The glycosyltransferase ST3GAL2 modulates virus proliferation and the inflammation response in porcine reproductive and respiratory syndrome virus infection

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Received: 26 January 2021 / Accepted: 10 May 2021 / Published online: 28 July 2021
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Abstract
β-galactoside α-2,3-sialyltransferase 2 (ST3GAL2) is a member of the sialyltransferase family that mediates terminal modification of glycoproteins and glycolipids. ST3GAL2 has been found to play a role in obesity, aging, and malignant diseases. In this study, we cloned porcine ST3GAL2 (pST3GAL2) from porcine alveolar macrophages (PAMs), and its role in porcine reproductive and respiratory syndrome virus (PRRSV) infection was investigated by transcriptome analysis. pST3GAL2 was found to be located in the Golgi apparatus, and it was expressed at high levels in PRRSV-infected PAMs. Overexpression of pST3GAL2 resulted in a slight increase in PRRSV proliferation, and the interaction between pST3GAL2 and GP2a of PRRSV was detected by coimmunoprecipitation and confocal microscopy. The expression of pro-inflammatory cytokines (IFN-β, IL-2, IL-6, IL-18, IL-1β and TNF-α) was significantly inhibited in pST3GAL2-overexpressing, PRRSV-infected cells and upregulated in PRRSV-infected pST3GAL2-knockout cells, while the pattern of expression of anti-inflammatory cytokines (IL-4 and IL-10) was diametrically opposite. Our results demonstrate that the regulation of pST3GAL2 plays an important role in PRRSV proliferation and functional alterations in virus-infected cells. These results contribute to our understanding of the role of β-galactoside α-2,3-sialyltransferase 2 in antiviral immunity.

Introduction
β-galactoside α-2,3-sialyltransferase 2 (ST3GAL2) is a sialyltransferase (ST) that transfers sialic acid from the activated donor CMP-sialic acid to the glycan terminus of a glycolipid or glycoprotein [1]. STs can be classified into four families according to the carbohydrate linkage they synthesize: the ST3Gal (α2,3-ST), ST6Gal (α2,6-ST), ST6GalNAc, and ST8Sia (α2,8-ST) [2]. Each of these families can be further classified into multiple subfamilies. ST3Gal has six different subfamilies, ST3Gal I-VI, which have been reported to play important roles in many biological processes, including cell signalling, cellular recognition, cell-cell and host-pathogen interactions, and metastasis of cancer [3].

Glycosylation, the addition of glycan units to the proteins and lipids, is a critical co-/post-translational modification that occurs in all eukaryotic cells [4–6]. Sialylation is one of the most important types of glycan processing, since sialic acid (Sia or Neu5Ac) is the outermost sugar of glycan chains. ST3GAL2, which contains two putative N-glycosylation sites (Asn92 and Asn211), is mainly responsible for GD1a and GT1b ganglioside biosynthesis in the brain [7]. Glycosylation of viral proteins is a common phenomenon [8, 9]. Glycoproteins are necessary for the proliferation of many viruses, recognition of host cells [10], and fusion of the viral envelope with the host cell membrane [11].

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped single-stranded positive-strand RNA [12, 13]. PRRSV infection often causes acute reproductive failure in sows and dyspnea in piglets, resulting in substantial economic losses each year [14]. The PRRSV genome is 15 kb long and contains eight open reading frames (ORFs). ORF1 encodes the viral non-structural proteins. ORF2-ORF7 encode structural proteins, and the expression
products of ORF2-5 are glycosylated envelope proteins (glycopolyprotein [GP]2-5). ORF6 and ORF7 encode the matrix protein (M) and the nucleocapsid protein (N) [15]. GP2a is one of the smallest structural proteins, composed of 256 amino acids [16], but it is important for virion structure and plays an important role in the process of cell entry and proliferation of PRRSV [17, 18]. Although the function of ST3GAL2 in many different cellular processes has been investigated, it remains unknown whether it regulates the proliferation of the enveloped virus. In this study, we cloned the porcine ST3GAL2 (pST3GAL2) gene from porcine alveolar macrophages. The effect of pST3GAL2 on the proliferation of PRRSV was also investigated. This study provides a basis for understanding the function of GP2a protein glycosylation in viral replication and activation of immune responses.

