EXPLORING ALTERNATIVE ONCOGENE-FREE MAMMALIAN HOSTS FOR ADENO-ASSOCIATED VIRUS PRODUCTION

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Gene therapy pose to tackle challenging diseases using viral and non-viral vectors to deliver genes of interest by focusing on the genetic modification of cells to produce a therapeutic effect. The need for this study was emphasized by the fact that transformed cell lines that contain oncogenes may pose serious risks to the patient if the oncogenes are incorporated in the final product. Human embryonic kidney 293 (HEK293) suspension cells are the most widely used host for rAAV, lentivirus, and other vector production. However, this cell line inherently expresses Adenovirus E1A/E1B oncogenes, which are necessary for both transformation and AAV production.

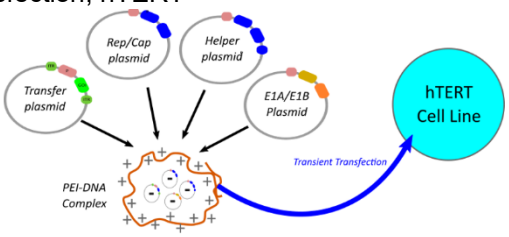

Figure 1 – Quadruple transient transfection to verify candidate cell lines capable of AAV production

Therefore, there is a critical need for development of alternative oncogene-free host cell lines that will be able to produce rAAV with reduced or eliminated oncogene incorporation. The purpose of this study is to discover and develop alternative oncogene-free host cell lines capable of stably producing rAAV vectors in a commercially scalable manner, and to characterize capsid contents in HEK293 and these alternative cell lines. Here, alternative methods to alter primary cell lines for continuous growth and support AAV production would be useful by using human telomerase reverse transcriptase (hTERT) instead of viral oncogenes for immortalization. To support stable AAV production, 6 candidates epithelial and fibroblast hTERT-derived cell lines were screened for their ability to produce AAV without oncogenes by transient transfection with polyethyleimine (PEIPro, Polyplus) or X-tremeGENE™ 9 (Roche) (Figure 1). Preliminary screenings have identified optimal DNA loading capacity and transfection efficiencies (TE) with single vector compared to control cell lines, HEK293 and HEK293T. Several batches of quadruple transfection were conducted to improve the efficiencies (%) and genome titer considering total DNA amount, 4 different plasmid ratios (helper: repcap: GFP: E1A/E1B) with different transfection reagent. hTERT-HME1 showed a decrease in transfection efficiency under reduced DNA total amount, but enhanced titer was observed. The batch with 1:1:1:1 plasmid ratio was 50% lower in TE (%) compared to 2.5:1:1:1, however enhanced genome titer was observed. UMB1949, a different hTERT-derived cell line got 80% higher in TE(%) under 1:1:1:1 plasmid ratio condition compared to 2.5:1:1:1 but interestingly, the genome titer has decreased. Overall, higher amount of total DNA seems to give higher TE(%) but the genome titer enhances with the lowest DNA amount. To characterize the purity of capsid contents (and therefore understand the potential safety profile of our vectors), a qPCR assay was developed looking at different targets. These targets include GFP(our titer), E1A, E1B (oncogenes), Rep, Cap (viral genes) and AmpR (antibiotic marker gene). The results reveal that While titre is the main component of capsids, AmpR, Rep and Cap are all detectible and withing 1-2 orders of magnitude of the main GFP titer. It is also reported that E1A/E1B oncogenes are detected to be very low in abundance when measured by qPCR method. However, this is likely due to truncation or partial encapsidation of the genes, revealing a potential weakness of the targeted, sequence-dependent qPCR approach. Ongoing work will focus on conducting ELISA for full and empty AAV capsids followed by western blot to detect protein of interest (VP1, VP2, VP3) if necessary. Future work involves design and construction of custom helper plasmids incorporating various combinations of conserved E1A/E1B sequences, and ideally no residual oncogene function. The customized plasmids will then be stably transfected into hTERT cell lines and screened in batches according to their cell type. Oncogene-free derived AAVs will be assessed according to their viral titer and confirmation of reduced or eliminated incorporation of oncogene sequences in the viral DNA sequence. In addition, a Nanopore-Sequencing approach will be developed to detect all capsid contents in a non-biased manner as in qPCR where the targets must be specified before measurement is done. This will likely reveal the exact portions of oncogenes (and other contaminants) that actually get encapsidated into our vectors in HEK293 and other alternative host cell lines.