DEVELOPMENT AND CHARACTERIZATION OF A VECTOR SYSTEM BASED ON THE SIMIAN ADENOVIRUS TYPE 25

Ozharovskaia TA✉, Popova O, Zubkova OV, Vavilova IV, Pochtovyi AA, Shcheblyakov DV, Gushchin VA, Logunov DYu, Gintsburg AL

Gamaleya National Research Center for Epidemiology and Microbiology of the Ministry of Health of the Russian Federation, Moscow, Russia

Technological versatility and the humoral and cellular immune response induction capacity have conditioned wide spread of adenoviral vectors as vaccine and gene therapy drugs. However, vaccination with Sputnik V made a significant portion of the population immune to the types 5 and 26 (Ad5 and Ad26) recombinant human adenovirus vectors, which are some of the most frequently used bases for candidate vaccines. Today, vaccine designers tend to select alternative adenovirus serotypes as platforms to develop vaccines against new pathogens on. A good example is simian adenovirus type 25 (SAd25), which belongs to subgroup E. It is genetically distant from Ad5 and exhibits extremely low seroprevalence in human beings, which makes it an appealing alternative vaccine vector. The purpose of this work was to design and study a new vaccine platform based on simian adenovirus type 25. We relied on the advanced methods of molecular biology and virology to construct and make recombinant adenoviruses; the phylogenetic analysis in the context of this study was enabled with bioinformatic methods. The resulting recombinant adenoviral vector can effectively replicate itself in the HEK293 cell line (human embryonic kidney cells). This work substantiates the expediency of further investigation into the SAd25 vector as a platform for development of the prevention vaccines against various infectious diseases.

Keywords: adenovirus vector, vaccine platform, recombinant adenovirus, simian adenovirus type 25

Funding: the work is the result of the effort under the "Development of a recombinant vaccines platform (including live vaccines) enabling design of the infectious diseases prevention vaccines" task set by the Ministry of Health of the Russian Federation.

Acknowledgments: we express our sincere gratitude to AE Nikonova, a researcher with the Laboratory of Molecular Biotechnology of the NF Gamaleya National Research Center for Epidemiology and Microbiology for her help in conducting the phylogenetic analysis of adenovirus hexon sequences, as well as to research laboratory assistant D.D. Kustova for her help in obtaining the whole genome sequences.

Author contribution: Ozharovskaia TA, Zubkova OV, Logunov DYu, Gintsburg AL — conceptualization and planning of the experimental part; Popova O, Zubkova OV — production of genetically engineered constructs and recombinant simian adenovirus type 25; Ozharovskaia TA, Vavilova IV — phylogenetic analysis, virological experiments; Pochtovyi AA — whole genome sequencing of adenoviruses; Ozharovskaia TA, Zubkova OV — article preparation and authoring, interpretation of the results; Shcheblyakov DV, Gushchin VA — manuscript editing; Gushchin VA — state assignment project management.

Compliance with ethical standards: the work was carried out in accordance with the principles of the Declaration of Helsinki.

✉ **Correspondence should be addressed:** Tatiana A. Ozharovskaia
Gamalei, 18, Moscow, 123098, Russia; t.ozh@yandex.ru

Received: 29.12.2022 **Accepted:** 20.01.2023 **Published online:** 28.02.2023

DOI: 10.24075/brsmu.2023.006

РАЗРАБОТКА И ХАРАКТЕРИСТИКА ВЕКТОРНОЙ СИСТЕМЫ НА ОСНОВЕ АДЕНОВИРУСА ОБЕЗЬЯН 25-ГО СЕРОТИПА

Т. А. Ожаровская✉, О. Попова, О. В. Зубкова, И. В. Вавилова, А. А. Почтовый, Д. В. Щебляков, В. А. Гущин, Д. Ю. Логунов, А. Л. Гинцбург

Национальный исследовательский центр эпидемиологии и микробиологии имени Н. Ф. Гамалеи Министерства здравоохранения Российской Федерации, Москва, Россия

В настоящее время аденовирусные векторы широко используют в качестве вакцинных и генотерапевтических препаратов благодаря их технологичности и способности индуцировать стойкий гуморальный и клеточный иммунный ответ. Однако одни из наиболее часто используемых векторов для разработки кандидатных вакцин — рекомбинантные аденовирусы человека 5-го и 26-го серотипов (Ad5 и Ad26) становятся менее привлекательными из-за высокого процента населения с иммунитетом к данным векторам, вызванным вакцинацией «Спутником V». На сегодняшний день появляется тенденция к выбору альтернативных серотипов аденовирусов в качестве платформ для разработки вакцин против новых патогенов. Например, аденовирус обезьян 25-го серотипа (SAd25), принадлежащий к подгруппе E, генетически отдален от Ad5 и проявляет крайне низкую серопревалентность у людей, что делает его привлекательным альтернативным вакцинным вектором. Целью работы было создать и исследовать новую вакцинную платформу на основе аденовируса обезьян 25-го серотипа. Для конструирования и получения рекомбинантных аденовирусов использовали современные молекулярно-биологические и вирусологические методы, для филогенетического анализа применяли биоинформатические методы. Полученный рекомбинантный аденовирусный вектор способен эффективно реплицироваться в производственной культуре клеток HEK293 (клетки почки эмбриона человека). Результаты работы обосновывают целесообразность дальнейшего исследования вектора SAd25 в качестве платформы для разработки вакцин для профилактики различных инфекционных заболеваний.

Ключевые слова: аденовирусный вектор, вакцинная платформа, рекомбинантный аденовирус, аденовирус обезьян 25-го серотипа

Финансирование: работа выполнена в рамках государственного задания Министерства здравоохранения Российской Федерации «Разработка технологической платформы для создания рекомбинантных, в том числе живых вакцин для профилактики инфекционных болезней».

Благодарности: выражаем искреннюю благодарность научному сотруднику А. Э. Никоновой из лаборатории молекулярной биотехнологии ФГБУ «НИЦЭМ им. Н. Ф. Гамалеи» за помощь в проведении филогенетического анализа последовательностей гексонов аденовирусов, а также лаборанту-исследователю Д. Д. Кустовой за помощь в получении полногеномных последовательностей.