Materials and methods

Cells, viruses, and antibodies

The porcine alveolar macrophage (PAM) cell line 3D4/21 was cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Biological Industries) and antibiotic-antimycotic solution. Human embryonic kidney (HEK) 293T cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% FBS, and an antibiotic-antimycotic mixture of 100 mg of streptomycin, 100 IU of penicillin, and 50 U of amphotericin B per ml. Both cell lines were maintained in a humidified 5% CO2 incubator at 37°C. A stock of PRRSV strain JXwn06 with a titer of 10^4 PFU/ml was used in this research. Anti-ST3GAL2 polyclonal antibodies were prepared by multiple immunizations of BALB/c mice with recombinant His-ST3GAL2 combined with mineral oil adjuvant. Monoclonal antibodies against PRRSV nsp2 were donated by Prof. Jun Han of China Agricultural University. Labeled antibodies used in the experiments were purchased from Cell Signaling Technology (CST). The internal reference antibody and secondary antibodies were purchased from Invitrogen.

Cloning of the complete porcine ST3GAL2 CDS

A pair of primers (Table 1) was designed based on the coding sequence (CDS) of the predicted pST3GAL2 sequence (NCBI reference sequence: XM_021093801.1). Total RNA was extracted from PAM cells using TRIzol Reagent (TaKaRa, China) according to the manufacturer’s instructions. RNA was reverse transcribed using a reverse transcription kit (TaKaRa). The complete CDS of porcine ST3GAL2 was obtained by RT-PCR amplification.

Plasmid construction

The pFlag-CMV2-ST3GAL2 and pMyc-CMV2-ST3GAL2 eukaryotic expression constructs were made using specific primers pairs (Table 1) containing sequences from the vector. These were used to amplify the ST3GAL2 gene, which was ligated into the pFlag-CMV2 and pMyc-CMV2 vectors using a one-step cloning kit (Vazyme, Nanjing, China).

Determination of virus titer

The virus titer was determined by the 50% tissue culture infective dose (TCID50) method. 3D4/21 cells were seeded in 96-well plates (1×10^4 cells/well), and after the cells adhered to the plate and had grown to about 50% confluence, they were incubated with serial 10-fold dilutions (100 µl) of the primary PRRSV stock, with two replicates of each dilution. (1-10^-6) was inoculated into the cells. Mock-infected cells were used as controls. The cells were incubated at 37°C for 7 days and TCID50 values were calculated by the Reed-Muench method.

Transcriptome sequencing and analysis

3D4/21 cells were grown in 6-well plates at an initial concentration of 10^6 cells/ml and were then inoculated with PRRSV at an MOI of 0.5 for 24 h. Virus-infected cells were washed twice with cold PBS and 1 ml of TRIzol Reagent was added. The treated cells were sent to the

Table 1 Primers used in amplification and plasmid construction

| Primer           | Accession no. | Primer sequence (5′-3′) | Product size | Use          |
|------------------|---------------|-------------------------|--------------|--------------|
| CMV2-ST3GAL2-F   | XM_021093801.1| CCAGTCTGACTCTAGA        | 1053 bp      | Flag-ST3GAL2 |
|                  |               | GGATCCATGAA             |              | Myc-ST3GAL2  |
|                  |               | TGCTCCCTGCGG            |              |              |
|                  |               | GTGT                    |              |              |
| CMV2-ST3GAL2-R   |               | CAGGGATGCCCACCAGCG      |              | Flag-ST3GAL2 |
|                  |               | GGATCCCATGTTCGAGCGTC    |              | Myc-ST3GAL2  |
|                  |               | TCGGTTGAGACCCCTCG       |              |              |

a Italics indicate restriction sites
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Guangzhou Gene Denovo Biotechnology Company for transcriptional sequencing. The obtained transcriptome data and a heat map of differentially expressed genes were analyzed using online software (https://software.broadinstitute.org/morpheus/).

Quantitative reverse transcription PCR (RT-qPCR)

Primers for quantitative real-time PCR were designed based on known sequences in the GenBank database, and their sequences are shown in Table 2. qPCR was carried out to determine the transcription levels of specific genes, using DBI Bioscience-2043 Bestar Sybr-Green qPCR master mix on an ABI 7500 Real-Time PCR System. The relative quantities of mRNA were calculated by the 2^ΔΔCT method, and the data were normalized to the expression level of the β-actin gene. The reported values were from three independent tests.

