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PEDV infection affects the expression of polyamine-related genes inhibiting viral proliferation

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is an alpha-coronavirus that causes epidemic diarrhea in swines. The mortality of PEDV infection in one-week-old piglets is extremely high, which causes a huge significant economic loss to the global pig husbandry and blocks its healthy development. There was a lack of adequate studies to elucidate pathogenic mechanism associated with PEDV infection. In the present study, we detected the expression profiles of polyamine metabolism associated genes in Vero cells infected with PEDV by RT-qPCR. It is shown that PAOX(acetylpolyamine oxidase), SMOX(spermine oxidase), SAT1(spermidine-spermine acetyltransferase 1), ODC1(ornithine decarboxylase 1), DHPS(deoxyhypusine synthase) and EIF5A(eukaryotic initiation factor 5A) were significantly upregulated. Through intervening SAT1 level in PEDV-infected Vero cells, it is identified that overexpression of SAT1 inhibited PEDV replication by reducing polyamine levels. Furthermore, polyamine depletion and upregulation were found to regulate the proliferation of PEDV. PEDV infection in Vero cells did not result in a significant change in the protein level of eIF5A, and in addition, the activated eIF5A did not affect the proliferation of PEDV. Our results provided new insights into the influence of polyamine metabolism on the proliferation of PEDV.

1. Introduction

PEDV was first discovered in Europe (Pensaert and de Bouck, 1978), and then spread worldwide. Since 2010, PEDV mutant strains have been detected in China (Wang et al., 2013), which has caused serious economic losses to the pig husbandry (Song and Park, 2012; Sun et al., 2012). PEDV infection mainly causes contact acute intestinal diseases in swine, including typical symptoms of watery diarrhea, vomiting and dehydration in newborn piglets, and the mortality can even reach 100% (Jung et al., 2015; Li et al., 2012; Song and Park, 2012). PEDV is a positive-sense, single-stranded RNA virus of the *Coronaviridae* family. The genome of PEDV is about 28 kb in length and contains 7 open reading frames that are responsible for encoding two polyproteins (1a and 1ab) (Kocherhans et al., 2001), four structural spike protein(S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) and an accessory protein (ORF3) (Lee, 2015; Song and Park, 2012).

Some organic small molecules in the cellular environment play a potential role in cell- virus interactions. Polyamines are positively charged aliphatic molecules found in all mammalian cells (Michael, 2016). They are small-sized and abundant metabolic regulators in the cellular environment that participate in the gene transcription, protein translation, cell growth, differentiation and other biological processes in cells (Gerner and Meyskens, 2004; Igarashi and Kashiwagi, 20102015; Mandal et al., 2013; Pegg, 2009). In eukaryotic cells, polyamines exist in the three forms of putrescine, spermidine and spermine (Pegg, 2016). They are synthesized as follows (Bachrach, 2010; Miller-Fleming et al., 2015): Ornithine is converted into putrescine by activating ODC1, and then it is transformed into spermidine and spermine in turn under the action of SRM(spermidine synthase) and SMS (spermine synthase) (Fig. 1). In addition, cells also strictly regulate intracellular spermidine and spermine levels through SAT1, SMOX and PAOX. Spermidine participates in the activation of eIF5A under the action of DHPS(deoxyhypusine synthase) and DOHH(deoxyhypusine hydroxylase). Polyamines are involved in the hypusine modification (Jao and Chen,

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2006; Zanelli et al., 2006), thereby inhibiting the initiation of translation (Park et al., 2010).

Previous studies have demonstrated that polyamines can affect the proliferation of RNA and DNA viruses, involving vaccinia virus (Hodgson and Williamson, 1975; Williamson, 1976), human cytomegalovirus (Gibson et al., 1984; Tyms and Williamson, 1982), herpes simplex virus (Tuomi et al., 1980; Yudovin-Farber et al., 2009), severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) (Firpo et al., 2021), et al. For example, spermine and spermidine mediate the activation of RNA polymerase and the inhibition of viral helicase to regulate the replication process of HCV (Korovina et al., 2012). Evidences have also shown that activated eIF5A can regulate expression levels of viral genes and thus mediate viral proliferation (Olsen et al., 2018) (Tables 1 and 2).

The pattern of metabolism between host cells and virus has been extensively characterized in recent years (Martin-Vicente et al., 2020; Mayer et al., 2019; Yan et al., 2019). So far, the influence of cellular polyamine metabolism on PEDV infection has not been reported. To clarify the interaction between cellular polyamine anabolic pathways and PEDV, this study detected expression levels of polyamine metabolism associated genes ODC1, SMS, SRM, SMOX, SAT1, PAOX, DHPS, DOHH and EIF5A in Vero cells infected with PEDV at different stages, aiming to determine the role of polyamine metabolism pathway in PEDV-infected Vero cells and the effect of polyamines on PEDV proliferation.

Table 1
Primers used for the detection of polyamine-related genes.

Primer	Sequence(5'-3')	Product length(bp)	Use
SAT1-F	AACAGTCTCCAACCCTCTTCAC	166	Detection for SAT1 gene
SAT1-R	GCTTTGGCATAGGATCAGAAAT		
ODC1-F	TGGGTGATTGGATGCTCTTTGA	173	Detection for ODC1 gene
ODC1-R	GGCGTCCTGTTCCTCTACTTCG		
SRM-F	TCCGCAGTAAGACCTATGGCAACG	176	Detection for SRM gene
SRM-R	CACCACCTCACGCAGGACACCT		
SMS-F	ACATGGATTGGTGTTGCTGGAC	157	Detection for SMS gene
SMS-R	TCCTCGCACTATGGGTGGTAAT		
DHPS-F	AGCAGGTTTCCGATCCTATTGATT	184	Detection for DHPS gene
DHPS-R	GTTCAGGCATCCGTGAGACCAT		
DOHH-F	CGATGACGGGGTCCGAGGAATAT	189	Detection for DOHH gene
DOHH-R	TCAAGCATGAGCTGGCCTACTGCC		
EIF5A-F	TGTTATGAGTTGACGGGCAGAT	160	Detection for EIF5A gene
EIF5A-R	CGTAAGAATGGCTTTGTGGTGC		
PAOX-F	ACTCAAGCCCAGCAATGAACCC	181	Detection for PAOX gene
PAOX-R	TGAGAAGCCTGTGAAGACCATCCA		
SMOX-F	CACCCAGCGACACGGTCACAAT	160	Detection for SMOX gene
SMOX-R	GAGGGCGACCATAATCACGACA		