Вклад авторов: Т. А. Ожаровская, О. В. Зубкова, Д. Ю. Логунов, А. Л. Гинцбург — концепция и план экспериментов; О. Попова, О. В. Зубкова — получение генно-инженерных конструкций и рекомбинантного аденовируса обезьян 25-го серотипа; Т. А. Ожаровская, И. В. Вавилова — филогенетический анализ, вирусологические эксперименты; А. А. Почтовый — полногеномное секвенирование аденовирусов; Т. А. Ожаровская, О. В. Зубкова — подготовка и написание статьи, интерпретация результатов; Д. В. Щебляков, В. А. Гущин — редактирование рукописи; В. А. Гущин — руководство проектом госзадания.

Соблюдение этических стандартов: работа проведена в соответствии с принципами Хельсинкской декларации.

✉ **Для корреспонденции:** Татьяна Андреевна Ожаровская
ул. Гамалеи, д. 18, г. Москва, 123098, Россия; t.ozh@yandex.ru

Статья получена: 29.12.2022 **Статья принята к печати:** 20.01.2023 **Опубликована онлайн:** 28.02.2023

DOI: 10.24075/vrgmu.2023.006

Recombinant adenoviral vectors (rAd) are a popular tool for gene delivery into mammalian cells; in particular, they are used in development of candidate vaccines [1–4]. Vaccines based on human adenovirus types 5 and 26 (Ad5 and Ad26, respectively) designed to counter Ebola and COVID-19 have proven to be effective [5, 6]. However, there is a need for new adenovirus vectors of other serotypes that can be alternatives to the existing ones, since vaccination of the large portion of the population translated into immunity to adenovirus vectors that can lower efficacy thereof. In this connection, human adenovirus types other than Ad5 and Ad26, as well as simian adenovirus serotypes, can facilitate development of the new drugs and heterologous prime-boost immunization patterns. There are over 80 serotypes of human adenoviruses; there are various approaches to their classification: they can be viewed as forming seven separate subgroups (A–G) or belonging to seven species of adenoviruses (*Human mastadenovirus A-G*) [7, 8].

Simian adenoviruses (SAd) are also being extensively investigated as vector vaccines. There is a considerably wide range of SAd-based adenoviral vectors currently being analyzed as vaccine candidates, including the chimpanzee adenoviruses (ChAd) ChAd3 [9], ChAd63 [10, 11], ChAd68 (also called SAd25) [12, 13], ChAd83 and ChAd155 [14, 15], as well as SAd22 [16], SAd23 [16, 17], SAd24 [16, 18]. These vectors have shown high immunogenicity in animal models and in clinical trials of candidate vaccines [13, 19, 20]. In addition, they are significantly similar to human adenoviruses biologically and exhibit low cross-activity of sera with virus-neutralizing antibodies to Ad5 and Ad26 [12, 21].

Simian adenovirus type 25 (SAd25) is one of the alternative serotypes used as a platform for therapeutic agents. It belongs to subgroup E, which makes it a potent vector in its own right and as part of heterologous immunization. In addition, antibodies to Ad2, Ad4, Ad5, Ad7, and Ad12 do not neutralize SAd25, therefore, this vector a promising vaccine platform candidate [12].

This study aimed to design a new vector platform based on SAd25 and study its biological and physical characteristics.

METHODS

Bacterial strains and cell lines

For this work, we used a laboratory strain of *Escherichia coli* DH5α (New England Biolabs; USA), an *E. coli* BJ5183 strain (Stratagene; USA) and the HEK293 cell line, human embryonic kidney cells containing the Ad5 region in the E1 genome (Russian Collection of Vertebrate Cell Lines; Russia). We cultivated HEK293 in the DMEM1× medium (Dulbecco's Modified Eagle Medium; HyClone, USA) supplemented with 5% fetal bovine serum (HyClone; USA) and 25 ml of sodium bicarbonate 7.5% (PanEco; Russia), in the presence of 146 mg of L-glutamine (PanEco; Russia) and a mixture of penicillin and streptomycin (PanEco; Russia), at 37 °C, with CO_2 at 5%.

SAd25 genome sequencing

The SAd25 wild-type (wt-) virus strain was obtained from the State Collection of Viruses of II–IV Pathogenicity Groups of the D.I. Ivanovsky Institute of Virology, part of the N. F. Gamaleya National Research Center of Epidemiology and Microbiology. This virus was passaged on HEK293 cells and purified by cesium chloride (CsCl) density gradient ultracentrifugation. We used the standard technique [22] to isolate the genomic DNA from purified wt-SAd25. The nucleotide sequence of wt-SAd25 was determined using whole genome sequencing on the Oxford MinION sequencer (Oxford Nanopore Technologies; UK).

Designing the recombinant adenovirus vectors

Firstly, to create the pArms-SAd25-EGFP plasmid, we PCR-amplified the left homology arm (nucleotides 1-537 and 3406–3801 in accordance with the wt-SAd25 genome) and the right homology arm (nucleotides 35969–36519). The expression cassette, consisting of the cytomegalovirus early promoter (CMV promoter), the enhanced green fluorescent protein (EGFP) gene and the SV40 virus polyadenylation signal, is located at the site of the E1 deletion (nucleotides 538–3405). For further linearization of the pArms-SAd25-EGFP plasmid a SwaI site was inserted between the homology arms.

To construct the plasmid pSAd25ΔE1-EGFP carrying the full-length SAd25 genome and the expression cassette with the EGFP gene at the site of the deleted E1 region of the genome, we used the method of homologous recombination between the linearized pArms-SAd25-EGFP plasmid and wt-SAd25 genomic DNA in *E. coli* cells of the *BJ5183* strain. The bacterial cells were transformed by electroporation with the MicroPulser (Bio-Rad, Hercules; USA), as prescribed by the manufacturer's instructions. We analyzed the resulting plasmid pSAd25ΔE1-EGFP with PCR and restriction mapping.

For the E3 region modification, we designed the pBS-E3-SAd25 plasmid that carried a fragment of the SAd25 genome (12804 nucleotides) cloned by NheI. The E3 region of the genome (nucleotides 27131–31051) was deleted using the NcoI and SalI sites and with the help of the homologous recombination technique. The final pSAd25-EGFP plasmid contained deletions in the E1 and E3 regions of the genome. We resorted to restriction mapping and whole genome sequencing to analyze the resulting molecular clone.

