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Notch signaling contributes to the expression of inflammatory cytokines induced by highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection in porcine alveolar macrophages

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Abstract

Notch signaling, an evolutionarily conserved signal pathway has emerged as a key signal pathway to regulate host immune response but the contribution of Notch signaling to immune response in pigs remains unknown. Infection of porcine alveolar macrophages (PAM) with porcine reproductive and respiratory syndrome virus (PRRSV) triggers expression of Jagged1 mRNA, suggesting that Notch signaling might play a role in the immune response to PRRSV infection. To further explore it, we examined the expression profile of Notch molecules in PAM following a highly pathogenic PRRSV (HP-PRRSV) strain infection. We demonstrated that HP-PRRSV infection resulted in the induction of Notch ligands (*Jagged1*, *Dll3*, *Dll4*), the transcription factor *RBP-J*, and the target gene *Hes1*, consistent with activation of Notch signaling. Next, using DAPT treatment and the knockdown of RBP-J illustrated that inhibition of activation of Notch signaling attenuated induction of the inflammatory cytokines (TNF- α and IL-1 β) instead of viral replication in PAM during HP-PRRSV infection. Furthermore, the knockdown of Jagged1, the most induced ligand not only inhibited activation of Notch signaling, but also reduced the expression of inflammatory cytokines without any influence in viral replication. Moreover, our data revealed that several signaling including NF- κ B, MAPK and Notch signaling contributed to the induction of Jagged1 in PAM during HP-PRRSV infection. In summary, these findings reveal that Notch as an important signaling pathway could contribute to the regulation of inflammatory response induced by HP-PRRSV infection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dci.2020.103690>.

Keywords

HP-PRRSV; Notch signaling; Porcine alveolar macrophages; Inflammatory response

1. Introduction

Notch is an evolutionarily conserved signal pathway from vertebrate to insects, and has an important role in cell differentiation, proliferation, survival, and tissue homeostasis. In mammals there are five ligands (Jagged1, Jagged2, Dll1, Dll3 and Dll4), each of which can bind to any of four receptors (Notch1, Notch2, Notch3 and Notch4) (Bray, 2006). In canonical Notch signaling, once Notch ligands bind to Notch receptors on adjacent cells, the signaling cascade will be triggered including: 1) the γ -secretase-dependent proteolytic release of the Notch intracellular domain (NICD) into the nucleus; 2) in the nucleus, NICD interacts recombinant-recognition-sequence-binding protein at the J κ site (RBP-J, also known as CSL or CBF1); 3) then, the interaction of NICD and RBP-J will convert RBP-J from a transcriptional suppressor to an activator through the recruitment of coactivator proteins, such as mastermind-like family proteins and CBP/p300; 4) finally, the target genes including *Hes1* will be activated by the RBP-J-associated transcriptional complex.

In the immune system, Notch signaling plays an important role in the development of lymphocytes (B cells and T cells) and in the regulation of T cell differentiation as well as its functions (Maillard et al., 2005; Tanigaki and Honjo, 2007; Radtke et al., 2010). Aside from its effect on the regulation of lymphocyte development, the interaction of Notch receptors and ligands can serve as important signaling between antigen presenting cells (APC) and T cell to promote T cell differentiation. For example, influenza virus H1N1 infection enhanced the expression of Dll1 in macrophages and the induction of Dll1 regulated IFN- γ production in CD4 and CD8 T cells (Ito et al., 2011). Moreover, respiratory syncytial virus (RSV) infection up-regulated Dll4 expression in dendritic cells (DC) to promote the development of a protective Th1 response in comparison to anti-Dll4 group (Schaller et al., 2007). In addition to its effect on T cell development and differentiation, Notch signaling involves in regulation of macrophage activation and implicates regulation of inflammation mediated by macrophages. For example, Notch signaling regulated TLR-induced activation of macrophages and the production of inflammatory cytokines. The reported mechanism was that early and direct cooperation between TLR and Notch signaling leads to Jagged1-RBP-J-mediated autoamplification of Notch signaling that could further contribute to the later phase of the TLR response (Foldi et al., 2010). Moreover, Japanese Encephalitis virus (JEV) infection induced expression of inflammatory cytokine by microglia through activation of Notch signaling. The underlined mechanism was that JEV-induced let-7a/b interacted with the Notch-TLR7 pathway to induce expression of TNF- α in microglia (Mukherjee et al., 2019). Though effect of Notch signaling on immune response has been extensively studied using mouse or human immune cells, yet how it does involve in regulation of immune response in pigs remains unknown.

Porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped single-stranded RNA virus that belongs to the genus Arterivirus, family Arteriviridae, order

Niclovirales (Cavanagh, 1997). There are two genotypes, known as genotype I (European-like) and genotype II (North American-like) PRRSV with approximately 60% identity at the genomic level (Allende et al., 1999). PRRSV, the pathogen of porcine reproductive and respiratory syndrome is a big threat to pig industry worldwide. In 2006, outbreak of highly pathogenic PRRSV (HP-PRRSV) isolate has been reported in China (Tian et al., 2007). Diseases caused by HP-PRRSV were characterized as high fever, high morbidity, and high mortality. PRRSV mainly infects monocyte/macrophage lineage and induces robust inflammatory response. In the lung phase, PRRSV primarily infects alveolar macrophages and causes robust inflammatory response characterized by severe pneumonia (Duan et al., 1997; van Reeth et al., 1999; Xiao et al., 2010; Zhou et al., 2008). Previous studies have demonstrated that TLRs, NF- κ B and MAPK signaling regulate the production of inflammatory cytokines by porcine alveolar macrophage during PRRSV infections (Bi et al., 2014; Du et al., 2016; Liu et al., 2017; Yu et al., 2017). Although Notch signaling has been well-documented to modulate the immune response, the effects of Notch signaling on immune responses including antiviral and inflammatory response to PRRSV infection are unknown.

Given that RNA-seq screening suggested an association between PRRSV infection and Jagged1 upregulation in PAM *in vitro* (Badaoui et al., 2014), we hypothesized that Notch signaling plays a role in the immune response to PRRSV infection. In this study, for the first time, we determined the expression profile of Notch molecules and the effects of Notch signaling on viral replication and the expression of inflammatory cytokines in porcine alveolar macrophages during HP-PRRSV infection. We identified Notch signaling contributed to regulation of the inflammatory cytokine expression but not viral replication in macrophages. These data contribute to understanding the immunopathogenesis of HP-PRRSV infection in pigs.

2. Materials and methods

2.1. Piglets

All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Veterinary Research Institute (IACUC No: Shvri-po-201606 0501) and were performed in compliance with the Guidelines on the Humane Treatment of Laboratory Animals (Ministry of Science and Technology of the People's Republic of China, Policy No.2006 398). Piglets (30–40 days old, Shanghai great white pig strain) were purchased from the Shanghai Academy of Agricultural Sciences (Shanghai, China). Furthermore, the piglets were all tested negative for PRRSV antibody by ELISA (IDEXX Laboratories, Westbrook, ME, USA), as well as for PRRSV, porcine circovirus type 2, classical swine fever virus, and swine influenza virus by qPCR.

2.2. Reagents

A NF- κ B inhibitor (Bay11–7082), a JNK inhibitor (SP600125), a MEK inhibitor (PD98059), a p38 inhibitor (SB203580), a γ -secretase inhibitor (DAPT) were purchased from Sigma (St. Louis, MO, USA). All of the inhibitors were reconstituted in dimethyl sulfoxide (DMSO) and stored in -80°C . Primary antibodies against RBP-J and Jagged1

were purchased from Cell Signaling Technology (CST, Danvers, Mass, USA). A rabbit polyclonal antibody against PRRSV N were generated using a synthetic peptide (peptide antigen: GPGKKNRKKNPKEKPHFPC). Mouse anti-actin antibody was purchased from Sigma.

