EXPRESSION OF ANTI-APOPTOTIC GENES TO ENHANCE rAAV PRODUCTION

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Key Words: CHO cells, CRISPR/Cas9, RMCE, anti-apoptosis, fed-batch, Ambr15.

Programmed cell death (apoptosis) is one of the main challenges in the development of a bioprocess for the production of recombinant adeno-associated virus (rAAVs). Apoptosis is triggered in mammalian cells under stressful conditions, such as nutrient depletion, high osmolality or accumulation of toxic by-products. When producing rAAVs, the expression of the corresponding viral genes triggers this molecular mechanism, reducing the time in which the cells remain productive. Overcoming apoptosis could increase productivity, improving scalability of the final process.

In this work, the apoptotic process was studied in Chinese hamster ovary (CHO) cells. Expressing human anti-apoptotic genes to delay the apoptotic response is a common practice often referenced in the literature with successful effects in increasing the final titre of, for instance, monoclonal antibodies in CHO cells (Zhang et al., 2018). The most prominent anti-apoptotic genes are part of the Bcl-2 family (Henry et al., 2020).

The study of anti-apoptotic genes is confounded by clonal effects when using standard random integration. Here, we used CRISPR/Cas9 and recombinase-mediated cassette exchange (RMCE) to develop isogenic cell lines expressing relevant anti-apoptotic genes from human, CHO and viral origin. We showed that all anti-apoptotic genes delayed apoptosis to different extents depending on their origin. Moreover, Bcl-2, Bcl-xL and Mcl-1 also improved specific and volumetric productivity of a model protein during a fed-batch run in Ambr 15.

Overall, we have demonstrated that expressing anti-apoptotic genes is a viable option to improve the longevity and productivity of the cell culture and identified the most promising candidate genes to implement in a rAAV production platform.

References

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