Research Square

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Activation of LXRα regulates cellular cholesterol homeostasis and inhibits infection of PRRSV

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Research Article

Keywords: PRRSV, LXRα, Cholesterol, Lipogenic Gene

Posted Date: September 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10786611/v1

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Additional Declarations: No competing interests reported.

Abstract

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is an important pathogen that seriously endanger the development of pig farming. Porcine alveolar macrophages (PAMs) are target cells of PRRSV, and the liver X receptor α (LXRα)-cholesterol metabolism axis can affect macrophage resistance to pathogenic infections by regulating cholesterol homeostasis. The present study demonstrates that GW3965, a synthetic ligand for LXRα, effectively inhibits PRRSV entry and replication. The antiviral mechanism is primarily attributed to the regulation of host cell cholesterol homeostasis. On one hand, activation of LXRα reduces cholesterol uptake by promoting the degradation of the low-density lipoprotein receptor (LDLR). On the other hand, it upregulates the expression of ATP-binding cassette transporter A1 (ABCA1), which facilitates intracellular cholesterol efflux. This process effectively deprives the virus of the lipid-rich environment essential for its replication. Furthermore, activation of LXRα significantly suppresses PRRSV-induced inflammatory responses, as evidenced by the reduced secretion of the NLRP3 inflammasome and associated inflammatory cytokines. In summary, our findings suggest that activation of LXRα exerts anti-PRRSV effects by remodeling cholesterol homeostasis and mitigating virus-induced inflammatory damage, highlighting its potential as a therapeutic agent against PRRSV infection.

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) caused by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a highly dangerous acute infectious disease in pigs, which has seriously affected the development of the global pig industry [1]. The lungs are the primary target organs of PRRSV, and pulmonary alveolar macrophages (PAMs) are target cells of PRRSV infection, which play a role in the process of lung anti pathogen infection [2]. They devour invading pathogens, kill lysosomes through phagocytosis, and activate acquired immunity through antigen presentation epidemic response, and cholesterol metabolism homeostasis is an important regulatory factor for macrophages to resist pathogenic infections [3].

Cholesterol, as an important component of the cell membrane, has functions of maintaining the integrity of the biofilm, regulating the fluidity and permeability of the cell membrane, forming lipid rafts and microstructural domains on the membrane [3]. Sun and Huang found that the use of cholesterol extractant methyl-β-Cyclodextrin pretreatment could eliminate cell membrane cholesterol, disrupt lipid raft structure, significantly inhibit PRRSV infection in a dose-dependent manner, while supplementing with exogenous cholesterol could partially restore the infectivity of PRRSV [4]. Therefore, pharmacological interventions targeting cholesterol metabolism hold promise as broad-spectrum antiviral strategies.

Liver X receptors (LXRs), including LXRα and LXRβ, are DNA-binding transcription factors belonging to the nuclear receptor superfamily that function as key sensors of lipid homeostasis [5]. Specifically, LXRα plays a central role in regulating cholesterol and lipid metabolism by modulating the expression of

multiple lipid-responsive genes upon activation[6]. For instance, activation of LXRα induces the E3 ubiquitin ligase (IDOL), which promotes low-density lipoprotein receptor (LDLR) degradation and subsequently reduces low-density lipoprotein (LDL) uptake [7]. Concurrently, LXRs upregulate ATP-binding cassette transporter A1 (ABCA1), thereby facilitating cholesterol efflux [6].

GW3965, a well-characterized synthetic LXRα agonist, is widely employed to study LXR biology and has been explored as a broad-spectrum antiviral agent due to its ability to potentiate cholesterol efflux. It has demonstrated antiviral efficacy against viruses such as hepatitis C virus (HCV) [8], Newcastle disease virus (NDV) [9], and bovine viral diarrhea virus (BVDV) [10]. Nevertheless, its potential activity against PRRSV infection has not yet been investigated.

In the present study, we demonstrated that GW3965 significantly inhibited PRRSV infection. Mechanistically, GW3965-mediated LXRα activation upregulated ABCA1 and downregulated LDLR protein levels, thereby promoting cholesterol efflux while limiting cholesterol uptake, ultimately restricting PRRSV replication by depleting host cholesterol pools. These findings revealed a previously unrecognized branch of LXRα-mediated cholesterol efflux and expanded our understanding of LXRα-dependent antiviral mechanisms. Collectively, our results offered new insights into targeting cholesterol homeostasis as a therapeutic strategy against PRRSV and provided a theoretical foundation for the development of cholesterol-targeting antiviral drugs.

Results

PRRSV can upregulate host cholesterol levels and promote infection

We investigated the effects of PRRSV infection on cholesterol levels using both *in vivo* and *in vitro* methods. We respectively used oil red O and Filipin dye to stain on PAMs in the lungs of infected and uninfected piglets. The staining results showed that there was lipid accumulation in the lung tissue of piglets infected with PRRSV(Fig. 1A), as well as a large amount of lipid accumulation cholesterol around the nucleus of PAMs in the lungs of piglets (Fig. 1C). We also infected PAMs with PRRSV to detect the total cholesterol content in PAMs, and we found cholesterol levels have been upregulated (Fig. 1B). Subsequently, we tested the toxicity of different concentrations of cholesterol on PAMs, and finally incubated PAMs with 50 ug/ml cholesterol for 2 hours before infecting them with PRRSV (Fig. 1D). We found that cholesterol can promote PRRSV infection (Fig. 1E). Together, these results indicated that PRRSV can upregulate host cholesterol levels and promote infection.

