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Design of live-attenuated animal vaccines based on pseudorabies virus platform

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Abstract

Pseudorabies virus (PRV) is a double-stranded DNA virus with a genome approximating 150 kb in size. PRV contains many non-essential genes that can be replaced with genes encoding heterogenous antigens without affecting viral propagation. With the ability to induce cellular, humoral and mucosal immune responses in the host, PRV is considered to be an ideal and potential live vector for generation of animal vaccines. In this review, we summarize the advances in attenuated recombinant PRVs and design of PRV-based live vaccines as well as the challenge of vaccine application.

Keywords: Recombinant pseudorabies virus, Live-attenuated vaccine, Swine, CRISPR/Cas9

Introduction

Pseudorabies virus (PRV) belongs to the members of *herpesiviridae* family, *Alpha-herpesvirinae* subfamily (Lefkowitz et al. 2018). The genome of PRV is a linear double strand-DNA of approximate 150 kb in length, containing a unique long region (UL), a unique short region (US), a terminal repeat sequence (TRS), and internal repeat sequences (IRS) (Klupp et al. 2004; Pomeranz et al. 2005). Nearly half of the PRV genome is non-essential for virus replication, such as TK, gE, gI, gG or gC, which can accommodate foreign sequence insertions without impairing virus propagation (Lei et al. 2016; Zhang et al. 2021). In addition, PRV has a wide range of hosts and can infect a variety of domestic and wild animals (Müller et al. 2011; Sun et al. 2016; Cheng et al. 2020). Particularly, PRV is a neurotropic virus that preferentially infects the nervous system and can establish long-term latent infection in vivo (Heldens et al. 2008; Freuling et al. 2017; Gu et al. 2018). Such infectious properties make PRV as a promising vector for generation of recombinant living vectored vaccines (Hu et al. 2015). In fact, it has been shown that multiple recombinant PRVs (rPRVs) expressing heterogenous antigens could successfully induce humoral or cellular immune responses in vivo. Herein, we review the current strategies for construction of rPRVs and the research progress using attenuated rPRVs as vaccine candidates.

Strategies for rPRV construction

Multiple methods have been used to successfully introduce foreign gene coding sequences into PRV genome. The early method for rPRV construction relies on the homologous recombination technique that is less efficient and time-consuming. Subsequently, the bacterial artificial chromosomes (BAC) technique provides an efficient method for generation of viral infectious clones (Jiang et al. 2010; Warden et al. 2011; Dunn et al. 2017). This method enables the insertion of PRV genome into BACs in *Escherichia coli* and facilitates mutagenesis of the viral genome by using the bacterial recombination mechanisms. Accordingly, recombination systems based on RecA, Red/ET, Cre/loxP, and FLP/FRT technology have been extensively developed to rapidly insert, delete and mutate specific sequences in BACs (Tischer et al. 2010; Tischer

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and Kaufer 2012; Grzesik et al. 2018). PRV-BAC clones containing the PRV genome were transfected into eukaryotic cells, and recombinant PRVs (rPRV) were then isolated and purified by plaque purification (Tan et al. 2017). However, due to the large genome of PRV, construction of rPRV by BAC method is still laborious.

The recently developed CRISPR/Cas9 system is a highly efficient technique for gene editing (Cong et al. 2013; Xue and Greene 2021). Guided by a single guide RNA (sgRNA), the Cas9 nuclease can edit target gene sequence by non-homologous terminal junction or homologous-mediated repair thereby leading to gene knockout or knock-in. In fact, the CRISPR/Cas9 gene editing system has been used for generation of vector-based vaccines (Okoli et al. 2018; Vilela et al. 2020) including manipulating genomes of large DNA viruses, such as PRV (Tang et al. 2016; Yu et al. 2017; Hubner et al. 2018). Generally, rPRV can be generated by co-transfection of a CRISPR/Cas9-gRNA plasmid and a donor plasmid containing the target gene sequence, and a fluorescent expression cassette into eukaryotic cells following infection of primary PRV. Eventually, rPRV expressing fluorescence was isolated and purified by plaque assay (Xu et al. 2015). Although fluorescence is easy for rPRV screening and purification, it is undesirable to contain the fluorescence protein in a live vector vaccine. To avoid it, the CRISPR/Cas9-gRNA plasmid and PRV genome can be co-transfected into eukaryotic cells, and rPRV will be isolated and purified by plaque assay and identified by PCR and sequencing (Bo et al. 2020) (Fig. 1). Thus, editing of PRV genome by CRISPR/Cas9 system shows great efficiency and simplicity and serves as a powerful tool for rPRV construction.

