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Capsid modifications of adeno-associated virus serotype 2

Shitik E.M.*, Shalik I.K., Yudkin D.V.

State Research Center of Virology and Biotechnology "Vector", Rospotrebnadzor, World-Class Genomic Research Center for Biological Safety and Technological Independence, Federal Scientific and Technical Program on the Development of Genetic Technologies, Koltsovo, Novosibirsk, Russia

** shitik.ekaterina@gmail.com*

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Motivation and Aim: Adeno-associated virus (AAV) is a non-enveloped virus of size 20–25 nm belonging to the parvovirus family that becomes a leading platform for the delivery of transgenes *in vivo*. In 2022 263 clinical trials of drugs based on recombinant AAV (rAAV) are being carried out, 32 of which have reached phase III [1]. rAAV is not a pathogen for human cells and does not have the ability to maintain its own life cycle in the absence of helper viruses – adenovirus or herpesvirus. At the same time, rAAV entry does not depend on the cell division cycle and the virus maintains the expression of the transgene for a long time in the form of an episome. At the beginning of the virus study, the nonspecific affinity of rAAV for different target cells was also highlighted as an additional advantage because of the possibility to develop vectors for the treatment of various diseases. However, with the beginning of clinical trials, it became clear that the ability of the virus to enter all tissues of the body significantly limits the possibility of its systemic administration. Hence, the aim of the study is to develop a versatile vector based on AAV serotype 2 that can be used to create a highly tropic delivery platform to target cells.

Methods and Algorithms: A capsid of adeno-associated virus is composed of three main proteins VP1, VP2 and VP3 that are translated through splicing from one mRNA (Fig. 1). The VP3 sequence is a common part of all of three proteins that determines the tropism of the virus [2]. Moreover, it determines the transduction efficiency because of tyrosine residues that can be phosphorylated in the cell cytoplasm [3]. The VP2 protein presented in a small amount has a free N-terminus in the region of which rather long sequences of amino acids can potentially be introduced without affecting the capsid assembly [4].

In this study the DNA sequence of VP3 protein of AAV serotype 2 in pDp2 plasmid [5] has been modified. To increase the transduction efficiency of AAV2 three codon substitution Y444F, Y500F and Y730F were made with overlap extension PCR. For all DNA regions to be changed it has been chosen a pair of forward and reverse primers complemented to one region. To eliminate natural viral tropism six codon substitution were also made with overlap extension PCR. Codons 484, 487, 532, 585 and 588 were changed to codons of aliphatic amino acid residues to eliminate the viral tropism to heparan sulfate, and codon 513 – to eliminate the tropism to α5β1 integrin. Furthermore, the VP2 protein sequence has been cloned to phMGFP plasmid (E642A, Promega) to express separately from other capsid proteins. In order to avoid expression from original pDp2 plasmid the start-codon of VP2 was destroyed without alteration of other proteins sequences also using overlap extension PCR.

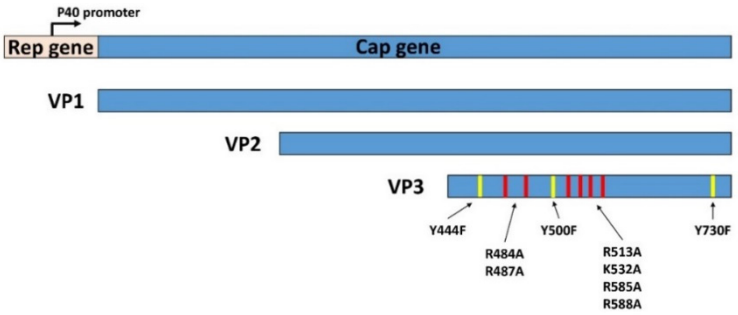

Fig. 1. Scheme of the Cap gene encoding all proteins of the AAV capsid. Proteins VP1, VP2 and VP3 are transcribed from p40 promoter. Yellow markers indicate codons to be changed to increase the efficiency of virus transduction. Red markers indicate codons to be changed to destroy viral natural tropism

To receive viral particles cell line HEK293A was transfected with the plasmids obtained. The titer of viral particles was analyzed using real-time PCR. All of viral particles contained sequence of EGFP protein as a transgene. An efficiency of transduction was analyzed with fluorescence microscopy on HeLa cell line and on a line of fibroblasts that had been immortalized in our laboratory.

Results: Three types of modified rAAV serotype 2 have been obtained. One type contains replacement tyrosine residues with phenylalanine. With fluorescence microscopy it has been shown more effective transduction of this type in comparison with wild type of rAAV2. The second type in addition to previous modification has been obtained using plasmid that contains the sequence of VP2 protein regulated with its own promoter. With real-time PCR and fluorescence microscopy it has been shown that this modification does not affect the assembly of the viral particles and can be used to introduce proteins of interest. Apart from two previous modifications, the third type contains substitutions R585A, R588A, R484A, R487A, K532A and R513A. An almost absence of fluorescence on cell lines compared to the second modified type has shown that these substitutions determine AAV serotype 2 tropism.

Further studies on the introduction of proteins of interest into the N-terminus of VP2 protein to increase the tropism of viral particles will allow us to evaluate the effectiveness and versatility of the platform.

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