Production of recombinant adenoviruses

We used the pSAd25-EGFP plasmid to obtain a pSAd25-EGFP recombinant adenovirus. HEK293 cells were seeded in six-well culture plates and incubated overnight to 80% confluence. To remove the bacterial part, we hydrolyzed the plasmid DNA with PacI and SspI restriction endonucleases and then transfected into HEK293 cells using the Lipofectamine 2000 reagent (Thermo Fisher Scientific; USA), as prescribed in the manual provided by the manufacturer. Once the CKX41 inverted microscope (Olympus; Japan) allowed a visual conformation of the viral cytopathic effect (CPE), the cells in the culture medium were subjected to three freeze-thaw cycles.

The Ad5-mCherry and Ad26-EGFP recombinant adenoviruses were obtained as described previously [23].

Accumulation and purification of recombinant adenoviruses

We produced the recombinant adenoviruses in the HEK293 cell line. The HEK293 cells were seeded into 10 culture dishes (diameter of 15 cm) in the amount of 15–17 × 10^6 per dish. The next day, a monolayer of cells with the confluence of 65–75% was infected with recombinant Ad at a dose of 107 PFU per dish. Two days later, when the CPE was at 90–100%, the infected cells were collected, concentrated by low-speed centrifugation, resuspended in buffer (0.01 M Tris-HCl pH 8.0, 0.01 M NaCl, 5 mM EDTA) and subjected to three freeze-thaw cycles to destroy cell and nuclear membranes and release the virus from the cells. Cell lysates were centrifuged at 5000 rpm for 10 minutes at room temperature, the precipitate removed afterwards.

Recombinant adenoviruses were purified in Optima XPN-90 (Beckman Coulter Inc.; USA) by double ultracentrifugation in a CsCl gradient (stepwise and equilibrium gradient).

We used PCR and whole genome sequencing to confirm purity and identity of the Ad. The titers of purified viruses were determined using 50% Tissue Culture Infectious Dose ($TCID_{50}$) assay on HEK293 cells [24]. The results were detected on days 10–12 after cell transduction.

Adenovirus particles quantification

We counted the particles using reagents from the Pico488 dsDNA quantification kit (Lumiprobe; USA). Each well of a 96-well microplate was filled with 10 µl of calibration solutions (in a known concentration) and solutions of the test sample. Next, 10 µl of lysis solution (TE buffer + 0.1% SDS) were added to the wells, mixed there and incubated for 5 minutes at room temperature. Then, we added 80 µl of TE solution and 100 µl of staining solution to the wells and left the mixtures to incubate for 5 minutes. After that, the fluorescence intensity was measured by a spectrofluorimeter at 480 nm excitation and 520 nm emission wavelengths. Next, a graph of the linear dependence of the fluorescence intensity of the calibration solutions (ordinate axis) on the concentration of viral particles (abscissa axis) was plotted.

Adenovirus genomes quantification

We isolated the total DNA using the Wizard Genomic DNA Purification Kit (Promega; USA). The number of genomes was determined by real-time PCR (RT PCR) using universal primers for all three studied serotypes (F: 5'GGCGGCTGGCGGTAGAG, R: 5'GCAACATCTGGAACCGCG). We used the qPCRmix-HS SYBR mixture (Evrogen; Russia) as prescribed by the manufacturer. Real-time PCR was performed in automatic mode on a CFX 96 Real-Time PCR Detection System (Bio-Rad; USA).

Phylogenetic analysis of the sequences of hexons, fibers and pentons of adenoviruses of various serotypes

Nucleotide sequences of the genomes of various serotypes of human adenoviruses (45 serotypes) and simian adenoviruses (5 serotypes) were taken from the NCBI database (USA). Using the Geneious Prime program, we aligned the amino acid sequences of the studied proteins of adenoviruses of various serotypes. The Neighbor-Joining method [25] enabled depiction of the evolutionary history [25]. The tree was drawn to scale, with branch lengths in the same units as the evolutionary distances used to build the phylogenetic tree. The evolutionary distances were calculated by the number of differences method and expressed as the number of amino acid differences per sequence. We used the MEGA 11 software to build the tree [26].

RESULTS

Comparison of hexons, pentons and fibers of different serotypes of human and simian adenoviruses

The structural proteins of adenovirus hexon, penton and fiber contain type-specific antigenic determinants that are targets for neutralizing antibodies. For this reason, when choosing an adenovirus serotype for the development of a new vector platform, it is important to take into account how these proteins of this serotype differ from similar proteins of already used recombinant adenoviruses, in particular from Ad5 and Ad26. With this in mind, after obtaining the results of whole genome sequencing of wt-SAd25 we performed a phylogenetic analysis of the amino acid sequences of hexons, pentons, and fibers of various serotypes and compared them to the SAd25 proteins (Fig. 1).

The analysis shows that hexon, penton and fiber of SAd25 are sufficiently distant evolutionarily from the same proteins of adenoviruses Ad5 (subgroup C) and Ad26 (subgroup D), on which the Sputnik V vaccine is based. Therefore, with a high degree of probability, virus-neutralizing antibodies generated by Ad5 or Ad26 vaccination targeting these proteins will not neutralize SAd25, and this vector may be a suitable candidate both for stand-alone use and for priming or boosting.

Construction of the vector based on simian adenovirus type 25

The recombinant SAd25-based replication-defective vector was constructed following the standard viral genome modification pattern used to create vectors based on Ad5 and Ad26 (Fig. 2A). Deletion of the E1 region turns the adenoviral genome into

