies specific for PPP1R11 (1:500, 20,263–1-AP, Proteintech, China), AREB6 (1:1000; A5600, ABclonal, USA), and β-actin (1:2000; AC006, ABclonal, USA) were used for immunoblotting. An Image Quant LAS4000 mini (GE Healthcare Life Sciences, USA) was used to detect protein expression.

### Core promoter analysis

To determine the core promoter, multiple fragments were systematically deleted. The 2000 bp upstream from the first start codon of the PPP1R11 gene was set as a target region for the core promoter of interest. The nuclear proteins of PK cells were extracted with the Nucleoprotein Extraction Kit (Beyotime). Oligos corresponding to the AREB6 binding sites of the PPP1R11 core promoter were synthesized and annealed into double strands. The DNA binding activity of the candidate transcription factor protein was detected by the LightShift® Chemiluminescent EMSA Kit (Pierce, USA). The 10 μg ovarian follicle nuclear extract was added to 20 fmol Biotin-labeled double-stranded oligonucleotides, 1× binding buffer, 2.5% Glycerol, 5 mM MgCl2, 50 ng Poly (dI·dC), 0.1 mM EDTA and 0.05% NP-40. The control group

### Transcription factor analysis

Based on the core promoter result, the BIOBASE software was used to predict the transcription factor and its binding sites, and then EMSA (electrophoretic mobility shift assay) and ChIP (chromatin immunoprecipitation) were used to confirm the binding of the transcription factor within the core promoter region.

### Electrophoretic mobility shift assays (EMSA)

The nuclear proteins of PK cells were extracted with the Nucleoprotein Extraction Kit (Beyotime). Oligos corresponding to the AREB6 binding sites of the PPP1R11 core promoter were synthesized and annealed into double strands. The DNA binding activity of the candidate transcription factor protein was detected by the LightShift® Chemiluminescent EMSA Kit (Pierce, USA). The 10 μg ovarian follicle nuclear extract was added to 20 fmol Biotin-labeled double-stranded oligonucleotides, 1× binding buffer, 2.5% Glycerol, 5 mM MgCl2, 50 ng Poly (dI·dC), 0.1 mM EDTA and 0.05% NP-40. The control group
was added 2 pmol of unlabeled competitor oligonucleotides. The mixtures were then incubated at 24°C for 20 min. The reactions were analyzed by electrophoresis in 5.5% polyacrylamide gels in 0.5 × TBE buffer at 180 V for 35 min, and then transferred to a nylon membrane. The dried nylon was scanned with a GE ImageQuant LAS4000 mini (GE-Healthcare, USA).

**Chromatin immunoprecipitation (ChIP) assay**

ChIP assays were performed using the EZ-ChIP™ Kit (Millipore, USA). Briefly, after crosslinking the chromatin with 1% formaldehyde at 37°C for 10 min and neutralizing it with glycine for 5 min at room temperature, PK cells were washed with cold PBS, scraped and collected on ice. Then, cells were harvested, lysed and sonicated. Nuclear lysates were sonicated 20 times for 10 s with 20 min intervals in ice water using Scientz-IID (Scientz, China). An equal amount of chromatin was immuno-precipitated at 4°C overnight with at least 1.5 μg of AREB6 (GTX105278, GeneTex, USA) and normal mouse IgG (Millipore) antibodies. Immuno-precipitated products were collected after incubation with Protein A + G coated magnetic beads. The beads were washed, and the bound chromatin was eluted in ChIP elution buffer. Then, the proteins were digested with Proteinase K for 4 h at 45°C. DNA from the immune-precipitated complex was amplified through PCR. The primer sequences were as follows: AREG6-ChIP-F, 5′-GGACGCCTCTTCCGCCAAAT-3′; AREG6-ChIP-R, 5′-AGCCAGGCTCCTCCACCTTTT-3′.

**MiRNA analysis**

The software TargetScan (http://www.targetscan.org/) was used to predict the miRNAs targeting PPP1R11, obtained two potential candidates: miR-181 and miR-34a. As the miR-181 has no effect on the activity of the fluorescent carrier after the pmiRGLO vector is inserted into the PPP1R11 3′-UTR, it is not a potential miRNA to target the gene PPP1R11, whereas results showed that miRNA-34a has a targeted relationship with the PPP1R11-3′-UTR region.

