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The immunogenicity of PRV ΔgE/TK/UL49.5 three-gene-deleted vaccine in mice

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Abstract

Background Pseudorabies (PR) caused by the re-emerging of pseudorabies virus (PRV) variant has outbroken among PRV vaccine immunized swine in many pig farms, which has caused serious social and economic consequences since the end of 2011. The PRV UL49.5 protein can inactivate the transporter associated with antigen processing (TAP), thereby downregulating the cell surface expression of swine leukocyte antigen class I (SLA-I) to evade host immune surveillance.

Methods In this study, based on the PRV ΔgE/TK strain, PRV ΔgE/TK/UL49.5 triple gene deletion strain was constructed through homologous recombination and deletion of the PRV UL49.5 gene by the Cre-LoxP system. Its growth curve and effect on SLA-I transcription level were determined. Preliminary studies were carried out on serum neutralizing antibody levels, IFN-γ and IL-4 cytokines levels in mice immunized with PRV ΔgE/TK/UL49.5, and the viral load and challenge protection in mice tissues after challenge.

Results The growth characteristics of PRV ΔgE/TK/UL49.5 strain were similar to those of PRV ΔgE/TK strain. The level of SLA-I was returned to normal after the deletion of PRV UL49.5 gene. The immunization of PRV ΔgE/TK/UL49.5 did not affect the weight gain of mice. Immunized mice could induce high levels of serum neutralization antibodies and immune cytokines, including IFN-γ and IL-4, which could provide complete protection against virulent PRV challenge. No obvious pathological damage was observed in lung, brain and trigeminal ganglion of mice immunized with PRV ΔgE/TK/UL49.5, and the tissue viral load was the lowest.

Conclusions PRV ΔgE/TK/UL49.5 strain can induce enhanced immunogenicity and had the potential to be used as a candidate strain.

Keywords Pseudorabies virus, gE/TK/UL49.5 deletion, Safety, Immunogenicity

Background

Pseudorabies (PR) is an acute infectious disease caused by pseudorabies virus (PRV) [1, 2]. Pig is the natural host and storage host of PRV. After PRV acute infection, pigs mainly establish latent infection in nerve tissue, and carry the virus for life in vivo, and do not show clinical symptoms [3–6]. When the body's immunity is reduced or stimulated by stress factors, the latently infected virus can be reactivated, re-produce infectious virus particles, and cause recurrent infection and excretion. At present, the main strategy for prevention and control of PR is still vaccination [7, 8]. Since 2011, a large-scale outbreak

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of pseudorabies has occurred in pigs immunized with Bartha-K61 vaccine or other classical vaccine strains in China, indicating that the traditional Bartha-K61 vaccine has been unable to provide complete protection against new PRV variant infection [9–12].

PRV belongs to the Herpesvirus family, the alphaherpesvirus subfamily, the genus Varicella virus, and is a linear double-stranded DNA virus. The gE and TK genes are the main virulence genes of PRV, and the deletion significantly reduces the virulence of the virus, but still maintains good immunogenicity. It has been confirmed that gE and TK genes are close to the virulence of PRV strains, and they do not affect the immunogenicity of the virus. Therefore, these genes are ideal targets for the study of genetically engineered vaccines. The current commercial live attenuated PRV vaccines are produced on the basis of deleting gE and TK [13, 14]. PRV UL49.5 protein, as a PRV immune escape protein, inactivates the transporter associated with antigen processing (TAP), resulting in the retention of swine leukocyte antigen class I (SLA-I) in the endoplasmic reticulum, thereby down-regulating the expression of SLA-I on the cell surface, thereby weakening the clearance of cytotoxic T lymphocytes to escape the host's immune surveillance [15, 16]. PRV immune escape protein plays a key role in the formation of latent infection. In this study, the PRV ΔgE/TK/UL49.5 gene deletion strain was constructed by homologous recombination and Cre-LoxP system. The safety and immunogenicity of PRV ΔgE/TK/UL49.5 strain in mice were preliminarily studied to evaluate its potential as a vaccine candidate strain.

Methods

Cells and viruses

African green monkey kidney (Vero), human embryonic kidney cells 293T (293T) and porcine kidney-15 (PK-15) cells were preserved by our laboratory and cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), streptomycin (100 μg/mL), and penicillin (100 IU/mL) at 37 °C in 5% CO_2. PRV QYY2012 strain was isolated from a piglet with neurological symptom in Henan province of China in 2012, and PRV ΔgE/TK strain was obtained by deletion of gE and TK genes based on PRV QYY2012 strain through homologous recombination and Cre/LoxP system. All viruses were propagated in Vero cells.

Experimental animal

6-week-old female Kunming mice were purchased from Henan Experimental Animal Center, Zhengzhou, China.

Construction of recombinant plasmid and virus

The PRV QYY2012 strain genome was used as the template for PCR amplification. The left and right homologous arms (L-arm (960 bp) and R-arm (896 bp)) of PRV UL49.5 gene were amplified by primers UL49.5L-F/R and UL49.5R-F/R, respectively, in which there was a LoxP site downstream of the L-arm and upstream of the R-arm. The L-arm contained *Kpn* I and *Hin*d III restriction sites, and the R-arm contained *Eco*R I and *Spe* I restriction sites. The digested products were purified by gel extraction and ligated with pBluescriptSK(-) vector (Hunan Keai Medical Equipment Co., Ltd.) to construct the recombinant plasmid pSK-UL49.5L/R. Using pEGFP vector as template, the CMV-EGFP-SV40 fragment was amplified by EGFP-F/R with primers containing *Hin*d III and *Eco*R I restriction sites. The digested products were ligated into the same digested pSK-UL49.5L/R vector to construct the recombinant plasmid pSK-UL49.5L/R-EGFP (Fig. 1).The recombinant plasmid was confirmed by DNA sequencing. All primers used to amplify the sequence are listed in Table 1.