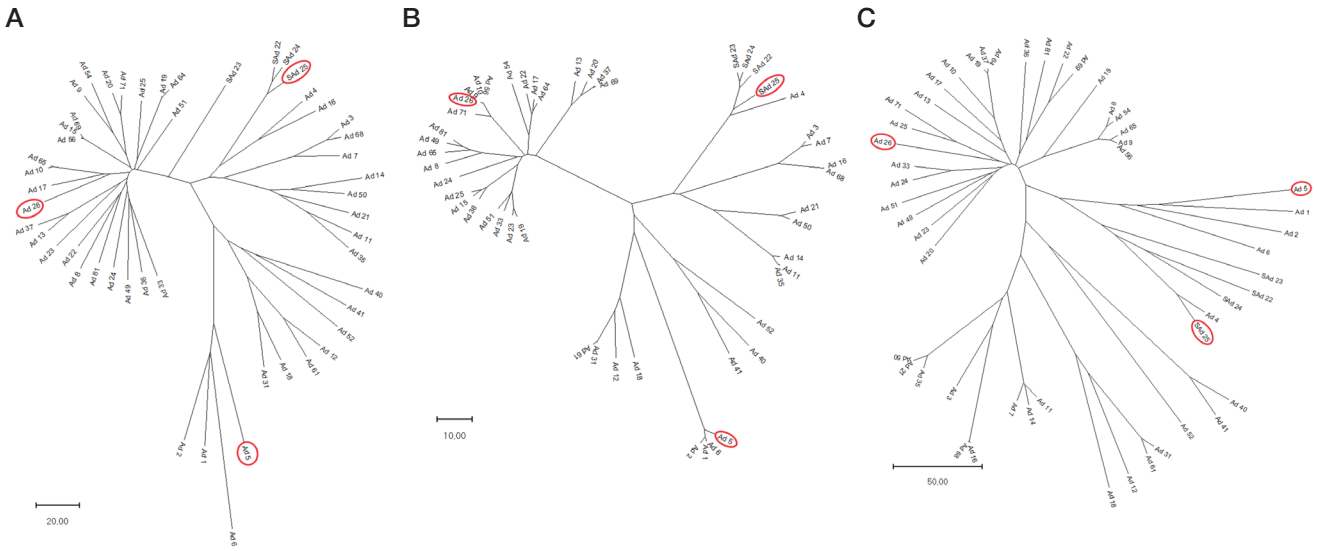

Fig. 1. Phylogenetic tree showing the relatedness of the hexon (**A**), penton (**B**) and fiber (**C**) sequences of adenoviruses of different serotypes. The tree was built based on the amino acid sequences of hexons of 45 human adenovirus serotypes and 5 simian adenovirus serotypes. The evolutionary distances were calculated based on the number of differences; they are given in as amino acid differences per sequence. Ad5, Ad26 and SAd25 are highlighted in *red*

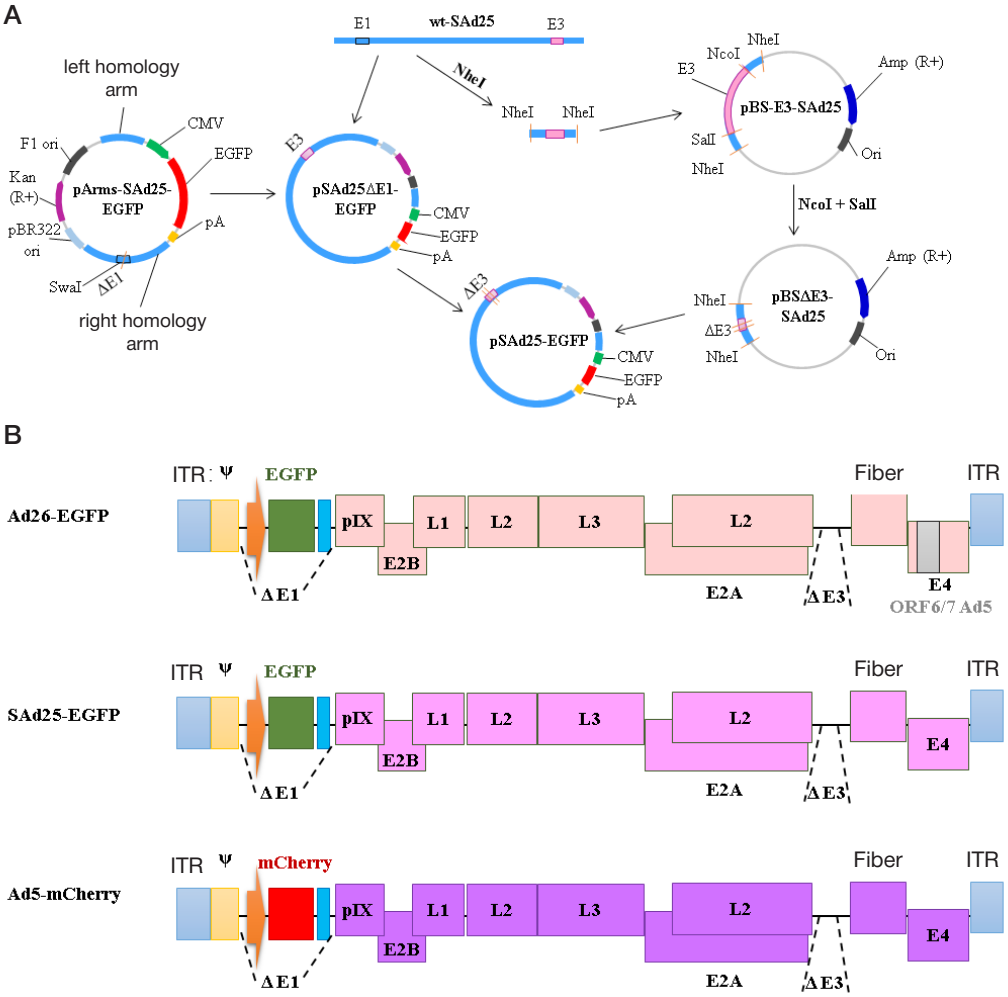

Fig. 2. Patterns of recombinant adenoviral vectors. **A**. Production of a plasmid construct containing the full-length genome of simian adenovirus type 25 and carrying the EGFP gene expression cassette. **B**. Genomes of recombinant replication-defective adenoviruses used in this study

a replication defective (it can only reproduce in a special cell line). The place of the deleted E1 region is occupied with a cassette carrying the reporter gene (CMV promoter, EGFP gene, polyadenylation signal). To increase the packing capacity, we have also deleted the E3 region. The plasmid carrying the recombinant SAd25-EGFP genome was engineered by homologous recombination in bacterial cells.

We used HEK293 cells transfected with pSAd25-EGFP plasmid with the vector part removed (containing the origin of replication (ori) and the kanamycin gene) to produce the infectious progeny of adenovirus. Despite the fact that HEK293 cells express proteins of the E1A region, which have some specificity, especially E1B55K, we have shown that this cell line efficiently assembles recombinant simian adenovirus type 25.

