The Glycosyltransferase ST3GAL2 Modulate the Proliferation and Inflammation Response in PRRSV Infection

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Abstract

The viral proliferation process of envelop viruses including invasion, assembly, and release process is impacted by glycosylation. Sialic acid modification mainly exists in the terminal glycosylation of proteins and lipids. In this study, the effect of porcine sialylglycosyltransferase (ST3GAL2) on virus proliferation, as well as regulation of inflammatory factor in PRRSV-infected 3D4/21 cell model were analyzed. The expression of ST3GAL2 was up-regulated in PRRSV-infected 3D4/21 cells. The expression of ST3GAL2 that promote the proliferation of PRRSV was determined by overexpression and interference experiment. The ST3GAL2 localizes to Golgi apparatus and interacts with PRRSV structural protein GP2a was verified by confocal microscopy and co-immnoprecipitation array, which may play roles in the assembly of PRRSV envelope and virus proliferation. The expression of ST3GAL2 also impacts the expression of inflammatory cytokines, which regulate cellular immunity and promote the immune escape of PRRSV from innate immunity. Our results highlighted a potential mechanism whereby ST3GAL2 promotes PRRSV proliferation and offers the anti-inflammatory role of ST3GAL2 in cellular immunity.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), is a single-stranded *positive*-sense RNA *virus* and has a surface envelope[51, 53]. PRRSV invasion mainly targets macrophages and monocytes in the blood and alveoli[16, 39]. The virus adheres to the phospholipid bimolecular membrane of the targeted cell, and binds to the corresponding receptor on the cell surface through a specific protein and enters the cell by endocytosis. Then, PRRSV removes the outer nucleocapsid and releases its own RNA nucleic acid. The host cell is used to generate a replication and transcription complex (RTC). RTC first synthesizes negative-strand full-length RNA and negative-strand sg mRNA and then synthesizes positive-strand RNA and positive-strand sg mRNA[17]. Finally, the newly synthesized RNA has viral activity through the action of the endoplasmic reticulum and nucleus and is excreted from the cell after being modified by the Golgi apparatus to obtain the surface envelope[6].

The large-scale popularity of PRRSV in the world has caused huge economic losses to the pig industry[15]. Because of its variability, it is difficult to be effectively suppressed. PRRSV itself is rich in glycosylation modification, and most of its cell surface binding proteins are glycoproteins[47], but there are few studies on PRRSV and related glycosylation enzymes. ST3GAL2 is a member of the sialyltransferase family that mediates terminal modification of glycoproteins and localizes to the Golgi of cells[14]. ST3GAL2 has been shown to affect sugar metabolism and tumor development in cells[45]. Its role in viral immunity and its mechanism is still worthy of our exploration and research. Therefore, studying the mechanism of ST3GAL2 protein in PRRSV infection is of great significance for preventing PRRS and further exploring the mechanism and scope of the ST3GAL2 gene. It also has a certain effect on the research of viral glycosylation and the healthy development of the pig industry.

Glycosylation is one of the most complex protein post-translational modifications[23]. The viral protein is also modified by glycosylation. The viral glycoprotein can mediate the entire infection process of its recognition, binding, and invasion of the host cell, and can also help the virus escape from the immune system[31].

Infected cells can also induce an immune response by recognizing glycoproteins on the surface of the viruses, producing cytokines and antibodies to kill them[2].

At the early stage of infection, the virus must attach to the cell utilizing receptors or attachment factors on the surface of the host cell[1]. In addition to attaching the virus to target cells, these early interactions are usually the key factors that determine the tropism and host range of the virus[41]. Probably because these viruses recognize glycans prominently displayed on the cell surface, sialic acid is widely used as the initial connection receptor for a large number of viruses[10]. Sialylation is a glycosylation modification at the end of the sugar chain[11]. The sialylation process is mainly mediated by the sialyltransferase (ST) family[38]. Sialyltransferase can specifically transfer the sialic acid residues on the substrate CMP-Neu5Ac to new glycosyl receptors, thereby terminally modifying the corresponding glycoproteins or glycolipids[27].

ST3Gal II belongs to the α-2,3 sialylglycosyltransferase, which is one of the four subfamily members of the sialyltransferase family[33]. It is highly expressed in the brain, and also expressed in the liver, heart, kidney, and lung. ST3Gal II has been reported to have a regulatory role in obesity and insulin resistance. ST3Gal II has high selectivity for the terminal galactose of the receptor disaccharide Galβ1-3GalNAc, has very low activity on Galβ1-3GlcNAc, and has no activity on Galβ1-4GlcNAc[32]. The absence of ST3Gal II leads to impaired systemic insulin sensitivity and reduces the ability of glucose to transfer to surrounding tissues in response to changes in glucose or insulin[37]. ST3GAL II is also considered to be related to tumors. It may promote tumorigenesis through the EGFR pathway[12]. The expression of ST3GAL II in renal cell carcinoma tissue is higher than that in non-tumor kidney tissue, suggesting that ST3GAL II may be involved in the process of tumor deterioration[40].

