GENERATION OF HELPER VIRUS-FREE ADENO-ASSOCIATED VIRAL VECTOR PACKAGING/PRODUCER CELL LINES BASED ON A HUMAN SUSPENSION CELL LINE

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The emerging number of clinical trials in the gene therapy field poses the challenge to the industry to produce viral vectors in a scalable, reproducible and cost-efficient manner. To address this issue, our CAP-GT platform comprises high density, serum free suspension cell lines that enable reproducible, scalable transfection and high titer production of viral vectors. An adeno-associated virus (AAV) based vector was the first approved gene therapy product in clinical applications. Attractive features of AAV as a gene therapy vector are e.g. its lack of pathogenicity and its ability to transduce dividing and non-dividing cells. Moving away from mainly targeting ultra-rare diseases and taking more common indications into focus will need to see significant improvements concerning productivity and consistent quality of AAV vector production in order to ensure supply. For this purpose, we are developing a helper virus-free packaging cell line that can easily be turned into a producer cell line by only one additional step of cell line development. Base of this packing cell line is the generation of a cell line with stable Tet-inducible expression of Rep proteins. Extensive screening of Rep expressing single cell clones resulted in clonal cell lines which produced high AAV titers upon induction and transfection of the missing components. In a next step, the adenoviral helper functions E2A and E4orf6 are introduced, due to their toxicity also under the control of a Tet-inducible promoter. In addition, the VA RNA is encoded by the same construct. Finally, a Tet-inducible capsid function and GFP as transgene flanked by the ITRs combined on one construct will be stably integrated resulting in a proof of principle producer cell line. This approach should enable a consistent quality production of AAV vectors that abolishes the drawbacks of transient transfection concerning reproducibility, consistency and high costs for GMP-grade DNA. Process optimization in regard to process duration, feeding strategy etc. is currently ongoing for further improving the vector yields.