The pig PPP1R11 3′-UTRs were amplified, double-digested with Pmel and XhoI, and then cloned into the pmirGLO vector (Promega, USA). Binding site mutants were generated using a Fast Site-Directed Mutagenesis Kit (TIANGEN, China) and mutagenic primers. The primer sequences were as follows: PPP1R11-3′-UTR-F, GGGTTAAAACCTTGATCCCTTCTCCAGC; PPP1R11-3′-UTR-R, CCGCTCGAGACACTCAATCCACACA; PPP1R11-3′-UTR-mut-F, CCAGCTTTCACGGGCTTTCTAGTGATCAGATTCCGGAACCTCG; PPP1R11-3′-UTR-mut-R, GACTAGGACCCAGGTGGAAGCTGGGGCTGTGGGAAACCCAGTTTAAC. The synthesized siRNA-PPP1R11 (50nM) and miRNA-34a mimics (50nM) (Ribobio, Guangzhou, Guangdong, China) were transfected into cells with Lipofectamine RNAiMAX (Invitrogen). After 24 h and 48 h, cells were harvested for qPCR assays and western blot assays, respectively.

**Apoptosis analysis**

PAMs were transfected with miR-34a mimics, NC (negative control) mimics, miR-34a inhibitor, NC inhibitor, pCDNA3.1-PPP1R11, pCDNA3.1-AREB6, pCDNA3.1, siRNA-PPPR11, siRNA-AREB6 or siRNA NC and harvested 48 h after transfection. Fluorescence-activated cell sorting (FACS) was used to measure apoptosis. The experiments were performed according to the manufacturer’s protocol of the Annexin V-FITC Apoptosis Detection Kit (Invitrogen, USA). Apoptosis analysis was analyzed by software FlowJo v10.0.7.

**Statistical analysis**

All results are presented as the mean ± SD. Each experiment had three biological replicates, in each of which contains three technical repeats. Two-tailed t-test was used when two groups were compared. Significant differences were evaluated using an independent-samples t-test. P< 0.05 was considered to be statistically significant.

**Results**

**PPP1R11 regulated the apoptosis of PAM cells challenged SS2**

We firstly tested the PPP1R11 gene expression in PAM cells after SS2 challenged. The qPCR analysis showed that PPP1R11 in PAM cells was significantly
up-regulated when compared the SS2 challenged samples to SS2 unchallenged samples (Figure 1(a)). Then, we tested whether PPP1R11 can affect the apoptosis of PAM cells. pCDNA3.1-PPP1R11, pCDNA3.1, siRNA-PPP1R11 or siRNA-NC was transfected into PAMs. PPP1R11 mRNA and protein expression levels were significantly increased when PAM cells were transfected with pCDNA3.1-PPP1R11 or suppressed when transfected with pCDNA3.1, siRNA-PPP1R11 or siRNA NC (Figure 1(b, c)). As shown in Figure 1(d), PPP1R11 overexpression suppressed apoptosis of PAM cells and PPP1R11 inhibition promoted PAM cell apoptosis.

**Transcription factor AREB6 regulates expression of PPP1R11**

To date, there have been no reports about porcine PPP1R11; thus, we identified its core promoter and regulated elements. A 2000 bp contig in 5’-flanking region of the porcine PPP1R11 gene was amplified by PCR. To determine the promoter region, seven promoter deletions were introduced upstream of the luciferase gene. Luciferase activity analysis in PK cells revealed that the 5’-flanking sequence from −225 to −200 bp was important for its transcriptional activity (Figure 2(a)).