2. Materials and methods

2.1. Cell, virus, and reagents

Vero cells were cultured in Dulbecco's modified eagle medium (DMEM, Gibco, Life Technologies, CA, USA)supplemented with 10% heat-inactivated fetal bovine serum in 5% CO_2 at 37 °C. The PEDV strain was previous isolated by and stored at our laboratory. Vero cells were treated with PEDV (MOI=0.01) at 37 °C for 1 h in 10% FBS DMEM, which allowed the viruses to enter the cells. Putrescine, spermidine(HY-B1776), spermine(HY-B1777), Diftuoromethylornithine(DFMO, HY-

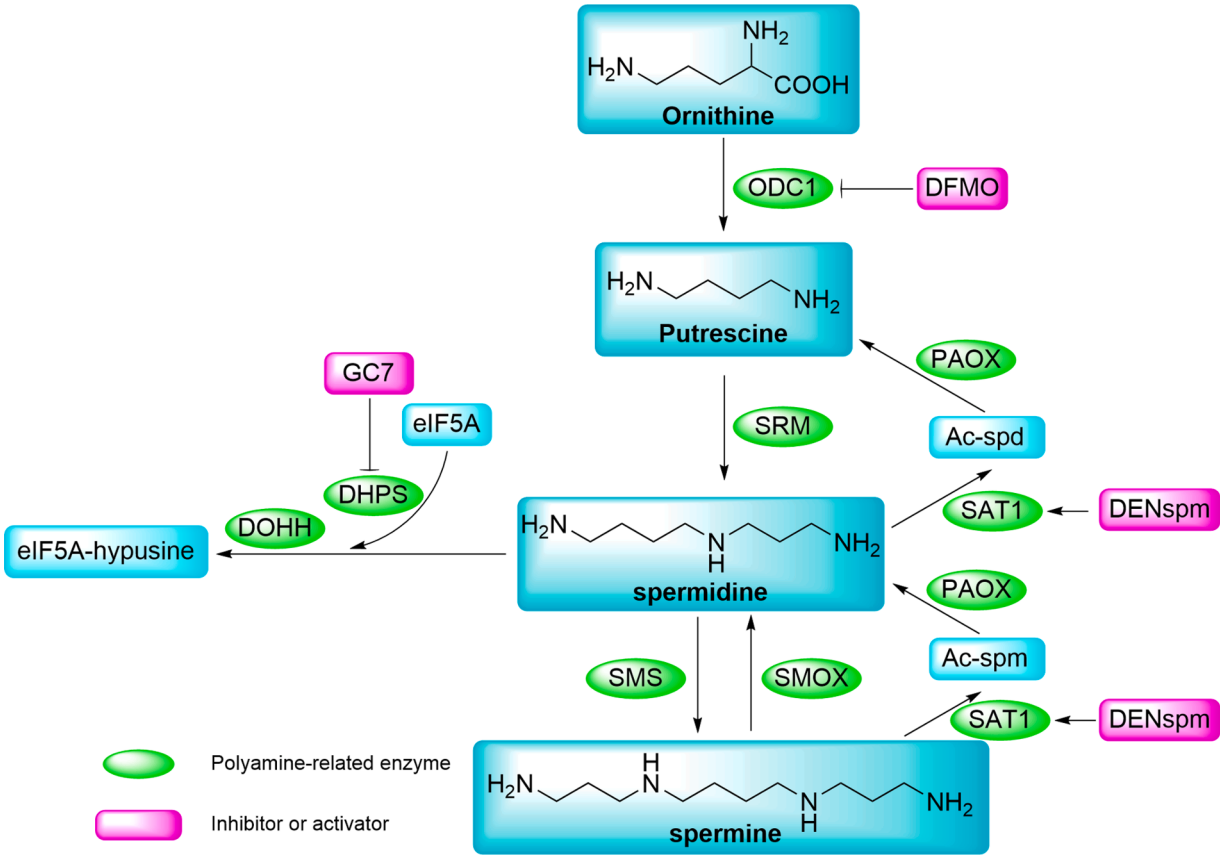

Fig. 1. Pathways related to intracellular biopolyamine metabolism.

Table 2
The siRNA used for silencing SAT1 genes.

Primer	Sequence(5'-3')	Use
siRNA-1	GGAGCTGGCTAAATATGAA	Silence SAT1 gene
siRNA-2	GGAATGAACCATCCATCAA	

130744)were purchased from MedChemExpress(MCE, Shanghai, China). All drugs were stored at -20 °C until further use.

2.2. RT-qPCR

The ODC1, SMS, SRM, SMOX, SAT1, PAOX, DHPS, DOHH and EIF5A sequences were obtained by NCBI. Related primers were designed by Primer 5.0 software. The cDNA template of vero cell was acquired by total RNA extraction kits(Feijie, Shanghai, China) and HisScript Reverse Transcriptase(Vazyme, Nanjing, China). Gene expression was detected with Quantitative Real-time PCR(RT-qPCR) and analyzed by the ΔΔct method. A 198 bp DNA fragment of the N gene was amplified with the primer sets of forward primer (qN306-F) 5′-CGCAAA-GACTGAACCCACTAAC-3′ and reverse primer (56R) 5′-TTGCCTCTGTTGTTACTTGGAGAT-3′.

