A novel Pseudorabies virus vaccine developed using HDR-CRISPR/Cas9 induces strong humoral and cellular immune response in mice

ABSTRACT

Outbreaks of Pseudorabies (PR) by numerous highly virulent and antigenic variant Pseudorabies virus (PRV) strains have been causing severe economic losses to the pig industry in China since 2011. However, current commercial vaccines are often unable to induce thorough protective immunity. In this study, a TK/gI/gE deleted recombinant PRV expressing GM-CSF was developed by using the HDR-CRISPR/Cas9 system. Here, a four-sgRNA along with the Cas9D10A targeting system was utilized for TK/gI/gE gene deletion and GM-CSF insertion. Our study showed that the four-sgRNA targeting system appeared to have higher knock-in efficiency for PRVs editing. The replication of the recombinant PRVs were slightly lower than that of the parental strain, but they appeared to have similar properties in terms of growth curves and plaque morphology. The mice vaccinated with the recombinant PRV expressing GM-CSF via intramuscular injection showed no obvious clinical symptoms, milder pathological lesions, and were completely protected against wild-type PRV challenge. When compared to the triple gene-deleted PRV, the gB antibodies and neutralizing antibody titers were improved and the immunized mice appeared to have lower viral load and higher mRNA levels of IL-2, IL-4, IL-6, and IFN-γ in spleens. Our study offers a novel approach for recombinant PRV construction, and the triple gene-deleted PRV expressing GM-CSF could serve as a promising vaccine candidate for PR control.