To further identify the transcription factor which binds to the core promoter of PPP1R11, the software BIOBASE was used to predict the transcription factor and its binding sites within the region −225 to −200 bp; the predicted results are shown in Figure 2(b). From those results, we have selected five candidates: Pax4 (paired box 4), p54, USF (upstream stimulating factor), C/EBP (CCAAT-enhancer-binding protein) and AREB6. We mutated the binding sites of those five candidates and transfected them into PK cells to detect fluorescence, and found there was no significant difference from their corresponding wild-type in four candidates: i.e., Pax4, p54, USF and C/EBP (Figure 2(c)). Those indicated that the four transcription factors are not key transcription factors regulating the

*Figure 1. PPP1R11 regulated the apoptosis of porcine alveolar macrophage (PAM) cells. (a) PPP1R11 mRNA levels were detected at 24 h after being challenged with SS2 (0.5 moi). (b) qPCR was used to detect PPP1R11 in PAM cells at 24 h after transfection with pCDNA3.1-PPP1R11, and pCDNA3.1 as control; with siRNA-PPP1R11, and siRNA NC, respectively. (c) Western blot and a densitometric analysis using software imageJ to detect PPP1R11 protein levels at 48 h after PAM cells transfection. (d) PAMs were transfected with pCDNA3.1-PPP1R11, pCDNA3.1, siRNA-PPP1R11 or siRNA negative control (NC), harvested and stained with anti-annexin V-propidium iodide, and analyzed by FACS at 48 h post transfection. The results were expressed as the mean ± S.E.M. (three independent replicates per group). ** p < 0.01.*
PPP1R11 gene. We found that the fluorescence activity increased significantly after the transfection of the AREB6 overexpression vector into PK cells compared to the PGL3-basic and pCDNA3.1 no-load vectors (Figure 3(a)), then identified point mutations in the AREB6-binding sites of the PPP1R11 promoter (Figure 3(b)) and observed that its fluorescence activity changed significantly (Figure 3(c)). Therefore, we conclude that AREB6 may be a key transcription factor regulating the PPP1R11 gene. Further, we overexpressed and knocked-down expression of AREB6 by the transfection with pCDNA3.1-AREB6 and siRNA-AREB6 (Figure 3(d), e), then checked the mRNA and protein expression levels of the target PPP1R11, and found that the expression of PPP1R11 was increased when AREB6 was overexpressed and was decreased by knockdown of AREB6 (Figure 3(f, g)). Those results further demonstrated that AREB6 is a key transcription factor for PPP1R11.

**Transcription factor AREB6 binds to PPP1R11 promoter**

ChIP analysis was performed to investigate whether AREB6 binds to the pig PPP1R11 gene promoter in vitro. A 244 bp DNA region from samples immunoprecipitated with an anti-AREB6 antibody was amplified, and no band from the anti-IgG or anti-OCT1 antibody precipitates was observed (Figure 4(a)). The results indicated that AREB6 binds to the pig PPP1R11 gene promoter region in vitro.

EMSA was used to further check the binding of AREB6 to PPP1R11 promoter in vitro, a strong gel-shift signal was detected when the AREB6-binding site probe was added in the PK cells nuclear extracts in lane 2. However, there was almost no gel-shift signal for the probes of the second or third AREB6-binding sites. The shift band signal generated using the probe for the AREB6-binding site was decreased.
when excess unlabeled competitor probe was added in lane 3, but was not affected by the same amount of mutant competitor probe in lane 4 (Figure 4(b)).

**Mir-34a directly targets PPP1R11**

Our prediction results showed that miR-34a may be a candidate miRNA to regulate PPP1R11. The miR-34a-binding seed sequences in PPP1R11 3′-UTR were also highly conserved in mammals (Figure 5(a)).
showed that miR-34a significantly inhibited the expression of PPP1R11 at the mRNA level and the protein level (Figure 5(c), d). We further investigated whether the expression of miR-34a changed when SS2 infected PAM cells, the results showed that miR-34a was significantly downregulated in PAM cells infected with SS2 (Figure 5(e)); meanwhile, the expression of PPP1R11 was upregulated (Figure 1).