Investigation of biological and physical properties of the new adenoviral vector

One of the important characteristics of a viral vector is the genetic stability of the adenovirus genome. We did a number of passages of the SAd25-EGFP adenovirus on the HEK293 cell line to determine the respective capability of the vector. The genomic DNA was isolated from passages 1, 5 and 10, and then analyzed with the help of whole genome sequencing. The analysis revealed no mutations in the genome; the genome sequences from all three passages had 100% identity. These results allow a conclusion that SAd25-EGFP recombinant adenovirus remains genetically stable after at least 10-fold in vitro passaging.

To compare the replication efficiency of the produced SAd25-EGFP adenovirus and the two recombinant adenoviruses of types Ad5 and Ad26 that were produced earlier at the N.F. Gamaleya National Research Center for Epidemiology and Microbiology, we used vectors expressing the genes for fluorescent proteins EGFP and mCherry (red fluorescent protein). Adenoviruses SAd25-EGFP, Ad5-mCherry, and Ad26-EGFP (Figure 2B shows the genome structures) were transduced into wells of a 96-well plate with HEK293 cells (5×10^4 cells/well) at a dose of 1×10^4 viral particles (v.p.), and incubated for 72 h. Olympus IX73 (Olympus; Japan) inverted microscope with a U-RFL-T fluorescent module (Olympus; Japan) enabled detection of viral plaques in all wells infected with different adenoviruses. Viral plaques found in cells infected with SAd25-EGFP were noticeably larger than those in cells infected with other adenoviruses. In addition, the former had the lysis zone clearly enlarged, which indicates a higher viral cytotoxicity (Fig. 3). These findings are consistent with the results obtained for another simian adenovirus, SAd23 [17].

To perform a more detailed analysis of the replication efficiency of the resulting adenoviral vector, as well as to compare it with other adenoviral vectors, we cultured SAd25-EGFP, Ad5-mCherry and Ad26-EGFP on ten culture dishes (150 mm), purified the cultures by ultracentrifugation and collected them in equal final volumes (2 ml). The final sample could contain virus particles without a genome (unpacked or empty), with a full genome (packed or complete), or various intermediate forms in between. Next, we carried out a physical

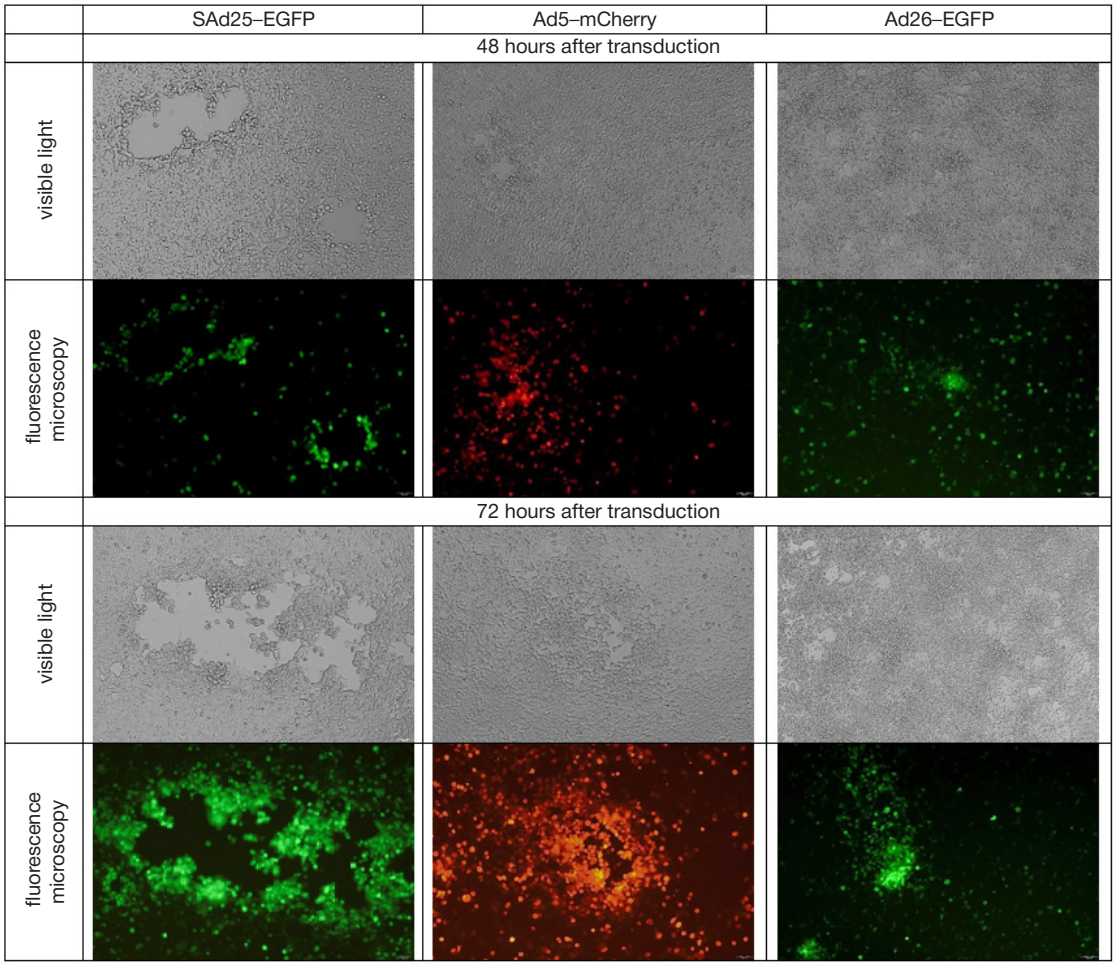

Fig. 3. Cytopathic effect of adenoviruses on HEK293 cells 48 and 72 hours after transduction

and biological characterization of the obtained adenoviruses (see Table).

The amount of SAd25-EGFP v.p. was similar to the amount of Ad5-mCherry and Ad26-EGFP v.p., but the infectious titer of SAd25-EGFP was lower than that of Ad5 and Ad26. The data also indicated that, compared to Ad5 and Ad26, the SAd25-EGFP sample contained more non-infectious viral particles. The yield of recombinant simian adenovirus was lower in comparison with Ad26-EGFP. Detection of more viral genomes for SAd25-EGFP indicates presence of unpackages viral DNA. Thus, these results show that recombinant SAd25 efficiently accumulates in HEK293 cells. A large number non-infectious viral particles and free viral DNA detected in the SAd25-EGFP samples necessitates the search for optimal conditions of cultivation and purification.