2.3. Cell culture and viruses

Porcine alveolar macrophages (PAM) were generated as shown in the previous study (Qi et al., 2017). Briefly, after euthanasia, the lungs of 10 pigs (male, 30–40 days old) were harvested and lavaged with ice-cold phosphate-buffered saline (PBS). The lung lavage fluid were centrifugated at 1200 rpm for 10 min at 4 °C. Then the isolated cells were suspended in RPMI 1640 medium (Thermo Fisher Scientific, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 mg/ml). Marc-145 cells were maintained in Dulbecco modified Eagle medium (DMEM, Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) at 37 °C in a 5% CO₂ atmosphere. The HP-PRRSV strain SH-PRRS01 (Qi et al., 2017) was stored in our Lab and was further propagated on Marc-145. The viruses were titrated using tissue culture infective dose 50 (TCID₅₀) as mentioned in the previous study (Shi et al., 2014).

2.4. The RNA interference (RNAi)

A total of 10⁶ PAM per well in 24 well-plates were transfected with 30 pmol of a mixture of RBP-J-specific siRNA (#1 siRBP-J, the target sequence: GCATTGCCTCAGGAACAAA; #2 siRBP-J, the target sequence: GGACAGAATTTCACTCCAA), Jagged1-specific siRNA (#1 siJagged1, the target sequence: GGTCAGAATTGTGACATAA; #2 siJagged1, the target sequence: GCAAGTGCCCTGAGGATTA; #3 siJagged1, the target sequence: CCCAGAGCCTGAACCGCAT), or negative control siRNA (GenePharma, Shanghai, China) by Lipofectamine RNAiMAX following the manufacture's instruction. After 48 h of transfection, cells were infected with HP-PRRSV in a multiplicity of infection (MOI) 1. After 24 h post-infections (hpi), samples were collected for further analysis.

2.5. Western blot analysis

Protein samples from PAM were prepared as previously described (Qiu et al., 2008). In brief, transferred membranes were prepared by using the samples and blocked with 5% skim milk (BD Difco™, Detroit, USA) for 1 h at room temperature. Primary antibodies were incubated with membrane for overnight at 4 °C [anti-RBP-J (1:1000, clone D10A4, CST), anti-Jagged1 (1:1000, clone 28H8, CST), anti-PRRSV N (1:1000), anti-actin (1: 10,000, clone C4, Sigma)]. Secondary antibodies were incubated for 1 h at room temperature [goat anti-mouse HRP (1:5,000, Abcam), goat anti-rabbit (1:10,000, Abcam)].

2.6. ELISA

The concentrations of porcine TNF- α and IL-1 β in supernatants harvested from PAM culture were measured by ELISA kit (R&D, Minneapolis, USA).

2.7. qRT-PCR analysis

Total RNA was extracted from PAM with RNAiso Reagent (Takara) and cDNA was prepared using the PrimeScript RT reagent kit (Takara). Gene expression was analyzed by qRT-PCR using SYBR Green qPCR Master Mix (Takara). Specific primers were shown in Table S1. The Calculation was performed as described in our previous study (Neal et al., 2017). Briefly, the expression of *GAPDH* was used as a reference. Relative expression is calculated as the percentage of *GAPDH* (2^{-Ct}) and fold induction (2^{-Ct}) relative to the mock group.

2.8. Statistical analysis

All data were analyzed with GraphPad Prism software (GraphPad Software, Inc.). An unpaired Student's t-test was used to determine significant differences. Values were considered statistically significant when $p < 0.05$. Data were given as mean (SD) as indicated; 'n' refers to the sample size.

3. Results

3.1. Expression profile of Notch molecules in porcine alveolar macrophages during HP-PRRSV infection

Since previous RNA-seq analysis revealed that PRRSV infection may induce expression of *jagged1* in porcine alveolar macrophages (PAM) (Badaoui et al., 2014), we first measured expression of Notch molecules including Notch receptors (1–4), ligands (*Dll1*, 3, 4, and *jagged1* and 2), the master regulator transcription factor RBP-J and the representative target *Hes1* in PAM following HP-PRRSV infection by using real-time PCR. During HP-PRRSV infection no Notch receptors were induced, of note, only *Notch1* was decreased in comparison to mock-infected PAM (Fig. 1A). In contrast, Notch ligands including *Jagged1*, *Dll3* and *Dll4* were induced (Fig. 1B), especially *Jagged1* expression with more than 40-fold increase. Moreover, PRRSV infection induced expression of *RBP-J* compared with that of mock-infected control. Consistent with the increased expression of Notch ligands and the transcription factor RBP-J, the target gene *Hes1* was robustly induced (Fig. 1C), suggesting that HP-PRRSV infection induces activation of Notch signaling in PAM. Collectively, these results reveal that HP-PRRSV infection could induce activation of Notch signaling in PAM, which is associated with the induction of Notch ligands, especially the increased *Jagged1*.