PRV-based live attenuated vaccines for viral diseases

Currently, many rPRVs expressing key antigens from animal viruses have been successfully constructed. A detailed summary of the constructs is listed in Table 1.

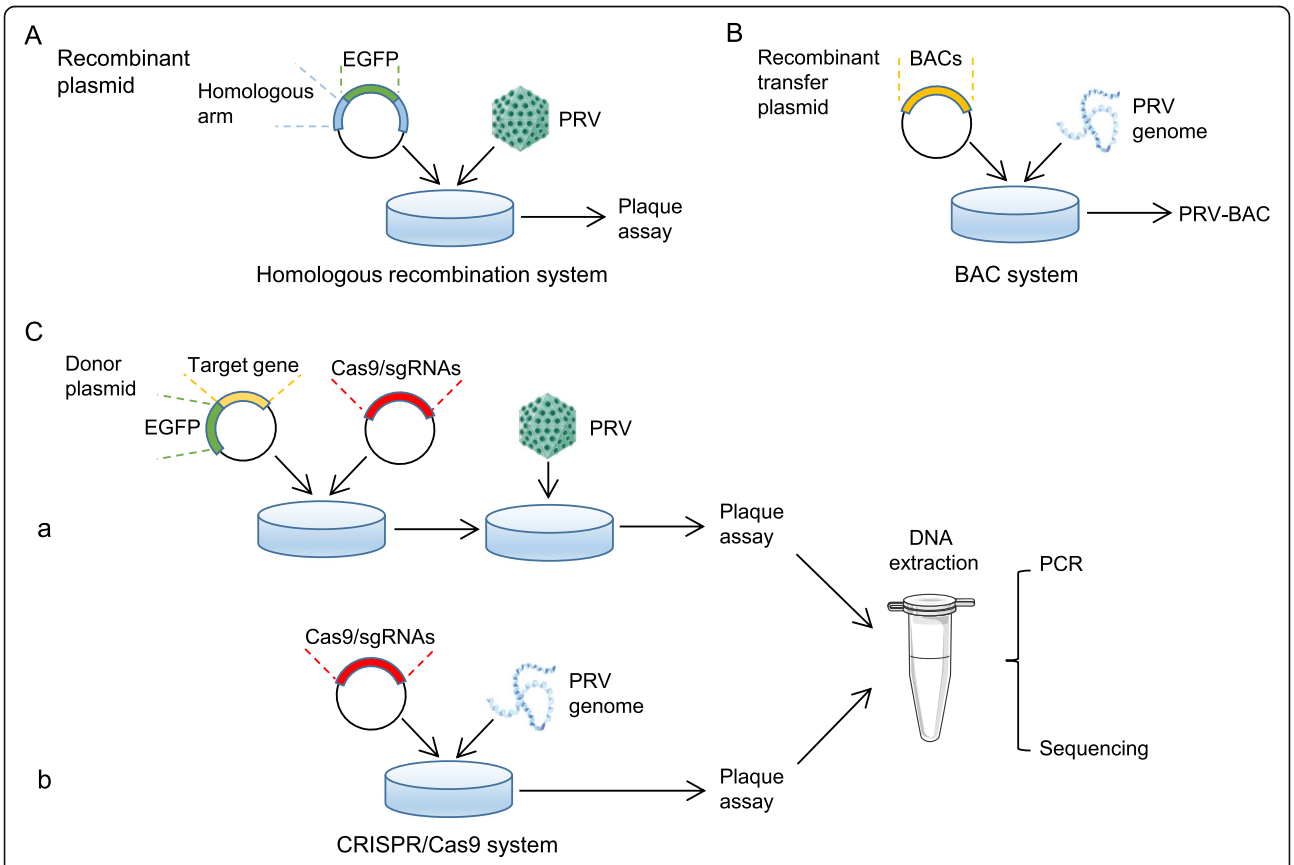

Fig. 1 Schematic diagrams of distinct strategies for rPRV generation. **A** Generation of rPRV by homologous recombination technology. The recombinant plasmids are transfected into cells and then infected with PRV. Subsequently, rPRV with fluorescent expression cassette is isolated and purified by plaque assay. **B** Generation of rPRV by BAC system. The recombinant transfer plasmids and PRV genome are co-transfected into cells to generate the PRV-BAC vector. Then PRV-BAC vector is transfected into cells to produce recombinant virus. **C** Generation of rPRV by CRISPR/Cas9 technology. **a** CRISPR/Cas9-gRNA plasmids and donor plasmids containing target gene sequences and fluorescent expression cassettes are co-transfected into cells for 24 h and infected with PRV for 24 h. rPRV with fluorescent expression cassette is then isolated and purified by plaque assay. **b** CRISPR/Cas9-gRNA plasmids and PRV genome are co-transfected into cells, and rPRV is then isolated and purified by plaque assay. Recombinant viruses are then verified by PCR and sequencing

