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CREATION OF NEW VARIANTS OF REP GENES

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Введение. Gene therapy is a promising approach for the treatment of hereditary and multifactorial diseases. Its main idea is to correct genetic defects by introducing therapeutic genes into somatic cells. Currently, viral vectors are often used for therapeutic gene delivery, and recombinant adeno-associated viral vectors (rAAV) are among the most promising of them. They are characterized by high delivery efficiency, stable gene expression, broad tropism, and nonpathogenicity for humans [1].

The production of rAAV is often accomplished by co-transfection of the GOI plasmid (carries the target gene), Rep/Cap plasmid (encodes replication and capsid proteins) and HELPER plasmid (contains adenovirus genes) into a suitable cell culture (e.g. HEK293). One of the main problems of rAAV production is the formation of by-products - “empty” capsids that lack the therapeutic gene. Empty capsids reduce the efficacy of therapy and may induce an undesirable immune response. Another problem is the low titer of vector particles, which negatively affects the cost of production and limits the availability of drugs for patients [1,2].

Rep proteins, which regulate genome replication and packaging, play a critical role in rAAV production. There have been studies showing that modified Rep variants increase titer and packaging efficiency, therefore their development is an important research goal. Rep gene variant libraries can be made by methods of directed evolution or rational design. The first approach provides wide diversity of variants, but requires complex screening due to the large volume and risk of lethal mutations. The second method provides compact libraries with targeted mutations, simplifying the search for effective variants [2,3].

Основная часть. New variants of Rep genes were created using rational design, specifically site-directed mutagenesis. PCR with mutagenic primers was used and DNA fragments were assembled using the Gibson method. The obtained Rep/Cap5 and Rep/Cap6 plasmids were transformed into E. coli cells and grown on selective medium. Then, clones were screened, and plasmid DNA sequences of selected clones were verified by Sanger sequencing. By this way we obtained eleven variants of Rep/Cap5 and Rep/Cap6 genetic constructs respectively.

The rAAV production was performed in HEK293 cells cultured in a shaker incubator under standard conditions (37°C, 5% CO_2). Transfection was done by adding a mixture of GFP, Helper and Rep/Cap plasmids with polyethylenimine (PEI) to the cell suspension. Nutrient supplements were added 24 hours after transfection, and 48 hours later, sampling for analytical studies was performed. For this purpose, the cell suspension was centrifuged, the supernatant was removed, and precipitates were obtained.

Precipitates were analyzed by quantitative PCR (qPCR) and enzyme-linked immunosorbent assay (ELISA). The number of vector particles per milliliter of cell suspension was determined by qPCR, and the total number of capsids was determined by ELISA. Based on these data, the percentage of full capsids containing the vector genome was calculated.

In the first screening repeat for Rep/Cap5 constructs, five out of eleven variants showed a significant increase in titer and proportion of complete capsids compared to the control (unmodified Rep/Cap5). Of the first five Rep/Cap6 variants two variants showed a similar improvement in parameters.

Выводы. In this work, we have chosen a method of library construction, namely rational design through site-directed mutagenesis, and obtained new variants of Rep genes. Transfection with the obtained Rep gene variants was performed and adeno-associated viral vectors were produced. The influence of the obtained Rep-gene variants on the titer and packaging of adeno-

associated viral vectors for the first repeat of transfection was assessed and the candidates that showed the best results were identified. Further work plan includes conducting 3 repeats of transfections to confirm the obtained results and analyzing the precipitates by western blot to assess the expression of Rep and Cap proteins.

Список использованных источников:

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