Activation of LXRα inhibits PRRSV infection and only affects the invasion and replication stages

GW3965 were commonly used exogenous agonists for studying the LXR pathway. Through cytotoxicity testing experiments, we determined 1 μM as the optimal concentration for GW3965 (Fig. 2A). To clarify the impact of activation of the LXR-ABCA1 pathway on PRRSV infection, we first treated PAMs with GW3965 agonist for 24 h before PRRSV infection, followed by PRRSV infection for 12 hours. The results showed that the genomic level of PRRSV (Fig. 2B) and PRRSV N protein levels (Fig. 2C) were significantly reduced under GW3965 treatment. Meanwhile, TCID50 assay was performed on the collected supernatant, and it was found that the viral titer in the cell supernatant of the GW3965 treated group was significantly reduced (Figure.2D). To further investigate the stage at which activation of LXRα affects PRRSV infection, PAMs were first treated with GW3965 for 2 h, followed by incubation with PRRSV (MOI = 0.1) at 4°C for 1 h to allow viral attachment. After removing the inoculum and washing three times with PBS, cell pellets were collected for total RNA extraction, and viral genomic RNA levels were analyzed by RT-qPCR. The results showed no significant difference in PRRSV genomic RNA levels between the GW3965 treated and control groups, indicating that activation of LXRα does not affect PRRSV attachment (Figure.2E).

Next, to determine whether activation of LXRα inhibits PRRSV entry, PAMs were treated with GW3965 for 2 h, followed by incubation with PRRSV at 37°C for 1 h. Cells were then collected, and qRT-PCR analysis revealed a significant reduction in viral RNA levels, suggesting that activation of LXRα treatment impairs PRRSV entry (Figure.2F).

Finally, to assess whether activation of LXRα inhibits PRRSV replication, cells were treated with GW3965 after PRRSV infection and harvested at 12 h. QRT-PCR results showed a significant decrease in viral genomic RNA levels, demonstrating that activation of LXRα also suppresses PRRSV replication (Figure.2G). In summary, these experimental results indicate that activation of LXRα can significantly inhibit PRRSV infection.

Activation of LXRα inhibits PRRSV infection by downregulating LDLR

To investigate the role of LDLR in PRRSV infection, we first examined its expression dynamics in PAMs following viral infection. The results showed that both the mRNA and protein levels of LDLR were significantly upregulated at 12, 24, and 36 hpi (Figure.3A, 3B). Supplementation with LDL, the primary ligand of LDLR, significantly enhanced PRRSV infection, suggesting that PRRSV may exploit LDLR-mediated cholesterol uptake to create a favorable environment for its replication (Figure.3C). Conversely, siRNA-mediated knockdown of LDLR in PAMs markedly suppressed PRRSV infection (Figure.3D,3E). Notably, treatment with GW3965 significantly reduced LDLR protein level, indicating that activation of LXRα inhibits PRRSV infection at least in part through downregulating LDLR protein levels (Figure.3F).

Activation of LXRα inhibits PRRSV infection by regulating cholesterol efflux pathway

Activation of the LXRα pathway by GW3965 significantly modulates the expression of multiple genes involved in lipid metabolism. In this study, we observed that GW3965 treatment not only upregulated the mRNA levels of LXRα and ABCA1 but also significantly increased the expression of sterol regulatory element-binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor gamma 1 (PPAR-γ1), and fatty acid synthase (FASN) (Figure.4A). The increase in ABCA1 protein expression was further confirmed by Western blot analysis. This pathway act synergistically with ABCA1 to enhance cholesterol efflux (Figure.4C). Filipin staining demonstrated that activation of LXRα significantly reversed the PRRSV-induced increase in intracellular cholesterol levels (Figure.4D). Importantly, cholesterol supplementation effectively rescued the antiviral effect of activation of LXRα, underscoring the critical role of cholesterol depletion in its mechanism of action.

Activation of LXR downregulates PRRSV-induced inflammatory cytokines and NLRP3 expression

PRRSV infection induces high levels of pro-inflammatory cytokine expression, which may contribute to inflammatory responses and the pathogenesis of viral infection. Numerous studies have demonstrated that GW3965 exerted potent anti-inflammatory effects in various disease models by modulating the production of inflammatory cytokines. To investigate the anti-inflammatory role of GW3965 during PRRSV infection, we examined the expression of pro-inflammatory cytokines in infected cells. QRT-PCR results showed that activation of LXRα significantly reduced the mRNA levels of IL-1β, IL-6, IL-8, and TNF-α induced by PRRSV infection at 12 hours post-infection(Figure.5A-5D). Consistently, ELISA assays revealed that the protein levels of IL-1β, IL-6, and TNF-α in the supernatants of PAMs were also significantly decreased following activation of LXRα (Figure.5E-5G). Furthermore, activation of LXRα downregulated the protein expression of NLRP3 (Figure.5H). These findings suggest that activation of LXRα attenuated PRRSV-induced inflammatory responses, potentially alleviating virus-associated immunopathology.

Discussion

The porcine reproductive and respiratory syndrome virus causes reproductive disorders in sows and respiratory diseases in pigs, seriously threatening the normal life course of pigs and the development of pig farming [11]. With the continuous evolution of PRRSV and the emergence of new highly pathogenic virus strains, studying the multiple infection mechanisms of PRRSV is still crucial for exploring antiviral mechanisms [12]. Therefore, cholesterol inevitably plays an important role in virus proliferation, and the relationship between viral infection and cholesterol homeostasis is inseparable. We have demonstrated through in vitro and in vivo experiments that PRRSV infection can lead to the accumulation of host cholesterol. The present study also demonstrated that activation of LXRα effectively inhibited PRRSV infection by remodeling host cholesterol homeostasis. The antiviral mechanism of activation of LXRα involved promoting the degradation of LDLR to inhibit cholesterol uptake, and activating the efflux to the

extracellular space through ABCA1 on the cell membrane. Meanwhile, activation of LXRα attenuated PRRSV-induced inflammatory responses, further contributing to its antiviral effects.