3.2. Effect of Notch signaling on the replication of HP-PRRSV in PAM

To understand the biological functions of Notch signaling during HP-PRRSV infection, we first tested the effect of Notch signaling on the replication of HP-PRRSV in PAM. We treated PAM with DAPT (a chemical inhibitor of γ -secretase) to suppress Notch signaling and compared the viral replication by TCID₅₀ and *Hes1* expression by qPCR between vehicle-treated and DAPT-treated groups during HP-PRRSV infection. There was no difference in the viral replication between vehicle-treated and DAPT-treated groups (Fig. 2A). Consistent with blockade of activation of Notch signaling during HP-PRRSV infection, DAPT treatment significantly inhibited expression of the target gene *Hes1* in comparison to vehicle-treated group (Fig. 2B). Furthermore, we examined the role of Notch signaling in the

replication of HP-PRRSV in PAM by knocking down expression of RBP-J, the master regulator transcription factor of the Notch signaling using RNA interference. The expression of RBP-J analyzed by Western blot and qPCR showed that we efficiently knocked down the expression of RBP-J (Fig. S1). Similarly, there was no difference in viral replication between NC and RNAi groups (Fig. 2C). Moreover, the knockdown decreased expression of *Hes1* compared with that in the NC group (Fig. 2D). Collectively, these results reveal that Notch signaling does not affect the replication of HP-PRRSV in PAM.

3.3. Effect of Notch signaling on inflammatory cytokine expression in PAM during HP-PRRSV infection

Previous studies have shown that Notch signaling contributes to regulation of inflammatory cytokine expression in macrophages under viral infection (Mukherjee et al., 2019) and proinflammatory stimuli (TLR ligands, inflammatory cytokines, and so on) (Foldi et al., 2010; Fung et al., 2007; Hu et al., 2008; Tsao et al., 2011), respectively. We next investigated the role of Notch signaling in the inflammatory cytokine expression in PAM during HP-PRRSV infection. Compared with the vehicle-treated group, DAPT treatment significantly reduced the protein and mRNA levels for the inflammatory cytokines, TNF- α and IL-1 β , which were induced in PAM during HP-PRRSV infection (Fig. 3). Furthermore, expression of TNF- α and IL-1 β in both protein and mRNA levels were also inhibited by the knockdown of RBP-J in PAM during HP-PRRSV infection in comparison to the NC-infected group (Fig. 4). Thus, these results reveal that Notch signaling contributes to regulation of inflammatory response to HP-PRRSV infection in PAM.

3.4. Induction of Notch ligand Jagged1 contributes to the inflammatory cytokine expression in PAM during HP-PRRSV infection

Having demonstrated that Notch signaling plays important role in regulation of inflammatory response to HP-PRRSV infection by the inhibition of γ -secretase and the RBP-J knockdown, we next sought to determine the effect of Notch ligands on the inflammatory cytokine expression during HP-PRRSV infection. Regarding that HP-PRRSV infection leads to robust induction of Jagged1, thus we investigated the role of Jagged1 in antiviral and inflammatory response to HP-PRRSV infection. First, we tested efficiency of the Jagged1 knockdown in protein and mRNA level by Western blot and qPCR, respectively. The results clearly showed that Jagged1 was efficiently knocked down by using the specific interfering RNA in comparison to the NC group (Fig. S2). Similarly, there was no difference in virus infection between NC and RNAi group (Fig. 5A), although the Jagged1 knockdown significantly decreased the expression of *Hes1* induced by HP-PRRSV infection in comparison to the NC-infected group (Fig. 5B). In contrast, the Jagged1 knockdown inhibited the induction of TNF- α (Fig. 5C and D) and IL-1 β (Fig. 5E and F) compared with those components in the NC-infected group. Overall, these results suggest that Jagged1-Notch signaling contributes to HP-PRRSV-induced inflammation in PAM.