The genomic DNA of PRV ΔgE/TK strain and pSK-UL49.5L/R-EGFP plasmid were co-transfected into a 6-well plate with monolayer 293 T cells by Lipofectamine® 2000 transfection reagent (Invitrogen). In a 6-well plate, 1 μg of PRV ΔgE/TK genome was added to each well, and the mass ratios of PRV ΔgE/TK genome and recombinant plasmid pSK-UL49.5L/R-EGFP were 1:2, 1:3 and 2:3, respectively, and the ratio of gene mass to the volume of transfection reagent Lipofectamine2000 was 1:2.5. When there was obvious cytopathic effect (CPE), the transfection mixture was collected, diluted to 10^{-3} at a tenfold ratio and inoculated into a 6-well plate with Vero cells. After adsorption for 2 h, it was replaced with DMEM culture medium containing 2% serum and

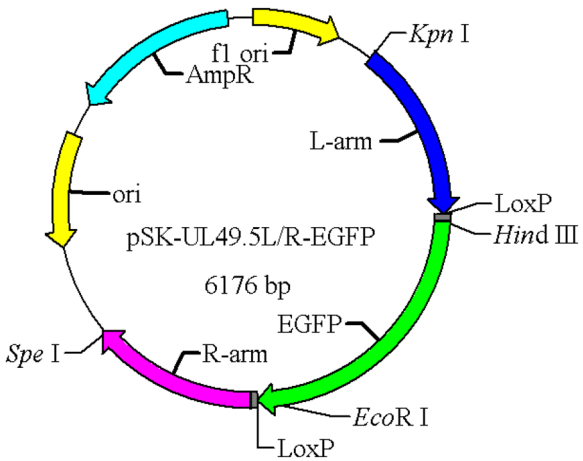

Fig. 1 pSK-UL49.5L/R-EGFP recombinant plasmid

Table 1 Primers that were used in this study

Primer	Sequence (5′ → 3′)	Restriction site	Expected product (bp)
UL49.5L-F	CGG*GGTACC*AGGCGGAGGAAGACGAGGCT	Kpn I	960
UL49.5L-R	CCC*AAGCTT*ATAACTTCGTATAATGTATGCTATACGAAGTTATGTCGCACCACCAACGGGAT	Hind III	
UL49.5R-F	CCG*GAATTC*ATAACTTCGTATAGCATACATTATACGAAGTTATTTCCCACTCGCTCGCCATGT	EcoR I	896
UL49.5R-R	CGC*ACTAGT*GAACGCGACCACGCACTCC	Spe I	
EGFP-F	CCC*AAGCTT*GGGACTTTCCATTGACG	Hind III	1452
EGFP-R	CCG*GAATTC*AATTTACGCGTTAAGATAC	EcoR I	
UL49.5-F	GATGTCGTATCCGGCGTC	–	853
UL49.5-R	TCGTAGGACGGGGCAGAC	–	
SLA-I-qF	CTTCCACTCGCAGCTCTTCT	–	113
SLA-I-qR	TAGATGCAGGGCTCGTACAC	–	
β-actin qF	TCCTGCGGCATCCACGAAAC	–	82
β-actin qR	CCGTGTTGGCGTAGAGGTCCTTG	–	

The underline and italics are the sequence of enzyme cutting sites, and the bold are the LoxP sequence

1% low-melting point agarose. After 48 h, the fluorescent lesions were observed under a fluorescence microscope. The fluorescent lesions were selected and placed into a centrifuge tube containing 1ml of DMEM medium, and plaque purification was carried out according to the above operations. The completely purified recombinant virus PRV ΔgE/TK/UL49.5/EGFP$^+$ strain was obtained through this plaque purification method. The genomic DNA of PRV ΔgE/TK/UL49.5/EGFP$^+$ strain and pcGlobin2-Cre plasmid were co-transfected into 293 T cells, and the recombinant virus PRV ΔgE/TK/UL49.5 strain without green fluorescent protein was obtained by plaque purification. PRV ΔgE/TK/UL49.5 strain was PCR identified with primers UL49.5-F/R and sequenced.

Growth characteristics of PRV ΔgE/TK/UL49.5 strain

To analyze the growth characteristics of different PRV strains in vitro, PRV QYY2012, PRV ΔgE/TK or PRV ΔgE/TK/UL49.5 were used to infect Vero cells in six-well cell plates at multiplicity of infection (MOI) of 1 respectively, and normal Vero cells were used as negative control. The cell supernatants were collected at 6, 12, 24, 36, 48, 60, and 72 h after infection, and the virus titers were determined by Reed-Muench method, and the one-step growth curves was drawn. Three replicates were set for each experiment. The virus solution collected at each time was diluted 10 times, and the dilutions of 10^{-1}–10^{-10} were respectively added to a 96-well plates covered with Vero cells, and used as a blank control. Place it in a 5% CO_2 incubator at 37 °C for 72 h to observe the diseased wells.

Effect of PRV UL49.5 on SLA-I transcription level

In order to detect the level of SLA-I transcription in PK-15 cells infected with different PRV strains, PRV ΔgE/TK/UL49.5, PRV ΔgE/TK and PRV QYY2012 were inoculated into PK-15 cells at MOI = 0.1, while the overexpression of pUL49.5 plasmid in PK-15 cells was used as control. The cells were collected at 6, 12, 18, and 24 h after infection or transfection, RNA was extracted according to the instructions of the RNA extraction kit, 3 μg of samples were reverse transcribed into cDNA according to the instructions of the reverse transcription kit. SLA-I was amplified by primer SLA-I-qF/R, porcine β-actin gene was used as internal reference, and the relative expression of SLA-I was calculated by $2^{-\Delta\Delta Ct}$ method. Three replicates were set for each experiment.