Confocal immunofluorescence

3D4/21 cells grown in 12-well plates until the cell density was about 50% and then transiently transfected with Golgi marker and ST3GAL2-Myc plasmids using polyetherimide (Sigma), following the manufacturer’s protocol. At 24 h posttransfection, the cells were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.3% Triton X-100 at room temperature for 15 min. The samples were incubated with 5% bovine serum albumin (BSA) for 1 hour and then incubated with 1:1000-diluted mouse Myc primary antibody at 4 °C overnight. Samples were incubated with PE-labeled fluorescent secondary antibody diluted 1:500 at room temperature in the dark for 1 h, followed by incubation with DAPI at room temperature in the dark for 10 minutes. After mounting the cells on a microscope slide with 50% glycerol, the localization of fluorescence was observed using a confocal microscope (Olympus).

Western blot analysis

After 24 h post-transfection, the cells were lysed with cell lysis buffer (Solarbio) and boiled in loading buffer for 10 min, and the proteins were separated by 12% SDS-PAGE and transferred to the PVDF membrane (Millipore). Membranes were blocked with 5% skimmed milk in 1×TBST (Tris-buffered saline with 0.05% Tween-20) for 1 h and incubated overnight at 4 °C with an antibody against pST-3GAL2 (1:500), PRRSV Nsp2 (1:2,000), β-actin (1:5,000) or with labeled antibodies (1:5,000). This was followed by washing and incubation with HRP-conjugated antibody for

Table 2 Primers used in quantitative real-time PCR

| Gene       | Accession no. | Primer sequence (5'-3')                                      | Product (bp) | Tm (°C) |
|------------|---------------|-------------------------------------------------------------|--------------|---------|
| IL-1β      | NM_214055.1   | GTCATCGTGCGCAGTGGAGAA GGTGAGGCCTTTCAGCATGT                 | 253          | 57      |
|            |               | GGTGAGGCCTTTCAGCATGT                                        |              |         |
| IL2        | NM_213861.1   | TACATGCCCAAGCAGGACTAC TAGACATTCTTTATACACAGTC               | 216          | 60.1    |
|            |               | TACATGCCCAAGCAGGACTAC TAGACATTCTTTATACACAGTC               |              |         |
| IL-6       | NM_214399.1   | GCTGTTCTGTGGATGGGCTA GCCAGTACCTCCCTTGCTGT                  | 294          | 58.3    |
|            |               | GCTGTTCTGTGGATGGGCTA GCCAGTACCTCCCTTGCTGT                  |              |         |
| IL-18      | NM_213997.1   | GCTGTGAAACCGGGAGCACAA AAACACCTGTTAGTGGTCTCCTT             | 192          | 60.8    |
|            |               | GCTGTGAAACCGGGAGCACAA AAACACCTGTTAGTGGTCTCCTT             |              |         |
| IFN-β      | NM_001003923.1| GGACGCAAGCCATTGGGATGT TGACGGTTTCATCAGCAGCA                 | 219          | 59.8    |
|            |               | GGACGCAAGCCATTGGGATGT TGACGGTTTCATCAGCAGCA                 |              |         |
| TNF-a      | NM_214022.1   | TGGCCCAAGGACTCACTACAA TCTTTCAGCTTCAGCAGGTT                 | 142          | 60.5    |
|            |               | TGGCCCAAGGACTCACTACAA TCTTTCAGCTTCAGCAGGTT                 |              |         |
| IL4        | NM_214123.1   | ACACAATGCGACACATACCT TTCAATGCGTGTCGA                      | 189          | 60.0    |
|            |               | ACACAATGCGACACATACCT TTCAATGCGTGTCGA                      |              |         |
| IL10       | NM_214040.1   | GCCCTTGAAACACAGGCCCA AAACACCTGTTAGTGGTCTC CTCTTCGCGGGCA   | 137          | 57.6    |
|            |               | GCCCTTGAAACACAGGCCCA AAACACCTGTTAGTGGTCTC CTCTTCGCGGGCA   |              |         |
| IL13       | NM_001113695  | CTGACCCAGACCATCAGA TCCGTTGGCGAAAAATCATCC                | 219          | 60.0    |
|            |               | CTGACCCAGACCATCAGA TCCGTTGGCGAAAAATCATCC                |              |         |
| PRRSV-N    | JX317649.1    | GCCCAAGAAAAACAGTACCC GGCAAGCCAGAAACTAAC                      | 256          | 58.4    |
|            |               | GCCCAAGAAAAACAGTACCC GGCAAGCCAGAAACTAAC                      |              |         |
| ST3GAL2    | XM_021093801.1| CTTCTCCACACGGGACATCGCG GCCGAATTCCTCCCCAGATGCGTG            | 254          | 58.6    |
|            |               | CTTCTCCACACGGGACATCGCG GCCGAATTCCTCCCCAGATGCGTG            |              |         |
| β-actin    | DQ452569.1    | GAACTCTGCAGGACATCCACGA CTGCGGTACTCTCCTGCTGCT              | 230          | 55.0    |
|            |               | GAACTCTGCAGGACATCCACGA CTGCGGTACTCTCCTGCTGCT              |              |         |
1 h at room temperature. Immunodetection was done using Pierce ECL Western Blotting Substrate (Thermo Scientific).

