DEVELOPMENT OF A TECHNOLOGY FOR EXPRESSION OF RECOMBINANT HUMAN ERYTHROPOIETIN IN CULTURED MAMMALIAN CELLS USING ALPHAVIRUS EXPRESSION SYSTEM

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Introduction. Currently there are no industrial eukaryotic expression systems other than transient expression from plasmids or expression from genes integrated into host genome. Both approaches (use of eukaryotic plasmids or chromosomal integration) suffer from poor scalability and often from poor yields. Although, in laboratory settings, effective means for transducing of cultured cells to express foreign proteins and for high-level transient expression were developed based on viral genomes. We thought to develop a scalable and suitable for industrial application technology for the production of recombinant human erythropoietin (EPO) in mammalian cell cultures using an expression vector based on the genome of RNA virus.

Materials and methods. We produced full-length cDNA copy of the genome of the Venezuelan equine encephalomyelitis virus (VEE). The copy of VEE genome was cloned into E.coli plasmid for the convenience of genetic engineering. Synthetic gene encoding the EPO was produced by the de novo synthesis from oligonucleotides. The expression cassette was produced for insertion of the EPO gene into the VEE genome. The genomic RNA of recombinant virus was produced and transfected into cultured mammalian cells.

Results and discussion. The genome of VEE (strain TC-83) was produced by ligation of long PCR-amplified cDNA fragments. The 5"- and 3"-ends of the VEE genome were assembled from oligonucleotides. The full-length copy of VEE genome (11473 bp) was cloned into plasmid pVEE/TC-83 under control of promoter of SP6 RNA polymerase. The expression cassette GFP-2A-EPO was designed. This expression cassette encodes a polyprotein consisting from sequences of green fluorescent protein (GFP), foot-and-mouth disease protease 2A, and EPO. The GFP was included into the expression cassette with intention of convenient observation of the intracellular replication of the VEE RNA. The foot-and-mouth disease protease 2A is a short aminoacid sequence (17 aa) which presence leads to cotranslational cleavage of the polyprotein into the GFP-2A and EPO parts. The EPO is thought to be liberated from the polyprotein by action of the 2A protease. The expression cassette GFP-2A-EPO was cloned into the pVEE/TC-83, resulting in development of the construct pVEE/GFP-2A-EPO which encodes the autonomously replicating viral genome fragment capable of producing GFP and EPO during intracellular replication. Viral RNA (VEE/GFP-2A-EPO RNA) was synthesized in vitro in a reaction catalyzed by the SP6 RNA polymerase and transfected into the cultured baby hamster kidney (BHK-21) cells. The transfection results indicate that the viral genome VEE/GFP-2A-EPO is viable and capable of production of heterologous proteins in the infected cells.

Conclusions. Further development of this strategy will result in a callable expression system for production of the recombinant EPO and other proteins in mammalian and insect cells.

Acknowledgments. This work was supported by the project "Development of a technology platform for production of recombinant proteins in mammalian cell culturesusing viral replicons" within the Target Program "Development of translational and personalized medicine to create the foundations of the biomedical industry in the Republic of Kazakhstan, 2014-2016 years".