2.3. Western blotting analysis

The method of protein quantification and western blotting assay were conducted as described previously (Ai et al., 2021). The cell lysate quantifed with the BCA Protein Assay Kit(Bio-Rad, Hercules, CA, USA) run on 12% polyacrylamide gels. Gels were transferred to nitrocellulose membrane. After blocked with 5% non-fat milk for 1 h at room temperature, the membrane was incubated overnight with rabbit primary antibody or mouse primary antibody. Gonjugated goat anti-rabbit IgG or conjugated goat anti-mouse IgG was used for the protein and the membrane was imaged by the Odyssey imaging system(LI-COR Biosciences).

2.4. $TCID_{50}$ test assay

In short, 2×10^4 vero cells were seeded in a 96-well plate and then cultured for 24 h. The ten-fold serially diluted virus solution was added to each well. Cytopathic effects(CPE) was examined for each well at 7 days post infection(dpi.). The virus titers was calculated using the Reed and Muench method.

2.5. Cytotoxicity assay

Cell Counting Kit 8 (Beyotime, Shanghai, China) was used to detect the cytotoxic effects of durgs. After the cells are treated accordingly, the plates are incubated in the incubator for an appropriate period of time. Add 10 μl of CCK-8 solution to each well at the appropriate time point (be careful not to generate air bubbles in the wells, they can affect the O. D. value readings). Incubate the plates in the incubator for 1–4 h and measure the absorbance at 450 nm using a plate reader.

2.6. siRNA interference

The siRNA of SAT1 gene was synthesized from RiboBio(RiboBio Co., Ltd, Guangzhou, China). The transfectionof siRNA was performed as described previously (Qiangyun Ai and Xiwei Lin, 2021). Confluent vero cells of 24-well plates were treated with 6 pmol siRNA using 2 μL RFect (primary cell small nucleic acid transfection reagent) for 72 h to analyze the inhibitory effect.

2.7. Thin layer chromatography(TLC) determination of polyamines

TLC is used to determine the level of intracellular polyamines (Madhubala, 1998). 250 μL 2% perchloric was added to vero cells which were collected and washed twice with PBS. After samples were incubated for 24 h at 4 °C, 200 μL supernatant of samples mixed with 400 μL 5 mg/ml dansyl chloride(Sigma-Aldrich, Shanghai, China) acetone, 200 μL saturated sodium bicarbonate, which was incubated for 16 h in the dark at room temperature. 100 μL 150 mg/ml proline(Sigma-Aldrich) was combined with samples for 30 min in the dark to clear excess dansyl chloride and then the dansylated polyamine was obtained using 500 μL toluene. The polyamine samples was added to the TLC plate (Dingguo, Beijing, China) at small points and the TLC plate was placed in a developing tank with developing solvent(2:3 cyclohexane/ethyl acetate) for 1 h, which was finally scanned and visualized under UV exposure.

3. Result

3.1. PEDV infection affected polyamine-related gene expression

To confirm the expression profiles of polyamine-related gene, the ODC1, SMS, SRM, SMOX, SAT1, PAOX, DHPS, DOHH and EIF5A transcriptional levels of vero cells following PEDV infection for 0, 4, 8 h, 12 and 20 h were detected. There were significant changes in ODC1, SAT1, PAOX and SMOX mRNA expression levels in the cells infected with PEDV, with a 2.7-fold, 4.8-fold, 1.8-fold and 2.8-fold increase at 20 h post infection(hpi.) (Fig. 2). The upregulation of ODC1 and SAT1 which were mainly responsible for the biosynthesis and degradation of polyamines implied the change of the level of intracellular polyamines. In addition, the levels of DHPS and EIF5A mRNA expression were also increased by 2.1 folds and 3.7 folds, respectively (Fig. 2). It was speculated that this may be a chain reaction following the alteration of cellular polyamine content.

3.2. SAT1 reduced the intracellular polyamine level during PEDV infection

To explore the effect of PEVD infection on cellular polyamine content, the TLC was used to detect the polyamine level of cell infected PEDV for 12, 24, and 36 h. The result showed that the level of intracellular polyamines reduced visibly following PEDV infection for 24 h (Fig. 3A and B). It was found previously that SAT1 was up-regulated by 4.8 folds according to the polyamine-related gene expression profiles. These results suggested that cells up regulated the expression of SAT1 to reduce the intracellular polyamine level during PEDV infection.

3.3. SAT1 inhibited PEDV proliferation relying on polyamine depletion

Previous result indicated the sharp increase of SAT1 expression of vero cells with PEDV infection. The next step is to investigate the effect of SAT1 on PEDV multiplication. The cytotoxicity assay of DENSpm(N1, N11-diethylnorspermine) presented that 1, 10, 50, and100 μM DENSpm did not affect vero cell vitality (Fig. 4A). We treated vero cell with escalating does of DENSpm for 16 h, and then detected the mRNA expression level of SAT1 and the level of polyamines of the cell. It was confirmed that 10, 50, and 100 μM DENSpm stimulated the expression of SAT1 upregulated 3.5, 8.9, and 7.2 folds (Fig. 4B). The result were verified by TLC (Fig. 4C and D).

The cells treated with DENSpm were infected with PEDV (MOI=0.01). The supernatants of cells was collected after 24 h post infection(dpi.) to detect the replication level of PEDV genes by Quantitative Real-time PCR(RT-qPCR). The cell extractions were used for western blotting to assess the expression level of PEDV-N protein. These results showed that the expression level of SAT1 was negatively correlated with PEDV titer (Fig. 4F), implicating the overexpression of SAT1 in restricting PEDV multiplication. Beside, we confirmed the redution of PEDV gene transcript levels and PEDV-N protein levels (Fig. 4F and G), which strongly indicated the SAT1 as a role in PEDV replication by