Table 1 Information of recombinant PRVs

Foreign Genes	Insertion sites (gene)	Neutralizing Antibodies	Dose for immunization	Host	Route of vaccination	PRV vector (strain)	Challenged virus (strain, dose)	Protection efficiency and/or clinical outcome	Reference
PCV2 ORF1-ORF2	Between gI and gE	Yes	10^5 $TCID_{50}$ (mice/pig)	Mice & Pig	Footpad	PRV Ea	NA	NA	(Ju et al. 2005; Qiu et al. 2005)
PCV2 ORF2 and IL-18	gG	Yes	10^7 $TCID_{50}$	Mice	Subcutaneous	PRV HB98	PCV2 (HN strain, 5×10^5 $TCID_{50}$)	Reduced viral load in animals	(Zheng et al. 2015)
PCV2b Cap, CSFV E2, and Erns-GM-CSF	TK, gG and gE	Yes	1.2×10^8 PFU	Pig	Intranasal& Subcutaneous	PRV Becker	PCV2 (PCV2b no. 40895, 2.275×10^4 PFU)	Better protection than Zoetis Fostera Gold PCV commercial vaccine	(Pavulraj et al. 2022)
PPV VP2	gI	Yes	5×10^5 $TCID_{50}$	Pig	Intramuscular	PRV Fa	PPV (SC1, $1 \times 10^{6.5}$ $TCID_{50}$)	96% protection ($n = 28$)	(Chen et al. 2011)
PPV VP2 and IL-6	gG	Yes	10^5 $TCID_{50}$	Mice	Intramuscular	PRV HB98	PPV (HN strain, 10^6 $TCID_{50}$)	90% protection ($n = 10$)	(Zheng et al. 2020)
JEV NS1	gG	Yes	10^5 PFU (mice/pig)	Mice & Pig	Intramuscular	PRV Ea	JEV (SA14–14-2, 10^5 PFU)	Developed good humoral and cellular immune response against JEV	(Xu et al. 2004)
JEV PrM-E	Between gI and gE	Yes	10^6 $TCID_{50}$	Mice	Intramuscular	PRV Ea	JEV (SX09S-01; 10^7 PFU)	80% ($n = 10$)	(Qian et al. 2015)
PRRSV GP5 and M	TK	No	10^7 PFU	Pig	Intramuscular & Intranasal	PRV Bartha-K61	PRRSV (CH-1a $10^{6.5}$ $TCID_{50}$)	Mild lesions in the lungs and kidneys	(Qiu et al. 2005)
PRRSV GP5m and M	Between gI and gE	Yes	10^5 PFU (mice)/ 10^6 PFU (pig)	Mice & Pig	Intramuscular	PRV Ea	PRRSV (YA1, 5×10^5 $TCID_{50}$)	No clinical signs	(Jiang et al. 2007)
PRRSV GP5 and M	TK, gI and gE	Yes	10^6 $TCID_{50}$	Mice	intramuscular	PRV XJ	NA	NA	(Zhao et al. 2022)
FMDV P12A and 3C	Between gI and gE	Yes	10^6 $TCID_{50}$	Pig	Intramuscular	PRV Ea	FMDV (O/ES/2001, 10^6 $TCID_{50}$)	60% ($n = 5$)	(Zhang et al. 2011)
SIV HA (H3N2)	TK	Yes	10^5 PFU	Mice	Intranasal	PRV Bartha-K61	SwHLJ74 (H3N2 strain, 10^5 $TCID_{50}$)	Mild pathological lesions limited in lung	(Tian et al. 2006)
SIV HA (H1N1)	gG	Yes	2×10^7 PFU	Pig	Intranasal	PRV Bartha-K61	SoIV (A/California/7/09 strain, 2×10^6 $TCID_{50}$)	The vaccinated animals were protected from clinical signs	(Klingbeil et al. 2014)
CSFV E2	Between gI and gE	Yes	10^6 $TCID_{50}$	Pig	Intramuscular	PRV TJ	CSFV (Shimen, 10^6TCID_{50})	100% ($n = 5$)	(Lei et al. 2016)
Brucella BP26	Between gI and gE	Yes	10^4 $TCID_{50}$	Mice	Intramuscular	PRV Ea	NA	NA	(Yao et al. 2015)
Toxoplasma gondii SAG1	Between PK and gE	Yes	6×10^5 PFU	Mice	Intramuscular	PRV Bartha-K61	50 virulent *T. gondii* trophozoites	60% ($n = 15$)	(Liu et al. 2008)
Toxoplasma gondii SAG1 and MIC3	gG	Yes	10^6 $TCID_{50}$	Mice	Intramuscular	PRV Ea	100 virulent *T. gondii* tachyzoites	66.7% protection ($n = 6$)	(Nie et al. 2011)
Schistosoma japonicum Sj26GST and SjFABP	Between PK and gG	Yes	6×10^5 PFU (mice)/ 1.2×10^6 PFU (sheep)	Mice & Sheep	Intramuscular	PRV Bartha-K61	40 ± 2 (mice) and 400 ± 2 (sheep) *S. japonicum*	Worm reduction 39.3% in mice and 48.5% in sheep	(Wei et al. 2010)