**Immunoprecipitation**

3D4/21 cells were cultivated in 60-mm plates and transfected with GP2a-Myc and Flag-ST3GAL2 plasmids. At 48 h post-transfection, RIPA containing 1% protease inhibitor (PMSF) was added for cell lysis. The cell lysates were incubated overnight at 4 °C with mouse anti-Flag agarose beads, washed three times with lysis buffer for 10 min each, and boiled for 5 min with protein loading buffer. The proteins bound to the beads were separated by SDS-PAGE and analyzed by Western blot.

**RNA interference**

To analyze the effects of knockdown of ST3GAL2 on PRRSV replication and cytokine expression, an siRNA assay was carried out using an siRNA against B4GALT5 (siB4GALT5) and a negative control (NC), which were synthesized by GenePharma (Tianjin, China; Table 3). 3D4/21 cells were seeded in 12-well plates (60–80% confluence) and transfected with siRNA (siST3GAL2) or NC at a final concentration of 50 nM using Lipofectamine 3000 (Invitrogen). The cells were infected with PRRSV at an MOI of 0.5 and harvested after 24 h. The gene expression levels were determined by quantitative real-time PCR (qRT-PCR) and Western blotting.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism software, and the significance of each gene in each group was analyzed by the two-way ANOVA method. Results are shown as the mean ± standard deviation. *P < 0.05* (*) indicates a statistically significant difference.

| Table 3 Primers used in small interfering RNA assay |
|---------------------------------------------------|
| siRNA | Abbreviation | Primer sequence (5′-3′) |
|--------|--------------|------------------------|
| Negative control | NC | F: UUCUCGGAACGUGUCAGCUTT |
| | | R: CACUGACAGUUGGGAGATT |
| siST3GAL2-2 | Sus-662 | F: CCCCUGGGACCUGUGUGGAGUTT |
| | | R: AUCCAAUAGUGCUAGGTT |

**Results**

**Upregulation of ST3GAL2 expression in 3D4/21 cells by PRRSV infection**

The RNA expression profiles of PRRSV-infected 3D4/21 cells were analyzed by high-throughput RNA sequencing (RNA-Seq), and the role of glycosyltransferase-associated genes on the pathogenesis of PRRSV was evaluated (Fig. 1A). We found that ST3GAL2 was the most strongly upregulated of the glycosyltransferase genes after PRRSV infection (Fig. 1A). To validate the results of the RNA-Seq data mining, pST3GAL2 mRNA from 3D4/21 cells infected with PRRSV at an MOI of 0.5 was detected by qRT-PCR. The results showed that the mRNA level of pST3GAL2 was elevated at 12 and 24 h after PRRSV infection (Fig. 1B), indicating that PRRSV infection led to upregulation of pST3GAL2. The changes in pST3GAL2 expression levels suggest its potential involvement in the proliferation of PRRSV.

**Localization of porcine ST3GAL2 in the Golgi apparatus**

It has been reported that many glycosyltransferases that are present within subcompartments of the Golgi are inextricably linked to the mechanisms that cells employ to direct the flow of proteins and lipids within this organelle [19]. To investigate the location of porcine ST3GAL2, 3D4/21 cells were co-transfected with recombinant plasmids (GFP-Golgi, GEP-LAME, or GFP-ER marker protein and ST3GAL2-Myc), as described in Materials and methods. The results showed that the fusion protein ST3GAL2-Myc co-localized with a Golgi marker protein (Fig. 2).