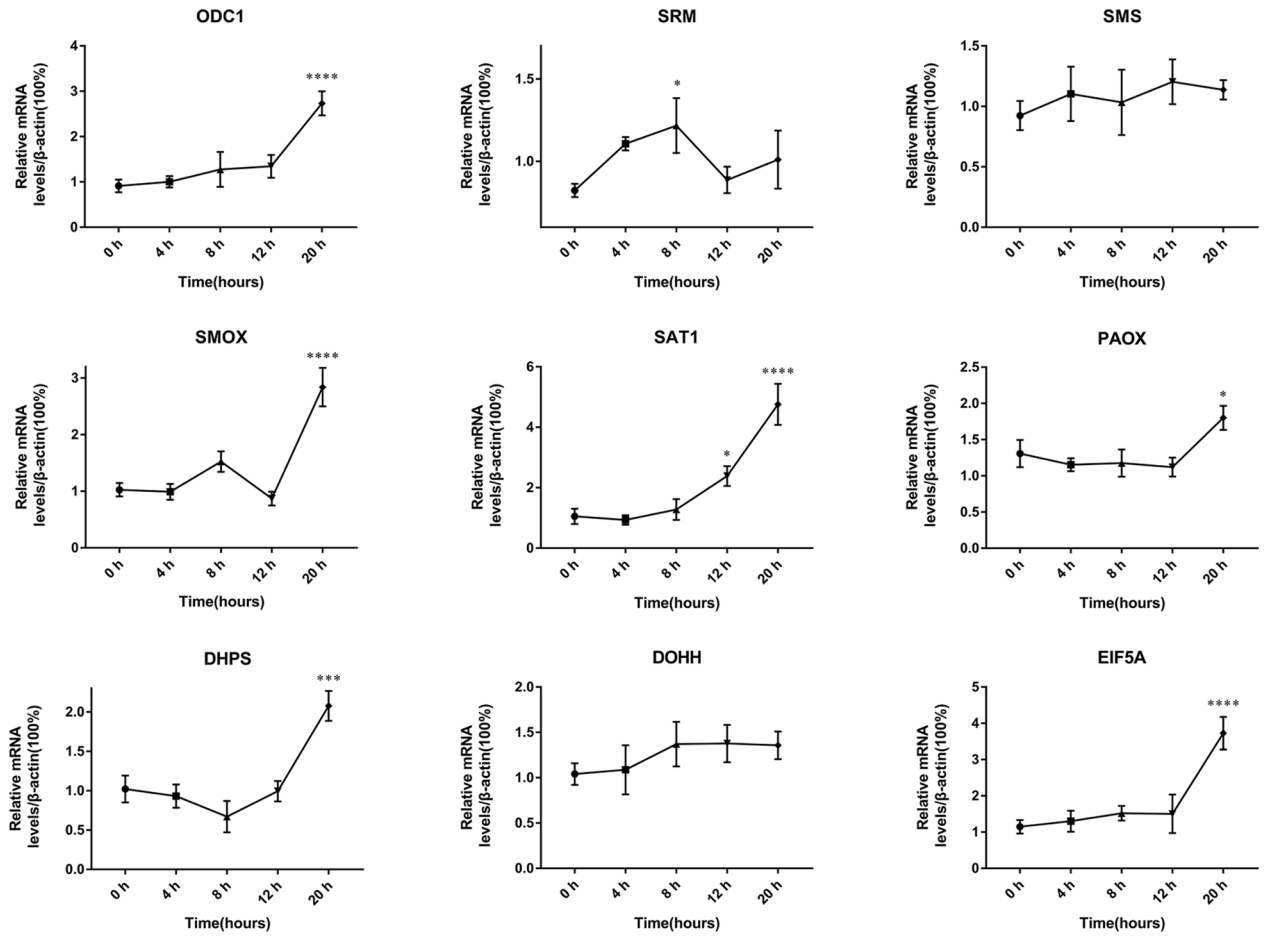

Fig. 2. Changes in the level of ODC1, SMS, SRM, SMOX, SAT1, PAOX, DHPS, DOHH and EIF5A gene expression after PEDV infection of vero cells. Significant differences are relative to the control group (0 h) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

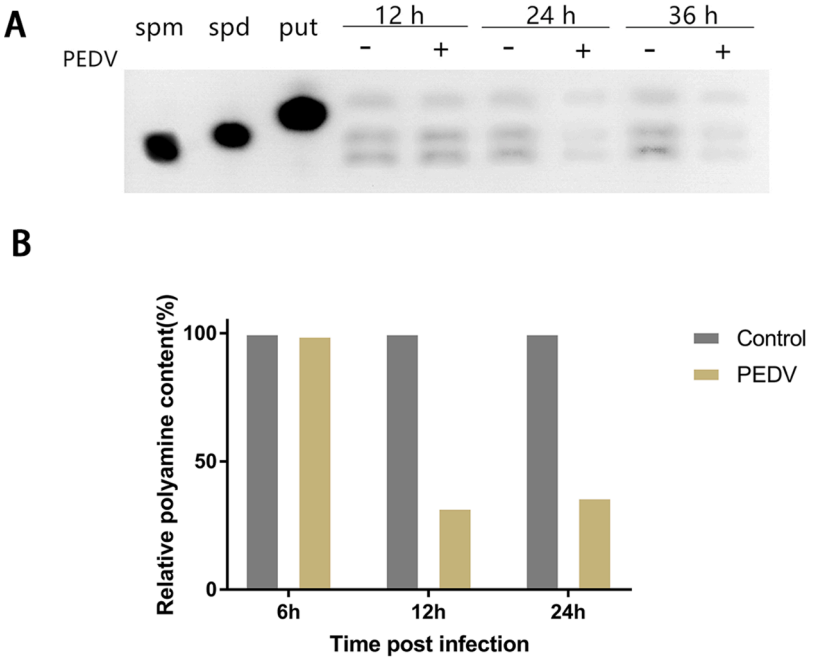

Fig. 3. (A)Vero cells were infected with PEDV (MOI = 0.01) for 12, 24 and 36 h. And then cells were collected, processed and analyzed by TLC. Put(putrescine), Spd (spermidine) and Spm(spermine) represent the standards of individual polyamine. (B)Relative polyamine contents were obtained through ImageJ.

