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Tissue expression of porcine transient receptor potential mucolipin protein channels and their differential responses to porcine reproductive and respiratory syndrome virus infection *in vitro*

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Abstract

Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) infection results in a serious disease, posing a huge economic threat to the global swine industry. The transient receptor potential mucolipin proteins (TRPMLs) have been shown to be strongly associated with virus infection and other physiological processes in humans, but their tissue distribution and responses to PRRSV in pigs remain unknown. **Material and Methods:** Quantitative reverse-transcription PCR analysis was undertaken to determine the optimal primer for TRPML expression detection and for quantifying that expression individually in different pig tissue samples. Meat Animal Research Center 145 (MARC-145) monkey kidney cells and the TRPML-specific activator mucolipin synthetic agonist 1 (ML-SA1) were used to reveal the relationship between TRPML and PRRSV-2 infection. **Results:** The best primers for each TRPML gene used in a fluorescence-based quantitative method were identified and TRPML1 was found to be highly expressed in the kidneys and liver of pigs, while TRPML2 and TRPML3 were observed to be primarily expressed in the kidney and spleen tissues. The expression of TRPML2 was upregulated with the rise in PRRSV-2 infection titre but not the expression of TRPML1 or TRPML3, and ML-SA1 inhibited PRRSV-2 in a dose-dependent manner. **Conclusion:** Our research revealed the gene expression of TRPMLs in pigs and identified that TRPML channels may act as key host factors against PRRSV infection, which could lead to new targets for the prevention and treatment of pig infectious diseases.

Keywords: pig diseases, PRRSV infection, TRPML expression, qRT-PCR, specific activator.

Introduction

Porcine reproductive and respiratory syndrome (PRRS), also known as porcine blue ear disease, is an acute contagious disease with typical temporal and spatial distribution characteristics. It is caused by porcine reproductive and respiratory syndrome virus (PRRSV), which has caused huge economic losses to the global swine industry (6, 9). The virus is an enveloped, single-stranded positive-sense RNA virus with a genome of approximately 15 kilobase pairs in length (2). According to the newest official classification given by the International Committee on Taxonomy of Viruses, PRRSVs are separated into two species – *Betaarterivirus suid 1* (PRRSV-1) and *Betaarterivirus suid 2* (PRRSV-2) – and only 50–60% sequence similarity is noted between the two species. Strains of each species are further classified into multiple genetic lineages, and all strains are prone to sequence diversification through the accumulation of point mutations and recombination of homologous genomic fragments (20). Numerous PRRSV variants have emerged in China, including JXA1, HuN4-F112, NADC30-like and TJM-F92, which has significantly increased the complexity and difficulty of Chinese PRRSV control. As a result of PRRSV genetic variation and the vast scale of pig breeding, China has become the country suffering the greatest economic losses in the swine industry (18, 30). Acute respiratory distress, foetal death, abortion in pregnant sows, reproductive failure and congenital infections in pregnant animals are the

primary clinical manifestations of PRRSV infection. These clinical signs are similar in both low- and high-virulence PRRSV infections and justify why PRRSV is regarded as one of the most significant economic threats in the swine industry (5). Although vaccination protection is still the major defence against PRRSV, commercial vaccines do not offer adequate protection because of the variety of strains of PRRSV (22). Therefore, an ongoing challenge for the pig industry is identifying new intracellular antiviral targets and developing effective antiviral medications against these targets.

As a type of ion channel that is widely distributed in the peripheral and central nervous systems, the transient receptor potential (TRP) superfamily is a requisite for the detection of or response to many external stimuli, such as light, sound and temperature (29). The transient receptor potential mucolipin protein (TRPML) subfamily consists of three homologous proteins in mammals – TRPML1, TRPML2 and TRPML3 – sharing approximately 40% identity of their amino acid sequences (21). In addition to mediating some non-selective cation outflow (such as calcium and sodium ions), TRPML channels as the major cation channel on late endosome or lysosome membranes have also been revealed to be strongly associated with endosomal pH homeostasis, membrane trafficking, lysosome maturation, membrane fusion and autophagy (12, 25). Since the change of calcium concentration in the vesicles plays an important role in the process of endosomal division, transport and fusion, TRPML channels are also known as endosomal membrane transporters because of their essential maintenance of calcium balance (8, 21).

