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# ВИРУСЫ

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# VIRUSES

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**Резюме.** Аденоассоциированный вирус принадлежит к роду *Dependoparvovirus* семейства *Parvoviridae*. Ценное свойство ААВ — его способность проникать как в делящиеся, так и неделящиеся клетки. Определенный серотип преимущественно проникает лишь в конкретную ткань или орган, то есть обладает своим уникальным тропизмом. Для того, чтоб использовать вирус в качестве переносчика нужных генов, требуется удалить часть его генетического материала и вставить нужный ген. Подобный механизм используется в генной терапии. Сегодня уже существует несколько препаратов на основе ААВ, которые получили одобрение от регулирующих органов для коммерческого использования у пациентов.

**Ключевые слова:** вирус, болезни, токсичность, экспрессия, трансген, ААВ.

**Resume.** Adeno-associated virus belongs to the Dependoparvovirus genus of the Parvoviridae family. A valuable property of AAV is its ability to penetrate both dividing and non-dividing cells. The certain serotype predominantly penetrates only a specific tissue or organ, that is, it has its own unique tropism. In order to use a virus as the desired genes carrier, it is necessary to remove some of its genetic material and insert the desired gene. The similar mechanism is used in the gene therapy. Nowadays, there are already several AAB-based drugs that have received regulatory approval for commercial use in patients.

**Keywords:** virus, diseases, toxicity, expression, transgene, AAV.

**Relevance.** We used to think of viruses as something deadly, especially now, after the coronavirus pandemic. However, almost any virus, including SARS-CoV-2, can be turned from the sworn enemy into the best health defender by removing dangerous genes from its DNA or RNA and replacing them with the necessary transgenes, whose protein products are so needed by malfunctioning cells. Recombinant viral vectors, therefore, represent the gene therapy basis and are the only effective way to introduce genes into the human cells, thanks to the penetration mechanisms and the virus spread in the cell honed by millions of evolution years. New treatment methods based on the functional genes introduction (or even full-fledged genome editing) can radically change existing therapeutic strategies and provide effective care for many now "hopeless" diseases.

**Aim:** to prove that viruses could help doctors to save lives.

**Objectives:**

1.To form the general population’s opinion about gene therapy mechanisms.

2.To highlight benefits of using viruses in medicine.

**Materials and methods.** Scientific literature, articles, Internet resources were analyzed.

**Results and their discussion.** A few drugs based on the AAV vectors have been

approved. The most famous are Zolgensma, Luxturna and Glybera. Many such drugs are undergoing clinical trials, including drugs for the treatment of cystic fibrosis, hemophilia, heart failure, lipoprotein lipase deficiency, and Parkinson's disease.

One of the first to use viruses for the brain anatomy study was James Papetz, the discoverer of the brain limbic system responsible for emotions. However, at that time, viral vectors had a fairly high toxicity greatly limited their use. But technologies are still developing and scientists are trying to get vectors actively used in optogenetics now, in the study of connections between neurons, gene expression and even in viral therapy. The ideal viral vector should have the following properties:

• production ease in large quantities and potentially infinite capacity for transgenes to increase the infection chances and deliver large-volume genetic products to the cell;

• non-invasiveness and convenience when injected into the animal's body (through the systemic circulation or directly into the brain);

• the ability to infect any cell type but to be active only in certain types;

• minimal toxicity to the body;

• regulation of transgene expression so that the genetic product amount, the beginning and end of the transgene expression in the cell can be controlled.

The adeno-associated virus is often used in gene therapy, as it has the qualities of the ideal viral vector. The adeno-associated virus belongs to the genus Dependoparvovirus of the family Parvoviridae. The genus name reflects its unusual life cycle, when the auxiliary virus presence is necessary for reproduction. The assistant virus, as a rule, is the adenovirus or herpesvirus family representatives.

