AP08856376 «Improvement of the Capripoxvirus-Based Vaccine Vector»

Project Supervisor, Ph.D. in Biology., Professor Chervyakova O.V.

Abstract

As a result of the proposed project, potential immunomodulatory genes of capripoxviruses will be identified, recombinant viruses with deletions of selected genes will be obtained, and their immunogenic activity will be studied. Optimization of vaccine vectors by enhancing immunogenicity will allow for improved vaccination protocols.

Objective and Tasks

Project Goal:To design an optimized capripoxvirus-based vector capable of inducing a long-lasting protective immune response.Objectives:Comparative genomic analysis of capripoxviruses and vaccinia virus to identify gene deletions that enhance immune response.Generation of recombinant capripoxviruses with knockouts of immunomodulatory genes.Assessment of foreign gene expression in recombinant capripoxviruses with immunomodulatory gene knockouts.Evaluation of protective efficacy of the optimized capripoxvirus vector expressing a key antigen of a selected pathogen.

Таблица 1. Vaccinia virus genes that, when removed, enhance immune response

Gene	Function
A41L	Chemokine binding protein
A46R	IL-1/TLR signaling inhibitor
B8R	Soluble IFN-g receptor-like protein
B16R	IL-1 beta receptor
B19R	IFN-alpha/beta receptor glycoprotein
C6L	Bcl-2-like protein, IFN-beta inhibitor
C12L	Serpin 1,2,3, IL-18-связывающий белок
F1L	Caspase-9 (apoptosis) inhibitor (mitochondrial-associated)
K7R	Host immune response repressor
N1L	Anti-apoptotic Bcl-2-like protein
N2L	Alpha amanatin target protein, Ингибитор IRF-3

Materials and Methods

Lumpy skin disease virus (LSDV) was propagated in TYA cells using PSP medium supplemented with 2% fetal bovine serum (FBS) for 7–10 days at 37°C in a 5% CO_2 atmosphere. Viral activity was determined by microtitration in 96-well plates. Viral titers were calculated using the Reed and Muench method and expressed as log $TCID_{50}$/mL.Recombinant viruses were generated via homologous recombination under conditions of transient dominant selection. Recombinants were selected by limiting dilution and plaque assays. Identification of recombinant viruses was performed by fluorescence microscopy and/or PCR.

Results and Discussion

Genomic analysis of poxviruses identified seven genes potentially influencing the immune response of the capripoxvirus vector. Recombinant LSDV strains with knockouts of genes LSDV005 or LSDV008 expressing foreign antigens were generated and characterized. It was found that deletion of immunomodulatory genes in the tested combinations did not affect virus replication in vitro. An exception was the double knockout of LSDV008 and LSDV066, which resulted in decreased replication during passaging.The recombinant LSDV strains effectively expressed inserted antigens in vitro in cell culture. The level of transgene expression was independent of the presence or absence of immunomodulatory genes in the viral genome.In mice, recombinant LSDV strains with knockouts of LSDV005 or LSDV008 expressing either interleukin-18 or rabies virus glycoprotein induced a cellular immune response. Antibody production was only observed in viruses with a deletion of the LSDV005 gene.Recombinant LSDV strains with knockouts of LSDV005 or LSDV008 expressing rabies virus glycoprotein provided full or partial protection against rabies infection, respectively.

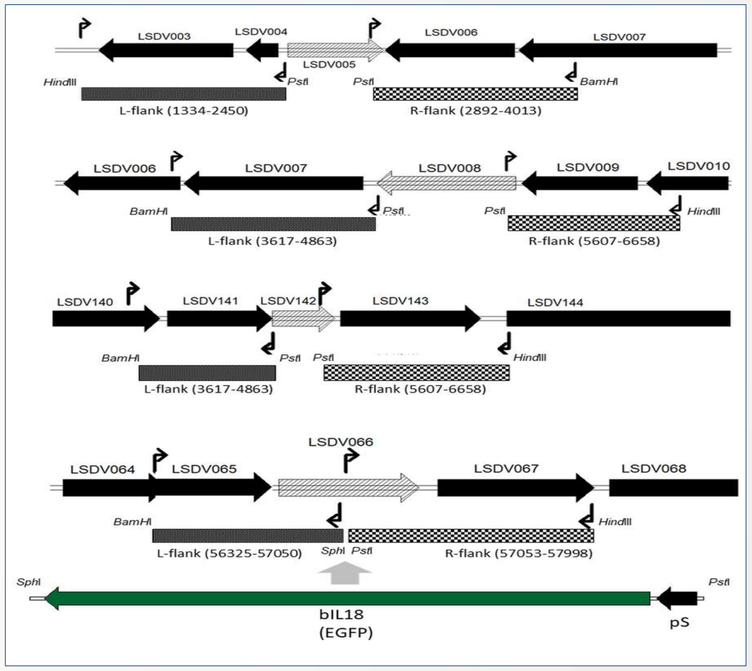

Figure 1. Scheme for obtaining PCR DNA fragments flanking the deleted LSDV genes for constructing integration plasmids

List of Published Works

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5. Chervyakova O., Issabek A., Tulendibayev A., Omarova Z., Sultankulova K., Orynbayev M. Development of safe vaccine against Lumpy skin disease: XXIII International Poxvirus, Asfarvirus and Iridovirus Conference (virtual), July 5-9, 2021, Philadelphia, Pennsylvania

Contacts

Chervyakova O.V., email: o.chervyakova@biosafety.kz