NA Not available

PRV-based vaccines for porcine circovirus-associated diseases (PCVD)

Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus-associated diseases (PCVD) that leads to immense economic losses in swine industry worldwide (Mankertz et al. 2004; Darwich and Mateu 2012; Meng 2013). PCV2 is a single-strand circular DNA virus with a tiny genome only approximately 1.7 kb in size and belongs to the members of the family *Circoviridae*. PCV2 has two major open reading frames (ORFs), ORF1 and ORF2, encoding Rep and capsid proteins, respectively (Mankertz et al. 2004; Shen et al. 2008; Masuda et al. 2018). It has been reported that rPRV expressing ORF1–ORF2 fusion protein induced high levels of antibodies against PRV and PCV2 in both immunized mice and pigs (Ju et al. 2005). To further improve the immunogenicity of the rPRV vaccine, a novel rPRV expressing PCV2 ORF2 and interleukin 18 (IL-18) was constructed (Zheng et al. 2015). Mice immunized with rPRV-ORF2-IL-18 twice produced specific antibodies against PCV2 and higher $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocyte counts in peripheral blood, indicating that expression of immunopotentiator such as IL-18 can largely enhance the immune responses of the host. Recently, a novel trigene deletion rPRV (PRVtmv) vaccine was constructed with expressing chimeric PCV2b-shell, CSFV-E2 and chimeric Erns-fused bovine granulocyte monocyte stimulating factor (Erns-GM-CSF) (Pavulraj et al. 2022). The PCV2b challenge showed that the PRVtmv vaccine produced better protection in immunized pigs than a commercial inactivated PCV2 vaccine. In addition, pigs immunized with PRVtmv vaccine also generated CSFV- and PRV-specific neutralizing antibodies, suggesting that PRVtmv vaccine could be a multivalent vaccine against multiple diseases. It is worthy to note that the insertion sites of ORF2 in the above two cases are different. Therefore, the insertion sites of the foreign antigen genes in rPRV genome might affect the efficiency of the vaccines.

PRV-based vaccines for porcine parvovirus infection

Porcine parvovirus infection is one of the major reasons for reproductive failure in pregnant sows (Ren et al. 2013; Meszaros et al. 2017). Capsid protein VP2, the major structural protein of the causative agent porcine parvovirus (PPV), is the key antigen that induces neutralizing antibodies (Xu et al. 2013; Ji et al. 2017). Thus, rPRV expressing VP2 of PPV was generated (Chen et al. 2011). Piglets vaccinated with rPRV-VP2 produced PRV-specific and PPV-specific humoral immune responses and significantly reduced mortality caused by PRV infection. In order to enhance the protective immune responses, rPRV expressing PPV VP2 and IL-6 fusion protein was further generated recently (Zheng et al. 2020). BALB/c mice inoculated with rPRV-VP2-IL6 via the intramuscular route produced specific antibodies against PPV and also maintain a strong specific lymphocyte proliferative response. Unfortunately, it only provided partial protection against PPV infection. This study indicates that the current strategies for PRV-based vector vaccines are not successful and further investigation is required to generate better vaccine candidates.