DISCUSSION

Simian adenoviruses have long been subjects of research. They are investigated to understand their capability to act as vectors for delivering target genes into cells. They have an undeniable advantage: human beings almost completely lack antibodies to common serotypes, including CAd63 and CAd68. The first vector based on a chimpanzee adenovirus was CAd68, which had the E1 region deleted [12]. In preclinical studies of the HIV-1 vaccine, this recombinant adenovirus was shown to be a highly immunogenic vaccine vector [20]. Later, a recombinant adenoviral vector based on simian adenovirus type Y25 was developed; subsequently, it was named ChAdOx1. The vaccine platform based thereon offers a good safety profile, as was shown in phase I–III clinical trials of the ChAdOx1-based vector vaccines designed for prevention of a number of diseases, including influenza, tuberculosis, malaria, prostate cancer, MERS, Zika fever and COVID-19 [27].

The SAd25 adenovirus has a good safety profile and high immunogenicity [27], but there are still no vaccine or gene therapy preparations based thereon that are used in the Russian Federation. Therefore, we have developed a vector platform based on SAd25 with the prospect of its further use as a vaccine platform.

At the first stage of our work, we performed a bioinformatic analysis of the wt-SAd25 genome sequence. We focused on the hexon, penton and fiber adenovirus sequences, i.e. the main proteins of the virion capsid. The epitopes in hexon are targets for neutralizing antibodies in vivo; they are recognized by cytotoxic T cells and serve as one of the parameters in allocation

Table. Biological and physical characteristics of recombinant adenoviruses

Serotype	$TCID_{50}$/ml	number of genomes/ml	number of v.p./ml	number of genomes/$TCID_{50}$	number of v.p./ $TCID_{50}$
SAd25-EGFP	$2{,}15 \times 10^{9}$	$1{,}79 \times 10^{12}$	$1{,}15 \times 10^{12}$	833	535
Ad5-mCherry	$4{,}64 \times 10^{9}$	$1{,}01 \times 10^{12}$	$1{,}02 \times 10^{12}$	218	220
Ad26-EGFP	$6{,}81 \times 10^{9}$	$1{,}88 \times 10^{12}$	$1{,}91 \times 10^{12}$	276	281

of adenoviruses into various serotypes [28]. Therefore, it was important to establish how far the SAd25 hexon is evolutionarily from other serotypes, including those used in the development of vaccines based on Ad5 and Ad26. As a result, we have shown that hexon, penton, and fiber of SAd25 are quite evolutionarily distant from these proteins of Ad5 and Ad26. Thus, our next task was to create a vector system based on SAd25.

There are several adenoviral vectors construction methods: cloning, obtaining a vector through cosmids, homologous recombination in bacteria and homologous recombination in eukaryotic cells [29]. Among them, the common method is the third one, which was also used in this work. This method includes obtaining a plasmid containing the adenovirus genome by homologous recombination of a shuttle vector carrying regions of homology with the adenovirus genome and genomic adenoviral DNA in *E. coli* BJ5183 cells.

Most of the engineered adenovirus-based vectors investigated as candidate vaccines are considered to be first-generation vectors, in which the E1 and E3 regions of the genome have been deleted. Deletion of the E1 region makes the adenovirus replication-defective, that is, unable to multiply in animal cells. This procedure significantly improves safety and reduces the likelihood of side effects. Deletion E3, another region of the genome, increases the possible insert size to 8 kb, which grants greater flexibility to design of the expression cassette. In addition, this deletion does not affect growth in the complementary cell line. In the recombinant adenoviral vector based on SAd25 that we produced, both of the mentioned regions were removed.

Clinical applicability requires design of a vector that will effectively replicate in a producer cell line. The cell line used in Russia to produce recombinant adenoviral vectors is HEK293, which is complementary and contains the E1 region of the Ad5 genome. But in the case of creating a vector with a deleted E1 region, there is a risk that this vector will form replicatively competent adenoviral particles during growth. However, SAd25 presents no such risk, since the percentage of homology of the nucleotide sequence of this region is not high enough and there will be no homologous recombination between the SAd25 genome and the E1 region in the HEK293 cell line.

To confirm the stability of the vaccine vector throughout the production process it is necessary to test the genetic stability of the recombinant viral vector. The test involves a number of passages of viral vectors that exceeds the level used in production; the usual amount is 5–10 passages [30]. The frequency of mutations in the genome of a replication-defective adenovirus is considered rare, however, this analysis is important for assessment of unchangeability of the expression cassette and, accordingly, expression of the target gene. We performed 10 passages of the obtained recombinant adenovirus SAd25-EGFP, after which the genomic DNA was analyzed by whole genome sequencing. The SAd25 vector was found to be genetically stable.

Further production and investigation of SAd25-based adenoviral vectors, including safety and immunogenicity studies thereof, and their comparison with the safety profile and immunogenicity of the already existing adenoviral vectors (including Ad5 and Ad26) will expand the panel of recombinant viruses that can be used as basis for development of drugs preventing a wide range of infectious diseases.

CONCLUSIONS

We produced a recombinant adenoviral vector based on simian adenovirus type 25 that has the expression cassette with the target gene (EGFP) inserted into the E1 region and E3 region of the genome removed to increase the potential genetic capacity. This recombinant adenovirus can efficiently replicate in the HEK293 cell line, which is used in production of the adenovirus-based drugs. It was noted that the phenotype of viral plaques in the obtained adenovirus differs from that peculiar to Ad5 and Ad26, since SAd25 plaques are larger and have a larger lysis zone. The data obtained indicate the possibility of further study of the adenoviral vector as a platform for prevention drugs.

