Development of mammalian host cell lines for rapid production of recombinant proteins

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Specific problem being addressed: The current pandemic highlighted the need to rapidly develop stable cell lines for the production of monoclonal antibodies and other therapeutics for clinical testing and beyond. Due to the complex glycosylation that is critical for their function, biotherapeutics and biosimilars are produced in mammalian hosts, primarily CHO cells. In recent years there are studies that use HEK293 cell lines are being explored and developed for production of many proteins where human-like glycosylation is essential. Other studies have shown that both the glutamine synthetase and the DHFR-systems can create stable cell lines in HEK293 cell lines. However, for an industrially viable process, high productivity of the clone is desirable. Also, to reduce the overall clone development timeline, it is imperative to create better host cells that exhibit improved properties such as increased secretion rate, instead of engineering post creation of a stable clone.

Project Summary: In recent years there are studies that use HEK293 cell line is being explored and developed for production of many proteins where human-like glycosylation is essential. Other studies have shown that both the glutamine synthetase and the DHFR-systems can create stable cell lines in HEK293 cell lines. However, for an industrially viable process, high productivity of the clone is desirable. Thus, many clones are required to be screened before a high-producer clone can be identified. While gene copy number can be increased through the amplification process in the presence of methotrexate in the DG44 host, this needs to be supported with both increased translation and enhanced ER processing for secretion of the recombinant proteins. Many cell engineering targets have thus been identified and patented in CHO cells, with a similar development required for the HEK293 cells. However, protein secretion is governed by complex pathways, modifying which requires multiple genes to be simultaneously targeted.

We have developed an alternate approach based on the concept of evolutionary adaptation, to increase the secretory capacity of the cells and achieve cells with higher titers. While adaptive laboratory evolution-based approaches have been successfully applied to engineer microbial cells for industrial application, their use in mammalian systems is limited. The proof of concept of our approach was demonstrated using rCHO cells that led to increase in both titers and productivity of the monoclonal antibody produced by these cells. We show that an ER stress inhibitor, tunicamycin, can be used to evolve cells such that they exhibit an increase in the ER capacity resulting in increased secretion of recombinant proteins. The approach was also demonstrated on another rCHO cell line. A patent has been filed on this process encompassing various mammalian cell lines. We next propose to use this approach to create HEK293 and CHOK1 host cells with improved secretory capacity that can be used for stable and transient production of biosimilars. We believe this approach can reduce the development time for high productivity stable and transient clones. The main objectives of the project include adaptation of HEK293 cells from adherent to suspension, adaptation of suspension cells to tunicamycin and demonstrating the application of the adapted cell line by transfection of a biosimilar.

Impact of this innovation: Rapid production of desired products is crucial for pharma companies and vaccine manufacturers. The host cell line developed in this project, will have increased secretory capacity and can be used to transfect various biosimilars, thus reducing the time for isolation of high-productivity clones. We hope to provide an alternate to various commercially available host cells with improved capabilities for production of biosimilars and recombinant proteins.

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