Both the adhesion and infection of PRRSV to PAM are dependent on the sialic acid on the surface of the viral envelope[13, 52]. In contrast, there are other types of viruses that mediate adhesion by recognizing sialic acid on the surface of host cells, such as some avian influenza virus, coronaviruses, and adenoviruses[8]. Viral proteins generally have different types of glycoproteins. Glycosylated viral proteins play an important role in many biological processes, such as identifying host cells, promoting membrane fusion, allowing viruses to enter cells, and mediating the immune escape of viruses[20]. The invasion of PRRSV virus into the host cell is carried out by the multimer on the envelope and the CD163 receptor on the host cell membrane[5]. PRRSV has four functional glycoproteins, GP2a, GP3, GP4 and GP5[9]. Among them, GP2a structural protein is one of the small structural proteins encoded by ORF2a, which is composed of 256 amino acids[44]. Previous studies have shown that the process of virus invasion into host cells is mediated by PRRSV structural protein GP2 binding to CD163 receptor on the surface of PAM[50]. GP2 can combine with two other small structural proteins, GP3 and GP4, to form a GP2-GP3-GP4 multimeric complex, and they are considered to be essential for viruses to enter susceptible host cells[30]. These proteins are all glycoproteins, suggesting that glycosylation of viral structural proteins may play an important role in the invasion and immunity of PRRSV[4].

As a member of the sialyltransferases, the role of Golgi apparatus targeted porcine ST3GAL2 in PRRSV infection remains elusive. In this study, the regulation mechanisms of porcine ST3GAL2 in PRRSV proliferation were investigated in detail.

Materials And Methods

2.1 Cells, viruses, and antibodies.

Porcine alveolar macrophage (PAM) cell line 3D4/21 and HEK-293T cells were cryopreserved in our laboratory and respectively cultured in RPMI 1640 medium and high sugar DMEM medium supplemented with 10% fetal bovine serum (FBS). Both cells were cultured in a humid 5% CO2 incubator at 37℃. The PRRSV-JXwn06 was used in our study with a titer of 10^4 PFU/ml. Anti-ST3GAL2 polyclonal antibodies were prepared by multiple immunizations of BALB/c mice with His-ST3GAL2 recombinant protein conjugated mineral oil adjuvant. Monoclonal antibodies against PRRSV nsp2 were donated by China Agricultural University. Goat anti-mouse and goat anti-rabbit secondary antibodies were purchased from Invitrogen. Labeled antibodies used in the experiments were purchased from Cell Signaling Technology (CST).

2.2 Cloning of complete porcine ST3GAL2 CDS

Design a pair of primers based on the predicted ST3GAL2 sequence as shown in (Table 1). TRIzol reagent was used to extract total RNA from cells and reverse transcriptase (TaKaRa) was used to reverse transcribe them into cDNA. Complete porcine ST3GAL2 CDS is obtained by RT-PCR amplification.

2.3 Plasmid construction

The ST3GAL2 gene was amplified using specific primer pairs with a common sequence of vectors (Table 2) and connected to pFlag-CMV2 vector and pET-28a vector using a one-step cloning kit (Vazyme, Nanjing, China). The FLAG-ST3GAL2 eukaryotic expression vector and pET-28a-ST3GAL2 prokaryotic expression vector were constructed.

2.4 Determination of virus titer

The virus titer was determined by 50% tissue culture infective dose (TCID50) method. 3D4/21 cells were seeded in 96-well plates（1×10^4 cells/well）. After the cells adhered to the wall and grew to about 50 %, PRRSV dilution（1-10^{-6}） was inoculated into the cells. The normal cells were used as a mock control. The cells were incubated at 37 °C for 7 days and TCID50 values were calculated by the Reed-Muench method.

2.5 Relative Fluorescence Quantitative PCR (RT-qPCR)

Based on the known sequence in GenBank, the primers were designed by Oligo 7.0 for the CDS region with a length of about 200bp (Table 3). TransStart Top Green qPCR SuperMix (+ Dye I / + Dye II) reagents were used to prepare the PCR system and the samples were detected by real-time fluorescence quantitative PCR instrument (ABI 7500). The relative transcription level of each gene was based on β-actin and calculated by GraphPad Prism software.

2.6 Confocal Immunofluorescence

3D4 / 21 or Hela cells were selected and seeded in a 12-well plate placed in cell slides, and cultured at 37 ℃ to a cell density of about 50%. Cells were co-transfected with Golgi-marker and ST3GAL2-MYC plasmids. After

incubation at 37 ° C for 24 h, cells in each well were fixed with 4% paraformaldehyde solution for 30 min and then permeated with 0.3% Triton-X 100 for 15 min. The samples were blocked with 5% BSA for 1 hour and then incubated with 1: 1000 diluted mouse Myc primary antibody at 4 ℃ overnight. Samples were incubated with PE-labeled fluorescent secondary antibody diluted 1: 500 at room temperature in the dark for 1 h. DAPI (10 μg / mL) was incubated at room temperature in the dark for 10 minutes. After mounting the slide with 50% glycerol, it was placed on a confocal microscope (Olympus) to observe the fluorescence localization.

2.7 Western blot analysis

Cells were lysed using RIPA cell lysate with protease inhibitor (PMSF). Cell protein samples were separated by 10% SDS-PAGE gel electrophoresis, and the protein was transferred from polyacrylamide gel to the PVDF membrane activated by soaking methanol by wet transfer method. The PVDF membrane was blocked with 5% skim milk at room temperature for 1 h. Dilute mouse-derived anti-ST3GAL2 serum antibody at a ratio of 1: 500, and incubate the PVDF membrane at 4 ℃ overnight. The rabbit anti-mouse secondary antibody was diluted 1: 5000 and incubated in a shaker at room temperature for 1 h. Immunoreactive bands were visualized with chemiluminescence detection kit (Thermo Scientific, Waltham, MA, USA).