In addition to the specific binding of cell surface receptors, many viruses, including severe acute respiratory syndrome coronavirus 2, influenza A virus, Dengue virus (DENV), Zika virus (ZIKV) and PRRSV, require the hijacking of receptor-mediated endocytosis in order to enter the host (1, 28). Correspondingly, several critical regulatory proteins on the endosomal membrane, particularly ion channels, may become key host defences against pathogen invasion. Recently, TRPML channels have been revealed to be a crucial host factor in the regulation of the invasion of enveloped RNA viruses because of their unique endosomal membrane location (15). Rinkenberger *et al.* (13) discovered that increasing TRPML2 or TRPML3 expression can improve the infectivity of enveloped RNA viruses by enhancing viral trafficking from early to late endosomes. Furthermore, our research group first found that compound mucolipin synthetic agonist 1 (ML-SA1) and SN-2, which are selective activators of TRPMLs, could prevent DENV2 and ZIKV entry by regulating late endosome acidification and fusion with lysosomes in a concentration-dependent manner (23, 24). Therefore, TRPML-mediated endosomal trafficking may provide novel antiviral targets or strategies for the prevention and control of diverse enveloped RNA viruses. To better understand TRPML gene expression in pig tissues and their differential responses to PRRSV infection, quantitative reverse-transcription PCR (qRT-PCR) analysis and cell culture were undertaken. Additionally, MARC-145 cells were employed to measure the response because prior research has shown that PPRSV specifically infects pigs and a small number of cell lines, including pulmonary alveolar macrophages (PAMs), the African green monkey kidney epithelial cell line MA-104, and its derivative MARC-145 cells (5). The experiments were to determine whether TRPML channels could be a potential therapeutic target in PRRSV infection, to provide new insights into virus-host interactions, and to suggest novel controlling measures for future PRRSV outbreaks.

Material and Methods

Animals, cells and viruses. Three weaned piglets, approximately two weeks old, were purchased from Zhengzhou University (Zhengzhou, China) and received food or water regularly to provide an adaptation period before the experiment. Cells of a monkey kidney line highly permissive for PRRSV infection – Meat Animal Research Center 145 (MARC-145) – were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin at a temperature of 37°C in a humidified 5% CO_2 incubator. The less pathogenic PRRSV strain BJ-4 belonging to the PRRSV-2 species (LP-PRRSV, GenBank accession No. AF331831.1) was kindly donated by Professor Beibei Chu from Henan Agricultural University (Zhengzhou, China). Viruses were propagated and titrated in MARC-145 cells by calculating the median tissue culture infective dose as previously described (27).

Cell viability assay. To determine the appropriate concentration of ML-SA1 on PRRSV infection, its cytotoxicity was detected by the cell-counting kit 8 (CCK-8) reagent in MARC-145 cells according to the manufacturer's instructions (Yeasen Biotechnology, Shanghai, China). First, MARC-145 cells were seeded into 96-well plates at a density of 5×10^3, and then incubated with various concentrations of ML-SA1 (from 0 μM to 200 μM) when the cells reached 80% cover. After 36 h of incubation, the cell culture medium was removed, and cells were cultured in 100 μL of fresh medium with 10 μL CCK-8 reagent in each well for 1.5 h at 37°C. Finally, the optical density at 450 nm was measured using an ELx 800 Absorbance Microplate Reader (Biotek Instruments, Winooski, VT, USA), and the toxic effect of ML-SA1 on MARC-145 cells was determined according to the instructions supplied with the CCK-8 reagent. Each experiment was repeated three times, and data analysis was carried out as proposed by the manufacturer of the microplate reader:

Cell viability (%) = ((As−Ab)/(Ac−Ab)) × 100,

where As is absorbance of the experimental well (absorbance of wells containing cells, medium, CCK-8

and test compound); Ab is blank well absorbance (absorbance of wells containing medium and CCK-8); and Ac is control well absorbance (absorbance of wells containing cells, medium and CCK-8).

Design of qRT-PCR primers. The coding sequences (CDS) of the three TRPML genes were first searched for in the sequences of three TRPMLs logged in the National Center for Biotechnology Information (NCBI) GenBank database. Then, the CDS of each TRPML gene was imported into Beacon Designer software (PREMIER Biosoft, San Francisco, CA, USA) and the analysis results offered three potential primers that could be used in qRT-PCR experiments for TRPML detection, which were screened (Table 1 and Table S1). Under the selection process of the software, the regions that exhibited significant cross homologies and template structures were avoided automatically during the designing of the primers. Further considering other factors that affect primer specificity, including guanine-cytosine content, self-dimer primers and secondary structure formation, we screened three pairs of primers with the highest score for each TRPML gene. Finally, the designed candidate primers were synthesised by Wuhan Tianyi Huiyuan Biotechnology (Wuhan, China) and stored at −20°C until use.