LDLR is a critical receptor for low-density lipoprotein (LDL) uptake and serves as a primary route for exogenous cholesterol acquisition [7]. Accumulating evidence has also identified multiple viruses better invade hosts with LDLR including hepatitis C virus (HCV) [8] and pseudorabies virus (PRV) [13], making it an attractive antiviral target. In this study, we observed that siRNA-mediated knockdown of LDLR in PAMs significantly suppressed PRRSV infection. Conversely, PRRSV infection upregulated LDLR protein expression, suggesting that PRRSV rely on LDLR to uptake LDL and enhance its own proliferation. Mechanistically, GW3965 treatment activated the LXR-IDOL axis, leading to LDLR degradation. These results indicated that activation of LXRα can limit cholesterol intake, thereby reducing the invasion and replication of PRRSV.

ABCA1, a well-characterized LXR target gene, plays a pivotal role in cholesterol efflux to lipid-poor apolipoproteins [6]. Our data showed that activation of LXRα significantly upregulated ABCA1 expression in PAMs. Given that cholesterol is an important component of lipid rafts, which are critical membrane microdomains for the assembly, budding, and invasion of viruses [4], we speculated that ABCA1 mediated efflux of plasma membrane cholesterol may hinder multiple stages of the PRRSV lifecycle. By reducing membrane cholesterol content, activation of LXRα not only prevented virus invasion, but might also disrupt the formation of a lipid microenvironment conducive to replication. This is consistent with the growing consensus that there is an intrinsic relationship between lipid metabolism and viral replication efficiency.

Beyond its effects on cholesterol metabolism, activation of LXRα significantly reduced the mRNA and protein levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in PRRSV-infected PAMs. The inflammasome and type I interferon (IFN-I) pathways are two fundamental innate immune mechanisms that protect the host against a broad range of microbial pathogens [14]. Inflammasomes are multiprotein complexes that sense intracellular danger signals, typically mediated by effector molecules like NLRP3 [15]. By dampening the NLRP3 inflammasome and downstream cytokine secretion, activation of LXRα may alleviate virus-induced tissue damage, complementing its direct antiviral activity.

In summary, our study revealed activation of LXRα targets host cholesterol homeostasis at multiple levels: limiting cholesterol uptake through LDLR degradation, and promoting plasma membrane cholesterol efflux via ABCA1 (Fig. 6). These findings not only expand the current understanding of LXR-mediated antiviral mechanisms but also highlight the therapeutic potential of targeting cholesterol dynamics in combating PRRSV and other lipid-dependent viruses.

Materials and Methods

Animals, Cells and viruses

Six five-week-old piglets were selected as experimental animals in the study. All the piglets were negative for antibodies and antigens of porcine circovirus type 2 (PCV2) and PRRSV, which purchased from Xilingjiao Breeding and Raising Center. PAMs were isolated from the lungs of piglets, which were cultured in 1640 (Corning, America) with 10% fetal bovine serum (Gibco, America) and 100 U/mL penicillin and 100 μg/mL streptomycin. HP-PRRSV strain SX-1 was isolated and preserved in the lab.

Animal experiment design

Six five-week-old piglets were randomly divided into two groups and were separately raised in different isolation rooms. All the piglets were negative for antibodies and antigens of PCV2 and PRRSV before the experiment. Each piglet in the PRRSV strain group received a challenge with PRRSV strain SX-1 (2×10^5 $TCID_{50}$) via intranasal administration (1 mL) and intramuscular injection (1 mL), while the control group received an equivalent volume of DMEM. All piglets were euthanized by intravenous injection of sodium pentobarbital (90 mg/kg) on the seven days post-infection (dpi). Different tissues and pulmonary alveolar macrophages were collected and preserved for subsequent use.

Agents, antibodies and siRNA

The synthetic LXR agonists GW3965, Filipin III and cholesterol were purchased from Sigma–Aldrich. The Propidium Iodide (PI) was purchased from Thermo Fisher Scientific. LDL were purchased from YEASEN. The antibodies used in this study were rabbit polyclonal antibodies against LDLR (Proteintech), β-actin (Proteintech), GAPDH (ABclonal), NLRP3(Bioss) and mouse monoclonal antibodies against ABCA1 (Abcam), PRRSV N (Shandong Academy of Agricultural Sciences, China). The goat anti-mouse IgG (NOVUS), and goat anti-rabbit IgG (NOVUS) were used in the studies. The siRNA sequences were as follows, NC-siRNA-F, 5'-UUCUCCGAACGUGUCACGUTT − 3', NC-siRNA-R, 5'-ACGUGACACGUUCGGAGAATT-3', LDLR-siRNA-F, 5'-GCAUCUCCUUGGACAACUTT-3', LDLR-siRNA-R, 5'-ACUUUCUCCAAGGAGAUGCTT − 3'.

Measurement of cellular cholesterol content

Cells were seeded at 1×10^6 cells per well suspension evenly on a 6-well plate, then infected with 0.1 MOI PRRSV and cultured for 12 hours. 0.2–0.3 mL lysis buffer were added to lyse the cells. Follow the instructions of Amplex Red Cholesterol and Cholesterol Ester Detection Kit (Beyotime, China) to measure the cholesterol content ,and used BCA protein assay (CWBIO, China) to detect the protein content of each sample as normalization.