AAV is so harmless that in addition to the inability to reproduce independently, it does not cause any diseases of humans and animals. Moreover, according to some studies, most people (>70%) have been infected with one or more AAV serotypes during their lifetime. (Serotype is the virus variant that differs from others by antigens on its protein envelope). The virus is quite small — its capsid is only about 25 nm in diameter. The capsid is extremely stable: resistant to short-term exposure to heat, acidic environment and proteases. The genome represented by single-stranded DNA is also very modest in size - only 4.7 thousand nucleotides.

This small genome contains a minimal number of genes. The rep gene (from the word replication) encodes proteins necessary for the virus reproduction and its further assembly inside the cell. The cap gene (from the word capsid) encodes capsid proteins. The AAV genome is framed by two T-shaped hairpins — inverted terminal repeats (ITR).

Valuable property of AAV is its ability to penetrate both dividing and non-dividing cells. At the first stage, the adeno-associated virus binds to the receptor on the cell surface. Different serotypes of AAV preferentially bind to their receptors characteristic of the certain cell types.

And this is an important property — the certain serotype mainly penetrates only into the specific tissue or organ so it has its own unique tropism.

The virus binding to the receptor triggers the penetration process into the cell. The endosome (a membrane vesicle containing a viral particle) moves in the cytoplasm along the cytoskeleton. Gradually, the environment in the endosome becomes acidified, which

leads to changes in the capsid necessary for further successful the cell infection. After exiting the endosome, the virus has two pathways. Either it enters the proteasome and is destroyed or transferred to the nucleus, where the viral genome is released from the capsid.

Proteins cannot be produced from the single-stranded viral genome yet. It is necessary to complete the second DNA chain. It is synthesized by the host cell DNA polymerase, using the ITR hairpin as a seed for the second chain construction.

Viral ITRs help not only to build the second DNA chain, but also contribute to intermolecular and intramolecular virus genomes recombination. As a result, ring DNA molecules are formed in the cell nucleus — episomes in this AAV genomes form is able to present in the nucleus for many years.

In addition, the AAV genome is embedded with the low frequency at the specific locus on human chromosome 19. This phenomenon is due to the similarity of the DNA sequences of this locus and the ITR virus. In AAV deprived of the rep gene, the ability to embed its genome is greatly reduced. Such viral genomes are present in cells in the episomes form.

If the mutation has occurred in the human gene leading to the disease development, the disease can be defeated or alleviated by the broken gene working copy. This is the virus occurrence.

The AAV capsid itself is the key to entering the cell and nucleus so we can safely use it. It remains only to replace the virus genes with the healthy genes.

Natural serotypes of AAV infect effectively the liver after intravenous administration. Due to this virus property, drugs for the treatment of hemophilia A and B familial hypercholesterolemia, ornithine transcarbamylase deficiency, mucopolysaccharidosis-IIIA and Kriegler-Nayyar syndrome are in clinical trials.

Serotypes AAV8 and AAV9 can infect effectively different muscles types throughout the body. This property makes them ideal vectors for the gene therapy of many muscle diseases. Thus, the clinic is actively investigating drugs for the treatment of Duchenne myodystrophy, dysferlinopathy, myotubular myopathy, Pompe disease. The infected muscle is able to serve as biofactory for the secreted therapeutic proteins production for the non-muscular diseases treatment. Although most heart diseases are polygenic and subject to environmental influences, gene therapy drugs for the heart failure treatment are being developed.

When it comes to the clinical trials, large amount of the high-quality viral drugs is required. Their production is non-trivial task.

Viruses do not reproduce themselves on their own, they need the cellular machinery help. Cell cultures are used to develop AAV. There are many options: the yeast culture use (Saccharomyces cerevisiae), insect cells (Spodoptera frugiperda butterfly Sf9 cell culture), human — HEK293, HeLa.

Firstly, we need to decide on the production method. There are a lot of variants: for cells growing attached to the substrate (large Petri dishes). For cultures that feel good in the suspension form and with constant stirring — flasks, sealed bags and much more. With an increase in the production scale, it is possible to switch to special bioreactors. With their help, we are able to monitor a whole panel of important parameters for the process. They

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make production more technologically advanced and allow to increase production volumes to several hundred liters of virus-containing suspension in one operation cycle.

