SYNTHETIC CELL LINES FOR RECOMBINANT AAV PRODUCTION

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Recombinant adeno-associated virus (rAAV) is one of the most commonly used vehicles for gene therapy due to its effectiveness in in-vivo gene delivery, long-term persistency, and safety. A number of rAAV based products have been approved by regulatory agencies for clinical use and generated much excitement for many AAV therapies to come. However, a very large dose of rAAV is required in clinical applications, and the manufacturing of large quantities of rAAV poses a significant challenge. Innovations in viral vector manufacturing are called for to increase vector titers in production and to enhance the quality of vectors, especially minimizing the amount of empty viral capsids devoid of their therapeutic payload.
Being a very small virus and having only two genes, *rep* and *cap*, in the genome, AAV also relies on co-infection of a helper virus, typically adenovirus, to replicate in the host cell. Current methods of rAAV manufacturing involve transfecting host cells with the rAAV genome, necessary viral genes for packaging virus along with necessary helper viral genes in plasmid(s), or by infecting host cells with recombinant herpes simplex virus or insect viruses which carries those rAAV component genes. Multi-plasmid transfection process poses challenges in scaling up, while viral infection-based process requires additional virus production. Furthermore, the residual vectors must be removed from the rAAV product.

Our aim is to bring into being a scalable process by designing a cell line which can be induced to produce rAAV vectors without resorting to helper virus infection or plasmid transfection, thereby eliminating cumbersome vector production, and removing the risk of helper virus contamination in the product. Additionally, through dynamic tuning of the induction profile, we aim to control the expression of different viral components and modulate the full-to-empty particle ratio of the vector to enhance the quality of the product.
To accomplish those aims, we replaced native transcriptional regulation of constituent viral genes with inducible promoters and integrated engineered viral genes into HEK293 cell genome using a synthetic biology approach. The essential components of making rAAV vectors were grouped into three modules: (1) a genome module containing a rAAV genome including the gene of interest; (2) a replication module providing the large Rep protein of AAV and helper proteins of adenovirus; (3) a packaging module for expressing capsid proteins and the small Rep protein. HEK293 cell lines with these three modules integrated into the genome were obtained and screened for rAAV productivity. To facilitate synthetic cell line screening, we also established an assay cell line which harbors *rep* and helper genes but has no viral genome nor *cap* gene. The resulting synthetic cell lines are capable of producing infectious viral particles upon induction. By controlling the induction profile of *rep*, helper and *cap* genes, the productivity of rAAV varies and the full-to-empty particle ratio of the product also shifts. The results give proof of our concept of scalable production of rAAV and modulating its quality through a synthetic cell line.

To better understand critical variables affecting rAAV production in our synthetic system, RNAseq and targeted proteomics were adopted to quantify both cellular and viral transcripts and proteins over the production cycle. Several hypotheses on the limiting factor of rAAV production were generated including competition of cellular resources and host cell antiviral response. Further enhancement of virus productivity was achieved by determination of the optimal induction conditions via design of experiments, and modulation of inhibitory host responses.

In conclusion, taking a systems design and synthetic biology approach, we have demonstrated a next-generation rAAV production system. This design framework incorporated only a minimal set of essential viral components, hence we employed multi-omics analysis and systems approach to guide our course to further enhancement in the design-build-test-learn cycle. The present work exemplifies the crucial role of synthetic biology to engineer next-generation gene therapy manufacturing platforms.