Indirect immunofluorescence assay

Cells were washed with PBS and fixed with 4% paraformaldehyde for 20 minutes at room temperature. After three washes with PBS, the cells were incubated with 0.3% Triton X-100 for 20 minutes, followed by blocking with 5% BSA for 1 hour. The cells were then incubated with primary antibody for 1 hour at room temperature. Following three washes with PBS, the cells were incubated with FITC-conjugated goat anti-mouse IgG ((Beyotime)) for 1 hour at room temperature. Next, the cells were stained with 50 μg/mL

Flipin dye for 2 hours. Nucleus were stained with 50 μg/mL PI for 10 minutes. The stained samples were subsequently examined using a confocal microscope.

RNA isolation and quantitative reverse transcription PCR (RT-qPCR)

Total RNA was extracted from cells in each treatment group using was extracted using RNA-easy Isolation Reagent (Vazyme) and then reverse transcribed into cDNA using HiScript III RT SuperMix (Vazyme) for qPCR kit instructions. The operation method and steps of Real time qPCR test shall be carried out according to the instruction manual of ChamQ Universal qPCR Master Mix (Vazyme). After the Real time qPCR reaction was completed, save the data and calculate the results using $2^{-\Delta\Delta Ct}$ method and drew the result graph using GraphPad Prism software. The primers used are shown in the table below (Table 1).

Table 1
RT-qPCR primer and probe sequences.

gene	Sequence (5’-3’)
TNF-α F	CACCACGCTCTTCTGCCTAC
TNF-α R	ACGGGCTTATCTGAGGTTTGAG
IL-1β F	TCTGCCCTGTACCCCAACTG
IL-1β R	CCCAGGAAGACGGGCTTT
IL-6 F	GAGGATACCACTCCCAACAGACC
IL-6 R	AAGTGCATCGTTGTTCATACA
IL-8 F	AGACTGGGTTGCAATGCACT
IL-8 R	TTCTTAGCACTCCTTGGCAAAA
ABCA1 F	GCAGAGGGATGAGGCTACT
ABCA1 R	CTGGATTTACTCGGTGGC
FASN F	TGGCTCAGGCGGAACTCA
FASN R	CGCAAGCGGGAAGGTGTA
PPARγ F	AATGCCATCAGGTTTGGG
PPARγ R	GCGGAAGGACTTTTATGTATG
SREBP-1 F	GCGACGGTGCCTCTGGTAGT
SREBP-1 R	CGCAAGACGGCGGATTTA
ORF7 F	ATGGCCAGCCAGTCAATCAGCT
ORF7 R	GGCAAACTAAACTCCACAGTGTAACTTA
LDLR F	GAGTCTTGGAGGTGTGACGG
LDLR R	CACAGACAAACTTTGGGGCG
β-acitn F	TCTGGCACCACACCTTCT
β-acitn R	GATCTGGGTCATCTTCTCAC

Western blotting

Cells from the different treatment groups were lysed using RIPA buffer supplemented with 1% protease inhibitors to obtain proteins. The obtained proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore). The membrane was blocked with 5% milk in TBST at room temperature for 2 h followed by incubation with the primary antibody (2 h) and the secondary antibody (1 h) at room

temperature, respectively. Immunoreactive bands were detected using a fully automated chemiluminescence image analyzer(Tanon, China). The antibody was diluted with TBST.

Cell viability analysis

Cell viability was determined using a CCK-8 assay kit (ABclonal). Cells were seeded into 96-well plates at 1×10^4 cells per well and treated with the reagents of different concentrations, when the cells reached 70–80% confluence. After 24 h, 10 μL of CCK-8 reagent was added to each well and incubated for 1 h at 37℃. Absorbance was measured at OD450 nm using a microplate reader and calculated cell survival rate. Cell survival rate = [OD (drug) - OD (blank)]/[OD (control) - OD (blank)] × 100%.

Cytokine ELISA

PAMs were incubated with GW3965 during PRRSV infection. The supernatant was collected at 12 hpi. The concentrations of IL-1β, IL-6 and TNF-α were determined using an enzyme-linked immunosorbent assay kit (Elabscience) according to the manufacturer's instructions.

Statistical analysis

All data in this paper were statistically analyzed using GraphPad Prism software, t-tests were used to analyze the differences between two groups of data, and One Way ANOVA was used to analyze the differences between three or more groups of data (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All experiments in this paper have blank controls and are repeated three times.

Declarations

Ethics approval and consent to participate

The experimental animals for this study were purchased from Jinan Xilinjiao Breeding Center with the manager's approval. This study was approved by the Institutional Animal Care and Use Committee of Shandong Academy of Agricultural Sciences (Approval Number: IASVM-2024-041). The registration date in the manuscript for the Clinical Trial Number is 2024.10.21. All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Funding

This project was supported by the National Natural Science Funds (32070178, 32402881), Natural Science Funds of Shandong Province (ZR2024QC023), New high schools 20 in Jinan of Shandong Province (202333065), Shandong Province Pig Industrial Technology System (SDAIT-08), and the Taishan Scholars Program.

Author Contribution

These authors contributed equally: Chenguang Zhu, Jiang Yu.Y.L. and J.W. conceived and supervised the project. C.Z., J.Y., F.H. and T.L. conducted the experiments. Y.Z., F.L. and J.L. contributed to the computational analysis. L.D., S.R., Z.C. and W.S. provided key reagents or analysis and facility support. C.Z wrote the manuscript with input from all other authors.

Acknowledgements

We thank all of the members of Key Laboratory of Bacteriology in Nanjing Agricultural University and Key Laboratory of Livestock and Poultry Multi-omics of MARA in Shandong Academy of Agricultural Sciences for helpful discussions.