Vaccination and challenge

Eighty 6-week-old healthy female Kunming mice were randomly divided into 4 groups with 20 mice in each group. The mice in groups 1–2 were hindlimb intramuscularly (i.m.) inoculated with 1×10^6 $TCID_{50}$ PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK, respectively. The mice in groups 3–4 were hindlimb intramuscularly (i.m.) inoculated with 100 μL DMEM in the non-immunized group (positive control group) and negative control group, respectively. The booster immunization with the same dose and inoculation route was performed 21 days post-immunization (dpi). The immunized mice were weighed at the first immunization, 21 days after the first immunization and 21 days after the booster immunization. The serum of mice were collected through the tail vein on 7,

14, 21, 28, 35, 42, and 49 days after the first immunization, and the serum neutralizing antibody test was used to detect the level of PRV-specific neutralizing antibody in mice. The serum of mice was collected 21 days after booster immunization, and the expression levels of cytokines IFN-γ and IL-4 in immunized mice were detected by ELISA. On day 21 after booster immunization, mice in groups 1–3 were hindlimb intramuscularly (i.m.) injected with 4×10^4 $TCID_{50}$ PRV QYY2012 strain through the hindlimb muscle, and mice in the negative control group 4 were injected with DMEM. The clinical response and survival rate of mice were observed and recorded daily for 14 consecutive days after challenge. On the 14th day after challenge, the mice were euthanized with CO_2 asphyxiation. The lung, brain and trigeminal ganglion of mice in each group were collected. The viral load was determined by qPCR, and histopathological analysis was performed.

Neutralizing antibody detection

Blood samples were collected from the tail of immunized mice at 7, 14, 21, 28, 35, 42, 49 days after the first immunization, and serum samples were tested for PRV specific neutralizing antibodies (NAbs) by virus serum neutralization test (NT) as previously described [28]. Briefly, the collected serum samples were inactivated at 56 °C for 30 min in a water bath, diluted with DMEM by two-fold serially, mixed with an equal volume of 200 $TCID_{50}$ PRV QYY2012 strain, incubated at 37 °C for 1 h, and 100 μL was inoculated into a 96-well plate covered with Vero cells, and incubated at 37 °C for 72 h. The neutralizing antibody titer was calculated by Reed-Muench method. Three replicates were set for each experiment.

Cytokine detection

On the 21st day after booster immunization, the peripheral blood of immunized mice was collected, centrifuged at 3500 r/min for 10 min, and the supernatant was taken. The expression levels of cytokines IFN-γ and IL-4 were detected by ELISA (mouse IFN-γ ELISA kit and mouse IL-4 ELISA kit, Jiangsu Enzyme Immunization Industrial Co., Ltd). Three replicates were set for each experiment.

Histopathological examinations and tissue viral load analysis

On the 14th day after challenge, the lung, brain and trigeminal ganglion of mice in each group were collected, and the viral load was determined by qPCR, and histopathological analysis was performed. Briefly, the collected lung, brain and trigeminal ganglion were fixed with 4% paraformaldehyde, and the histopathological sections were prepared by paraffin embedding. The pathological changes of each tissue were observed by optical microscope after HE staining. Tissue virus load was determined by designing specific primers gE-qF/R based on PRVgE gene and detecting the copy number of tissue virus genome by real-time quantitative PCR (qPCR). The upstream primer gE-qF: 5′-CTTCCACTC GCAGCTCTTCT-3′, and the downstream primer gE-qR: 5′-TAGATGCAGGGCTCGTACAC-3′.

Statistical analysis

The data were plotted by GraphPad Prism 9.0 software (San Diego, California USA, www.graphpad.com), and the data were expressed as mean ± standard deviation (SD). SPSS27 software was used to analyze the variance of the experimental data. For all experiments, $p<0.05$ was considered significant difference (*), and $p<0.01$ was considered extremely significant difference (**).

Results

Generation of gE/TK/UL49.5-deleted recombinant virus

PRV ΔgE/TK strain genomic DNA and pSK-UL49.5L/R-EGFP plasmid were co-transfected into 293 T cells, and the green fluorescent plaque was screened. After plaque purification, PRV ΔgE/TK/UL49.5/$EGFP^+$ strain was obtained (Fig. 2A, B). Then the genomic DNA of PRV ΔgE/TK/UL49.5/$EGFP^+$ strain and pcGlobin2-Cre plasmid were co-transfected into 293 T cells, and PRV ΔgE/TK/UL49.5 strain without green fluorescence was screened (Fig. 2C, D). PCR identification was performed using primers UL49.5-F/R. The amplified band of PRV ΔgE/TK/UL49.5 was 379 bp due to deletion of 474 bp fragment of UL49.5 gene. PRV ΔgE/TK/UL49.5/$EGFP^+$ strain, due to the deletion of 474 bp fragment of UL49.5 gene, inserted EGFP gene length 1521 bp and amplified band 1900 bp. The amplified band of PRV QYY2012 strain was 853 bp. The PRV ΔgE/TK/UL49.5 strain was successfully constructed as confirmed by PCR and sequencing (Fig. 2E).

The one-step growth curves of PRV strains

To evaluate the growth characteristics of PRV ΔgE/TK/UL49.5 strain in vitro, the replication of virus in Vero cells was analyzed byone-step growth curve. Compared with PRV QYY2012 and PRV ΔgE/TK strains, there was no significant difference in proliferation rate and virus titer of PRV ΔgE/TK/UL49.5 strain (Fig. 3). The results showed that the deletion of PRV UL49.5 gene did not affect its growth characteristics and had a good ability of proliferation in vitro.