Analysis by qRT-PCR. To quantify the specific gene expression of the tissues or cells, the intracellular total RNA from pig tissues or MARC-145 cells was extracted using the TRIzol reagent according to the manufacturer's (R401-01, Vazyme, Nanjing, China) protocol. After that, the concentrations and quality of purified RNA were first identified using a B-500 ultramicro UV-Vis spectrophotometer (Shanghai Yuanxi Instrument, Shanghai, China), and then 0.5–1 μg of total RNA was used as the template for complimentary DNA (cDNA) synthesis carried out with the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). Next, a quantitative fluorescence reaction with a volume of 20 μL was performed, containing 10 μL of Bestar SYBR green qRT-PCR master mix (DBI Bioscience, Ludwigshafen, Germany), 1 μL of cDNA template, 0.5 μL of forward and reverse primer and 8 μL double-distilled H_2O. The qRT-PCR primers used for gene expression testing and their specific sequence information are shown in Table 1. Finally, the threshold cycle values were determined with a LightCycler 96 instrument (Roche Life Science, Basel, Switzerland), and the relative gene expression level was analysed *via* the comparative method ($\Delta\Delta C_T$). Experiments were carried out independently in triplicate with duplicate real-time PCRs.

Table 1. Primers used in this experiment

Primer name	Primer type	Primer sequence (5′ to 3′)	Product size (base pairs)
Pig β-actin	Forward	CGGGACATCAAGGAGAAGC	132
	Reverse	CTCGTTGCCGATGGTGATG	
Pig TRPML1	Forward	CAGTTACAAGAACCTCAC	111
	Reverse	CAGTCAGGGATTTCATTG	
Pig TRPML2	Forward	AATCAGTATCATCGTCTA	140
	Reverse	ATATTCAGTGTCTCATTAG	
Pig TRPML3	Forward	TTACTTGGTCTGGCTGTT	129
	Reverse	TCCTCTTGGTAATGCTTAATTG	
Monkey GAPDH	Forward	TCAACGACCACTTTGTCAAGCTCA	97
	Reverse	GCTGGTGGTCCAGGGGTCTTACT	
Monkey TRPML1	Forward	CGGATGACACCTTCGCAGCCTAC	108
	Reverse	ACGCATACCGGCCCAGTGACAC	
Monkey TRPML2	Forward	CTGTGGCTGGATTGTCTTAGG	121
	Reverse	CTGGATTTGGGCAAAGGTTG	
Monkey TRPML3	Forward	CCAGAAATTGAAACTGAGTGTT	205
	Reverse	ATGTTATAGTCAGAGTAAAGTC	
PRRSV ORF5	Forward	GAAATGCTTGACCGCGGGCT	261 (16)
	Reverse	GTGTCAAGGAAATGGCTTGT	

TRPML – transient receptor potential mucolipin gene; GAPDH – glyceraldehyde 3-phosphate dehydrogenase; ORF – open reading frame

Specificity analysis of porcine TRPML qRT-PCR primers and detection of TRPML expression in different pig tissues by identified primers. In general, the melting curve is an efficient way to measure the specificity of qPCR primers, and a single distinct peak in each curve for each set of primers suggests that the primer specificity is good (26). In order to determine the specificity of the qPCR primers designed for the TRPML genes, first the recombinant TRPML plasmids were diluted 100 and 1,000 times, and then the diluted plasmid was used as the positive control; the negative control did not contain a template. Finally, the best primers with a single distinct peak in each curve and the lowest detection value in the negative control group were selected. Meanwhile, based on the identified qRT-PCR primers, qRT-PCR was initially used to check the expression of three TRPMLs in a variety of pig tissues, including the heart, liver, spleen, lung, kidney, stomach, bladder, and intestines. Data are expressed as ratio to TRPML mRNA expression in heart tissues after being adjusted to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

PRRSV infection and ML-SA1 treatment in MARC-145 cells. First, MARC-145 cells with a 60% confluence were infected with PRRSV at a multiplicity of infection (MOI) of 0.1 or 1 using the same volume of fresh media as a control. After 36 h post infection, the cell morphology was recorded by an M-shot fluorescent microscope, and the mRNA level of PRRSV or TRPML channels was assessed by qRT-PCR. To further investigate whether TRPML channels are directly associated to PRRSV infection, ML-SA1, a specific TRPML agonist, was employed. MARC-145 cells were first incubated with 10 μM or 50 μM of ML-SA1 using the same volume of DMSO as a control, respectively. After 36 h post infection, the cells were washed three times with ice PBS. Finally, the intracellular PRRSV RNA were analysed by qRT-PCR, and the cell morphology was recorded at a magnification of 100× by an M-shot fluorescence microscope (scale bars 100 μm).

