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Construction of an attenuated goatpox virus AV41 strain by deleting the TK gene and ORF8-18

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ABSTRACT

Goatpox virus (GTPV) is prevalent in goats and is associated with high mortality. This virus causes fever, skin nodules, lesions in the respiratory and lymph node enlargement. Considering the safety risks and side effects of vaccination with attenuated live GPTV vaccine strain AV41, an attenuated goatpox virus (GTPV-TK-ORF), was constructed by deleting non-essential gene fragments without affecting replication and related to the virulence and immunomodulatory functions of the goatpox virus AV41 strain (GTPV-AV41) using homologous recombination and the Cre (Cyclization Recombination Enzyme)/Loxp system. The results of both in vivo and in vitro experiments demonstrated that GTPV-TK-ORF was safer than wild type GTPV-AV41, possessed satisfactory immunogenicity, and could protect goats from a virulent GTPV-AV40 infection. Moreover, the IFN-γ, GTPV-specific antibody, and neutralizing antibody levels in the GTPV-TK-ORF-immunized group were significantly higher than that in the normal saline control group following immunization ($P < 0.01$). Thus, GTPV-TK-ORF may be used as a potential novel vaccine and viral vector with good safety and immunogenicity.

1. Introduction

GTPV, the causative agent of goatpox, is one of the most serious infectious diseases associated with high morbidity and mortality in goats (Kitching, 2003). In China, goats were mainly inoculated with the vaccine strain GTPV-AV41, which was derived from the GPTV-AV40 strain isolated from Qinghai province in 1959. The GTPV-AV41 strain was created via the continuous passage of the GPTV-AV40 strain at a temperature of 30 °C in goat and sheep testis cells in 1985 by Shaohua Wang et al. from the China Institute of Veterinary Drugs Control. Although this attenuated vaccine exhibited higher immunity to GTPV, it created local pock lesions following inoculation and caused secondary pock infections that led to death in severe cases. In particular, the attenuated vaccine also caused serious side effects (e.g., miscarriage in pregnant goats and secondary pock infections) in southern Chinese provinces, which limited its application. Therefore, it is especially important to develop vaccines with fewer side effects to effectively control goatpox (Bowden et al., 2008).

Recombinant fowlpox (Du et al., 2015a; Zhu et al., 2017) and vaccinia viruses (Du et al., 2015b) that express heterologous proteins have been constructed in our laboratory, and several genes have been deleted from vaccinia virus (Li et al., 2016, 2017) using homologous recombination and Cre/Loxp specific knockout technology. A prerequisite for the construction of the gene-deleted GPTV includes the selection of regions non-essential for viral replication. Multiple studies have shown that the deletion of the TK gene, which is a non-essential gene for viral replication but is a virulence-associated gene, does not impact poxvirus replication in vitro but can reduce the viral virulence without impacting its immunogenicity (Cetre-Sossah et al., 2017; Zhang et al., 2016).

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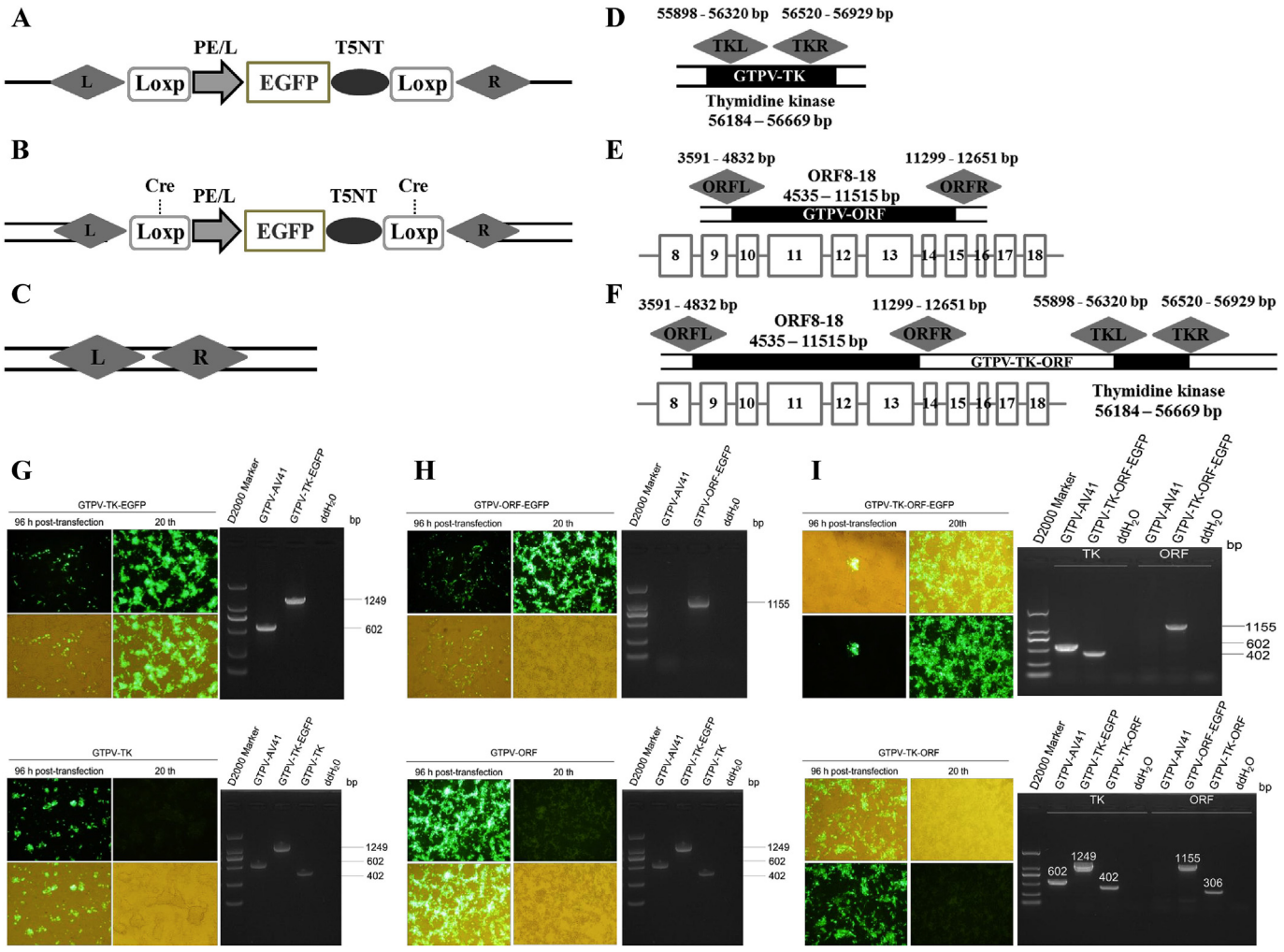