2.8 Immunoprecipitations

3D4 / 21 cells were plated in 6-well plates at a concentration of 2×10^6 / well. After the cell density reached about 70%, GP2a-myc and Flag-ST3GAL2 were co-transfected to 3D4 / 21 cells. After culturing for 48 h, RIPA containing 1% protease inhibitor (PMSF) was added for cell lysis. Mouse anti-Flag agarose beads (beads) were added to the samples and placed at 4°Covernight. Add an appropriate amount of 5 × loading buffer and boil for 10 min to prepare protein sample. The proteins bound to the beads were separated by SDS-PAGE and determined by Western blot.

2.9 RNA interference

The interference sequence siST3GAL2 was synthesized by GenePharma (Shanghai, China). The specific sequence is shown in Table 3-2. Centrifuge the tube containing the RNA Oligo dry powder at 3000 rpm for 2 min. According to the instructions, add 1 mL of DEPC water to dilute the powder. When 3D4 / 21 cells grow to 80%, siRNA and NC control are transfected with Lipofectamine 3000 to a final concentration of 50 nmol. After 24 hours of transfection with siRNA-ST3GAL2, the PRRSV was partially inoculated, and the cells were collected and cultured for 24 hours. The cells were collected for subsequent detection experiments.

Statistical Analysis

Statistical analysis was performed by GraphPad Prism software, and the significance of each gene in each group was analyzed by two-way ANOVA method. The statistical results were shown by mean ± standard deviation (mean ± SEM). $P < 0.05$ (*) indicates statistically significant difference.

Results

3.1 ST3GAL2 localizes to Golgi apparatus and up-regulated after PRRSV infection

We analyzed the transcriptome sequencing data of PRRSV-infected 3D4/21 cells, and made a heat map of the selected glycosylation modification genes (Fig. 1a). We found that after PRRSV infected 3D4/21 cells, the expression of the ST3GAL2 gene was significantly up-regulated. To further verify the results, we detected the expression of the ST3GAL2 gene in 3D4/21 cells inoculated with PRRSV by fluorescent quantitative PCR (Fig. 1b). Consistent with the previous results, the ST3GAL2 gene continued to be up-regulated in the early and mid-term of PRRSV infection. Many glycosyltransferases are specifically distributed on the Golgi apparatus to facilitate post-translational modification of proteins. As a type II transmembrane protein, the subcellular localization of porcine ST3GAL2 in cells was further verified by immunofluorescence experiments (Fig. 1c). An obvious co-localization phenomenon was observed in co-transfected of ST3GAL2-Myc and Golgi fluorescent marker plasmids, but no obvious co-localization phenomenon with endoplasmic reticulum and lysosomal marker protein. In conclusion, STGAL2, as a sialyltransferase, is localized on the Golgi of cells and is upregulated during PRRSV infection, which may be related to sialylation modification of viral envelop protein.

3.2 ST3GAL2 promotes PRRSV proliferation

We transfected Myc-ST3GAL2 plasmid into PRRSV infected 3D4/21 cells to study the effects of PRRSV proliferation by ST3GAL2 overexpression. We first verified the transcription and expression changes of ST3GAL2 in 3D4/21 cells by fluorescent quantitative PCR and Western blotting (Fig. 2a, b). Then we detected the transcription of PRRSV N structural protein mRNA and the expression changes of nsp2 non-structural protein (Fig. 2c, d). The supernatant of the experimental group and the control group were collected in the same way to detect the changes of virus titer (Fig. 2e). It was found that overexpression of ST3GAL2 promoted PRRSV proliferation.

After that, we transfected siRNA into PRRSV infected 3D4 / 21 cells to knock down the expression of ST3GAL2. First, the transcription and expression changes of ST3GAL2 after a knockdown by siRNA were verified (Fig. 2f, g). Then, fluorescence quantitative PCR and Western blot were used to detect the transcription of PRRSV N structural protein mRNA and the expression change of nsp2 non-structural protein (Fig. 2h, i). The supernatant of the experimental group and the control group were collected in the same way to detect the changes of virus titer (Fig. 2j). It was found that knocking down ST3GAL2 would inhibit PRRSV proliferation. The above results indicated that ST3GAL2 positively regulates PRRSV proliferation.

3.3 Interaction betweenporcine ST3GAL2 and PRRSV structural protein GP2a

According to previous research, we found that the expression of ST3GAL2 is proportional to the proliferation of PRRSV. As sialyltransferases that ST3GAL2 can modify proteins on the surface of cells or viruses to mediate their recognition. So we speculated that ST3GAL2 may modify the glycoproteins on the surface of PRRSV. Through confocal and immunoprecipitation methods, we verified the interaction between ST3GAL2 and PRRSV envelope glycoprotein (GP2a-5). We found that both ST3GAL2 and GP2a structural protein can be normally expressed and the co-localization of them was detected (Fig. 3a), while GP3-5 did not have a localization relationship with ST3GAL2 (Fig. 3b). Further, we conducted Co-IP experiments on ST3GAL2 and GP2a. Through immunoprecipitation experiments, we confirmed the interaction between ST3GAL2 and GP2a structural protein (Fig. 3c).