The deletion of UL49.5 gene upregulates the transcriptional level of SLA-I

In order to investigate the effect of PRV UL49.5 gene on antigen presentation, total RNA was extracted from

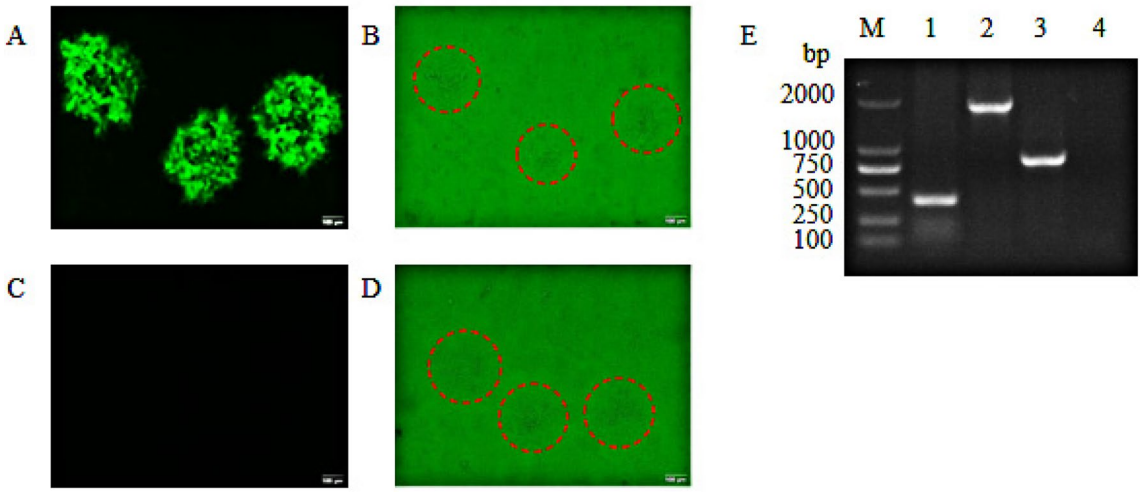

Fig. 2 Purification and identification of PRV strains. (A) Plaque purification of PRV ΔgE/TK/UL49.5/EGFP$^+$ strain for fluorescence visual field. (B) Plaque purification of PRV ΔgE/TK/UL49.5/EGFP$^+$ strain for bright visual field, red circles represent the plaque of PRV ΔgE/TK/UL49.5/EGFP$^+$. (C) Plaque purification of PRV ΔgE/TK/UL49.5 strain for fluorescence visual field. (D) Plaque purification of PRV ΔgE/TK/UL49.5 strain for bright visual field, red circles represent the plaque of PRV ΔgE/TK/UL49.5. (E) PCR analysis of different PRV strains with primers UL49.5-F/R. Lane 1, PRV ΔgE/TK/UL49.5; lane 2, PRV ΔgE/TK/UL49.5/EGFP$^+$; lane 3, PRV QYY2012; lane 4, negative control; M, DL2000 DNA Marker

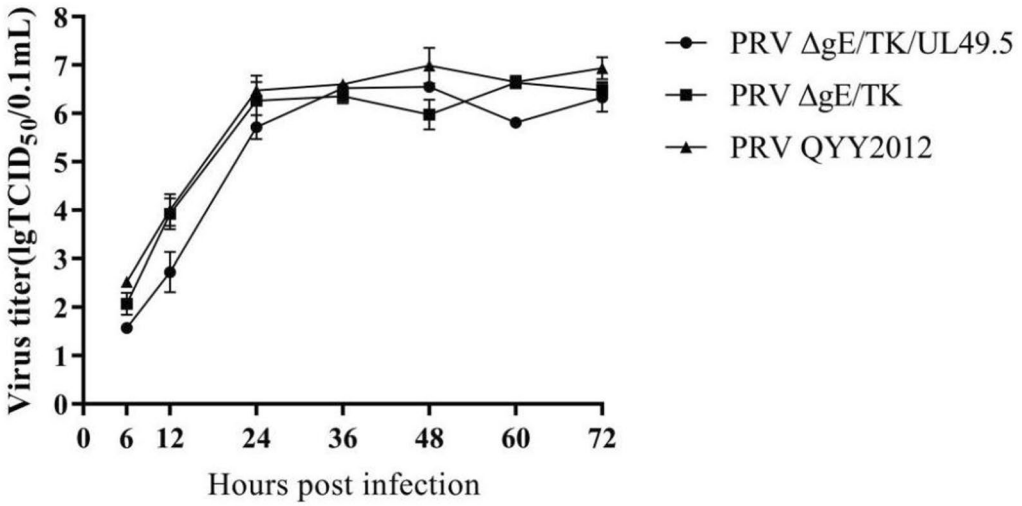

Fig. 3 The one-step growth curves of different PRV strains. Vero cells were inoculated with PRV QYY2012, PRV ΔgE/TK and PRV ΔgE/TK/UL49.5 strains. The cell culture supernatants were collected at different time points to calculate the $TCID_{50}$ of each virus

PK-15 cells infected with different PRV strains, and the transcription level of swine leukocyte antigen class I (SLA-I) was determined by RT-qPCR. The results showed that the transcriptional down-regulation of SLA-I by pUL49.5 was gradually obvious with the passage of time at 6 h after transfection. The down-regulation effect of PRV ΔgE/TK/UL49.5 strain on SLA-I transcription level was significantly lower than that of PRV QYY2012 strain at 12 h ($p=0.011$), and significantly lower than that of PRV QYY2012 strain ($p=0.001$) and PRV ΔgE/TK strain ($p=0.034$) at 18 h (Fig. 4). The results showed that the protein encoded by PRV UL49.5 gene could reduce the transcription level of SLA-I in PK-15 cells, and the down-regulation of SLA-I transcription level in PK-15 cells decreased after infection with PRV ΔgE/TK/UL49.5 strain.

