ENHANCING rAAV PRODUCTION BY HEK293 CELLS VIA METABOLIC PROFILING

Michela Pulix, University of Manchester and Cobra Biologics, UK
michela.pulix@cobrabio.com,
Vera Lukashchuk, Cobra Biologics, Keele, UK
George Prout, Cobra Biologics, Keele, UK
Suzanne Podmore, Cobra Biologics, Keele, UK
Kevin Bowes, Cobra Biologics, Keele, UK
Daniel Smith, Cobra Biologics, Keele, UK
Alan James Dickson, University of Manchester, Manchester, UK

Key Words: rAAV, HEK293, Viral vectors.

Viral vector manufacturing is expensive and time-consuming. Demand for rAAV-based vectors has risen massively in the past decade and continues to rise thanks to urgent healthcare supply demand. The industry is, however, currently missing a cost-effective and robust manufacturing strategy. One of the major downsides of rAAV production is the high percentage of “empty” vector particles being produced and harvested. In addition to complicating downstream purification processes, this characteristic limits the efficiency of rAAV manufacture and presents uncertainties for scale-up.

Efficiency of the manufacturing process is largely dependent on the productivity of the production cell line. Much emphasis has been put into understanding the effects of recombinant protein production on mammalian cell lines (e.g., CHO, HeLa, HEK293) but relatively little is known about the effects of viral vector production on cell biology and behaviour. Over the years, many clones have been derived, isolated and engineered from HEK293 to induce improvements in productivity and efficiency. However, the high cost of production and licensing, the expression of potentially undesired elements (e.g., T-antigen) and regulatory approval processes for next generation cell lines, hinders their use in clinical manufacturing. Increased understanding of HEK293 in relation to existing processes and process control offers realistic opportunity to enhance the efficiency of rAAV manufacturing.

Our aim is to identify and understand the critical parameters that contribute to setting the productivity in HEK293 cells (in terms of final yield and abundance of full capsids), ranging from the metabolic requirements prior to and during viral vector production, to cell culture parameter optimisation to maintain the cells in an optimal state of health.

We tested several commercially available media for rAAV9 production and selected the candidate that provided the best yield and quality of viral vector. With this medium as our baseline, we investigated the metabolism during a period of culture via extracellular metabolic profiling of control and rAAV producing cells. The analysis revealed the rapid use of several amino acids over the first 24 hr post-inoculation and the subsequent generation of metabolites indicative of metabolic profiles associated with cell growth. rAAV9 producing cells show lower rates of amino acid and glucose consumption than control cells but the profile of metabolism was not significantly changed as a result of transfection/production of rAAV9. These data were used to design medium supplements and the effect of supplement addition on cell proliferation, viability and rAAV production/quality was assessed. Specific combinations of amino acids generated an increased cell density (up to $9.3x10^6$ cells/mL at 5 days post-inoculation compared to $4.4x10^6$ cells/mL for cells in non-supplemented medium). This was associated with retention of improved viability in the presence of the supplement. In addition, the metabolic profiling we undertook indicated the build-up of potentially toxic/growth inhibitory metabolites during the period of stock cell preparations prior to setting up transfections. In various dilution experiments we were able to optimise the pre-treatment, cell density and dilution protocol to generate predictable and reproducible efficiencies of transfection, cell growth and rAAV production.

Overall, our data contributes metabolic insights to process conditions that generate HEK293 cells of appropriate health and defined parameters to robust and enhanced production of rAAV, providing work schemes that are also appropriate to the manufacture of further types of viral vectors.