3.4 Effects of porcine ST3GAL2 on the expression of inflammatory factors

During PRRSV infection, the secretion of many immune molecules will also affect the replication of the viruses. So we next investigated whether ST3GAL2 can also affect PRRSV proliferation by regulating the secretory pathway of immune molecules. We transfected Myc-ST3GAL2 to overexpress or siST3GAL2 to knock down ST3GAL2 gene expression and inoculated the cells with 0.5 MOI of PRRSV virus. Fluorescence quantitative PCR was used to detect the transcription of pro-inflammatory factors. It was found that in PRRSV-infected cells, ST3GAL2 inhibited the expression of pro-inflammatory factors involved in immune regulation (Fig. 4a, b). In the same way, we found that ST3GAL2 promoted the expression of anti-inflammatory factors in PRRSV-infected cells (Fig. 4c, d). It indicates that ST3GAL2 may inhibit the inflammatory response by regulating the expression of inflammatory factors and promote the proliferation of PRRSV.

Discussion

Our work showed that porcine ST3GAL2 was a type II transmembrane protein, localized on the Golgi apparatus of 3D4 / 21 cells. The expression of porcine ST3GAL2 was up-regulated in PRRSV infected 3D4 / 21 cells. Porcine ST3GAL2 may participate in the glycosylation modification of the PRRSV envelope protein GP2a and promote the proliferation of PRRSV. This process may be related to the formation and assembly of the viral envelope on the Golgi apparatus of the host cell. ST3GAL2 may increase the expression of anti-inflammatory factors (IL-4, IL-10) and down-regulate the expression of pro-inflammatory factors (IL-1β, IL-2, IL-6, IL-18, IFN-β, TNF-α) to resist inflammation. The relationship between ST3GAL2 and inflammatory factors and the interaction regulation mechanism needs to be further studied.

Glycosylation is a very common modification of proteins and lipids, and most glycosylation reactions occur in the Golgi apparatus[18]. Although the initial transfer of sugar to glycoproteins or glycolipids is carried out on the ER or ER membrane, the subsequent addition of different sugar groups to form a mature glycan occurs in the Golgi apparatus[28]. The Golgi membrane is covered with glycosyltransferases, glycosidases, and nucleotide sugar transporters[35]. The sequence is usually from the cis-Golgi to the trans-Golgi network, so that each enzyme activity can function on specific substrates generated earlier in the pathway[36]. Based on this characteristic, we verified the subcellular localization of porcine ST3GAL2 sialyltransferases on 3D4 / 21 cells and found that porcine ST3GAL2 is also localized on the Golgi body of the cell.

PRRSV has strong recognition and binding activity for the surface receptors of porcine alveolar macrophages, so it can target PAM to achieve massive proliferation and large-scale infection, causing serious damage to the host's lymphatic system and respiratory tract[22]. The body's immune response is triggered, and a series of related genes are regulated to cause differential expression[19]. At the same time, in order to promote its own proliferation, the viruses will also regulate the synthesis of some proteins to assemble into new viruses[48]. Through different methods, we verified that when PRRSV infects 3D4/21 cells, the transcription level of ST3GAL2 is significantly increased, indicating that ST3GAL2 may be related to the immune response triggered by PRRSV.

In the early stage of infection, the virus mainly attaches to the cell surface by binding to the corresponding receptor or attachment factor on the host cell surface[43]. Sialic acid is used by a large number of viruses as the initial connection receptor, especially the sialic acid on the surface of the virus, which can be recognized by the surface receptors of many cells and mediate the virus to invade the cells[46]. Therefore, in the next experiments, we verified the effect of sialyltransferase STGAL2 on PRRSV proliferation. We found that after overexpressing ST3GAL2 in 3D4/21 cells, the proliferation of PRRSV was significantly up-regulated compared to the control group. However, after transfecting ST3GAL2 specific siRNA to interfere with its expression, the viral load of PRRSV decreased significantly. These results indicated that ST3GAL2 expression was positively correlated with PRRSV proliferation.

GP2a, 3, 4, 5 are the structural proteins of PRRSV, they can be modified by glycosylation, and can also measure the process of virus replication[29]. GP2a structural protein is one of the small structural proteins encoded by ORF2a[49]. Previous studies have shown that GP2 can bind to the virus-cell surface receptor CD163 to help the virus invade the host cell[7]. GP2 forms a GP2-GP3-GP4 multimeric complex with two other small structural proteins, GP3, and GP4, which are believed to be critical for viruses to enter susceptible host cells[3]. In this study, we found that unlike GP3-5 glycoproteins, ST3GAL2, and GP2a have a co-localization and interaction relationship. PRRSV, as an enveloped virus, assembles in the cytoplasm and germinates on the Golgi to obtain the surface envelope, including several structural glycoproteins of PRRSV[17]. Therefore, we speculate that ST3GAL2 may play a role in the formation of the PRRSV envelope by modifying GP2a.