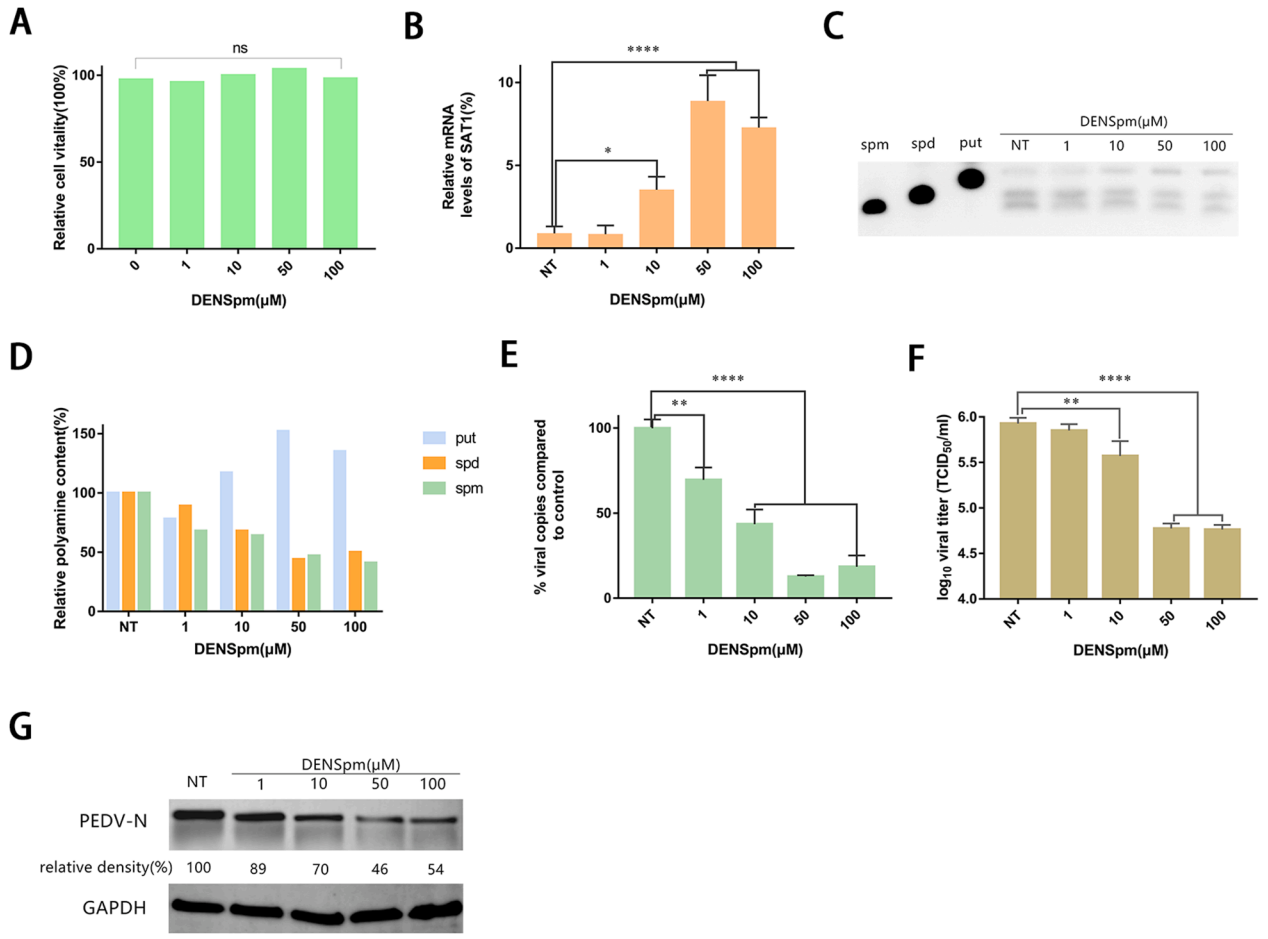

Fig. 4. (A)The cytotoxicity of DENSpm in veros was measured by Cell Counting Kit. (B)The determination of SAT1 levels in veros treated with DENSpm for 16 h by RT-qPCR. (C)The polyamine content were shown by thin-film chromatography. (D)Relative polyamine level was analyzed. (E-G)Veros treated with 1, 10, and 100 μM DENSpm for 16 h were infected PEDV at an MOI of 0.01 for 24 h and then the genomes copies of PEDV were measured and analyzed. The comparison between the value of DENSpm-treated group and non-treated group was shown(E). Concurrently, the viral titers(F) and the PEDV-N protein levels(G) were measured respectively by RT-qPCR and $TCID_{50}$ Test Assay. Significant differences are represented by black asterisks($^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$).

affecting the polyamine level.

3.4. Knock down of SAT1 gene promoted PEDV proliferation

Next, we try to further ask whether SAT1 affects PEDV replication. First, the better siRNA-1 of silencing SAT1 gene was chosen to transfect Vero cell according the result of knockdown efficiency (Fig. 5A). Moreover, the ability of siRNA-1 to down-regulate SAT1 due to siRNA-2 was reconfirmed at the protein level, avoiding a potential off-target effect in the knockdown studies (Fig. 5B). After 72 h, cells were infected with PEDV (MOI=0.01) and the PEDV titer were measured at 24 h post infection. It was found that the PEDV titer of siRNA-1 group increased (Fig. 5D). The same trend was observed at the viral copies level and PEDV-N protein level (Fig. 5C and E).

3.5. Spermidine and spermine facilitate PEDV proliferation

It was knowed that the upregulation of SAT1 expression level results in the reduction of spermidine and spermine of cells. We further investigate the effect of individual polyamines on PEDV replication. The result showed that 4 and 2 mM DFMO(difluoromethornithine) significantly contributed to the depletion of intracellular polyamine and the reduction of PEDV titer (Fig. 6 A–C), implying that polyamines participate the process of PEDV replication. Putrescine, spermidine and spermine is not cytotoxic to vero cells at the specified concentration of 5, 10, 20 and 40 μM (Fig. 6 D). We choosed 2 mM DFMO to treat vero cells for 72 h, and add 10 μM individual polyamines for the PEDV infection. It was found that spermidine and spermine promote PEDV multiplication according to the determination of the titers of PEDV, viral copies and PEDV-N protein levels (Fig. 6 E–G) (Fig. 7).

