DEVELOPMENT OF NON-INTEGRATIVE AUTONOMOUSLY REPLICATING VECTORS FOR EPIGENETIC REPROGRAMMING OF SOMATIC CELLS

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INTRODUCTION.

Induced pluripotent stem cells (iPSC) are of paramount importance for the regenerative medicine [1]. This work has been aimed at creating technology for the production of iPSC using non-pathogenic vectors, which replication is not accompanied by changes in the cellular genome. Alphaviruses such as Venezuelan equine encephalitis virus (VEE), Sindbis virus (SIN), etc. are attractive vectors for gene delivery into cultured cells and for gene delivery *in vivo*. Alphaviruses can infect wide range of mammalian or insect cells, including cell lines permitted for production of products for human use. Their replication occurs strictly in the cytoplasm of infected cells. When utilized as vectors, alphaviral genomes drive production of heterologous proteins to very high levels. The idea of the project is to utilize the genome of well-described alphavirus VEE as vector for delivery and expression of the human pluripotency factors used for epigenetic reprogramming of somatic cells, namely Nanog, Oct3/4, Sox2, Klf4, and c-Myc. To date, no non-integrative viral vectors for epigenetic reprogramming of cells are available, except for the system utilizing the genome of negative strand RNA-virus, Sendai paramyxovirus [2].

MATERIALS AND METHODS.

We used collection of plasmids in which parts of the Venezuelan equine encephalomyelitis genome were previously cloned. Missing fragments of the genome (5'-UTR and 3'-UTR) were synthesized *de novo*. Gene of the human pluripotency factor NANOG (950 bp) was synthesized *de novo* from 60-mer oligonucleotides.

RESULTS AND DISCUSSION.

Full-length 11474 bp - long cDNA copy of the VEE genome was produced and the cDNA copy was cloned into E.coli plasmid. The autonomously replicating fragment (replicon) of the VEE genome was produced in which the VEE structural protein genes are replaced by the GFP gene. Thus the GFP gene is placed under control of the 26S subgenomic RNA promoter. The total length of the replicon-containing plasmid (pRepVEE-GFP) is 10221 bp. The gene for the human pluripotency factor NANOG (950 bp) was synthesized de novo.

CONCLUSIONS.

We constructed a fragment of genome of the Venezuelan equine encephalomyelitis virus and placed gene of marker protein GFP under control of subgenomic promoter. We also synthesized *de novo* gene of the human pluripotency factor NANOG to replace GFP at later stages of the project.

ACKNOWLEDGMENTS.

This work was supported by a grant from the Ministry of Education and Science of the Republic of Kazakhstan.

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