In the process of virus infecting cells, macrophages can secrete a variety of cytokines to enhance phagocytosis and mediate inflammation to exert antiviral effects[25]. Interleukins, interferons, etc. are inflammation-related cytokines, some of which play a role in promoting inflammation[21]. Most of these pro-inflammatory factors are the objects of glycosylation, and the corresponding biological functions are obtained through the action of glycosyltransferases[34]. Anti-inflammatory activity of autoreactive IgG antibodies due to sialylation of Fc glycans[42]. The sialylated Fc selectively binds to type II FcR, inducing the production of IL-33 in regulatory macrophage[26]. IL-33 may activate Th2 helper T cells, trigger the release of anti-inflammatory cytokines, and play an anti-inflammatory role[24]. Therefore, the expression of anti-inflammatory factors may also be related to sialylation to some extent. Based on the above materials, we found that porcine ST3GAL2 may be modified by glycosylation to down-regulate part of the glycosylated pro-inflammatory factors and up-regulate the expression of inflammatory factors. The correlation between ST3GAL2 and inflammatory factors needs to be further explored, and the interaction regulation mechanism between them also needs to be verified by detecting intermediate regulatory factors such as Fc receptors or related transcription factors in its pathway.

Overall, this study initially clarified the regulatory role of ST3GAL2 in PRRSV proliferation and cellular immunity, providing a basis for virus prevention and discovery of the mechanism and scope of ST3GAL2. However, there are still many problems to be explored in the subsequent research, such as the regulatory mechanism of increased expression of ST3GAL2 during PRRSV infection, the mechanism of action of the ST3GAL2 gene, and cellular inflammatory factors. It is believed that with the continuous advancement of research, the exploration of β-galactose-α-1,3- sialyltransferases in the field of the inflammatory response and immune regulation will be more in-depth.

Declarations

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Author Contributions

Conceived and designed the experiments: JH H. Performed the experiments: YN S, YY G, XY L, RQ S, M Z, Z T, UE S, LL Z and JH H. Analyzed the data: JQ W, JH H. Contributed reagents/materials /analysis tools: JH H. Wrote the paper: JQ W, and JH H.

Author Conflict of Interest

The authors declare no competing interests.

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Tables

Table 1 Primers of ST3GAL2 for clone

Primers	Accession No.	Primer Sequence (5¢- 3¢)	Tm (°C)
ST3GAL2-T	XM_021093801.1	F:GCCGAACCATGAAGTGCTCCC	64
		R: GCTCAGTTGCCTCGGTAGACCT	

Table 2 Primers of ST3GAL2 for expression

Primers	Accession No.	Primer Sequence (5¢- 3¢)	Tm (°C)
pET-28a-ST3GAL2	XM_021093801.1	F:GCAAATGGGTCGCGGATCCATGGCCACCTTGCCCTACCTGGA	68
		R:GGAGCTCGAATTCGGATCCTCAGTTGCCTCGGTAGACCTCGAT	
CMV2-ST3GAL2	XM_021093801.1	F:CCAGTCGACTCTAGAGGATCCATGAAGTGCTCCCTGCGGGTGT	68
		R:CAGGGATGCCACCCGGGATCCTCAGTTGCCTCGGTAGACCTCG	

Table 3 Real-time PCR primers

Gene	Accession No.	Primer Sequence (5¢- 3¢)	Tm (°C)
PRRSV-N	AF353511.1	F: GCCCAGCAAAACCAGTCC	58.4
		R: GCGTTGGCAGACTAAACT	
ST3GAL2	XM_021093801.1	F:CCTCTCCACAGGGCAGATCCG	58.6
		R:CCTCTCCACAGGGCAGATCCG	
β-actin (ACTB)	DQ452569.1	F: CGAGACCTTCAACACCCCAG	60.0
		R: TTCTCCTTGATGTCCCGCAC	

Table 4 Primers for siRNA of ST3GAL2

siRNA	Abbreviation	Primer Sequence (5¢- 3¢)
siST3GAL2-1	Sus-89	F:GCAUGGCCACCUUGCCCUATT
		R:UAGGGCAAGGUGGCCAUGCTT
siST3GAL2-2	Sus-662	F:CCCUGGACCUAAUGUGGAUTT
		R:AUCCACAUUAGGUCCAGGGTT

Figures

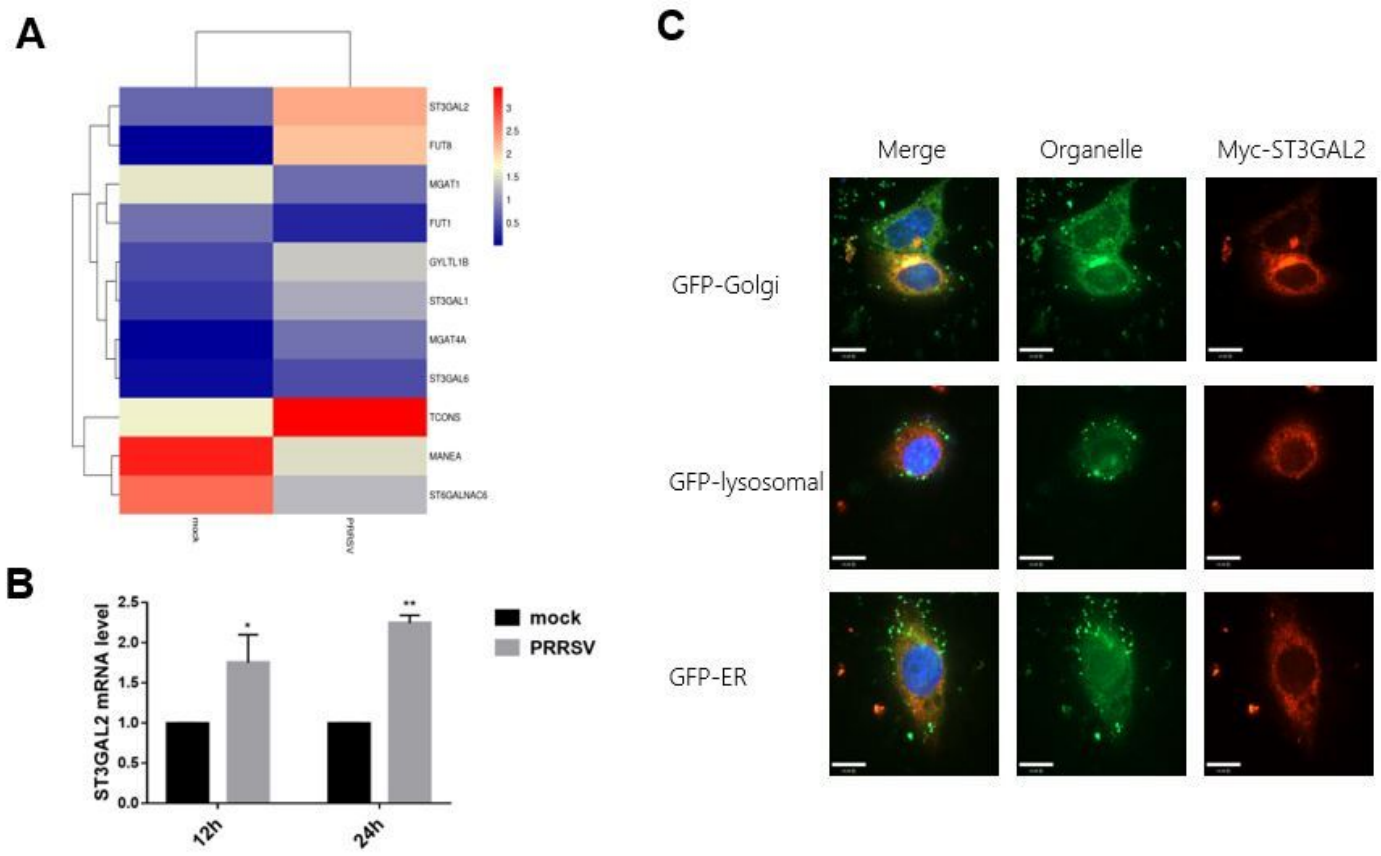

Figure 1

PRRSV infection promotes ST3GAL2 expression in 3D4/21 cells and subcellular localization of porcine ST3GAL2 (a) Heat map of differentially expressed genes related to glycosyltransferase in PRRSV infected 3D4/21 cells. (b) Changes of transcriptional levels of ST3GAL2 at 12 hours and 24 hours after viral infection. (c) Subcellular localization analysis of porcine ST3GAL2 in 3D4/21 cells（bar：14 μm）