**Effect of ST3GAL2 on PRRSV proliferation**

To examine whether pST3GAL2 affects PRRSV proliferation, we transfected 3D4/21 cells with Myc-ST3GAL2 and then infected the cells with PRRSV at an MOI of 0.5. qPCR showed that pST3GAL2 overexpression resulted in increased transcription of the PRRSV N gene (Fig. 3A). The level of Nsp2 protein observed in a Western blot assay was significantly higher in PRRSV-infected cells overexpressing pST3GAL2 (Fig. 3B). Compared to the control sample, the virus titer in pST3GAL2-transfected cells was also higher (Fig. 3C), suggesting that the upregulation of pST3GAL2 contributed to the proliferation of PRRSV in 3D4/21cells. To further examine the influence of pST3GAL2 on PRRSV proliferation, a small-interfering RNA assay for silencing pST3GAL2 expression was performed. qPCR and Western blot analysis revealed that pST3GAL2 expression was inhibited significantly by pST3GAL2 siRNA (Fig. 3D and E).
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mRNA expression of the PRRSV N gene was significantly downregulated in siRNA-transfected cells (Fig. 3F). Western blot analysis showed similar results for the expression of the PRRSV Nsp2 protein (Fig. 3G). The virus titers were also significantly reduced (Fig. 3H), indicating that silencing of pST3GAL2 inhibited the proliferation of PRRSV in 3D4/21 cells.

**Interaction of porcine ST3GAL2 with GP2a of PRRSV**

Virus-receptor interactions are critical for viruses to successfully invade host cells. Sialylated glycans present on the apical surface of host cells can function as receptors for viral proteins [20, 21]. However, the relationship between pST3GAL2 and PRRSV structural proteins is unclear. Confocal immunofluorescence was carried out to verify the co-localization of pST3GAL2 and PRRSV structural proteins (GP2a, GP3, GP4 and GP5). The images showed that pST3GAL2 and GP2a co-localized in the cytoplasm, but no obvious co-localization was observed with GP3, GP4 and GP5 (Fig. 4A). Co-immunoprecipitation (co-IP) was also carried out to verify the interaction between pST3GAL2 and GP2a. Expression plasmids encoding individual Myc-tagged GP2a proteins were co-transfected with plasmids encoding Flag-tagged pST3GAL2, and the results indicated that GP2a interacts with pST3GAL2 (Fig. 4B).

**Effects of porcine ST3GAL2 on the expression of inflammatory factors**

During PRRSV infection, the secretion of many inflammatory factors can affect virus proliferation [22, 23]. Therefore, we investigated whether ST3GAL2 can also affect PRRSV proliferation by regulating inflammatory factors. 3D4/21 cells were transfected with the Myc-ST3GAL2 plasmid or pST3GAL2 siRNA and then infected with PRRSV at an MOI of 0.5, and the expression of inflammatory factors produced by macrophages was analyzed by qRT-PCR. The results showed that ST3GAL2 inhibited the expression of pro-inflammatory factors involved in immune regulation (Fig. 5A and B) by promoting their expression in PRRSV-infected cells (Fig. 5C and D). This suggests that ST3GAL2 inhibits the inflammatory response by regulating the expression of inflammatory factors to promote PRRSV proliferation.
Discussion

Glycosyltransferases are a large family of enzymes that are responsible for glycosylation of proteins, and viruses exploit the host-cell machinery to glycosylate their proteins during replication [24]. ST3GAL2 transfers sialic acid preferentially to the three positions of galactose on the Galβ1-3GalNAc terminus of the gangliosides GM1 and GD1b to synthesize GD1a and GT1b, respectively. Deletion of ST3GAL2 might be involved in the development of late-onset obesity and insulin resistance [1]. Expression of ST3Gal-II transgenes induces abnormal protein glycosylation, suggesting that ST3GAL2 might be involved in the development of late-onset obesity and insulin resistance [1]. Expression of ST3Gal-II transgenes induces abnormal protein glycosylation, suggesting that ST3GAL2 might be involved in the development of late-onset obesity and insulin resistance [1]. 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Up to now, most studies of ST3GAL2 have concentrated on humans and mice, and no studies on pigs (Sus scrofa) were available.

The glycoproteins of enveloped viruses utilize glycosyltransferases for their glycosylation in the host cell. To explore the relationship between ST3GAL2 and the proliferation of enveloped viruses, we measured the transcription levels of ST3GAL2 in PRRSV-infected 3D4/21 cells. As shown in Figure 1A and B, PRRSV infection upregulated ST3GAL2 expression in 3D4/21 cells, suggesting that ST3GAL2 might be involved in the regulation of viral proliferation in 3D4/21 cells.

