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Novel pseudorabies virus variant with defects in TK, gE and gI protects growing pigs against lethal challenge

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ABSTRACT

One of the distinct features of the emerging Chinese pseudorabies virus (PRV) variant is its ability to cause severe neurological signs and high mortality in growing pigs in Bartha-K61-vaccinated pig farms. Either single- or multiple-gene-deleted live vaccine candidates have been developed; however, none was evaluated thoroughly in growing pigs. Here, we generated rSMXΔgI/gEΔTK, an attenuated PRV variant with defects in TK, gI and gE genes. The growth kinetics of the attenuated virus was similar to the wild type (wt) strain. It was safe for 1-day-old piglets. Twenty one-day-old weaned pigs were immunized intramuscularly either with $10^{6.0}$ $TCID_{50}$ of rSMXΔgI/gEΔTK or one dose of commercial Bartha-K61 vaccine, or with DMEM, and were challenged intranasally with $10^{7.0}$ $TCID_{50}$ wt virus at 28 days post vaccination. rSMXΔgI/gEΔTK elicited higher level neutralization antibody against both PRV variant SMX and Bartha-K61 strain, while Bartha-K61 vaccine elicited lower neutralization activity of antibody against SMX. After challenge, all pigs in rSMXΔgI/gEΔTK group survived without any clinical signs, while unvaccinated group showed 100% mortality, and Bartha-K61 group showed severe respiratory symptoms and 3 out of 5 pigs exhibited severe neurological signs. Pigs in rSMXΔgI/gEΔTK group gained significantly higher body weight and diminished viral excretion titer and period, compared with Bartha-K61 group. Furthermore, the safety and efficacy of rSMXΔgI/gEΔTK was also evaluated in sheep and compared with local vaccine in growing pigs. These data suggest that the attenuated strain rSMXΔgI/gEΔTK is a promising live marker vaccine candidate for PR control in the context of emerging PRV variants.

1. Introduction

Pseudorabies virus (PRV), a member of Alphaherpesvirinae subfamily and Varicellovirus genus, is the causative agent of pseudorabies (PR), or Aujeszkys' disease [1]. It can infect most livestock and wild animals, and swine is the natural reservoir. Infected pigs manifest various clinical symptoms, including fatal infection in suckling pigs, respiratory symptoms and growth retardation in growing pigs, and reproductive failure in adults [1,2]. PRV infection has been globally distributed, resulting in enormous economic losses [2,3]. Owing to the implementation of effective marker vaccine and the so-called DIVA ("differentiating infected from vaccinated animals") strategy, PR control and eradication have achieved remarkable success. Several countries declared PRV free from domestic pig populations [4–6]. In China, PR has been well controlled in the last decade as well [7,8]. However, since late 2011, PR has recurred in major pig-production provinces, where Bartha-K61 vaccine was intensively used, characterized by abortion storm in pregnant sows and high mortality in piglets [9,10]. According to recent studies, novel PRV variants showed enhanced pathogenicity [9–11]. Notably, severe neurological symptoms appeared in growing pigs of more than 35 days old, resulting high morbidity and mortality [11,12]. PRV variants show remarkable sequence divergence compared with classic strains [11,12]. Further, phylogeny analysis suggested that PRV variants belong to same genotype with Chinese PRV strains while American and European PRV strains form another genotype [12,13]. Evidences also indicated that Bartha-K61 live vaccine was incapable of providing sufficient protection against novel variants [9,14,15]. Thus, it is urgent to develop new vaccines to control circulating PRV variant in pig population.

Recently, several vaccine candidates derived from novel PRV variant have been developed. Wang et al. [14] reported that a