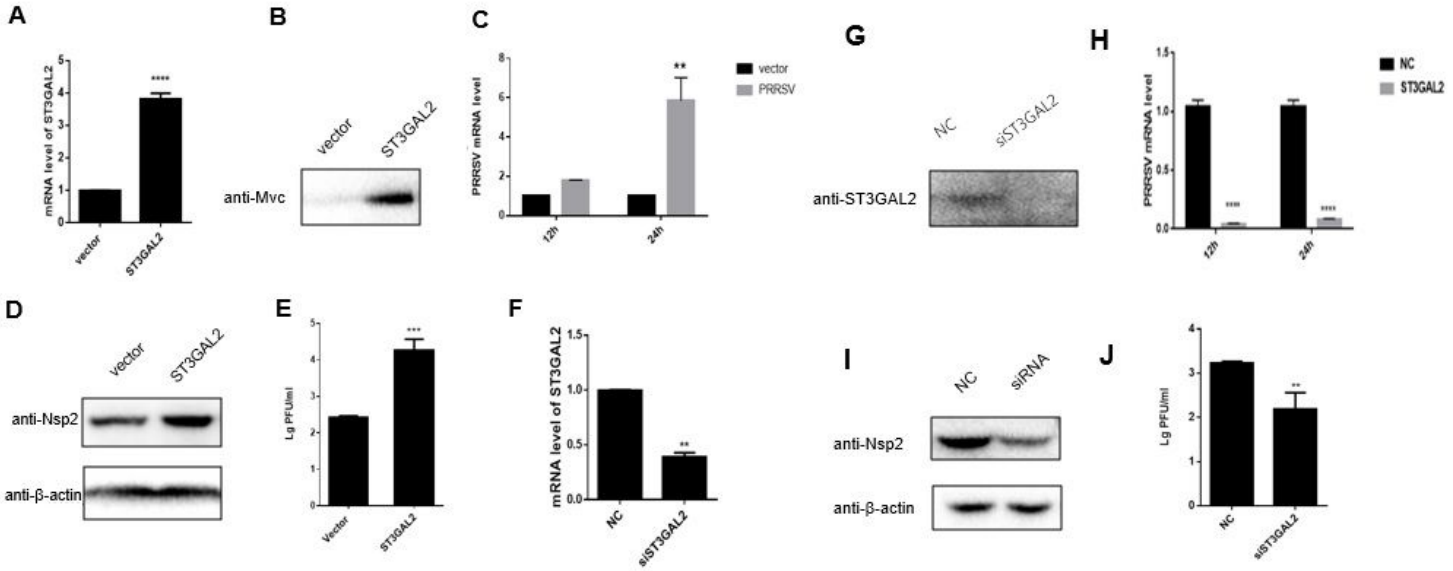

Figure 2

ST3GAL2 promotes the proliferation of PRRSV in 3D4/21 cells. (a) Verification of transcriptional levels of ST3GAL2 after overexpression. (b) Verification of protein expression of ST3GAL2 after overexpression. (c) ST3GAL2 overexpression plasmid was transfected into 3D4/21 cells, and PRRSV was inoculated after 24 hours. Transcriptional level of PRRSV N protein was detected by RT-qPCR. (d) PRRSV nsp2 protein expression was detected by Western-blot. (e) Viral proliferation was detected by TCID50. (f) SiRNA interference sequence targeting ST3GAL2 was transfected into 3D4/21 cells and the transcriptional level of ST3GAL2 was detected by RT-qPCR. (g) The expression level of ST3GAL2 was detected by Western-blot. (h) SiRNA interference sequence targeting ST3GAL2 was transfected into 3D4/21 cells. PRRSV virus was inoculated after24 hours and transcriptional of PRRSV N protein was detected by RT-qPCR. (i) PRRSV nsp2 protein expression was detected by Western-blot. (j) Viral proliferation was detected by TCID50.