3.5. Insight into the regulation of Jagged1 expression during HP-PRRSV infection in PAM

Given that Jagged1 is the most up-regulated ligand and contributes to inflammatory responses during HP-PRRSV infection, we next sought to determine mechanisms responsible for the induction of Jagged1 during HP-PRRSV infection. Previous studies have

reported that NF- κ B, MAPK and Notch signaling contribute to the regulation of expression of Jagged1 (Foldi et al., 2010; Tsao et al., 2011). Coincidentally, except Notch signaling, NF- κ B and MAPK have been reported to regulate PRRSV-induced inflammation (Bi et al., 2014; Du et al., 2016; Liu et al., 2017; Yu et al., 2017). Thus, we investigated the role of these signaling in the induction of Jagged1 during HP-PRRSV infection. First, we dissected effect of NF- κ B on the induction of Jagged1 by treatment of Bay11-7802, a NF- κ B inhibitor. In comparison to the vehicle-treated group, Bay11-7802 treatment significantly reduced expression of Jagged1 (Fig. 6A) by the infected PAM. Interestingly, Bay11-7802 treatment slightly trended to increase PRRSV infection in PAM compared with Vehicle-treated control (Fig. S3). Furthermore, we used the specific inhibitors including SP600125 (a JNK inhibitor), SB203580 (a p38 inhibitor), and PD98059 (a MEK inhibitor) to determine the role of MAPK in induction of Jagged1. Inhibition of JNK, p38, or MEK significantly diminished expression of Jagged1 (Fig. 6B) without inhibition of viral replication in comparison to the vehicle-treated group (Fig. S3). Lastly, we determined the role of Notch signaling in the induction of Jagged1 by DAPT treatment and RBP-J RNAi, respectively. Consistently, both DAPT treatment and RBP-J RNAi reduced expression of Jagged1 in comparison to control (Fig. 6C and D). Overall, these results reveal that HP-PRRSV infection affected Jagged1 expression, in part, through NF- κ B, MAPK and Notch signaling.

4. Discussion

This study provides novel information regarding the expression profile of Notch molecules and the effects of Notch signaling on HP-PRRSV infection in PAM. We demonstrate that HP-PRRSV infection induces expression of the target gene *Hes1*, consistent with activation of Notch signaling which is associated with induction of Notch ligands including Jagged1, Dll3 and Dll4 as well as the transcription factor RBP-J but interestingly, not the Notch receptors. We further establish that the effects of Notch signaling on the regulation of inflammatory response but not viral replication in PAM during HP-PRRSV infection. By targeting three different signaling molecules, we show that: 1) blockade of γ -secretase by DAPT inhibits production of TNF- α and IL-1 β but not viral replication; 2) the knockdown of RBP-J reduces production of TNF- α and IL-1 β but not viral replication; 3) the knockdown of Jagged1, the most robust induced ligand attenuates expression of TNF- α and IL-1 β but not viral replication. Moreover, we demonstrate that NF- κ B, MAPK and Notch signaling are involved in the regulation of Jagged1 in PAM during HP-PRRSV. Collectively, our data identify Notch as an important signaling pathway that could contribute to the regulation of inflammatory response induced by HP-PRRSV infection.

Our results are supported by previous findings (Badaoui. et al., 2014), in which we demonstrated that HP-PRRSV infection induces robust expression of Jagged1 in PAM. Except for Jagged1, we also found the induction of Dll3 and Dll4 in HP-PRRSV infected PAM. In comparison, Jagged1 is the most induced ligand among those three, while none of the receptors are induced and Notch1 is somewhat downregulated by HP-PRRSV infection. Furthermore, we demonstrated that HP-PRRSV infection significantly upregulates the Notch target gene *Hes1*, consistent with activation of Notch signaling. Thus, our data show that HP-PRRSV infection could activate Notch signaling which associated with the induction of certain ligands, especially upregulation of Jagged1.