PRV-based vaccines for Japanese encephalitis

JEV is a zoonotic pathogen and causes viral encephalitis with a serious public health problem in Asia, western Pacific countries, and northern Australia (Campbell et al. 2011). In swine, JEV infection generally leads to reproductive disorders with abortion and weak piglets (Yun and Lee 2014). Japanese encephalitis virus (JEV) contains a positive-sense RNA genome within a host-derived membrane and is classified within the family *Flaviviridae* (Laureti et al. 2018). The JEV genome encodes 3 structural proteins (C, PrM/M, E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Unni et al. 2011; Duong et al. 2017). Among them, PrM, E, and NS1 are glycosylated and are capable of inducing protective immunity (Li et al. 2012; Li et al. 2013). An early study reported rPRV expressing the NS1 protein of JEV immunization produced JEV-specific humoral and cellular immune responses in immunized animals (Xu et al. 2004). Comparably, a rPRV expressing PrM-E of JEV also induced a high level of antibodies against PRV and JEV (Qian et al. 2015). Following a lethal dose of JEV (SX09S-01) infection, the rPRV-PrM-E immunization provided 80% survival protection in mice. Although rPRV-JEV NS1 and rPRV-PrM-E can induce protection against JEV infection in mice, both vaccine candidates-elicited JEV-specific immune responses are lower than that of the inactivated JEV vaccine. Also, it is essential to know the immunogenicity and protective effect of PRV-based JEV vaccine in pigs.

PRV-based vaccines for porcine reproductive and respiratory syndrome (PRRS)

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of PRRS, which is an enveloped, positive-strand RNA virus that belongs to the family *Aterviridae* (Guo et al. 2018). PRRSV infection generally causes severe reproductive failure in sows and respiratory distress in piglets and growing pigs, leading to tremendous economic losses worldwide (Lunney et al. 2016). The genome of PRRSV is approximately 15 kb and contains 9 open reading frames (ORFs) including ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF6, and ORF7 (Bautista et al. 1996). Among them, ORF5 and ORF6 encode envelope glycoprotein GP5 and

non-glycosylated membrane protein M respectively (Verheije et al. 2002; Veit et al. 2014), two major membrane-associated proteins that are associated together as disulfide-linked heterodimers in the virus particle (Jiang et al. 2006; Wang et al. 2017). In 2005, an attenuated rPRV, rPRV-GP5, was developed that expresses the GP5 protein of PRRSV. Immunization of the rPRV-GP5 provides significant protection against clinical symptoms and reduces pathogenic lesions caused by PRRSV challenge in vaccinated pigs (Qiu et al. 2005). To improve the protective efficacy of rPRV-GP5, a Pan DR T-helper cell epitope (PADRE) sequence was introduced between the N-terminal and the neutralizing GP5 epitope. Compared to that of rPRV-GP5, the modified rPRV-GP5 elicited higher levels of PRRSV-specific neutralizing antibodies and cellular immune responses than the rPRV-GP5. In addition, another rPRV named rPRV-GP5m-M that expresses modified GP5 and M proteins of PRRSV was also constructed (Jiang et al. 2007). Consequently, mice immunized with rPRV-GP5m-M produced humoral immune responses specific to PRV and provided complete protection against lethal PRV infection. Meanwhile, high levels of neutralizing antibodies to PRRSV and lymphocyte proliferation responses were observed in the immunized animals. In comparison to the commercial inactivated PRRSV vaccine, rPRV-GP5m-M immunized animals generated higher PRRSV-specific neutralizing antibodies as well as the lymphocyte proliferation responses, showing great potential for better protection against PRRSV infection. Notably, the NADC-30-like PRRSV has become the dominant strain in the field in recent years. A rPRV expressing NADC30-like PRRSV GP5 and M proteins was then generated by using PRV variant strain (XJ) as a backbone (Zhao et al. 2022). Mice immunized with rPRV-NC56 produced PRV and NADC30-like PRRSV-specific humoral and cellular immune responses, suggesting that rPRV-NC56 could be a candidate vaccine for protection against NADC30-like PRRSV infection. For decades, while several attenuated vaccines have been developed to prevent PRRSV infection in recent years, the prevalence of PRRSV infection in pigs still remains relatively high levels (Du et al. 2017). Hence, it is worthy to generate novel vaccines such as PRV-based vaccine against PRRSV infection and rPRV-GP5m-M might be a good candidate vaccine for PRRSV.

PRV-based vaccines for foot-and-mouth disease (FMD)

FMD can be induced by foot-and-mouth disease virus (FMDV) infection in all cloven-hoofed animals including cattle, sheep, goats, pigs, and buffalo (Singh et al. 2019), which is mainly characterized by vesicular lesions of the mouth, nose, and feet (Grubman and Baxt 2004). FMDV is a positive single-stranded RNA virus with a genome of about 8.5 kb and belongs to the family of *Picornaviride* (Domingo et al. 2002). The VP1 protein of FMDV has been identified that contains most T- and B-cell epitopes to induce neutralizing antibodies (Diaz-San Segundo et al. 2017). Indeed, rPRV expressing VP1 of FMDV (rPRV-VP1) elicited high-level neutralizing antibody response to both FMDV and PRV as well as strong cytotoxic T lymphocyte (CTL) response against FMD in vaccinated pigs (Li et al. 2008). In addition, FMDV P12A and 3C genes have also been used widely on genetically engineered FMDV vaccine (Joyappa et al. 2009; Lyons et al. 2016). Accordingly, piglets vaccinated twice with rPRV expressing P12A and 3C (rPRV-P12A-3C) produced higher neutralizing antibodies after 15 days of booster immunization (Zhang et al. 2011). However, compared to the commercially available inactivated FMD vaccine, rPRV-P12A-3C did not provide a strong defense against FMDV infection although it still elicited significant FMDV-specific lymphocyte proliferative response in piglets. The immunized piglets also showed mild clinical signs and delayed appearance of blistering lesions possibly due to the low neutralizing antibodies induced by rPRV-P12A-3C. Thus, combined expression of P12A-3C and other adjuvant proteins might be helpful to enhance the immunogenicity and protection of PRV-based vaccines.