Data Availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Figures

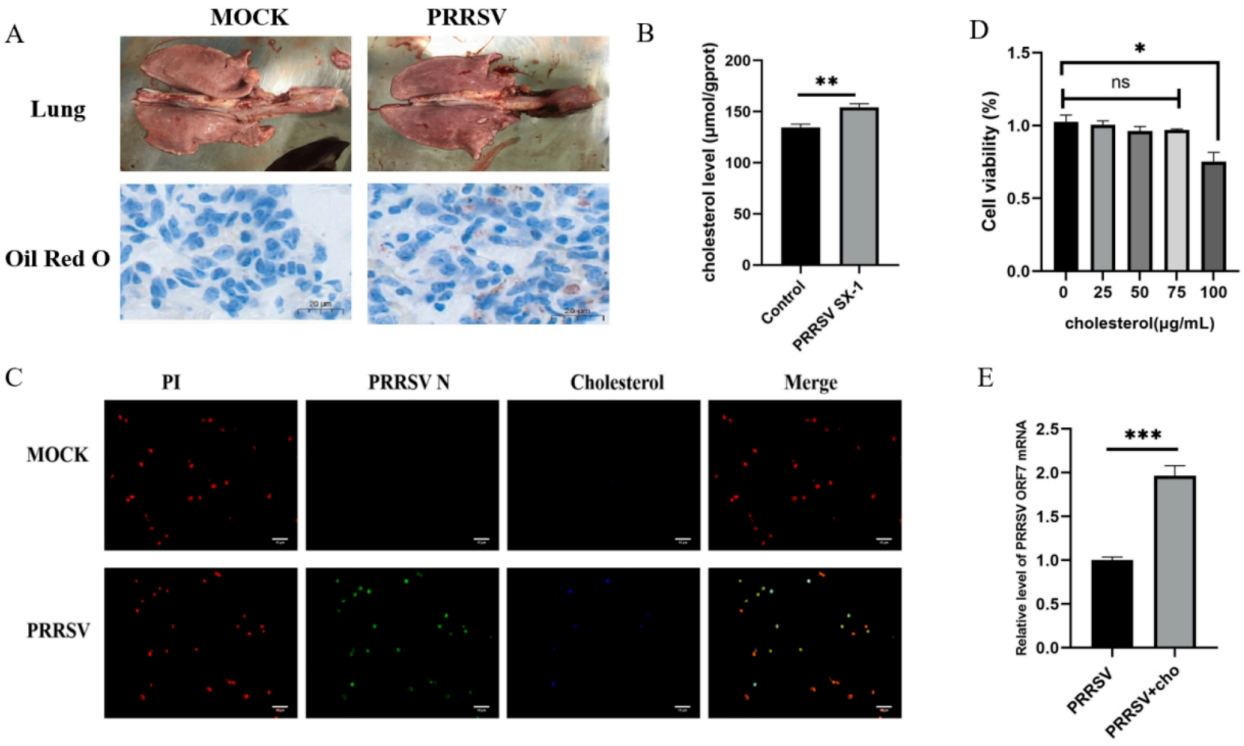


Figure 1

PRRSV can upregulate host cholesterol levels and promote infection. (A) We performed autopsy on piglets infected with PRRSV on the seventh day to obtain complete lungs. The affected areas were taken for Oil Red O staining (the nuclei were stained blue and the lipids were stained red). (B) Cells were infected with PRRSV for 12 hours to detect the total cholesterol content, and used BCA protein assay to detect the protein content of each sample as a correction. (C) Flipin staining was performed on PAMs in the lungs of infected and uninfected piglets, with red staining showing the nuclear region, green representing PRRSV N protein, and blue staining showing cholesterol distribution. (D) CCK8 assay to evaluate the effect of different cholesterol concentrations on cell viability. (E)Incubate PAMs with 50 μg/ml cholesterol for 2 hours, and then infect with PRRSV for 12 hours to detect viral gene levels. The presented results represent the means and standard deviations of data from three independent experiments (*, $p < 0.05$; **, $p < 0.01$; * * *, $p < 0.01$).