Fig. 1. Screening and identification of the gene deleted GTPVs. (A) Structure of the shuttle plasmid. (B) Recombinant goatpox virus. (A screening marker EGFP was inserted at the ORF8-18 site of GTPV and the recombinant GTPV with deletion of the ORF8-18 but containing the EGFP gene was obtained by green fluorescent plaque screening). (C) Gene-deleted GTPV. (The recombinant rGTPV-ORF-EGFP was generated through Cre/Loxp system to remove the EGFP gene. TK gene was knocked out in the same manner. Ultimately, the genes deleted strains GTPV-TK, GTPV-ORF, and GTPV-TK-ORF were obtained). (D) The genomic structure of the gene-deleted virus GTPV-TK, (E) GTPV-ORF and (F) GTPV-TK-ORF. The images represent the results of these recombinant viruses and gene-deleted viruses at 72 h post-transfection, as well as the twelfth round of plaque screening (magnification 200 ×). (G) The screening processes and identification of rGTPV-TK-EGFP and GTPV-TK. The 402 bp fragments can be amplified. (H) The screening processes and identification of rGTPV-ORF-EGFP and GTPV-ORF. The 306 bp fragments can be amplified. (I) The screening processes and identification of rGTPV-TK-ORF-EGFP and GTPV-TK-ORF. The 402 bp and 306 bp fragments can be amplified.

Using a preliminary prediction of the genomic functions of GTPV-AV41, there are five genes responsible for coordinating the host's immune response (one participates in the metabolism of nucleic acids; and one controls virus-host interactions; and two inhibitor apoptosis proteins) and two incomplete ORFs in 11 ORF encoding products from GTPV ORF8-18. Since these 11 ORF transcriptional regions are all far from the central coding region, the 11 ORFs may be non-essential genes for viral replication. Moreover, deleting these 11 ORFs does not appear to have a substantial impact on viral survival and significantly reduces viral virulence within the host.

In this study, the GTPV-TK-ORF strain was produced in Vero cells by deleting the TK gene and ORF8-18. Vero cells were used because goat testis cells are highly complicated to prepare and GTPV could also replicate in Vero cells, which are cell line approved for human viral vaccine use and possess good security. The gene-deleted strain, GTPV-TK-ORF, was constructed using homologous recombination and the Cre/Loxp system. The genetic stability, growth characteristics, and virulence of the gene-deleted strain GTPV-TK-ORF were evaluated using in vitro genetic stability, growth curve, pock development, cellular proliferation, and cytotoxicity assays. The safety and immunogenicity of the gene-deleted strain, GTPV-TK-ORF, was assessed by measuring the body temperature, pock size, neutralizing antibody titer, and protective efficiency in Jilin white goats.

2. Materials and methods

2.1. Cells, viruses, and animals

OA3.TS, BHK-21, HEK-293, Marc-145, MDCK, and Vero cells were purchased from the China Center for Type Culture Collection (CCTCC). All cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Hyclone, Beijing, China) with 10% fetal bovine serum (FBS) (Hyclone, Beijing, China), and penicillin (10,000 U/mL)/1% streptomycin (10 mg/mL) (Hyclone, Beijing, China). The attenuated vaccine (GTPV-AV41) and virulent strain were both obtained from the Institute of Virology at the Chinese Center for Disease Control and Prevention.

One-year-old Jilin White goats were purchased from the Experimental Animal Center of Academy of Military Medical Sciences of China. The animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Chinese