USING OF ALPHAVIRUS CAPSID MUTANT AS A VECTOR FOR THE PRODUCTION OF RECOMBINANT PROTEINS

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Introduction. Recombinant DNA technology is used in various ways in a healthcare. By the genetic engineering techniques recombinant protein with pharmaceutical activity aka biopharmaceuticals are produced. The most of approved drugs are produced by mammalian cells. Because, in order to function properly in human organism, recombinant proteins have to pass through glycosilation which can be performed in mammalian cells. In this research we investigated alphaviral vector based on Venezuelan encephalitis virus (VEEV) as possible gene delivery system for mammalian cells transduction.

Methods. In this work VEEV, GFP containing TC-83 vaccine non-cytopathic strain was applied. For virus replication BHK-21 cells were used. Standard molecular cloning technique was used for plasmid roduction. Plasmid DNA was purified by gradient separation in CsCl. It was linearized by MluI restrictase, and RNA was synthesized by using SP6 RNA polymerase and capping analog. Transfection of BHK-21 cells was performed by Lipofectamine 2000. Virus replication was measured by TCID50 titration technique on fluorescence microscope.

Results.Virus titer was measured by TCID50. Initial pathogen dose was 105 FFU/ml, 24 hours later it became 2,8 * 105 FFU/ml., on the third day 48 hours later titer was 1,6 * 104 FFU/ml, 72 hours later virus concentration reached 1,5 * 106 FFU/ml. We compared cytopathic effect of original TC-83 with mutation and one of TC-83 virus without mutation. BHK-21 monolayer, infected by TC-83 strain with mutation, was viable 5 days after infection, whereas cells, infected by original TC-83 were 100% dead 48 hours after infection.

Summary. As a result of investigation, TC-83 strain with mutation in capsid appeared to be much less cytopathic contrary to original TC-83. During replication on BHK-21 cells, virus reaches maximum titer 72 hours after initial infection. Due to reduced cytopathic effect in mutated TC-83, this strain is potentially effective vector for mammalian cells transduction with recombinant gene.