The immunogenicity of PRV ΔgE/TK/UL49.5 in mice

The mice were weighed 21 days after the first immunization and 21 days after the second immunization. The

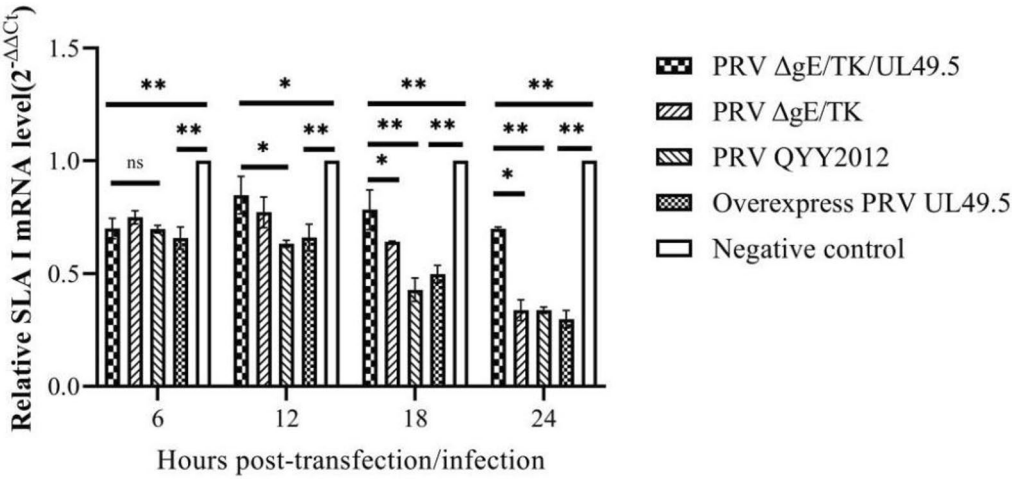

Fig. 4 The effect of PRV UL49.5 on SLA-I mRNA level in PK-15 cells. PK-15 cells were infected with PRV ΔgE/TK/UL49.5, PRV ΔgE/TK and PRV QYY2012 strain respectively, and pUL49.5 plasmid was overexpressed in PK-15 cells. The cells were collected at 6, 12, 18, and 24 h after infection or transfection. RNA was extracted and detected by RT-qPCR with the primer SLA-I qF/R. Data was presented as mean ± SD. * $p < 0.05$, ** $p < 0.01$

results showed that the mice were in good mental state, normal appetite and no itching symptoms after immunization. There was no significant difference in weight gain between the groups after two immunizations (Table 2). The results showed that PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK strains were safe to mice and did not affect the weight gain of mice.

The serum of mice was collected weekly after immunization, and the specific neutralizing antibody level of PRV was detected by serum neutralization antibody test. The results showed that the neutralizing antibody levels of mice immunized with PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK groups reached the highest at 21 days after booster immunization, and the neutralizing antibody level of mice immunized with PRV ΔgE/TK/UL49.5 group was higher than that of PRV ΔgE/TK group, but there was no significant difference. The negative control group did not induce serum neutralizing antibodies during the experiment (Fig. 5A).

At 21 days after booster immunization, the serum of mice was collected to detect the expression levels of IFN-γ and IL-4. The results showed that the expression level of IFN-γ in peripheral blood of PRV ΔgE/TK/UL49.5 immunized mice was significantly higher than that of PRV ΔgE/TK immunized group ($p = 0.041$), which was significantly higher than that of negative control group ($p = 0.001$), and the expression level of IL-4 was significantly higher than that of negative control group ($p = 0.001$) (Fig. 5B, C). The results showed that PRV ΔgE/TK/UL49.5 could induce cellular immune response in mice.

Protection of mice against virulent challenge

At 21 days after booster immunization, PRV QYY2012 was used for challenge. The results showed that the mice in the positive control group showed typical PRV symptoms such as intermittent excitement, loss of appetite, turning circles, arched back, itching and hair clutter on the 3rd day after challenge, and all died within 1 week after challenge. The protection rates of mice immunized with PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK were 100% (9/9) and 88.9% (8/9), respectively (Fig. 6).

On the 14th day after challenge, the lung, brain and trigeminal ganglion of mice in each group were collected for histopathological analysis. The results showed that the positive control group showed typical histopathological lesions, manifested as infiltration of lymphocytes and glial cells in brain tissue and trigeminal ganglion.

Table 2 Weight gain of mice with different groups by intramuscular injection (g)

Immune group	Pre-immunization	Body weight at 21 days after immunization	Body weight 42 days after immunization	Average weight gain after 1st immunization	Average weight gain after 2nd immunization
PRV ΔgE/TK/UL49.5	24.08 ± 1.59	34.79 ± 3.32	40.74 ± 2.65	10.71	5.95
PRV ΔgE/TK	24.07 ± 1.38	34.14 ± 1.90	39.95 ± 2.84	10.07	5.81
Negative control	25.12 ± 2.01	36.36 ± 3.09	41.82 ± 3.04	11.23	5.46