References

1. Tatsis N, Ertl HCJ. Adenoviruses as vaccine vectors. Mol Ther. 2004; 10 (4): 616–29.
2. Appaiahgari MB, Vrati S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. Expert Opin Biol Ther. 2015; 15 (3): 337–51.
3. Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis. 2017; 4 (2): 43–63.
4. Trapnell BC, Gorziglia M. Gene therapy using adenoviral vectors. Curr Opin Biotechnol. 1994; 5 (6): 617–25.
5. Dolzhikova IV, Tokarskaya EA, Dzharullaeva AS, Tukhvatulin AI, Shcheblyakov DV, Voronina OL, et al. Virus-Vectored Ebola Vaccines. Acta Naturae. 2017; 9 (3): 4–11.
6. Lundstrom K. Viral Vectors for COVID-19 Vaccine Development. Viruses. 2021; 13 (2): 317.
7. Henaff D, Salinas S, Kremer EJ. An adenovirus traffic update: From receptor engagement to the nuclear pore. Future Microbiol. 2011; 6 (2): 179–92.
8. ICTV. The ICTV report virus taxonomy: the classification and nomenclature of viruses. 2022. Available from: https://ictv.global/taxonomy/.
9. Tapia MD, Sow SO, Ndiaye BP, Mbaye KD, Thiongane A, Ndour CT, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2020; 20 (6): 707–18.
10. O'Hara GA, Duncan CJA, Ewer KJ, Collins KA, Elias SC, Halstead FD, et al. Clinical Assessment of a Recombinant Simian Adenovirus ChAd63: A Potent New Vaccine Vector. J Infect Dis. 2012; 205 (5): 772–81.
11. Younis BM, Osman M, Khalil EAG, Santoro F, Furini S, Wiggins R, et al. Safety and immunogenicity of ChAd63-KH vaccine in post-kala-azar dermal leishmaniasis patients in Sudan. Mol Ther. 2021; 29 (7): 2366–77.
12. Farina SF, Gao GP, Xiang ZQ, Rux JJ, Burnett RM, Alvira MR, et al. Replication-defective vector based on a chimpanzee adenovirus. J Virol. 2001; 75 (23): 11603–13.
13. Zhou D, Cun A, Li Y, Xiang Z, Ertl HCJ. A Chimpanzee-Origin Adenovirus Vector Expressing the Rabies Virus Glycoprotein as an Oral Vaccine against Inhalation Infection with Rabies Virus. Mol Ther. 2006; 14 (5): 662–72.
14. Napolitano F, Merone R, Abbate A, Ammendola V, Horncastle E, Lanzaro F, et al. A next generation vaccine against human rabies based on a single dose of a chimpanzee adenovirus vector serotype C. PLoS Negl Trop Dis. 2020; 14 (7): e0008459.
15. de Jong R, Stockhofe-Zurwieden N, Bonsing J, Wang KF, Vandepaer S, Bouzya B, et al. ChAd155-RSV vaccine is immunogenic and efficacious against bovine RSV infection-induced disease in young calves. Nat Commun. 2022; 13 (1): 6142.
16. Roy S, Gao G, Lu Y, Zhou X, Lock M, Calcedo R, et al.

Characterization of a family of chimpanzee adenoviruses and development of molecular clones for gene transfer vectors. Hum Gene Ther. 2004; 15 (5): 519–30.
17. Luo S, Zhang P, Ma X, Wang Q, Lu J, Liu B, et al. A rapid strategy for constructing novel simian adenovirus vectors with high viral titer and expressing highly antigenic proteins applicable for vaccine development. Virus Res. 2019; 268: 1–10.
18. Belousova N, Mikheeva G, Xiong C, Soghomonian S, Young D, Le Roux L, et al. Development of a targeted gene vector platform based on simian adenovirus serotype 24. J Virol. 2010; 84 (19): 10087–101.
19. Bauza K, Malinauskas T, Pfander C, Anar B, Jones EY, Billker O, et al. Efficacy of a plasmodium vivax malaria vaccine using ChAd63 and modified vaccinia Ankara expressing thrombospondin-related anonymous protein as assessed with transgenic plasmodium berghei parasites. Infect Immun. 2014; 82 (3): 1277–86.
20. Fitzgerald JC, Gao GP, Reyes-Sandoval A, Pavlakis GN, Xiang ZQ, Wlazlo AP, et al. A simian replication-defective adenoviral recombinant vaccine to HIV-1 Gag. J Immunol. 2003; 170 (3): 1416–22.
21. Roy S, Medina-Jaszek A, Wilson MJ, Sandhu A, Calcedo R, Lin J, et al. Creation of a panel of vectors based on ape adenovirus isolates. J Gene Med. 2011; 13 (1): 17–25.
22. Davis AR, Wivel NA, Palladino JL, Tao L, Wilson JM. Construction of adenoviral vectors. Mol Biotechnol. 2001; 18 (1): 63–70.
23. Logunov DY, Zubkova OV, Karyagina-Zhulina AS, Shuvalova EA, Karpov AP, Shmarov MM, et al. Identification of HI-Like Loop in CELO adenovirus fiber for incorporation of receptor binding motifs. J Virol. 2007; 81 (18): 9641–52.
24. Kanegae Y, Makimura M, Saito I. A simple and efficient method for purification of infectious recombinant adenovirus. Jpn J Med Sci Biol. 1994; 47 (3): 157–66.
25. N Saitou, M Nei. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4 (4): 406–25. DOI: 10.1093/oxfordjournals.molbev.a040454.
26. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018; 35 (6): 1547–9.
27. Folegatti PM, Jenkin D, Morris S, Gilbert S, Kim D, Robertson JS, et al. Vaccines based on the replication-deficient simian adenoviral vector ChAdOx1: Standardized template with key considerations for a risk/benefit assessment. Vaccine. 2022; 40 (35): 5248–62.
28. Ebner K, Pinsker W, Lion T. Comparative sequence analysis of the hexon gene in the entire spectrum of human adenovirus serotypes: phylogenetic, taxonomic, and clinical implications. J Virol. 2005; 79 (20): 12635–42.
29. Zhang W, Ehrhardt A. Getting genetic access to natural adenovirus genomes to explore vector diversity. Virus Genes. 2017; 53 (5): 675–83.
30. Fernandes P, Silva AC, Coroadinha AS, Alves PM. Upstream bioprocess for adenovirus vectors. In: Adenoviral vectors for gene therapy. Elsevier, 2016; p. 139–61.