Glycoprotein (GP) 2a is a minor structural protein of PRRSV and is important for binding cell receptors [26]. GP2a and GP4 of PRRSV serve as the viral attachment proteins that are responsible for mediating interactions with CD163 for virus entry into the susceptible host cell [27]. The GP2a protein is incorporated into virions as a heteromultimeric complex together with the GP3, GP4 and E proteins, and this complex is required to render particles infectious [28].
Moreover, GP2a has a conserved glycosylation site in different isolates that may play a role in virus particle production [29]. In this study, we investigated whether ST3GAL2 modifies GP2a and has an antiviral effect. The experimental results (Fig. 2 and 4) show that ST3GAL2 interacted with GP2a and was located in the Golgi apparatus. It has been reported that the PRRSV virion is assembled in the cytoplasm and buds into the Golgi to obtain its envelope, which includes GP2a [30]. While the N-linked glycans normally present on the GP2a protein are not essential for particle formation [29], several studies have shown that the oligosaccharides can shield critical epitopes from immune recognition [31, 32]. Such an effect might explain why no monoclonal antibodies have been obtained against the PRRSV GP2a protein. Therefore, it has been speculated that GP2a might accumulate in the Golgi and be galactosylated by ST3GAL2. Macrophages are widely distributed innate immune cells that play diverse roles in various physiological and pathological processes [33]. During infection, a variety of cytokines are secreted by macrophages to enhance the phagocytosis and mediate inflammation to exert antiviral effects [28].

Macrophages can thus have pro- and anti-inflammatory properties, depending on the disease stage and the signals they receive [34]. Most pro-inflammatory factors, including interferon (IFN-α), interleukin (IL-1β, IL-6, IL-18), and tumor necrosis factor (TNF-α), are glycoprotein molecules [35]. These molecules can be glycosylated by glycosyltransferases, which affect the corresponding biological functions in macrophage-mediated immunity [34]. Anti-inflammatory factors, including interleukins (IL-4, IL-10, IL-33), can be sialylated. The sialylation of Fc glycans can cause a transformation of IgG to a form that inhibits inflammation [36]. Otherwise, the sialylated Fc selectively binds to type II FcR, inducing the production of IL-33 in regulatory macrophages [37]. Th2 helper T cells may be activated by IL-33, resulting in the release of anti-inflammatory cytokines [38]. Our experimental results suggested that the transcription levels of pro-inflammatory cytokines secreted by macrophage cells had different degrees of downregulation in 3D4/21 cells overexpressing Flag-B4GALT5 that were then infected with PRRSV at an MOI of 0.5, while there was an opposite effect on anti-inflammatory cytokines (Fig. 5). Therefore, we
speculated that, in the process of viral infection, ST3GAL2 downregulates the expression of pro-inflammatory factors (IL6, IL-18, IL-1β, IFN-β and TNF-α) to promote viral infection while upregulating the expression of anti-inflammatory factors (IL-4, IL-10 and IL-13) to resist viral infection. However, β-galactose-α-2,3-sialyltransferases might be involved in the sialylation of glycoproteins. The regulatory mechanism needs to be verified by analyzing intermediate regulatory factors such as Fc receptors or related transcription factors in its pathway. All of these speculations need to be verified by experiments.

In general, studies on β-galactose-α-2,3-sialyltransferases will enhance our understanding of the glycosylation of proteins. However, more challenges will be faced. The process of glycosylation modification is dynamic [39, 40], and the connection of glycans is varied [41]. Although there has been substantial research on the role of β-galactose-α-2,3-sialyltransferases in tumor formation and migration [3, 42], other functions of α-2,3-sialyltransferases are less well understood. With the continuous advancement in research on inflammatory responses and immune regulation, we hope that the important function of β-galactose-α-2,3-sialyltransferases will be explored.
Fig. 5 Porcine ST3GAL2 regulates transcription of inflammatory factors in PRRSV-infected 3D4/21 cells. (A and B) Inhibition of pro-inflammatory factors and promotion of anti-inflammatory factors by overexpression of ST3GAL2 in 3D4/21 cells. PRRSV-infected, ST3GAL2-overexpressing 3D4/21 cells were collected at 24 h post-infection, and mRNA levels of pro- and anti-inflammatory factors produced by macrophages were measured by qRT-PCR. (C and D) Promotion of the transcription of pro-inflammatory factors and suppression of the transcription of anti-inflammatory factors by silencing of ST3GAL2. 3D4/21 cells were transfected with 50 nM of ST3GAL2 siRNA or negative control siRNA, and the expression levels of pro- and anti-inflammatory factors were examined at 48 h post-transfection by qRT-PCR. Differences were taken to be statistically significant if the P-value was less than 0.05 (*, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001).
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