3.6. PEDV replication is not required for hypusinated eIF5A

It has been reported that eIF5A modified by hydroxyl putrescine lysine is also involved in the replication of a number of RNA viruses. We decided to text whether active eIF5A plays a role in the life cycle of PEDV. The result of western blotting showed that there was no apparent increase in eIF5A protein expression of vero cells infected PEDV, in spite of remarkable up regulation of eIF5A gene in transcriptional level. Vero cells were treated with 1 μM or 10 μM GC7 (inhibiting the hypusination of EIF5A), incubated for 24 h and infected with PEDV(MOI=0.01). The vero samples were harvest for the assessment of PEDV titers and PEDV-N protein expression. These results indicated that there was no significant distinction in viral titer and the protein expression of PEDV-N between 1 μM GC7 group, 10 μM GC7 group and control group, suggesting that PEDV replication was not affected by hypusinated eIF5A.

4. Discussion

The mechanism of interaction between host cell polyamine metabolism and viruses has been extensively studied these years (Huang et al., 2020; Mounce et al., 2016a). Biopolyamines, including putrescine,

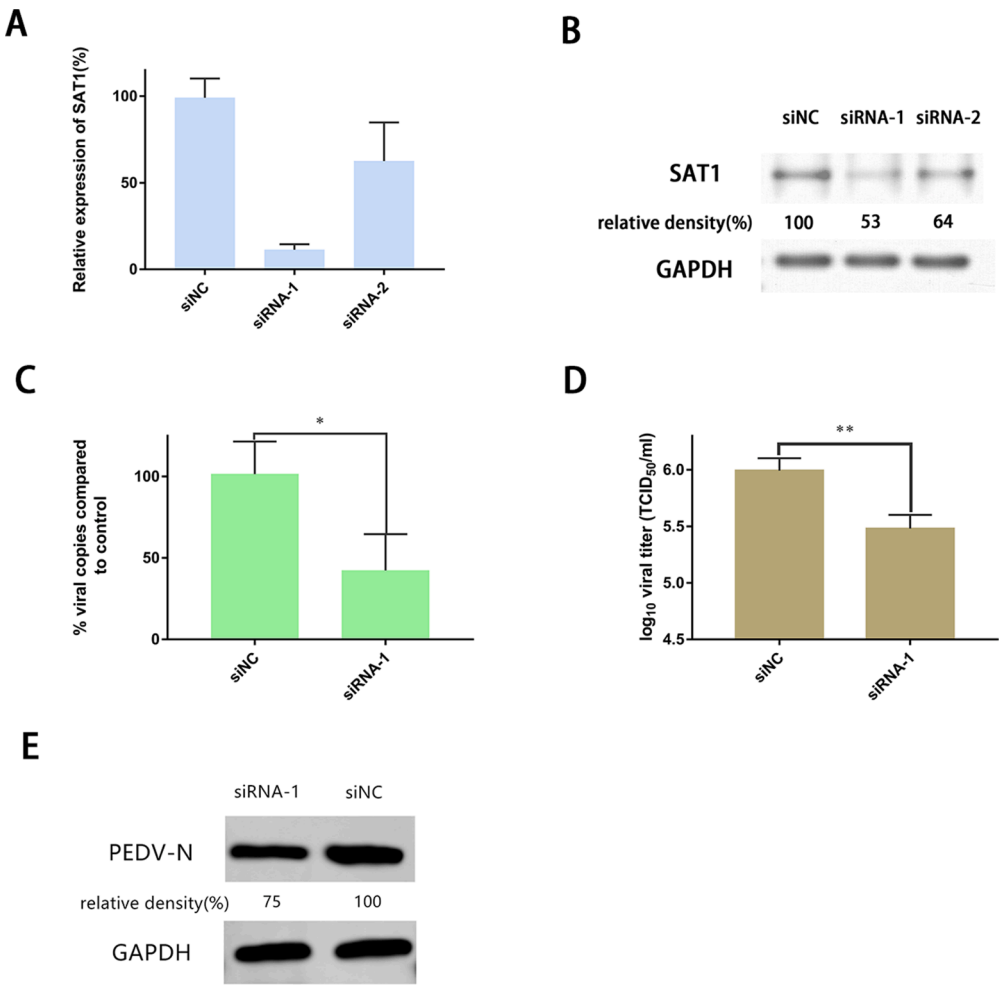

Fig. 5. (A) Test for the knockdown efficiency of two SAT1 siRNAs. (B) Verification for the knockdown efficiency of two SAT1 siRNAs by western blot assays. (C–E) Vero cells were treated with 6 pmol siRNA-1 using 2 μL RFect siRNA transfection reagent for 72 h and infected with PEDV for 24 h. The viral copies(C), viral titers (D) and PEDV-N protein levels (E) were detected.

spermidine, and spermine, have been extensively investigated about the potential functions for virus infection and transmission. Early researchers identified a specific link between polyamines and viral proliferation (Clarke and Tyms, 1991; Colombatto et al., 1989; Fukuma and Cohen, 1975; Gibson and Roizman, 1971; Lanzer and Holowczak, 1975). At first, researchers found the presence of polyamine in the viral particles of two densonucleosis viruses, which was responsible to the neutralization of viral DNA (Kelly et al., 1977). Studies in recent years have further elucidated the role of polyamines in the life cycle of virus. Intracellular polyamine depletion was able to interfere the crucial processes of Zika and Chikungunya viruses generation on account of damage to the function of viral polymerse (Mounce et al., 2016b). HBc protein expression of Hepatitis B virus also was regulated by polyamines (Mao et al., 2020). Some researchers have found that polyamines are involved in the binding process of enterovirus and host cell (Kicmal et al., 2019). And polyamines interfered with the entry of nunyavirus to reduce the production of infectious viral particles (Mastrodomenico et al., 2020).