To generate the viral particles plasmid DNA encoding the information necessary for the virus assembly is introduced into producing cells. The most popular protocol involves simultaneous cells infection with three plasmids:

• Plasmid with the therapeutic gene that will be packaged into the virus and delivered to the cells.

• Plasmid encoding rep- and cap-genes of the AAV itself. It is necessary for the capsid proteins synthesis and its subsequent assembly.

• Plasmid with the helper virus genes.

Instead of the third plasmid, the helper virus itself (adenovirus or herpesvirus) can be added, as in the earlier protocols case for the AAV particles production.

Production of AAV using HEK293 human cell culture as producer cells.

Three plasmids mixture is delivered into the cell (the plasmid with the therapeutic gene, the plasmid encoding rep/cap genes, and the plasmid with the helper virus genes). The necessary proteins production for the viral particles assembly begins. The DNA containing the therapeutic gene is packaged in the ready-made AAV capsid.

The cells begin to produce viral particles, after that they must be collected and cleaned.

At first, the raw material is collected. It can be the cellular environment if viruses are secreted by cells mainly into the liquid around. And it might be the cells themselves if most of the viral particles accumulate inside. As a rule, it depends on the AAV specific serotype. In order to destroy cell membranes and release the virus, cells are subjected to numerous freeze-thaw cycles, ultrasonic treatment or exposure to detergents.

Now the main task is to clean the viral particles from the numerous impurities in the suspension. The raw material contains intracellular free DNA and RNA, proteins and enzymes, large fragments of cell membranes and much more.

There are many cleaning methods. Improved versions of already used techniques and fundamentally new approaches are constantly appearing. All of them differ in levels of specificity, efficiency and cost. Conventionally, purification methods are divided into serotype-specific and universal. The first category includes affinity chromatography based on the capsids specific recognition by antibodies. The second is ultracentrifugation in density gradients of iodixanol or caesium chloride solutions, precipitation with polyethylene glycol or ammonium sulfate, ion exchange chromatography and many other methods based on the general physical properties of viral particles.

Today the main barrier to the widespread use of the gene therapy drugs is extremely high cost. Zolgensma is the most expensive medicine in the world ($2.1 million per dose). "Luxturna“ costs $850 thousand. This price is equally due to complex expensive production, long time-consuming development and a small number of patients.

The plasmids large number production of the high degree purification, the cell cultures maintenance, expensive reagents, the complex process of cleaning viral particles and the analytical techniques development for the drugs characterization lead to such prices. The cost is also affected by serious viral drug quality control. To let the drug enter the market,

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studies are being conducted on its toxicity, safety, bio-distribution and effectiveness. At the moment, the giants of AAV production around the world are working to reduce the drugs cost, developing more effective protocols for the AAV assembly and purification.

The immune response is also a serious problem. Potentially, immune reactions are possible to influence the viral capsid, its genome and the transgene protein product. Drugs based on AAV can be prevented by neutralizing antibodies to its capsid. By binding to viruses in the bloodstream, antibodies prevent the penetration of viral particles into cells, which leads to low efficacy of the drug. At the moment, screening of patients for the presence of antibodies to the AAV serotype used is mandatory. If they are detected, such a patient is excluded from clinical trials. New capsids are being developed that will not be recognized by neutralizing antibodies. After the delivery of therapeutic doses of AAV, a humoral immune response develops rapidly, which subsequently prevents the repeated administration of the drug. That is why most gene therapy drugs based on AAV are designed for a single administration. To suppress the T-cell immune response in patients, pharmacological suppression with steroids is used.

**Conclusions:**

1. The medicine future may be behind the dangerous viruses transformation into useful and safe drugs that will salvage the world from incurable diseases.

2. In future SARS-CoV-2 is likely to be used as a recombinant vector for the treatment of lung diseases, for example. Biology and medicine will achieve significant results in taming viruses soon, and dangerous infectious agents will guard the mankind health.

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