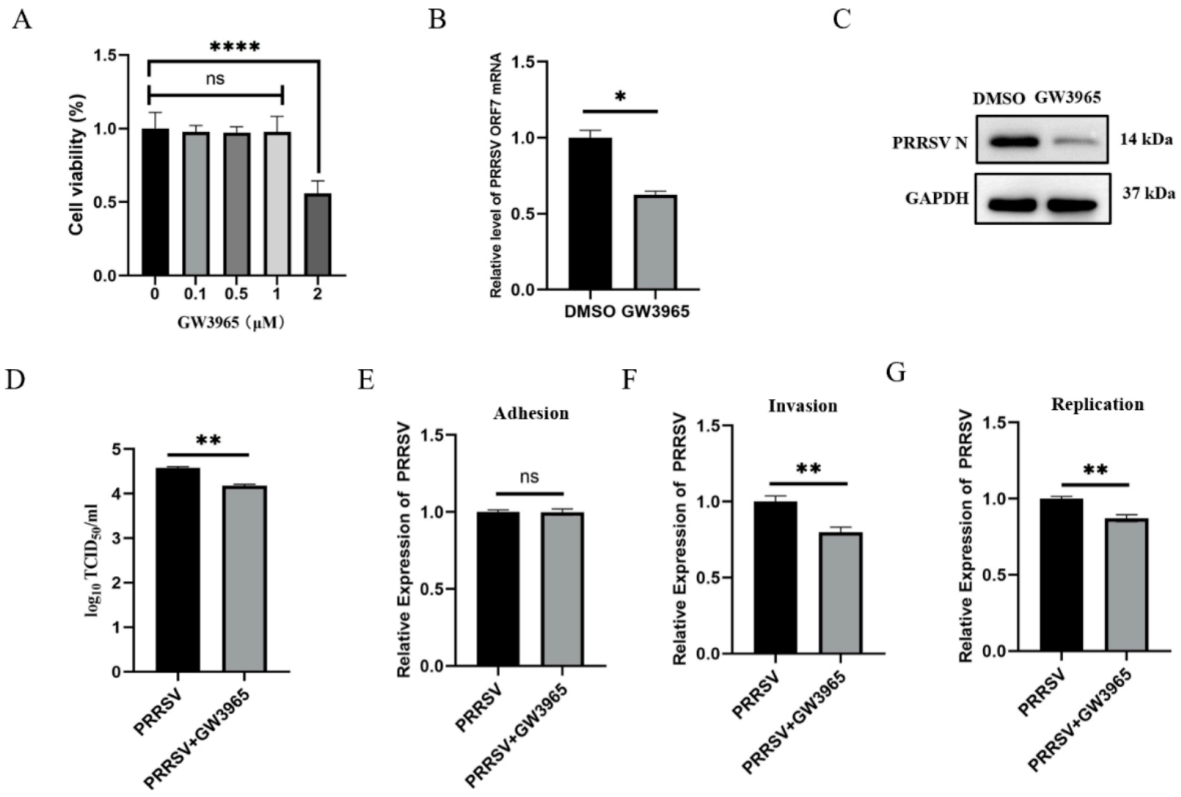


Figure 2

Activation of LXRα inhibits PRRSV infection and only affects the invasion and replication stages. (A) To detect the toxicity of GW3965 on PAMs, cells were incubated at different concentrations (0.1, 0.5, 1, 2 μM) for 24 hours, and then the cell survival rate was measured using CCK-8 reagent. (B) PAMs were treated with GW3965 and untreated, followed by PRRSV (0.1MOI) infection of cells. After 12 hours, samples were collected to detect the viral genome level (C) to detect the PRRSV N protein level (D) to detect $TCID_{50}$ in the cell supernatant. (E) Detect the effect of GW3965 on PRRSV adhesion. (F) PRRSV invasion. (G) PRRSV replication. The presented results represent the means and standard deviations of data from three independent experiments (*, $p < 0.05$; **, $p < 0.01$; * * *, $p < 0.01$).

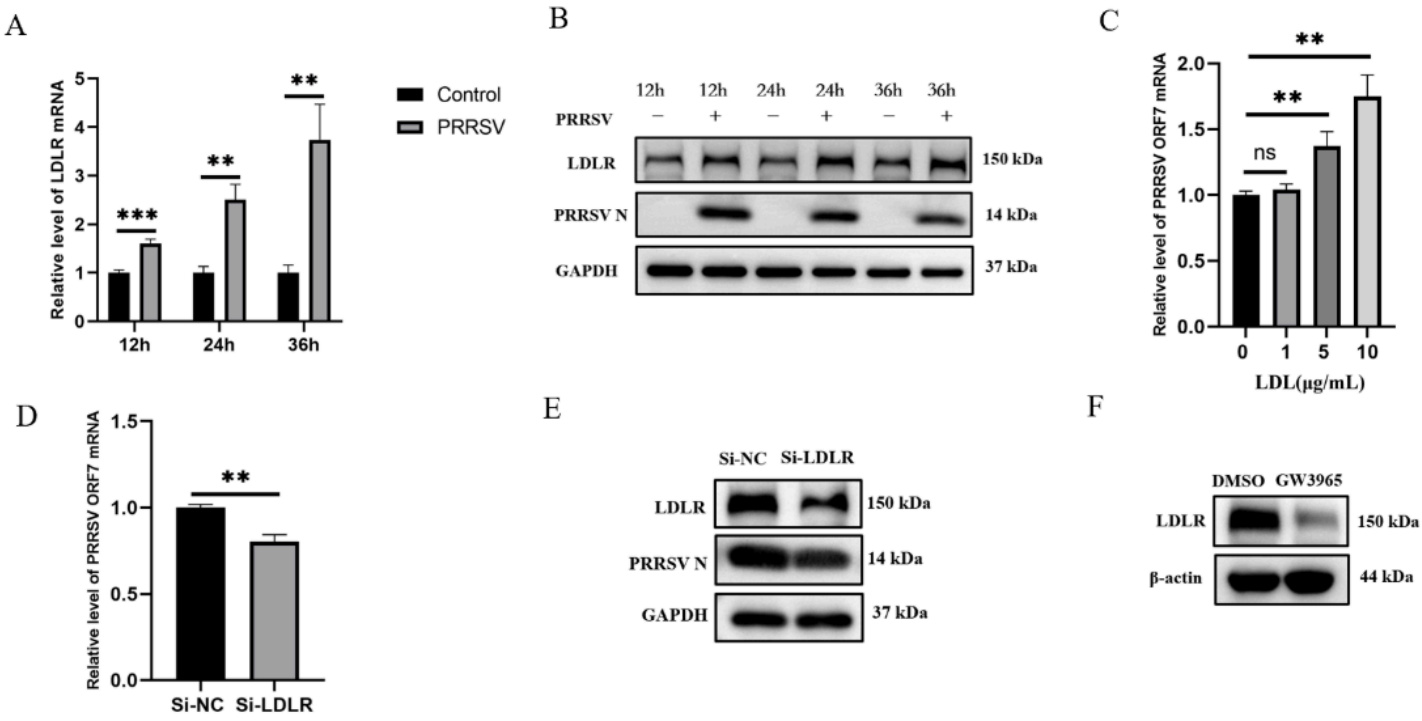


Figure 3

Activation of LXRα inhibits PRRSV infection by downregulating LDLR. (A) Infection of PAM with PRRSV at different time points for adoption detection of LDLR gene and (B) protein levels. (C) Administer PAMs with different concentrations of LDL and infect PRRSV for 12 hours, followed by testing for viral gene levels. (D) After knocking down LDLR with small interfering RNA, virus genes and (E) N protein levels were detected 12 hours after PRRSV infection. (F) Detect LDLR protein levels after treating PAMs with 1μM GW3965 for 24 hours. The presented results represent the means and standard deviations of data from three independent experiments (*, $p < 0.05$; **, $p < 0.01$; * * *, $p < 0.01$).