Some viruses potentially have associated mechanisms, regulating intracellular polyamine metabolism to establish more advantageous and supportive conditions for reproduction. Cytomegalovirus infection results in the increase in spermidine and spermine via up regulation of ornithine decarboxylase(ODC1) in fibroblasts (Garnett, 1988). Recently, the research showed the polyamine level was elevated in porcine alveolar macrophages(PAM) during ASFV infection depend on ARG1-polyamine pathway (Ai et al., 2021). There was also alternative mechanism for virus to respond to the regulation of polyamine metabolism in host cells. Coxsackievirus B3 is resistant to drastic change in polyamine content in the host cell environment through the enhanced hydrolytic activity of mutant 2A and 3C protease (Dial et al., 2019; Hulsebosch and Mounce, 2021). And the mutations of nsP1 and the opal stop codon, which was able to enhance the activity of membrane binding and methyltransferase and facilitate downstream translation, conferred chikungunya virus resistance to polyamine depletion in BHK-21 cells (Mounce et al., 2017).

However, host cells develop corresponding countermeasure. Least studies pay attention to the regulation of polyamine metabolism in the host cells responding to viral infection. Hela and L-cells reduced cellular spermine and spermidime levels via the regulation of polyamine metabolism after infection of herpes simplex virus (McCormick, 1978; McCormick and Newton, 1975). When HCV core proteins and NS5A are expressed in large amounts in transient in Huh7 cells, ODC1, SAT1 and SMOX expression levels were subsequently altered, led to the reduction of intracellular polyamine concentration (Smirnova et al., 2017). In addition, PAM reduces the level of spermidine and spermine via SAT1 to respond to porcine reproductive and respiratory syndrome virus (PRRSV) infection (Zhou et al., 2020).

Here, we understand that the expression levels of ODC1, SAT1, SMOX and PAOX increase in vero cell from 12 to 20 h after PEDV infection. The level of intracellular polyaminehave have been known to

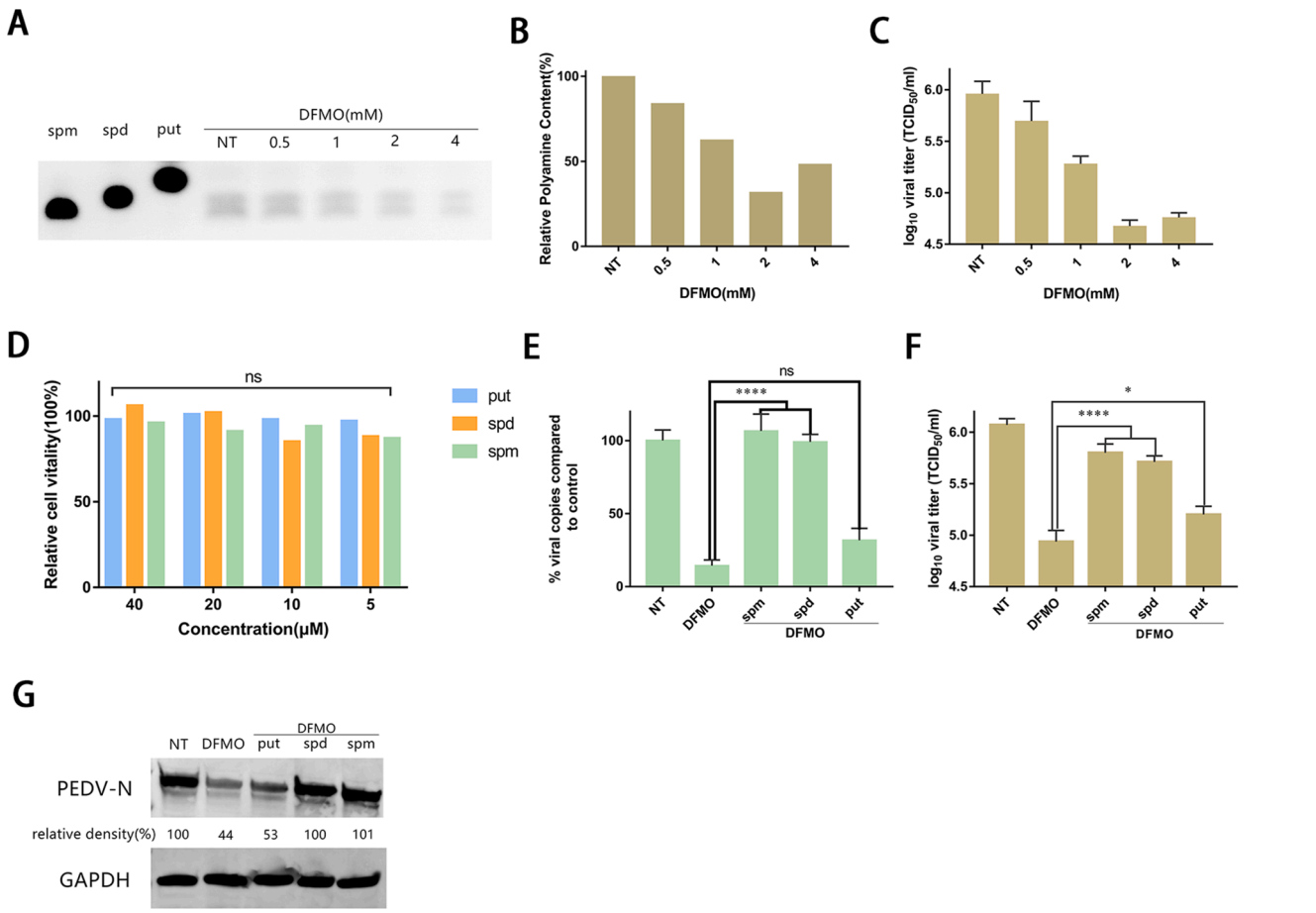

Fig. 6. (A,B)The polyamine level of vero cells treated with DFMO for 72 h was comfirmed by TLC(A) and relative polyamine content was quantitated by ImageJ(B). (C)DFMO-treated vero cells were infected with PEDV at an MOI of 0.01 for 24 h and cell culture medium was collected to detect the titers of PEDV. (D)The cytotoxicity of DFMO. (E–G)10 μM putrescine, spermidine, spermine and DFMO-treated vero cells incubated for 24 h, respectively followed by the infection with PEDV for 24 h. The viral copies(E), viral titers(F) and PEDV-N protein levels(G) were detected.

decline after 12 h of infection. ODC1 gene encodes ODC in charge of an essential step in facilitating putrescine biosynthesis in cell (Lenis et al., 2017). SAT1, PAOX, and SMOX are critical enzymes in the polyamine metabolic pathway, which are responsible for the catabolism of spermine and spermidine (Firpo and Mounce, 2020; Mandal et al., 2013). Our studied showed that upregulation of SAT1 contributes to depleting the polyamines of host vero cells to limit the proliferation of PEDV.