Statistical analysis. All data were obtained from at least three independent experiments for quantitative analyses. These were performed in GraphPad Prism software (La Jolla, CA, USA). Graphical representations of the analysis results were created using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA). The experimental results were expressed as means ± standard deviation and significance of statistical analysis was calculated by the two-tailed Student's *t*-test.

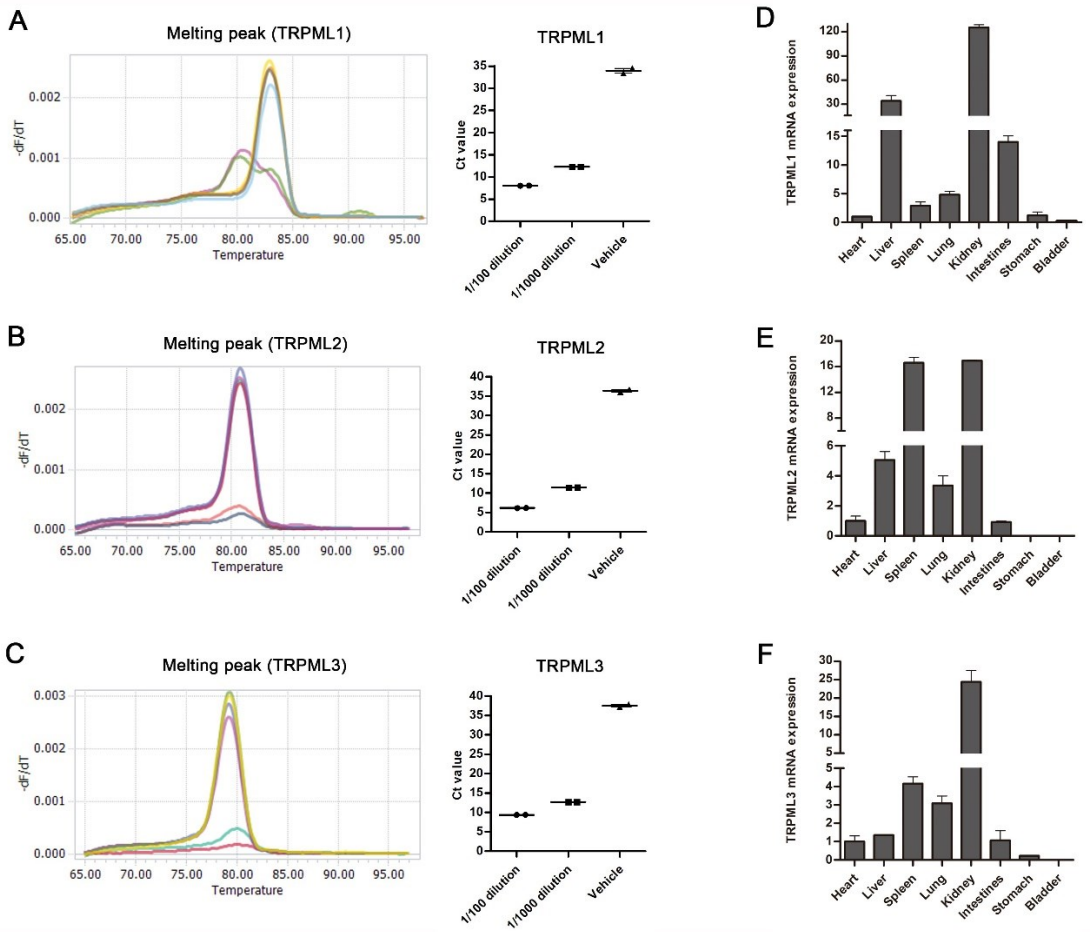

Fig. 1. Identification of primer specificity for transient receptor potential mucolipin (TRPML) genes in quantitative reverse-transcription PCR (qRT-PCR) and their distribution in peripheral porcine tissues. A – Melting curves of qRT-PCR for TRPML1 using the designed primers, and relative threshold cycle (Ct) values of TRPML1 obtained with different templates; 1/100(0) dilution – dilution of the constructed TRPML1 plasmid; vehicle – negative control containing no template; B – Melting curves of qRT-PCR for TRPML2 using the designed primers, and relative Ct values of TRPML2 obtained with different templates; C – Melting curves of qRT-PCR for TRPML3 using the designed primers, and relative Ct values of TRPML3 obtained with different templates; D–F – levels of porcine TRPML1, TRPML2 and TRPML3 messenger RNA (mRNA) in different porcine tissues determined by qRT-PCR. Data are presented as the means ± standard deviation