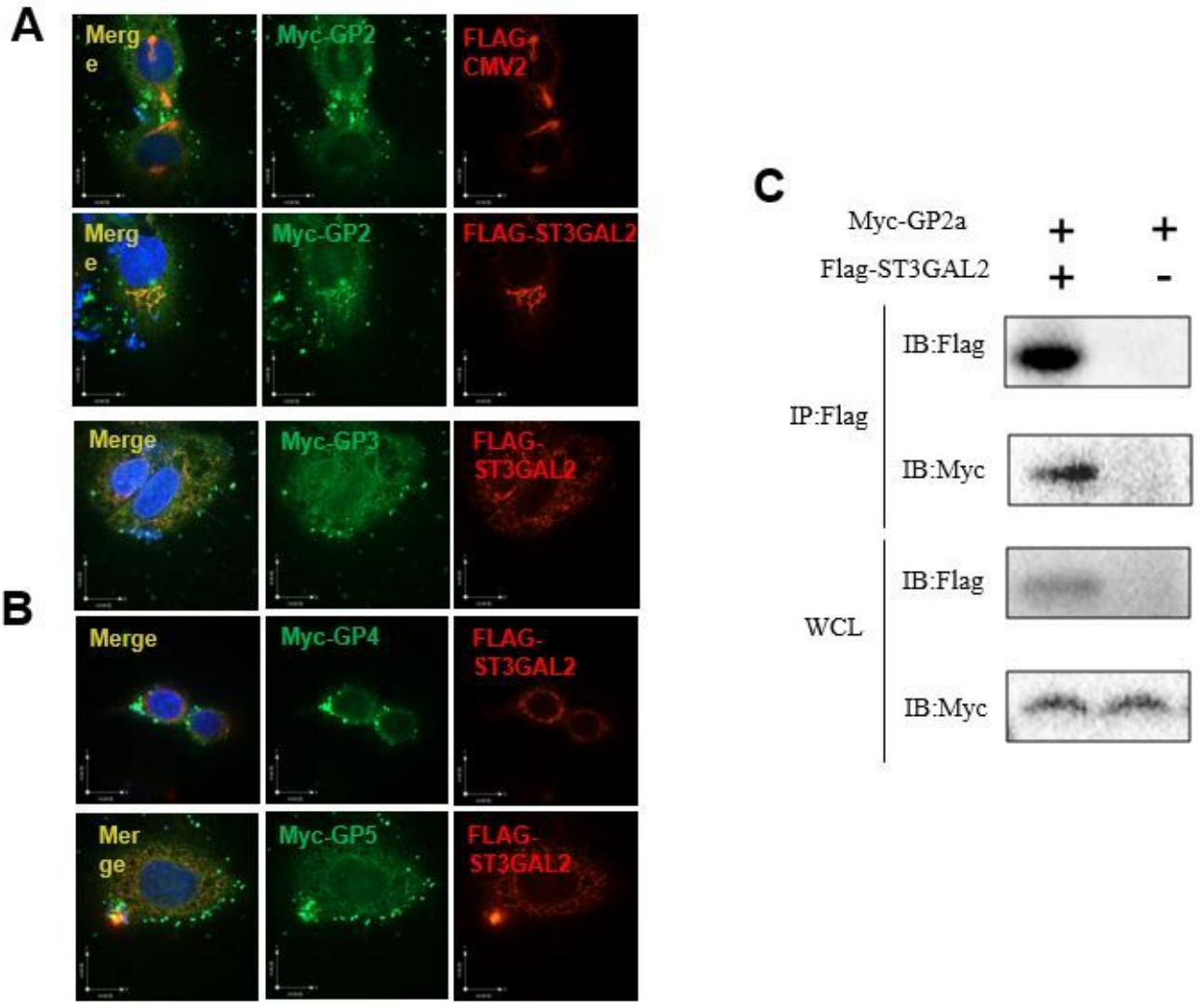

Figure 3

Interaction between porcine ST3GAL2 and PRRSV GP2a protein. (a) 3D4/21 was transfected with ST3GAL2 and PRRSV GP2a overexpression plasmids, then co-localization between ST3GAL2 and PRRSV GP2a (bar: 14 microns) was detected by confocal detection. (b) 3D4/21 was transfected with ST3GAL2 and PRRSV GP3, GP4, and GP5 overexpression plasmids. No co-localization between ST3GAL2 and PRRSV GP3, GP4 and GP5 (bar: 14 microns) were detected by confocal detection. (c) 3D4/21 was transfected with ST3GAL2 and PRRSV

GP2a overexpression plasmids. Interaction between ST3GAL2 and PRRSV GP2a was detected by immunoprecipitation.

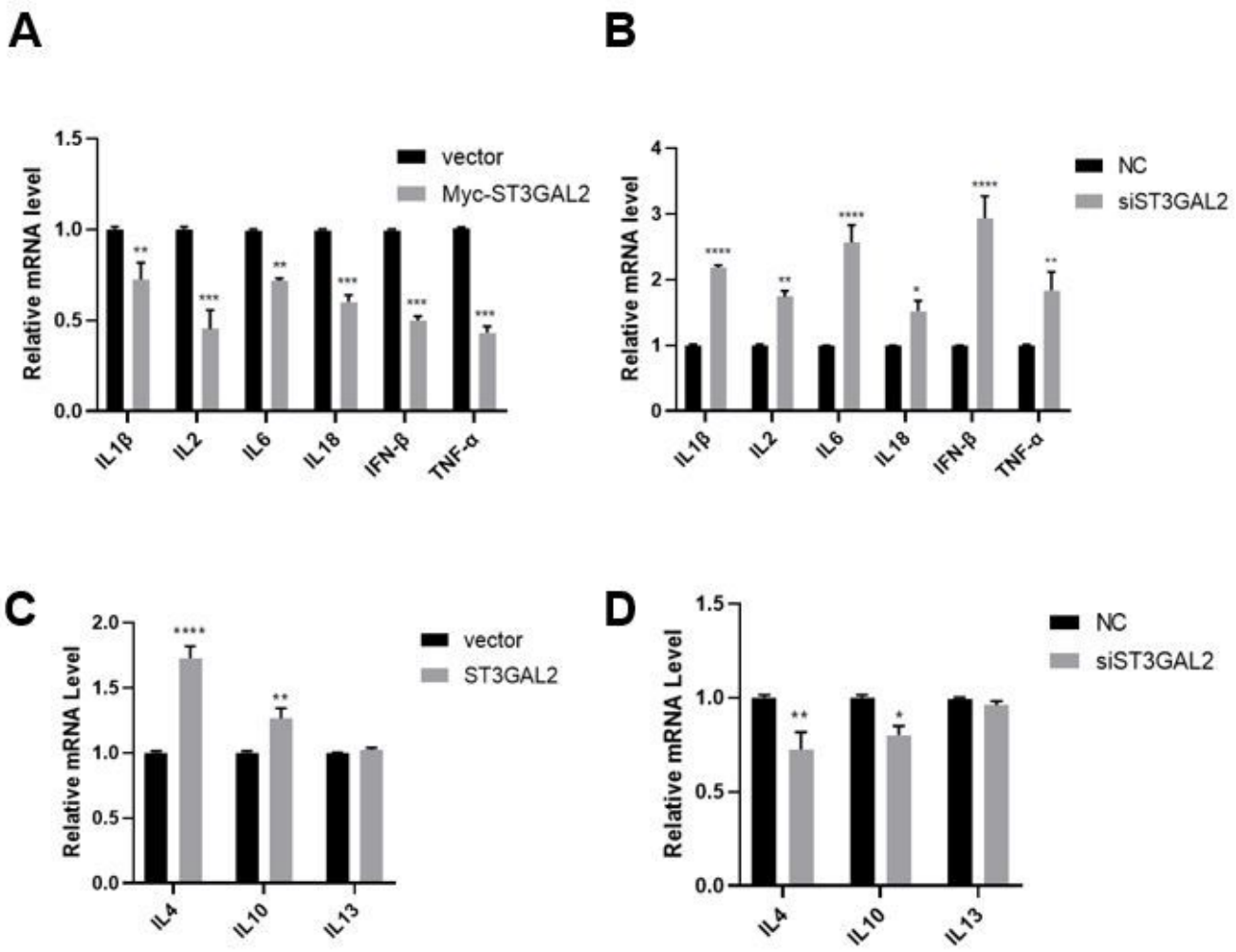

Figure 4

Porcine ST3GAL2 regulates inflammatory factors transcription in PRRSV infected 3D4/21 cells. (a). Overexpression of ST3GAL2 inhibited expression of IFN, Proinflammatory cytokines. (b). Interference with ST3GAL2 promoted the expression of interferons and proinflammatory factors. (c). Overexpression of ST3GAL2 promoted expression of IL-10. (d). SiRNA of ST3GAL2 inhibited the expression of IL-10.