PRV-based vaccines for swine influenza

Influenza virus is an enveloped RNA virus that consists of negative single-stranded RNA, which belongs to type A influenza virus and is the member of the family *Orthomyxoviridae* (Lefkowitz et al. 2018). Swine is susceptible to the infection of both avian and/or human influenza A viruses (Sun et al. 2020), which facilitates genomic reassortment among viruses from multiple host species, making swine as mixing vessels for influenza A viruses and a source of emergence for novel recombinant viruses. The hemagglutinin (HA) glycoprotein is the major surface glycoprotein and is the major immunogen of all influenza viruses, which can induce subtype-specific protective cellular and humoral immune responses in animals (de Vries and Rimmelzwaan 2016). Therefore, a rPRV expressing the HA gene from H3N2 subtype of SIV (rPRV-H3N2 HA) was constructed (Tian et al. 2006). Mice immunized intranasally with the rPRV-H3N2 HA produced HA antibodies at 3 weeks post-vaccination, while no vaccine virus was isolated from vaccinated mice. When the immunized mice were challenged with porcine H3N2 virus (A/Swine/Heilongjiang/74/2000), only slight pathological damage was observed in the lungs. More recently, a rPRV expressing codon-optimized H1N1 HA was also generated by BAC technology (Klingbeil et al. 2014). Single immunization of pigs with rPRV vaccine expressing the modified HA

gene induced high levels of HA-specific antibodies. The immunized pigs did not show clinical signs after swine H1N1 virus infection, showing that the rPRV-HA vaccines are safe and can protect swine from influenza virus infection. Notably, optimization of the exogenous protein codon can enhance the immune effect of rPRV-HA vaccines. Given that there are numerous subtypes of influenza viruses, it is necessary to verify whether the rPRV-HA vaccine is also effective to other subtypes of swine influenza viruses.

PRV-based vaccines for classical swine fever (CSF)

CSF generally leads to considerable economic loss in the pig industry worldwide (Xu et al. 2020), which is caused by infection of classical swine fever virus (CSFV), an enveloped, positive single-stranded RNA virus that belongs to the genus *Pestivirus* of the family *Flaviviridae* (Beer et al. 2015). The structural glycoprotein E2 of CSFV is a determinant for viral entry and the major protective antigen inducing neutralizing antibodies against CSFV (Van Gennip et al. 2004; Risatti et al. 2005; Huang et al. 2014). Recently, a recombinant variant PRV with gE/gI/TK deletion and E2 protein expression was generated and its safety and immunogenicity were evaluated in pigs (Lei et al. 2016). No clinical signs or virus shedding were observed in pigs immunized with different doses of rPRVTJ-delgE/gI/TK-E2. Importantly, the immunized pigs produced anti-PRV or anti-CSFV neutralizing antibodies and were completely protected against the lethal infection with either CSFV or variant PRV, demonstrating that rPRVTJ-delgE/gI/TK-E2 is a promising bivalent viral vaccine candidate against CSFV and PRV coinfections. Further studies are needed to compare the immunogenicity and protection efficiency of rPRVTJ-delgE/gI/TK-E2 and the current commercially CSFV vaccine. Given that CSFV chimeric vaccines and E2 subunit vaccines do not provide the desired safety profile (Wei et al. 2021), the optimized rPRV-based vaccines may have better application prospect in clinical.