Results

Primer specificity and differentiation of expression of TRPMLs in porcine tissue samples. The melting curve of the primer which was ultimately chosen had a single peak when the template had a dilution of 1:100 or 1:1,000 of the recombinant plasmids (Figs 1A–C). The best primer for TRPML1, TRPML2 or TRPML3 displayed the lowest detection value in the negative control group (Figs S1–S3). Significantly, expression of TRPML1 was notably low in other tissues, particularly the bladder, but was substantial in the kidney and liver tissue (Fig. 1D). Whereas, transient receptor potential mucolipins 2 and 3 were mostly expressed in the spleen besides the kidney tissue, in contrast to the tissue in which TRPML1 was expressed (Figs 1E and F).

Expression of three TRPML proteins in response to PRRSV infection in MARC-145 cells. Based on the close relationship between TRPML channels and pathogen invasion, the effect of PRRSV infection on three TRPML expression was further explored. Both the cytopathic effect (CPE) caused by PRRSV infection and the expression of PRRSV open reading frame (ORF) 5 showed a dose-dependent increase, indicating that PRRSV successfully infected MARC-145 cells (Figs 2A and 2B). Surprisingly, we further discovered that PRRSV infection significantly reduced the expression of TRPML1 or TRPML3 in MARC-145 cells by qRT-PCR, whereas it increased the expression of TRPML2, suggesting that TRPML channels can collectively respond to PRRSV infection differently (Fig. 2C).

Effect of TRPML specific activator ML-SA1 on PRRSV infection in MARC-145 cells. Because the toxic effect of ML-SA1 on MARC-145 cells is yet unknown, a CCK-8 assay was initially used. The results revealed no cytotoxicity of ML-SA1 to the MARC-145 cell line, even at concentrations up to 100 μM (Fig. 3A). Then, the inhibitory ability of ML-SA1 against PRRSV infection was evaluated using qRT-PCR assays. Both the CPE caused by PRRSV infection and the expression of PRRSV ORF5 were shown to be suppressed dose dependently, and ML-SA1 with a concentration of 50 μM inhibited approximately 40% of PRRSV RNA (Figs 3B and 3C). These data suggest that ML-SA1, as a selective activator of TRPMLs, is a potent inhibitor of PRRSV *in vitro*.