**miR-34a and AREB6 regulate the apoptosis of PAM cells**

To further confirm their regulatory relationships above, we performed similar experiments in primary PAM cells. qPCR analyses revealed that PPP1R11 mRNA expression level was significantly downregulated after miR-34a mimic was transfected into PAM cells, whereas inhibition of miR-34a significantly increased PPP1R11 mRNA expression level in PAM cells (Figure 6(a)). Western blot analyses showed that miR-34a mimic significantly inhibited PPP1R11 protein expression level, whereas inhibition of miR-34a significantly increased PPP1R11 protein expression level in PAM cells (Figure 6(b)). Further, qPCR and Western blot data from PAM cells with overexpression and knockdown of AREB6 revealed that transcription factor AREB6 positively regulated the expression of PPP1R11 (Figure 6(c), d)). Our results demonstrated that PPP1R11 expression was significantly regulated by miR-34a and the transcription
factor AREB6. Thus, miR-34a and AREB6 may affect PAM apoptosis through regulate PPP1R11. Subsequently, we investigated the effect of miR-34a and AREB6 on the apoptosis of PAM cells. As expected, miR-34a is positively correlated with apoptosis whereas AREB6 is negatively correlated (Figure 6(e, f)). These results indicated that miR-34a and AREB6 regulate PAM cells apoptosis through PPP1R11.

Discussion

This study confirmed that SS2 significantly induced PPP1R11 gene expression in PAM cells, as our previous work showed its significant expressions in pigs after SS2 infection. [3] Then we identified the core promoter and transcription factor of porcine gene PPP1R11 in PK cells. PK cells are one of the cell lines, little used to explore the Streptococcus-host cell interactions[35]. In contrast, PAM cells are often used to study the Streptococcus-host cell interactions [22,24–27,36]. As PAM cells are isolated from the lung of sacrificed piglets, therefore we used PK cells to study the normal regulation of PPP1R11 gene, then to further study the function performances in PAM cells challenged with SS2.

Because there has no report about transcription factor of porcine PPP1R11, and here from the predicted candidates of transcription factors, we selected five candidates which have relatively higher fractional number (Figure 2(b)) and have been commonly reported upon in the literature. Transcription factor USF regulates many different genes and numerous biological processes directly [37], the C/EBP has been found to regulate IL-6 and IL-1β response through an NF-κB response element, which is a marker pathway that regulates immune responses[38], PAX4 is a member of the PAX family of transcription factors, play important roles in development, pathology, and cancer [39], and p54 has been shown to promote castration-resistant prostate cancer growth[40]. From the above evidence, the PPP1R11 expression was regulated by transcription factor AREB6 in both PK and PAM cells. Several papers have reported that transcription factors are involved in the pathological process [10,40,41]. AREB6 was also
confirmed to physically bind to the PPP3CC promoter. The elucidation of the AREB6-PPP3CC axis reveals novel insights into the molecular mechanisms underlying the progression of glioma and provides a rationale for the development of clinical anticancer intervention strategies based on targeting the AREB6-PPP3CC axis.[42]. Those are supported our results that AREB6 is the core promoter of PPP1R11 expression and involved in SS2 pathogenesis.

Previous papers indicated that miRNAs play a vital role in the process of bacterial infection.[43] Up to date, few miRNAs for PPP1R11 and for SS2 pathogen, as well as interactions between bacterial small RNAs (sRNAs) and host miRNAs have been reported [44–47]. We identified and confirmed that...
**PPP1R11** is a target gene of miR-34a. miR-34a may promote apoptosis and may play a function in aging [48]. Here we found that miR-34a increases apoptosis of PAM cells and **PPP1R11** suppresses apoptosis of PAM cells. Thus, miR-34a might involve regulating PAM cell apoptosis through targeting **PPP1R11**.

In conclusion, this study identified the core promoter of porcine gene **PPP1R11** and its regulation elements, including transcription factors AREB6 and miR-34a, which significantly regulated **PPP1R11**. And that **PPP1R11** could inhibit PAM cell apoptosis, further found that miR-34a is positively correlated with apoptosis whereas AREB6 is negatively correlated with apoptosis. Our research indicates that **PPP1R11** is involved in the pathogenesis of the SS2 infection by suppressing PAM cell apoptosis and this process is regulated by AREB6 and miR-34a. In future, promising work would be interaction PPP1R11 with PP1, inflammation analysis and related aspects, such as IRF-7 or IFN-alpha.

**Author Contributions**

G.L., S.M. and Q.S.M. designed the study; J.W., M.Q. and H. W. performed lab work and statistical analysis; X.P. and G. L. drafted the manuscript; J.W., S.M. and Q.S.M. reviewed the manuscript; all authors approved the paper.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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