Furthermore, inhibition of Notch signaling by treatment of DAPT and the knockdown of RBP-J, respectively profoundly attenuated production on the inflammatory cytokines while there was no effect on the viral replication. Consistently, the induction of *Hes1* was inhibited either by the blocking of Notch signaling with DAPT treatment or the knockdown of RBP-J. Previous studies by using human and mouse macrophages have shown that induction of Jagged1 under stimulation of TLR ligands regulates activation of Notch signaling and promotes expression of inflammatory cytokines (Foldi et al., 2010). In this study, we demonstrate that induction of Jagged1 in porcine alveolar macrophages during HP-PRRSV infection not only contributes to activation of Notch signaling, but also induction of the inflammatory cytokines. Thus, our results reveal that activation of Notch signaling contributes to production of the inflammatory cytokines instead of the viral replication in PAM during HP-PRRSV infection.

Inflammation induced by HP-PRRSV infection contributes to the development of pneumonia (Liu et al., 2010; van Reeth et al., 1999). In pig lungs, alveolar macrophages are the primary target cells of PRRSV. Then, the infected alveolar macrophages robust produce the inflammatory cytokines like TNF- α and IL-1 β . It has been shown that several crucial signaling including NF- κ B and MAPK involve in regulation of inflammatory response to HP-PRRSV infection (Bi et al., 2014; Du et al., 2016; Liu et al., 2017; Yu et al., 2017). Here our study by using the *in vitro* cell model demonstrates that Notch signaling triggered by the adjacent cell interaction, plays an important role in the regulation of inflammatory cytokine expression during HP-PRRSV infection. *In vivo*, it should not be excluded that induction of Notch ligands in porcine alveolar macrophages by HP-PRRSV infection could trigger Notch signaling by binding to receptors in neighbor cells of pig lungs including macrophages themselves, T cells, epithelial cells and so on. Since absence of Notch signaling has not affected viral containment by PAM, our data favor the hypothesize that Notch pathway activation could be important to lung inflammation and the development of pneumonia induced by HP-PRRSV infection, rather than viral control. If this hypothesis is confirmed by future studies, blockade of Notch signaling could be beneficial to host during HP-PRRSV infection.

Although NF- κ B has been implicated in regulation of Jagged1 expression, previous study highlighted a difference in expression of Jagged1 between human and mouse macrophages under TLR stimulation. In mouse macrophages, TLR-induced Jagged1 is largely independent of NF- κ B; in contrast, induction of Jagged1 in human macrophages is dependent of NF- κ B (Foldi et al., 2010). Here we demonstrate that NF- κ B promotes Jagged1 expression in PAM in response to HP-PRRSV infection. Because HP-PRRSV infection and TLR stimulations do not represent equivalent conditions, we cannot conclude that Human and porcine macrophages have similar mechanisms for regulation of Jagged1; however, TLR stimulation by LPS results in the major NF- κ B activation. In this respect PAM regulate Jagged1 expression similarly to human macrophages and not like mouse macrophages. Future studies using identical set of conditions will be needed to definitively address this point.

In summary, our study for the first time demonstrates that HP-PRRSV infection activates Notch signaling in porcine alveolar macrophages, which is associated with induction of

Notch ligands. Furthermore, we provide the evidences to demonstrate that Notch signaling plays a crucial role in the regulation of inflammatory cytokine expression instead of viral replication, in porcine alveolar macrophages during HP-PRRSV infection. Overall, this study could provide a novel mechanism for understanding immunopathogenesis induced by HP-PRRSV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Expression profile of Notch molecules in porcine alveolar macrophages (PAM) during HP-PRRSV infections. PAM were infected with HP-PRRSV (MOI 1) for 24 h, then real-time qPCR was performed and the expression levels of Notch receptors (A), Notch ligands (B), the transcription factor *RBP-J* (C), and the target gene *Hes1* (D) were evaluated. Relative expression is normalized to *GAPDH*. Fold induction as compared to mock-infected samples, the dotted line is representative of 1 fold for mock samples. Data are shown as mean (SD) with n = 5–8 pigs per group. * < 0.05; ** < 0.01.