Литература

1. Tatsis N, Ertl HCJ. Adenoviruses as vaccine vectors. Mol Ther. 2004; 10 (4): 616–29.
2. Appaiahgari MB, Vrati S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. Expert Opin Biol Ther. 2015; 15 (3): 337–51.
3. Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis. 2017; 4 (2): 43–63.
4. Trapnell BC, Gorziglia M. Gene therapy using adenoviral vectors. Curr Opin Biotechnol. 1994; 5 (6): 617–25.
5. Dolzhikova IV, Tokarskaya EA, Dzharullaeva AS, Tukhvatulin AI, Shcheblyakov DV, Voronina OL, et al. Virus-Vectored Ebola Vaccines. Acta Naturae. 2017; 9 (3): 4–11.
6. Lundstrom K. Viral Vectors for COVID-19 Vaccine Development. Viruses. 2021; 13 (2): 317.
7. Henaff D, Salinas S, Kremer EJ. An adenovirus traffic update: From receptor engagement to the nuclear pore. Future Microbiol. 2011; 6 (2): 179–92.
8. ICTV. The ICTV report virus taxonomy: the classification and nomenclature of viruses. 2022. Available from: https://ictv.global/taxonomy/.
9. Tapia MD, Sow SO, Ndiaye BP, Mbaye KD, Thiongane A, Ndour CT, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2020; 20 (6): 707–18.
10. O'Hara GA, Duncan CJA, Ewer KJ, Collins KA, Elias SC, Halstead FD, et al. Clinical Assessment of a Recombinant Simian Adenovirus ChAd63: A Potent New Vaccine Vector. J Infect Dis. 2012; 205 (5): 772–81.
11. Younis BM, Osman M, Khalil EAG, Santoro F, Furini S, Wiggins R, et al. Safety and immunogenicity of ChAd63-KH vaccine in post-kala-azar dermal leishmaniasis patients in Sudan. Mol Ther. 2021; 29 (7): 2366–77.
12. Farina SF, Gao GP, Xiang ZQ, Rux JJ, Burnett RM, Alvira MR, et al. Replication-defective vector based on a chimpanzee adenovirus. J Virol. 2001; 75 (23): 11603–13.
13. Zhou D, Cun A, Li Y, Xiang Z, Ertl HCJ. A Chimpanzee-Origin Adenovirus Vector Expressing the Rabies Virus Glycoprotein as an Oral Vaccine against Inhalation Infection with Rabies Virus. Mol Ther. 2006; 14 (5): 662–72.
14. Napolitano F, Merone R, Abbate A, Ammendola V, Horncastle E, Lanzaro F, et al. A next generation vaccine against human rabies based on a single dose of a chimpanzee adenovirus vector serotype C. PLoS Negl Trop Dis. 2020; 14 (7): e0008459.
15. de Jong R, Stockhofe-Zurwieden N, Bonsing J, Wang KF, Vandepaer S, Bouzya B, et al. ChAd155-RSV vaccine is immunogenic and efficacious against bovine RSV infection-induced disease in young calves. Nat Commun. 2022; 13 (1): 6142.
16. Roy S, Gao G, Lu Y, Zhou X, Lock M, Calcedo R, et al. Characterization of a family of chimpanzee adenoviruses and development of molecular clones for gene transfer vectors. Hum Gene Ther. 2004; 15 (5): 519–30.
17. Luo S, Zhang P, Ma X, Wang Q, Lu J, Liu B, et al. A rapid strategy for constructing novel simian adenovirus vectors with high viral titer and expressing highly antigenic proteins applicable for vaccine development. Virus Res. 2019; 268: 1–10.
18. Belousova N, Mikheeva G, Xiong C, Soghomonian S, Young D, Le Roux L, et al. Development of a targeted gene vector platform based on simian adenovirus serotype 24. J Virol. 2010; 84 (19): 10087–101.
19. Bauza K, Malinauskas T, Pfander C, Anar B, Jones EY, Billker O, et al. Efficacy of a plasmodium vivax malaria vaccine using ChAd63 and modified vaccinia Ankara expressing thrombospondin-related anonymous protein as assessed with transgenic plasmodium berghei parasites. Infect Immun. 2014; 82 (3): 1277–86.
20. Fitzgerald JC, Gao GP, Reyes-Sandoval A, Pavlakis GN, Xiang ZQ, Wlazlo AP, et al. A simian replication-defective adenoviral recombinant vaccine to HIV-1 Gag. J Immunol. 2003; 170 (3): 1416–22.
21. Roy S, Medina-Jaszek A, Wilson MJ, Sandhu A, Calcedo R, Lin J, et al. Creation of a panel of vectors based on ape adenovirus isolates. J Gene Med. 2011; 13 (1): 17–25.
22. Davis AR, Wivel NA, Palladino JL, Tao L, Wilson JM. Construction of adenoviral vectors. Mol Biotechnol. 2001; 18 (1): 63–70.
23. Logunov DY, Zubkova OV, Karyagina-Zhulina AS, Shuvalova EA, Karpov AP, Shmarov MM, et al. Identification of HI-Like Loop in CELO adenovirus fiber for incorporation of receptor binding motifs. J Virol. 2007; 81 (18): 9641–52.
24. Kanegae Y, Makimura M, Saito I. A simple and efficient method for purification of infectious recombinant adenovirus. Jpn J Med Sci Biol. 1994; 47 (3): 157–66.

25. N Saitou, M Nei. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4 (4): 406–25. DOI: 10.1093/oxfordjournals.molbev.a040454.
26. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018; 35 (6): 1547–9.
27. Folegatti PM, Jenkin D, Morris S, Gilbert S, Kim D, Robertson JS, et al. Vaccines based on the replication-deficient simian adenoviral vector ChAdOx1: Standardized template with key considerations for a risk/benefit assessment. Vaccine. 2022; 40 (35): 5248–62.
28. Ebner K, Pinsker W, Lion T. Comparative sequence analysis of the hexon gene in the entire spectrum of human adenovirus serotypes: phylogenetic, taxonomic, and clinical implications. J Virol. 2005; 79 (20): 12635–42.
29. Zhang W, Ehrhardt A. Getting genetic access to natural adenovirus genomes to explore vector diversity. Virus Genes. 2017; 53 (5): 675–83.
30. Fernandes P, Silva AC, Coroadinha AS, Alves PM. Upstream bioprocess for adenovirus vectors. In: Adenoviral vectors for gene therapy. Elsevier, 2016; p. 139–61.