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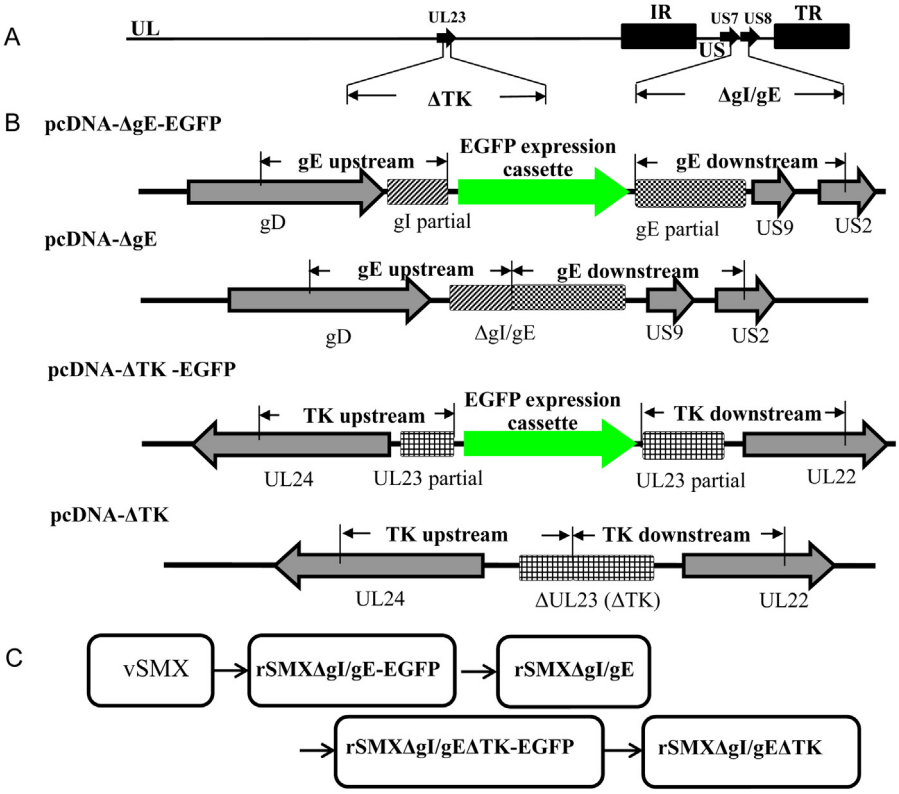

Fig. 1. Construction strategy of recombinant viruses based on PRV variant SMX. (A) PRV genome arrangement and relative loci of UL23 (TK), US7 (gI), and US8 (gE). (B) Construction of transfer plasmids, including target deletion region, upstream, downstream fragments and inserted EGFP expression cassette. All transfer plasmids used pcDNA as backbone. (C) Flowchart to generate SMXΔgI/gEΔTK virus. rSMXΔgI/gE-EGFP, rSMXΔgI/gE, rSMXΔgI/gEΔTK-EGFP, and rSMXΔgI/gEΔTK were obtained sequentially. UL, unique long region; US, unique short region; IR, internal repeat; TR, terminal repeat.

gE-deleted PRV, named as rPRVTJ-delgE, was safe and protected 7-weeks-old pigs from 10^5 $TCID_{50}$ of PRV TJ challenge. Gu et al. [15] generated inactivated vZJ01ΔgE/gI and showed that booster immunization provided good protection efficacy. Most recently, Zhang et al. [16] reported a TK/gE/gI triple genes deleted virus, and showed complete protection in piglets.

Here, we described the construction of a live attenuated PRV variant strain carrying two deletions in genome. The growth kinetics of the mutant strain was compared with wt strain. Its safety was tested in 1-day-old piglets. Immune responses of vaccinated pigs were analyzed by seroconversion, and serum neutralization test. Protection efficacy was evaluated thoroughly in growing pigs including clinical symptoms, body weight gains, virus excretion, mortality rate, and histopathological examination. The attenuated virus was also assessed in sheep and compared with commercial vaccines generated by local strains. The data suggest that the rSMXΔgI/gEΔTK is a promising live marker vaccine candidate for PR variant control in pig herds.

2. Materials and methods

2.1. Virus and cells

PRV strain SMX was isolated from neonatal piglet showing severe neurological disorder, including tremble, convulsion and opisthotonus, in May 2012 in a PR-outbreak pig farm which kept commercial Bartha-K61 vaccine as routine vaccination procedure. All viruses were propagated in PK-15 cells in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, CA).

2.2. Construction of transfer vector for gI/gE and TK deletion

The upstream and downstream homologous fragments were amplified by PCR using primers listed in Supplementary Table 1. Fragments were ligated together at *Xho* I site, and inserted into *Hin*d III and *Xba* I sites of the pcDNA3.1 vector (Fig. 1). The EGFP expression cassette was amplified from pEGFP-C1 collapse and inserted into gI/gE-deletion and TK-deletion vectors (Fig. 1).

2.3. Generation of mutant viruses

PRV SMX viral DNA was extracted [17]. Three micrograms of transfer plasmid and 3 μg of PRV genome DNA, with a molar ratio of 20:1, were co-transfected with Lipofectamine 2000 (Life, CA). Pure recombinant viruses were plaque purified, and confirmed by sequencing (Shanghai Shenggong, China).

Restriction fragment length polymorphism (RFLP) and Southern blot were employed to confirm the deletion of gI/gE and TK genes. The genomic DNA of SMX, rSMXΔgEΔTK, and Bartha-K61 were digested by *Bam*H I, and RFLP patterns were analyzed in 0.8% agarose gel. Southern blot was carried out by DIG High Prime DNA Labeling and Detection Starter Kit I (Roche, Germany) with specific gE or TK probes.

2.4. Growth kinetics

Growth kinetics was determined by one-step growth curve and plaque size calculation as described earlier [18].

2.5. Animal experiments

All the animal experiments were approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural