EXPRESSION OF ANTI-APOPTOTIC GENES TO ENHANCE rAAV PRODUCTION

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Recombinant Adeno-associated viruses (rAAV) are becoming popular as viral vector delivery systems for gene therapy. Currently, two rAAV-based products are available on the market and several clinical trials are on-going with promising results for diseases such as hemophilia or muscular dystrophy[1,2]. The most common method for manufacturing rAAV products is by triple transfection with a plasmid containing the gene of interested flanked by ITR sequences, a plasmid with adenovirus helper genes and a plasmid with AAV helper genes. The scalability of transfection processes has been disputed for a long time. However, in rAAV production it might be the only feasible option until a proficient stable cell line is developed solving all current challenges including, but not limited to: low cell density at transfection and interruption of cell replication. Another bottleneck in all cell culture-based process is the duration of the culture. Typically, the viable cell density increases until a stationary phase is reached in which, after a specific amount of time depending on the cell line, apoptosis begins and viability rapidly falls.

In rAAV production processes, apoptosis is triggered after transfection by AAV helper genes (mostly Rep78 and Rep 52) which are toxic for mammalian cells, reducing the duration of the cell culture decreasing the productivity of the process[3,4] . To overcome this challenge, several anti-apoptotic genes from different origins, such as human (bcl-2, bcl-xL and mcl-1), hamster (bcl-2 and bcl-xL) and viral genes (bhrf-1, p25 and vBcl-2), have been expressed in this study as well as a recombinant protein-based product to mimic the production of rAAV. Even though some genes were already reported in the literature, a detailed comparison could not be performed as random integration was used in the development of most of the cell lines. In this novel study, targeted integration was used to develop isogenic cell lines with one copy of both the anti-apoptotic gene and the protein product. As expected, the expression of anti-apoptotic genes increased the duration of the cell culture proving its potential as a tool to enhance the performance of rAAV production processes. Consistent with the literature, some genes were also able to improve the specific productivity of the protein product by a significant amount. In fact, one gene that was not studied previously outperformed the other candidates. Regarding the metabolism, consumption rates of glucose and production rates of lactate were analyzed to have a better understanding of the effect of these genes on the metabolism of the mammalian cell. Considering all the available results, we propose the use of anti-apoptotic genes to enhance the production of rAAV with mammalian cells.

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