PRV-based live attenuated vaccines for bacterial or parasitic diseases

PRV-based vaccine for *brucellosis*

Brucella is a zoonotic bacteria that infect domestic animals including cattle, sheep, swine and human (Ye et al. 2015; Glowacka et al. 2018). Currently, attenuated live vaccines are used for vaccination to protect animals against *Brucella* infection. However, production of attenuated live vaccines cannot avoid to culture live *Brucella,* which is a disadvantage of commercial vaccines. While the smooth live attenuated vaccines present biosafety risks, inactivated vaccines only offer low protection (Lalsiamthara and Lee 2017). Hence, generation of novel and safe vaccines for *Brucella* are much desired. The BP26 protein of *Brucella* is a highly conserved soluble cellular protein that can reduce bacterial infection when mice were immunized with the BP26 and Tf proteins (Yang et al. 2007). As such, rPRV expressing BP26 was generated and its immunogenicity was evaluated in mice (Yao et al. 2015). At 6 weeks post-vaccination, rPRV-BP26 induced a two-fold titer of antibody against BP26 and produced a high titer of PRV neutralizing antibody. In addition, immunized mice showed strong lymphocyte proliferative responses and the IFN-γ induction induced by rPRV-BP26 compared with that infected with the parent viruses. However, no *Brucella* challenge was performed against the rPRV-BP26 vaccine in these studies, raising the concerns of the efficiency of rPRV-BP26. In addition, it is also necessary to verify the protective effect of rPRV vector vaccines in pigs or sheep.

PRV-based vaccine for *toxoplasmosis*

Toxoplasma gondii is an important food-borne parasite, which infects various mammals and birds as well as human (Lourido 2019; Zhao and Ewald 2020). Owing to the low efficacy of inactivated and live attenuated vaccines, there are currently no approved vaccines and therefore there is a need to develop novel vaccines against *Toxoplasma gondii* infection (Warner et al. 2021). SAG1 induces humoral and cellular immune responses, is highly conserved in *T. gondii* strains, and is a major vaccine candidate antigen (Windeck and Gross 1996; Zhang et al. 2007). A rPRV expressing TgSAG1 protein of *Toxoplasma gondii* was generated (Liu et al. 2008). BALB/c mice vaccinated with rPRV-TgSAG1 produced a high level of specific antibody responses against *T. gondii* lysate antigen, and a strong splenocyte proliferation response. As a result, the levels of IFN-γ and IL-2 generated by T cells from immunized mice were significantly elevated in vitro, showing a strong cytotoxic T-cell response. Besides TgSAG1, the micronemal protein MIC3 expressed in all three infectious stages of *T. gondii* can also elicit early and powerful immune responses (Ismael et al. 2009). Therefore, rPRV expressing TgSAG1 or TgMIC3 proteins of *T. gondii* were generated and immunized BALB/c mice (Nie et al. 2011). Consequently, mice jointly immunized with rPRV-SAG1 and rPRV-MIC3 cocktail produce even higher *T. gondii*-specific IgG antibodies and lymphocyte proliferative responses, conferring more efficient protection against *T. gondii* challenge. These studies suggest that immunization of rPRV vaccines expressing different antigens according to a cocktail method can provide better effective protection. Hence, combined PRV vector vaccines expressing different antigens is also a good option for rPRV vaccine immunization.

PRV-based vaccine for schistosomiasis (*S. japonicum*)

S. japonicum is a zoonotic parasite that can infect several mammalian hosts (Gryseels 2012). The glutathione S-

transferase (Sj26GST) and fatty acid-binding protein (SjFABP) of *S. japonicum* were used to test their protective efficacy in laboratory animals (Tang et al. 2019). Thus, three recombinant viruses rPRV/Sj26GST, rPRV/SjFABP, and rPRV/Sj26GST-SjFABP were constructed and evaluated in mice and sheep against *S. japonicum* challenge (Wei et al. 2010). Not surprisingly, rPRV/Sj26GST-SjFABP provided significant protection in mice and sheep, indicating that combined antigens immunization provides much effective protection from *Schistosoma japonicum* infection. Similarly, immunization of rPRV/Sj26GST and rPRV/SjFABP together may provide better protection.

Concluding remarks

With the advantages of large foreign gene volume, good safety, wide host range, and low application cost, PRV has been used as a viral vector to express a variety of key foreign antigens of animal viruses, bacteria, and parasites, which have been successfully studied in the laboratory (Wei et al. 2010; Yao et al. 2015; Zheng et al. 2020). Another advantage is that PRV can be amplified on a wide range of cell lines with high viral titers. rPRVs modified at non-essential gene locations have similar characteristics to wild-type virus in terms of growth curve, morphogenesis, and virus plaque sizes (Klingbeil et al. 2015; Lei et al. 2016; Zheng et al. 2020). Also, PRV-based vaccines can be immunized via multiple manners including intranasal, subcutaneous, intravenous, and intramuscular inoculation. Furthermore, rPRV is able to induce both outstanding humoral and/or cellular immune responses and cytotoxic T lymphocytes responses that are crucial for control of pathogens in immunized animals. In addition, it is possible to optimize foreign gene codons or to construct rPRV expressing foreign genes of pathogens and fusion proteins such as IL-6 or IL-18 to enhance the immune effects (Zheng et al. 2015; Zheng et al. 2020; Chowdhury et al. 2021). Moreover, a cocktail immunization of rPRV expression multiple antigens can induce strong immune responses, which would be easier for application of multivalent or polyvalent vaccines (Pavulraj et al. 2022). Taken together, PRV as a viral backbone could provide a potential vaccine option for multiple animals other than pigs.