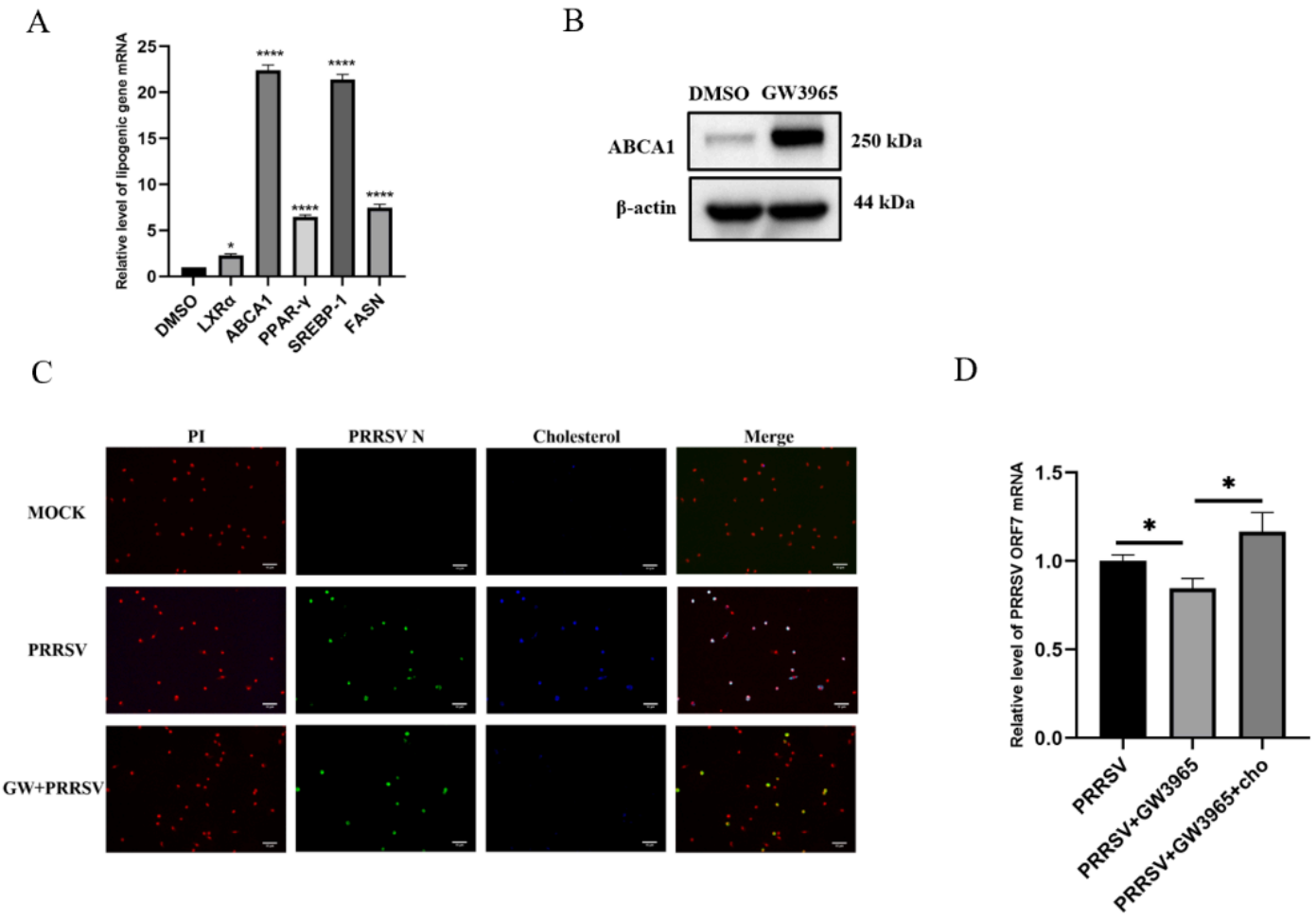


Figure 4

Activation of LXRα inhibits PRRSV infection by regulating cholesterol efflux pathway. (A) Detection of RNA level changes of various lipid genes , (B) the protein level of ABCA1 in PAMs treated with GW3965 for 24 hours. (C) Flipin staining was performed on PAMs with PRRSV and GW3965 treatment or not and red staining showing the nuclear region, green representing PRRSV N protein, and blue staining showing cholesterol distribution. (D) Cells were treated with or without GW3965 for 24 h. Subsequently, the cells were washed with PBS and incubated with medium alone or medium containing water-soluble cholesterol (50 μg/mL) for 30 min. The cells were infected with PRRSV for 12 h, then the level of PRRSV RNA was measured by qRT-PCR. The presented results represent the means and standard deviations of data from three independent experiments (*, $p < 0.05$; **, $p < 0.01$; * * *, $p < 0.01$).