Fig. 2.

Effect of Notch signaling on viral replication in PAM during HP-PRRSV infection. PAM were pretreated with DAPT (10 μ M) for 1 h, and then infected with HP PRRSV (MOI 1) for 24 h with DAPT (10 μ M). Supernatants were harvested and tittered by TCID₅₀ assay (A). Cells were harvested, and then the expression of the target gene *Hes1* was evaluated by qPCR (B). Furthermore, PAM were transfected with RBP-J siRNA and negative control (NC) siRNA. After 48 h, cells were infected with HP-PRRSV (MOI 1) for 24 h. Supernatants were harvested and tittered by TCID₅₀ assay (C). Cells were harvested, then expression of the target gene *Hes1* was evaluated by qPCR (D). Data are shown as mean (SD) with n = 3–8 pigs per group. NS, not statistically significant; * < 0.05; ** < 0.01.

Fig. 3.

Blockade of Notch signaling with DAPT treatment inhibits expression of the inflammatory cytokines induced by HP-PRRSV infection. PAM were pretreated with DAPT (10 μ M) for 1 h, then infected with HP-PRRSV (MOI 1) for 24 h with DAPT (10 μ M). Supernatants were harvested, and the cytokine levels were measured by using ELISA (A and B). Cells were harvested, and then the expression of the inflammatory cytokines was evaluated by qPCR (C and D). Data are shown as mean (SD) with n = 3–8 pigs per group. * < 0.05; ** < 0.01.

Fig. 4.

The knockdown of RBP-J inhibits expression of the inflammatory cytokines induced by HP-PRRSV infection. PAM were transfected with RBP-J siRNA and negative control (NC) siRNA. After 48 h, cells were infected with HP-PRRSV (MOI 1) for 24 h. Supernatants were harvested, then the cytokine levels were measured by ELISA (A and B). Cells were harvested, then expression of the inflammatory cytokines was evaluated by qPCR (C and D). Data are shown as mean (SD) with $n = 3-8$ pigs per group. * < 0.05 ; ** < 0.01 .

Fig. 5. Effect of induced Jagged1 on activation of Notch signaling, viral replication, expression of the inflammatory cytokines in PAM during HP-PRRSV infection. PAM were transfected with Jagged1 siRNA and negative control (NC) siRNA. After 48 h, cells were infected with HP-PRRSV (MOI 1) for 24 h. Supernatants were harvested, then the viruses were titrated by TCID₅₀ assay (A) and the inflammatory cytokines (TNF- α and IL-1 β) at protein level were analyzed by ELISA (C and E, respectively). Cells were harvested, then expression of the target gene *Hes1* (B), TNF- α (D) and IL-1 β (F) was evaluated by qPCR. Data are shown as mean (SD) with n = 3–10 pigs per group. NS, not statistically significant; * < 0.05; ** < 0.01; *** < 0.001.

Fig. 6.

Effect of the NF- κ B, MAPK and Notch signaling on induction of Jagged1 in PAM during HP-PRRSV infection. PAM were pretreated with DAPT (10 μ M), Bay11-7082 (1 μ M), SP600125 (10 μ M), SB203580 (10 μ M) or PD98059 (10 μ M) for 1 h, then infected with HP-PRRSV (MOI 1) for 24 h in the presence of those inhibitors or vehicle. Cells were harvested, then expression of Jagged1 was evaluated by qPCR. To note that treatment with the Bay11-7082 (A), SP600125 (B), SB203580 (B), PD98059 (B) and DAPT (C) inhibited expression of Jagged1 induced by HP-PRRSV infections in comparison to vehicle-treated and infected cells. Furthermore, (D) qPCR analysis showed that inhibition of Notch signaling by the knockdown of RBP-J inhibited expression of Jagged1 compared with that in siNC-infected cells. The samples were prepared as shown in Fig. 2. Data are shown as mean (SD) with n = 3–7 pigs per group. * < 0.05; ** < 0.01; *** < 0.001.