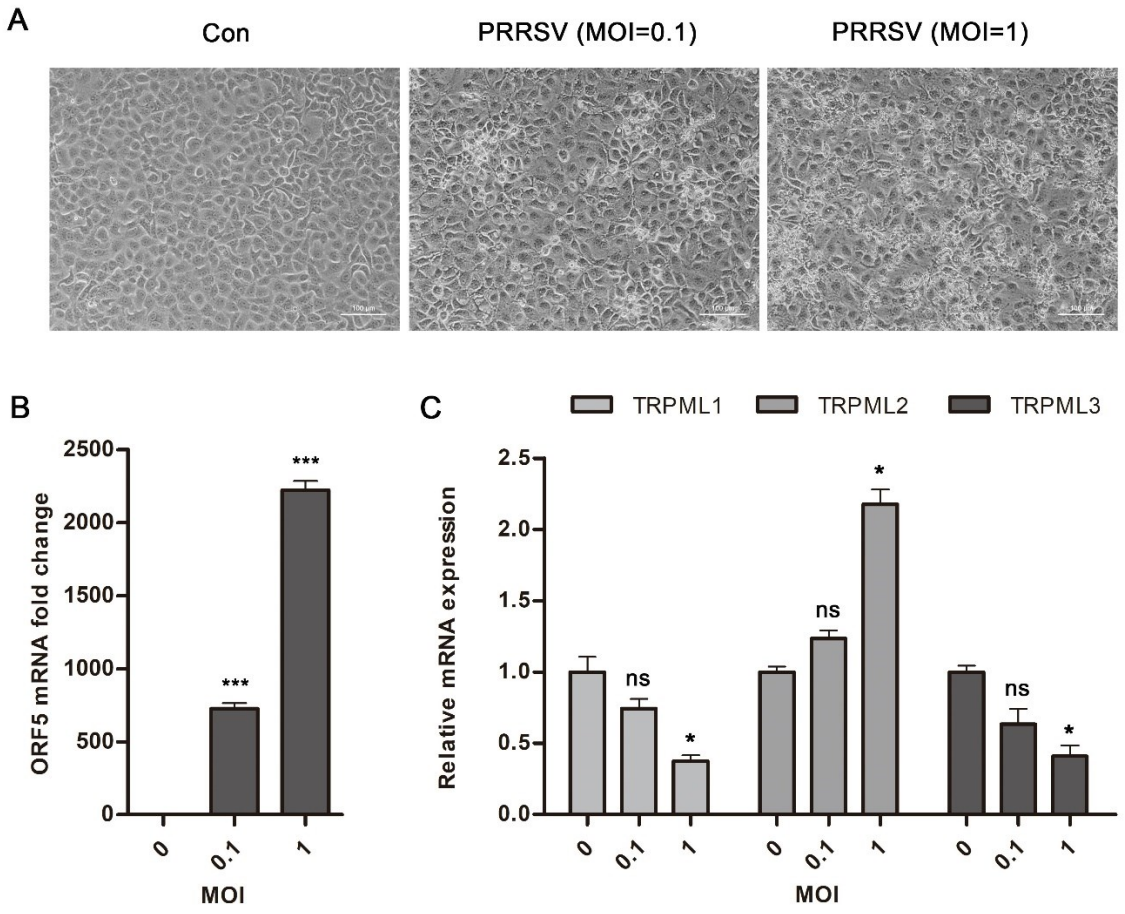

Fig. 2. Effects of porcine reproductive and respiratory syndrome virus (PRRSV) infection of Meat Animal Research Center 145 (MARC-145) monkey kidney cells on the expression of three TRPML proteins. A – Cell morphology with increased multiplicity of infection (MOI) of PRRSV infection. Morphology of MARC-145 cells at a magnification of 100× (scale bars 100 μm); B – Messenger RNA level of PRRSV open reading frame 5 assessed by quantitative reverse-transcription PCR analysis; C – Differential intracellular expression of three TRPML proteins regulated by PRRSV infection. For quantitative analyses, all data were obtained from at least three independent experiments and data are presented as the means ± standard deviation; Con – MARC-145 cells without virus infection; * – P-value < 0.05; ** – P-value < 0.01; *** – P-value < 0.001; ns – no significance

