Development of a regulated rAAV packaging cell line enabling scalable and cost-efficient vector manufacturing

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Introduction

Triple-plasmid transfection remains the dominant platform for recombinant adeno-associated virus (rAAV) production, but its reliance on GMP-grade plasmids, variable transfection efficiencies, reagent toxicity, and high costs present major limitations for scalable and reproducible manufacturing. To overcome these constraints, we developed regulated rAAV packaging and producer cell lines derived from NBX-Eng-HEK293, a clonal, GMP-banked HEK293 suspension cell line previously shown to outperform commercially available systems for rAAV production.

Methods

NBX-Eng-HEK293 cells were first engineered with a gene regulatory network system (RNS) responsive to an exogenously supplied activator, enabling tight and reversible control of potentially cytotoxic viral functions, including AAV rep and adenoviral helper genes. The regulatory system was extensively characterized, demonstrating stable inducible transgene expression over multiple cell generations. Subsequently, AAV rep and adenoviral helper genes were stably integrated under RNS control, generating a regulated rAAV packaging cell line. Finally, capsid genes and the gene of interest (GOI) were stably integrated, resulting in a fully regulated rAAV producer cell line.

Results

Inducible control of all rAAV production elements was validated by quantitative PCR and Western blot analysis, demonstrating robust repression in the absence of inducer and rapid, coordinated activation upon induction. Vector production was demonstrated across multiple capsid serotypes and GOI, confirming platform flexibility.

Comprehensive genomic and phenotypic characterization was performed to assess long-term stability and manufacturing suitability. Transgene insertion sites, DNA methylation signatures and cellular gene-expression profiles were characterized, demonstrating high genomic integrity and phenotypic consistency across extended passaging.

Conclusion

In conclusion, we report the development of a stable, inducible rAAV producer cell line that eliminates the need for transient transfection while enabling controlled, scalable, and cost-efficient rAAV manufacturing. This regulated producer platform provides a robust foundation for the next generation of high-quality viral vector therapeutics.