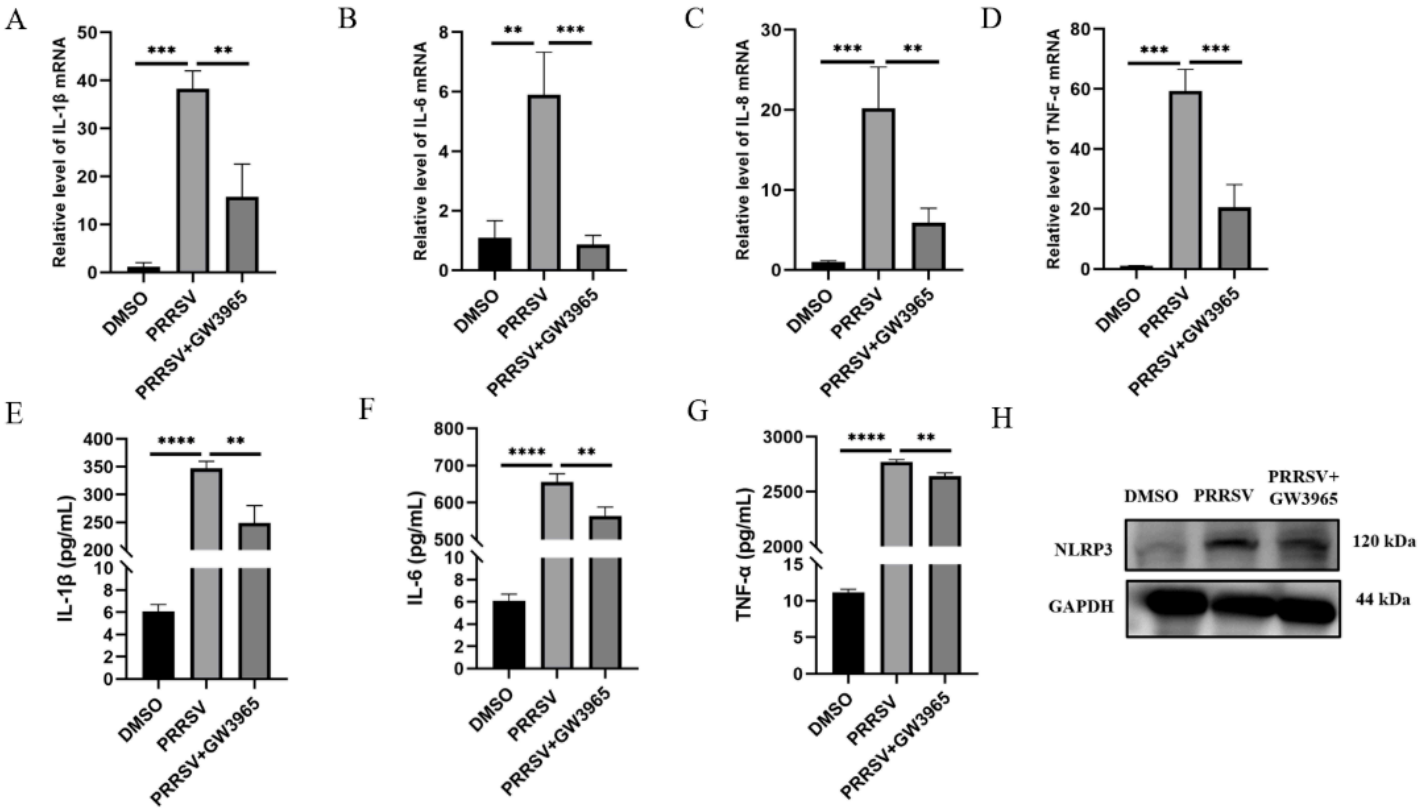


Figure 5

Activation of LXRα downregulates PRRSV-induced inflammatory cytokines and NLRP3 expression.(A) PAMs were infected by PRRSV and incubated with GW3965. The cells were collected at 12 h post-treatment. The relative expression level of (A) IL-1β, (B) IL-6, (C) IL-8 and (D) TNF-α were determined by qRT-PCR . Elisa analysis of the impact of GW3965 on the protein expression of (E) IL-1β, (F) IL-6, and (G) TNF-α in PAMs infected with PRRSV. (H) WB analysis of the impact of GW3965 on the NLRP3 protein in PAMs infected with PRRSV. The presented results represent the means and standard deviations of data from three independent experiments (*, $p < 0.05$; **, $p < 0.01$; * * *, $p < 0.01$).

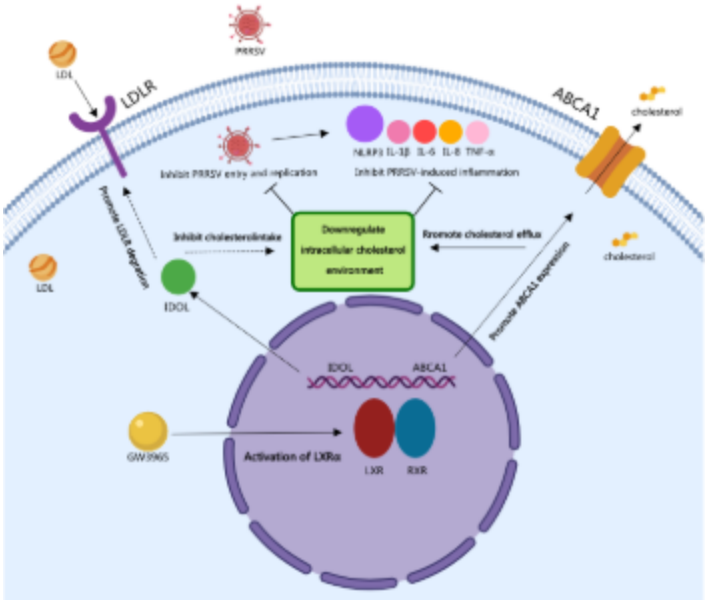


Figure 6

Schematic diagram of the mechanism of activating LXRα to inhibit PRRSV infection and inflammation.

Supplementary Files

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- NEWWBoriginalimage2.zip