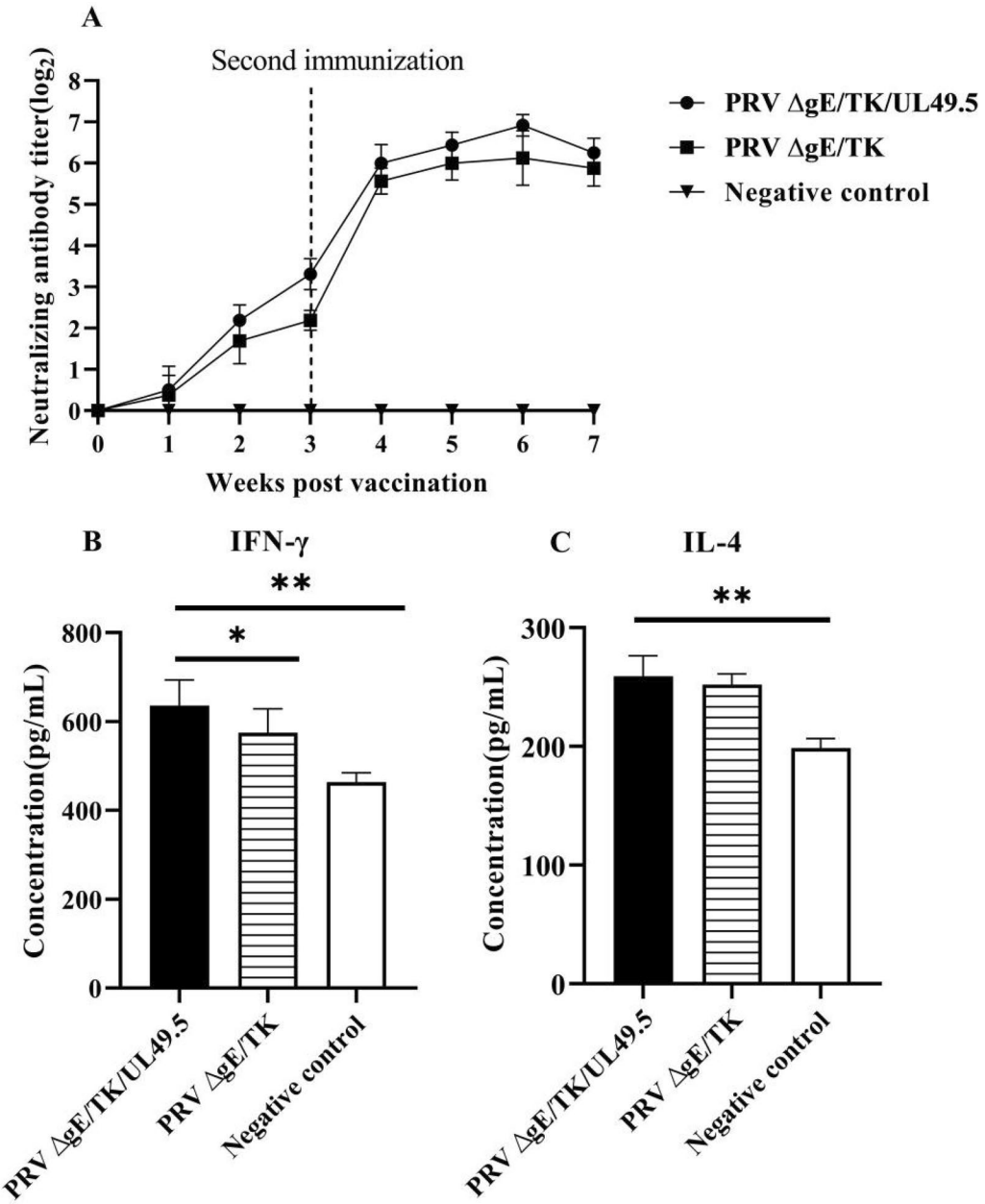

Fig. 5 The immunogenicity of PRV ΔgE/TK/UL49.5 in mice. (A) Neutralizing antibody titer in serum of immunized mice. (B) The expression levels of cytokines IFN-γ in peripheral serum of immunized mice were detected by ELISA 21 days after booster immunization. (C) The expression levels of cytokines IL-4 in peripheral serum of immunized mice were detected by ELISA 21 days after booster immunization. Data was presented as mean ± SD.* $p < 0.05$, ** $p < 0.01$

Pulmonary hemorrhage, congestion, pulmonary interstitial widened alveolar septum and alveolar wall thickening in varying degrees, accompanied by inflammatory cell infiltration. There was a small amount of lymphocyte infiltration in the brain tissue and trigeminal ganglion of the PRV ΔgE/TK group. No histopathological changes were observed in PRV ΔgE/TK/UL49.5 immunized mice (Fig. 7). The results showed that compared with PRV ΔgE/TK, PRV ΔgE/TK/UL49.5 could more effectively reduce organ lesions.

On the 14th day after challenge, the lung, brain and trigeminal ganglion of immunized mice were collected and the virus copy number was detected by qPCR. The results showed that the viral load of PRV ΔgE/TK/UL49.5

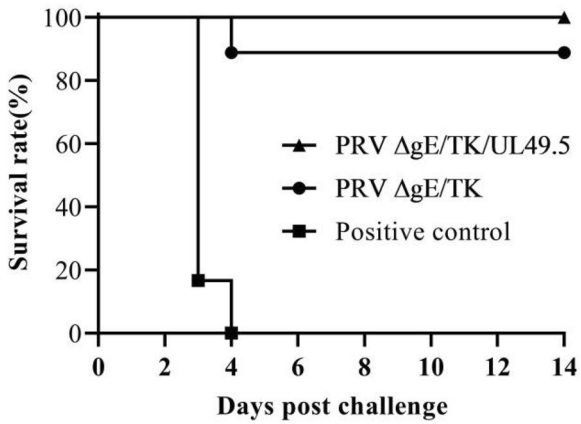

Fig. 6 Survival curves for mice after virulent challenge with PRV QYY2012

immunized group was lower than that of PRV ΔgE/TK immunized group, but the difference was not significant. The viral load of mice immunized with PRV ΔgE/TK/UL49.5 was significantly lower than that of the positive control group (PRV ΔgE/TK/UL49.5, lung vs. positive control group, lung, $p=0.002$, PRV ΔgE/TK/UL49.5, brain vs. positive control group, brain, $p=0.001$, PRV ΔgE/TK/UL49.5, TG vs. positive control group, TG, $p=0.003$) (Fig. 8).