In addition, eIF5A which is a only cellular protein requiring modification rely on spermidine plays the potential function of replication of certain viruses (Zanelli and Valentini, 2007). Early studies showed that eIF5A has an impact on Rev trans-activation and nuclear export of RNA, interfering with HIV-1(human immunodeficiency virus type 1) replication (Andrus et al., 1998; Bevec et al., 1996; Liu et al., 2011). The hypusinated eIF5A was required for the activity of IRES, which mediated the translation initiation of HIV-1, HTLV-1(human T-cell lymphotropic virus type 1) and MMTV(mouse mammary tumor virus) mRNA (Caceres et al., 2016). The infection of dengue virus has stimulated hight expression of eIF5A in C6/36 cells (Shih et al., 2010), however, subsequent studies showed that the eIF5A did not have a direct effect on the proliferation of dengue virus. The accumulation of EBOV VP30 protein was affected by the hypusination of eIF5A (Olsen et al., 2016). Our data showed that eIF5A and DHPS transcript levels were upregulated by 3.7 folds and 2.1 folds during PEDV infection, implying hypusinated eIF5A may affect the multiplication of PEDV. However, our subsequent experiments showed that there was no change in the protein level of eIF5A after 24 h of infection. And it was confirmed that the treatment of GC7 barely affected the proliferation of PEDV according to the result of viral copies, titers and the PEDV-N protein expression levels.

5. Conclusions

In summary, we investigated the regulation of polyamine-related genes expression in vero cells infected PEDV. Host cells reduce polyamine level via upregulation of SAT1. Spermidine and spermine promote PEDV replication. We then confirmed that PEDV proliferation is independent of hypusinated eIF5A. Our studies characterized the pattern of polyamine metabolism in vero cells infected with PEDV and confirmed the role of polyamine in the proliferation of PEDV. These results revealed the potential therapeutic value of the drugs targeting polyamine metabolic pathways for PEDV infection. During the long-term infection and anti-infection process between host cells and virus, we hypothesize that host cells have evolved a special immune mechanism, which is mean that host cells downregulate the intracellular polyamine levels through the stimulation with certain structural and nonstructural proteins of PEDV. This conjecture requires further study, the result of which will contribute to the development of antiviral drugs or vaccine adjuvants for PEDV.

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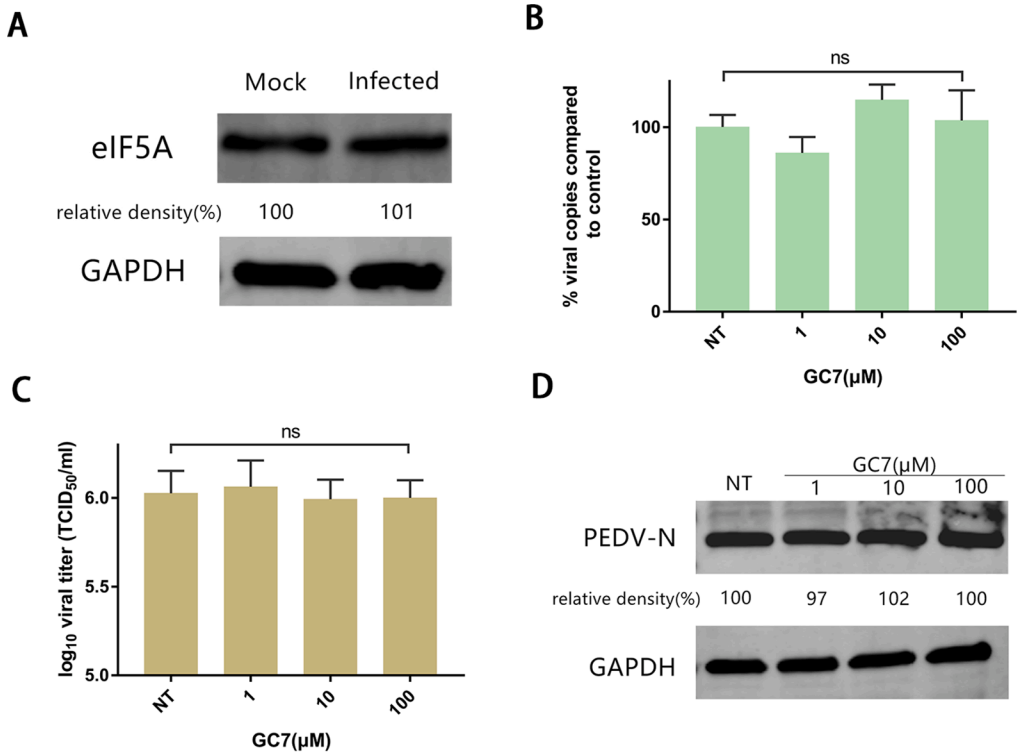

Fig. 7. (A)The eIF5A protein level of vero cells infected with PEDV for 24 h was detected by western blot analysis. (B–D)Vero cells were treated with 1 μM, 10 μM and 100 μM for 24 h respectively and infected with PEDV for 24 h. The viral copies(B), viral titers(C) and PEDV-N protein levels(D) were detected.

CRediT authorship contribution statement

Hangao Xie: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Qiangyun Ai:** Validation, Resources, Data curation, Investigation, Writing – review & editing. **Tiezhu Tong:** Resources, Methodology, Investigation, Writing – review & editing. **Ming Liao:** Supervision, Project administration, Visualization, Writing – review & editing. **Huiying Fan:** Conceptualization, Funding acquisition, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interest.

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