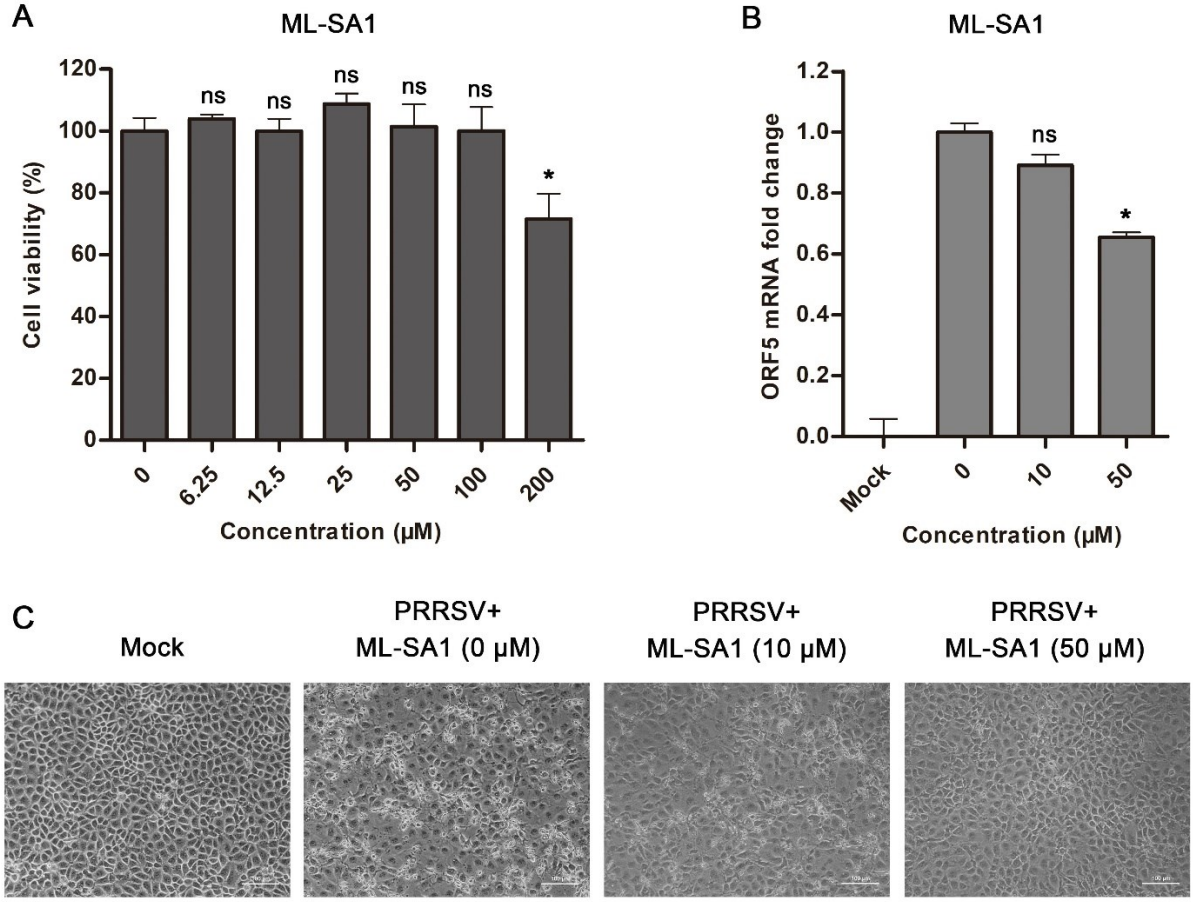

Fig. 3. Inhibitory activity of mucolipin synthetic agonist 1 (ML-SA1) against porcine reproductive and respiratory syndrome virus (PRRSV) in Meat Animal Research Center 145 (MARC-145) monkey kidney cells. A – Cytotoxicity of ML-SA1 in MARC-145 cells. Viability of cells treated with concentrations of ML-SA1 from 0 μM to 200 μM measured by a cell-counting kit 8 assay; B – Messenger RNA level of PRRSV open reading frame 5 with different concentrations of ML-SA1 treatment in MARC-145 cells incubated with 10 μM or 50 μM of ML-SA1; C – MARC-145 cell morphology in PRRSV infection after addition of different concentrations of ML-SA1 and incubation with 10 μM or 50 μM ML-SA1. For quantitative analyses, all data were obtained from at least three independent experiments and data are presented as the means ± standard deviation; Mock –MARC-145 cells without virus or ML-SA1 treatment; * – P-value < 0.05; ** – P-value < 0.01; *** – P-value < 0.001; ns – no significance

Discussion

The primary cause of porcine reproductive and respiratory syndrome is PRRSV, and its causing of significant mortality of newborn piglets and respiratory dysfunction in pigs of all ages cannot be disregarded. For instance, the annual economic losses caused by PRRSV in the United States reach $664 million (10). The virus' characteristics of fast transmission speed and strong mutation ability, the limited cross-protection afforded by vaccines, and antibody-dependent enhancement effect are what make finding new intracellular antiviral targets and strategies an important undertaking for the swine industry worldwide (14). Recently, TRPML channels – the main non-selective cation channels on endosome or lysosome membranes – have been linked to innate immune pathways and the control of viral invasion, primarily because of their high expression in immune cells and their distinct subcellular localisation (13, 23). However, it is still unknown how pig TRPML channels are distributed in tissues and how they react to pathogen invasion. Considering that PRRSV entry into the host also needs clathrin-mediated endocytosis and TRPML channels also play a significant regulatory role in endosomal homeostasis and membrane trafficking, TRPML channels were predicted to be related to PRRSV invasion (7, 21). By using qRT-PCR, we discovered that the three TRPML genes were expressed differently in eight porcine tissues, and that PRRSV infection resulted in the different regulation of TRPML gene expression. These findings suggest that TRPML channels may be expected to provide new ideas for the prevention and treatment of porcine diseases.