Discussion

Pseudorabies is an acute infectious disease of pigs caused by pseudorabies virus. Pigs of different ages can be infected and show different clinical symptoms after infected with PRV. Newborn piglets showed a significant increase in body temperature after infection, accompanied by neurological symptoms. Pregnant sows showed abortion, stillbirth, mummified fetus after infection. Fattening pigs showed dyspnea, slow weight gain and neurological symptoms [17, 18]. Boars showed infertility. Vaccination is still the main measure to prevent pseudorabies, among which PRV gene deletion attenuated live vaccine is the most widely used and effective. The gE and TK genes are the main virulence genes of PRV, and the virulence of the virus is significantly reduced after deletion, which does not affect the replication and immunogenicity of the virus [19–22]. The protein encoded by PRV UL49.5 gene is a TAP inhibitor, which can downregulate the expression of SLA-I molecules on the surface of infected cells and assist the virus to escape cellular immunity [23, 24].

In this study, the safety and immunogenicity of the PRV ΔgE/TK/UL49.5 strain in mice were studied. Compared with PRV QYY2012 and PRV ΔgE/TK strains, PRV ΔgE/TK/UL49.5 strain had similar growth characteristics and good in vitro proliferation ability on Vero cells. The protein encoded by PRV UL49.5 gene can reduce the transcription level of SLA-I in PK-15 cells. The mortality rate of piglets and mice infected with PRV variant was generally higher. Pigs, rabbits and mice can be selected for PRV animal model studies. Although pigs are the natural host of PRV, mice are often used as experimental animals for preliminary studies considering cost factors. Mice were considered to be more susceptible to PRV infection than swine in Cong's study, because mice infected with the gE/gI-deleted PRV mutant rPRVTJ-delgE/gI showed high morbidity and mortality, but infected swine still had no clinical symptoms [25]. Therefore, mice may be

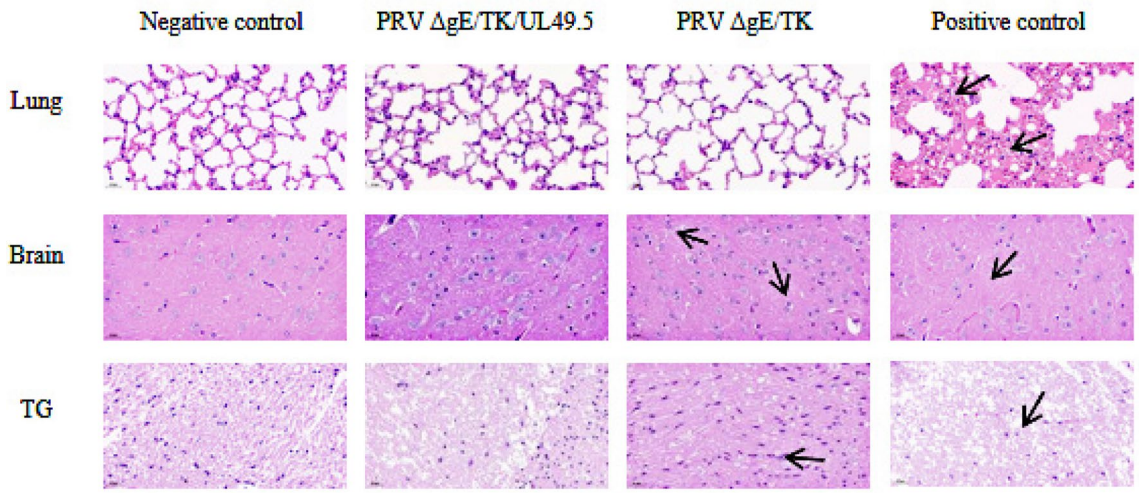

Fig. 7 Histopathological changes of mice in intramuscular immunization group after challenge (400×).The position indicated by the arrow is the lesion

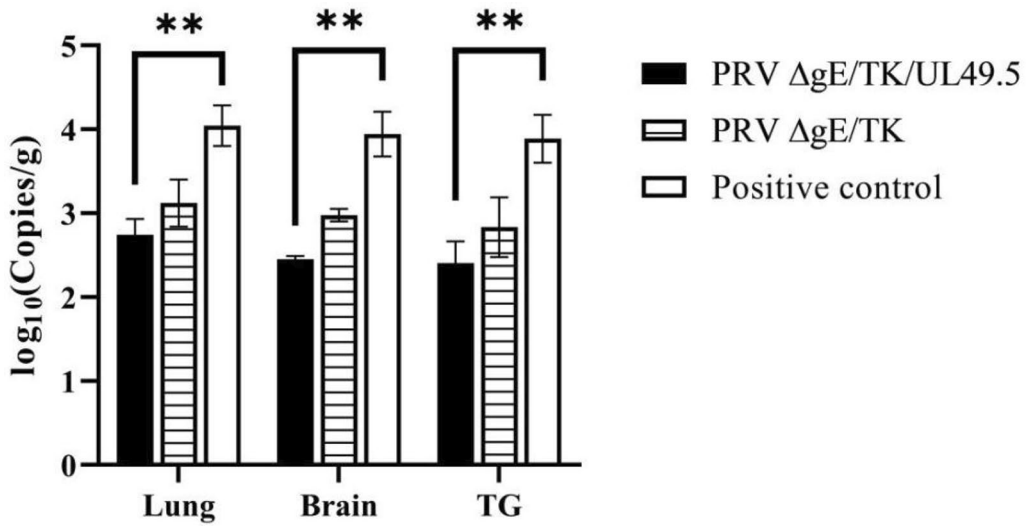

Fig. 8 Viral load in different tissue of mice in each group post challenge. Data was presented as mean ± SD. * $p < 0.05$, ** $p < 0.01$

more suitable for evaluating the pathogenicity of PRV. Alphaherpesvirus has a wide range of hosts, and different hosts can be infected in experiments. Detailed pathogenesis analysis can be carried out in mice infected with PRV virus [26], but the immune response, pathology, virulence and reactivation tendency of different hosts may be different [27].

