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INTEGRATION OF TRANSGENES FOR THE ESTABLISHMENT OF AN AAV-PRODUCING CELL LINE

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Introduction. Gene therapy is a set of biotechnological and medical approaches aimed at achieving a therapeutic effect by modifying the genome of human somatic cells to correct mutations in DNA structure. The delivery of a therapeutic gene can be accomplished through various methods, which can be broadly classified into two major groups: non-viral and viral delivery systems. Each method has its advantages and limitations: non-viral systems are characterized by transient expression, lack of immunogenicity, lack of tissue specificity, scalability, etc., while viral systems exhibit opposite characteristics. Currently, the most widely used and promising delivery method is recombinant adeno-associated viral vectors (rAAV) [1].

The production of rAAV carrying a therapeutic gene typically relies on transient transfection, involving three genetic constructs that encode helper virus genes, REP/CAP genes, and the gene of interest (GOI). This method is widely used due to its flexibility in optimization; however, it is expensive and challenging to scale up. Consequently, there is a growing demand for the development of stable cell lines that integrate all necessary genes for viral particle production.

To ensure efficient rAAV production, it is essential to achieve high-copy integration of the GOI into the genome of the cell line, which was the objective of this study. Methods such as nucleofection and CRISPR/Cas-based approaches were not considered in this work, as the focus was on achieving high-copy integration through lentiviral vectors (rLV) and the Sleeping Beauty transposon system [2,3].

Main Part. First, the necessary genetic constructs were obtained using restriction-ligation techniques: a transfer plasmid containing GOI for rLV assembly and a donor plasmid with GOI for the Sleeping Beauty system. These plasmids were then used for transfection of the HEK293 (Human Embryonic Kidney 293) cell line in the case of the transposon system. For rLV, viral particles were first produced, followed by the transduction of HEK293 cells. Since the gene of interest was green fluorescent protein (GFP), integration was assessed using flow cytometry. The selected cell population was further enriched via cell sorting.

The resulting cell pools were evaluated for rAAV production using qPCR. The results showed that cell pools obtained via rLV exhibited low rAAV titers. This was presumably due to the structure of the rAAV transfer cassette, which includes repetitive, GC-rich sequences within the ITR regions. These structures likely hinder rLV particle packaging. As a result, work with rLV was discontinued, and efforts were focused on the cell pool obtained using the Sleeping Beauty transposon system.

The cell pool generated through double transfection (donor plasmid with GOI and a plasmid encoding transposase) was monoclonally sorted based on fluorescence. The resulting monoclonal cell lines were tested for rAAV production using qPCR. The data revealed that some monoclonal lines exhibited virus titers six times higher than the general cell pool. To assess stability, 15 leading monoclonal lines were selected. After 30 passages, only two monoclonal lines demonstrated stable rAAV production. Additional analyses of expression levels and copy number are planned for these monoclonal lines.

Conclusions. This study successfully generated a set of genetic constructs for GFP integration, and among the two tested methods, the Sleeping Beauty transposon system demonstrated the highest efficiency. Using this approach, two monoclonal cell lines capable of sustained rAAV production over 30 passages were obtained. To further enhance rAAV production efficiency, additional rounds of GOI integration into the leading monoclonal lines via the Sleeping Beauty system are being considered.

References:

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