The quantitative PCR has quickly become an indispensable tool in scientific research and clinical diagnosis (4). However, it has some shortcomings, such as the high instrument cost and requirement for specific primer design. To facilitate the detection and quantification of the expression of three TRPML genes in pigs by qRT-PCR, we first acquired the complete CDS of porcine TRPML channels and constructed the recombinant plasmid by molecular cloning (Fig. S4). Then, Beacon Designer software created three possible primers for each TRPML gene, and their specificity was assessed using the recombinant plasmid as a template for qRT-PCR. The design requirements of quantitative PCR

primers exploit the melting curve as a useful tool for assessing their specificity (26). Adopting the curve as a criterion, of the three possible primers we selected the one that had a single melting curve peak when the template was each diluted plasmid and a messy melting peak when the negative control did not contain a template. In fact, the values measured by the other two primers were rather accurate when the concentration of three TRPML genes in solution was high. However, when the concentration was extremely low, it was easy to see the difference between the three primers, particularly in the relevant Ct values. From the three developed primers, we selected the one with the highest specificity and lowest value for negative control detection to be a reaction component for the subsequent tissue expression detection of the three TRPML genes.

We investigated the expression of the three TRPML genes in heart, liver, spleen, lung, kidney, stomach, bladder, and intestine tissue by qRT-PCR. Significantly, TRPML1, TRPML2 and TRPML3 were all most highly expressed in the kidney tissues of pigs. However, TRPML1 was next most widely distributed in the liver tissues, while both TRPML2 and TRPML3 were in second-highest concentration in the spleen. According to our previous reports, the distribution of all three TRPML genes in mice is primarily concentrated in the lung and spleen tissues, which is inconsistent with their distribution in pigs (25). A further difference between TRPML1 and the pair of TRPML2 and 3, besides their prevalence in tissue types, is that TRPML2 and TRPML3 but not TRPML1 can control the entry of various RNA viruses through their channel activity, as we and others have shown (13, 23). It is of great significance to investigate the effects of TRPML regulation on the activities of various tissues, particularly the spleen tissue, which is crucial for controlling pathogen invasion and modulating immunity and in which the high expression of TRPML2 and TRPML3 in porcine samples may be elucidative of their differentiation from TRPML1 in their action in the pig.

Following the tissue expression evaluation, we investigated the TRPML expression in MARC-145 cells exposed to PRRSV infection by using CPE and qRT-PCR. Surprisingly, we discovered that TRPML1 and TRPML3 expression in MARC-145 cells was considerably downregulated under PRRSV infection, but that TRPML2 expression was not. According to prior research, toll-like receptor stimulation may increase the expression of TRPML2 in murine macrophage RAW 264.7, but not that of TRPML1 or TRPML3, which was consistent with the alterations in porcine TRPML expression brought on by PRRSV infection (19). Currently, large numbers of small chemical activators that target TRPML channels have been found using high-throughput library screening (HTS) and patch-clamp approaches, including isoindoledione (ML-SA1), thiophene sulfonamide (MK6-83) and isooxazoline (SN-2) (11). Among these, compound ML-SA1 has no clear effect on TRPC, TRPV, TRPM, TRPA, and other TRP channels while being a universal activator that can simultaneously activate three TRPML channel subtypes in humans (17). To further verify whether the activities of TRPML channels are closely related to PRRSV infection, we also evaluated the effect of different concentrations of ML-SA1 on PRRSV replication in MARC-145 cells. Interestingly, this compound suppressed PRRSV concentration dependently at noncytotoxic concentrations, which indicates that it may function as a general antiviral agent. Together, these data clarify the regulation of TRPML expression by PRRSV infection and reveals the potential of TRPML channels as novel candidate molecular targets for antiviral therapy, which provides a new strategy for the treatment of pig diseases. Certainly, it is not enough to measure PRRSV-related changes only from the RNA level and cell morphological changes, and further techniques like Western blot and plaque assays are still required to support any claim of a correlation between TRPML expression and infection with this virus. While extensive literature has noted that lysosomal acidification and protease activity changes mediated by TRPML channels are closely related to viral entry, further work on the deep mechanism between TRPML or its specific activators and PRRSV infection is still needed (3, 31).

Conclusion

Our study created a quantitative PCR primer with high specificity for each TRPML gene, and utilising the produced primers in a qRT-PCR, we were able to determine that TRPML1 was strongly expressed in the liver and kidneys of pigs, but that TRPML2 and TRPML3 were predominantly expressed in the spleen and kidney tissues. Additionally, with the rise in PRRSV infection titres, the expression of all three TRPML genes changed dose dependently in MARC-145 cells, indicating that TRPML channels are probably crucial for PRRSV regulation. Finally, we discovered that the TRPML-specific activator ML-SA1 suppressed PRRSV infection with effect proportional to the dose, suggesting that it may function as a general antiviral agent. Our research findings collectively suggest TRPML channels as possible antiviral targets for PRRSV infection, which may provide a new strategy for the treatment of pig diseases.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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