Although PRV vector vaccines have shown great potential, there is still no rPRV-based vaccine commercially licensed in any countries so far due to the challenge in the clinical application of PRV vector vaccines. In pigs, one concern is that maternal antibodies may impair the immunization of rPRV (Wang et al. 2020b). TK- or gE-deleted PRV vaccines could reduce maternal antibodies interference (Kit et al. 1993; Pomorska-Mól et al. 2010). Although Bartha-K61 vaccine are still the mostly used vaccine to protects pigs for PRV, it is clear that emergence of variant PRVs in Bartha-K61 immunized pig farms has become an issue currently (An et al. 2013). In fact, while PRV-Bartha-K61 strain can provide complete protection against challenge with classical strain, several studies have shown that PRV-Bartha-K61 strain only partial protection against challenge with variant strains (JS-2012, HeN1) (An et al. 2013; Tong et al. 2015). Importantly, recent studies have shown that variant PRV can directly infect humans, causing severe neurological and respiratory damage, which has increased the concern about the biosafety of variant PRV (Yang et al. 2019; Wang et al. 2020a). In addition, the genetic stability of exogenous genes in PRV genome is also critical for live vector vaccines, which needs to be monitored by continuous passages. Finally, further understanding the improvement of the exogenous antigen levels and enhancement of immune responses mediated by rPRV also contribute to the vaccine application (Bouard et al. 2009; Rauch et al. 2018; Abid et al. 2019).

The bio-safety of the attenuated live vaccines for highly pathogenic agents is always a critical issue. For example, African swine fever virus (ASFV) infection can cause a devastating and economically significant disease in both domestic and wild swine (Galindo and Alonso 2017; Wang et al. 2021a; Wang et al. 2021b). Unfortunately, an effective vaccine for ASF is still not available (Dixon et al. 2019). Although attenuated live vaccines for ASFV have been reported recently, the bio-safety concerns make it difficult to apply commercially (Huang et al. 2021; Liu et al. 2021; Yang et al. 2021). Additionally, the source of primary macrophages for ASFV propagation also limits the production of live attenuated vaccines. Considering the advantages of rPRV, recombinant viruses expressing the ASFV antigens would be a possible way for generation of ASFV vaccine. In fact, PRV has been used to express ASFV antigens (Feng et al. 2020). As combined immunization of recombinant vaccinia virus expressing key ASFV antigens could induce the production of specific antibodies against ASFV (Jancovich et al. 2018), it is also possible that combination of multiple rPRV expressing distinct ASFV antigens may be an effective strategy for generation of novel ASF vaccines. In summary, PRV has shown great potential for development of live vector-based vaccines in animals, providing useful tools for prevention and control of animal infectious diseases.

Abbreviations

PRV: Pseudorabies virus; UL: Unique long region; US: Unique short region; TRS: Terminal repeat sequence; IRS: Internal repeat sequences; rPRVs: Recombinant PRVs; BAC: Bacterial artificial chromosomes; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; sgRNA: Single guide RNA; PCR: Polymerase chain reaction; PCVD: Porcine circovirus-associated diseases; PCV2: Porcine circovirus type 2; ORFs: Open reading frames; IL-18: Interleukin 18; PPV: Porcine parvovirus; JEV: Japanese

encephalitis virus; PRRS: Porcine reproductive and respiratory syndrome; PRRSV: Porcine reproductive and respiratory syndrome virus; PADRE: Pan DR T-helper cell epitope; FMD: Foot-and-mouth disease; FMDV: Foot-and-mouth disease virus; rPRV-VP1: rPRV expressing VP1 of FMDV; rPRV-P12A-3C: rPRV expressing P12A and 3C; HA: Hemagglutinin; rPRV-H3N2 HA: rPRV expressing the HA gene from H3N2 subtype of SIV; CSF: Classical swine fever; CSFV: Classical swine fever virus; Sj26GST: *S. japonicum* glutathione S-transferase; SjFABP: *S. japonicum* fatty acid-binding protein; ASFV: African swine fever virus.

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The authors declare that they have no competing interests.

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