Interferon (IFN) is a family of active glycoproteins secreted by cells, which has antiviral, anti-tumor and immunomodulatory effects. INF-γ is a key component of cell-mediated immunity. It can act on T lymphocytes to enhance the ability of T cells to assist in antibody production and cytotoxic T cell production; it can act on B lymphocytes to promote B lymphocyte proliferation, differentiation and antibody production, and enhance humoral immune response. The level of INF-γ in the body has been used as one of the indicators for evaluating vaccine-induced cellular immune response. In immune response, Th cells play an important role in cellular immunity and humoral immunity, and have auxiliary and regulatory functions. Th cells are divided into Th0, Th1 and Th2. Th1 assists cellular immunity, and cellular immunity is mainly manifested as CTL-mediated cytotoxicity and TDTH-mediated delayed-type hypersensitivity. Activated Th1 secretes cytokines, the most important of which are IL-2, IFN-γ, TNF-α, etc. These cytokines can induce CTL cytotoxicity and TDTH activation. Th2 assists humoral immunity, and Th2 secretes cytokines such as IL-4, IL-5, IL-6, and IL-10, which mainly promote B cell proliferation and differentiation into plasma cells, secrete specific antibodies, improves mucosal immunity, and mediate humoral immunity. INF-γ is a Th1 type cytokine, and IL-4 is a Th2 type cytokine.

The results of weight gain in mice showed that PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK strains were safe to mice and did not affect the weight gain of mice. The results of serum neutralizing antibody test showed that the neutralizing antibody level of PRV ΔgE/TK/UL49.5 immunized mice reached the highest at 21 days after booster immunization, and was higher than that of PRV ΔgE/TK group. The levels of cytokines in peripheral blood of mice were determined by ELISA. The results showed that the expression level of IFN-γ in peripheral blood of PRV ΔgE/TK/UL49.5 immunized mice was significantly higher than that of PRV ΔgE/TK immunized group ($p=0.041$), and the expression level of IL-4 was significantly higher than that of negative control group ($p=0.001$). The results of challenge protection test showed that the protection rates of mice immunized with PRV ΔgE/TK/UL49.5 and PRV ΔgE/TK were 100% (9/9) and 88.9% (8/9), respectively. All the mice in the positive control group died, indicating that the mice immunized with PRV ΔgE/TK/UL49.5 could provide complete protection against the virulent challenge of PRV QYY2012 strain, and the protection was better than that of PRV ΔgE/TK strain. No pathological damage was observed in the tissues of mice immunized with PRV ΔgE/TK/UL49.5 and the tissue toxicity was the lowest. These results suggest that PRV ΔgE/TK/UL49.5 as a vaccine can provide better protection for mice against challenge with emerging PRV. PRV US3 is the virulence gene of PRV. Its product US3 protein kinase has multiple functions, such as inhibiting apoptosis, regulating host immune response, and inhibiting antigen presentation by down-regulating the expression level of SLA-I molecules. A comparison of the immunogenicity of PRV ΔgE/TK/US3 and PRV ΔgE/TK/UL49.5 will be performed in later experiments. The results of this study showed that

PRV ΔgE/TK/UL49.5 was safe for susceptible mice and had good protection against attacks by virulent PRV, providing a reference for the subsequent immune protection of PRV ΔgE/TK/UL49.5 in pigs. The immune efficacy of PRV ΔgE/TK/UL49.5 in pigs and its comparison with commercial vaccines need to be further studied.

Conclusion

The gE/TK/UL49.5 gene deletion PRV variant was constructed by homologous recombination and Cre/LoxP system, and it was proved that PRV ΔgE/TK/UL49.5 could increase SLA-I transcription level. The safety and immunogenicity of PRV ΔgE/TK/UL49.5 in mice were evaluated. PRV ΔgE/TK/UL49.5 has strong cellular immunity and humoral immunity, which is expected to become an effective live-attenuated vaccine against PRV variant, and provide a reference for the prevention and control of PR. The immune efficacy of PRV ΔgE/TK/UL49.5 in swine and the difference in protection between PRV ΔgE/TK/UL49.5 and commercial vaccines will be determined in further studies.

Abbreviations

PRV	Pseudorabies virus
TAP	Transporter associated with antigen processing
SLA-I	Swine leukocyte antigen class I
MOI	Multiplicity of infection
i.m.	Intramuscularly
dpi	Days post-immunization
NAbs	Neutralizing antibodies

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Author contributions

C.D.: Conceptualization, Methodology, Data curation, Investigation, Writing—original draft, Writing—review & editing. Y.S.: Conceptualization, Methodology, Data curation, Writing—original draft, Writing—review & editing. X.Z.: Conceptualization, Methodology, Supervision, Writing—review & editing. M.S.: Methodology, Writing—review & editing. H.Y.: Methodology, Writing—review & editing. X.Z.: Methodology, Writing—review & editing. S.L.: Methodology, Writing—review & editing. Y.L.: Methodology, Supervision, Writing—review & editing. X.Y.: Methodology, Supervision, Writing—review & editing. L.Y.: Methodology, Supervision, Writing—review & editing. L.C.: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing—review & editing.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Henan Agricultural University (HNND2018030810).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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