INTRACELLULAR SECRETION ANALYSIS OF THERAPEUTIC ANTIBODIES IN ENGINEERED HIGHPRODUCIBLE CHO CELLS

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The Chinese Hamster Ovary (CHO) cell is the most commonly used cell line for the production of therapeutic recombinant proteins. The improvements in target gene amplification and culture method have contributed in achieving a very high productivity. Some studies have focused on post-translational secretion processes, and overexpression of proteins which work in the secretion pathway successfully increased the productivity [1]. However, those studies were performed based on the knowledge obtained from the normal, adherent cultured cells, and the detailed secretion processes of recombinant proteins in engineered, suspension cultured cells is still unclear. To clarify problems and to find new targets for a more efficient establishment of high producers, the basic analyses about the secretion in engineered, high-producible CHO cells were performed.

CHO-HcD6 cells, a high producer of humanized IgG1 derived from CHO-K1 cells, were cultured in a shaking flask with a serum free medium. To analyze the IgG secretion rate within the cells, chase assay was performed. Cells were incubated with protein synthesis inhibitor cycloheximide for several hours, and the amount of IgG secreted to the medium and remained in the cells were measured by quantitative western blotting method. It was found that, when the cells were treated with cycloheximide, the amount of IgG in the medium seemed to reach a plateau at around 4 hours after the inhibition (Figure 1, upper panel). IgG in the cells decreased until 4-6 hours, but was stable from 6 to 8 hours after the inhibition (Figure 1, lower panel). These results indicate that IgG could not be fully secreted to the medium even though some of them still remained in the cells [2].

To analyze the localization of such remained IgG within the cells, immunofluorescent microscopy against human IgG, endoplasmic reticulum (ER) and Golgi apparatus was performed. Even after the inhibition of protein synthesis, IgG were seen in many cells. Most IgG co-localized within the ER, but little within the Golgi apparatus. Finally, the maturation and the aggregation of IgG were analyzed by Size Exclusion Chromatography (SEC). When cells were not treated with cycloheximide, most IgG in the cells formed tetrameric full bodies. Even after the incubation with cycloheximide, the IgG that remained in the cells seemed to form full bodies. Some aggregations of heavy chains were detected regardless of the addition of cycloheximide, however the amount was much less than that of full bodies.

In summary, secretion speed, localization and maturation of IgG in the high producer CHO cells were analyzed. These brief analyses were useful to find problems in the secretion system. The cells could not fully secrete IgG within a few hours, and the IgG remained in the ER for a long time. However, many of these remained IgG seemed to form full bodies. The improvement in transportation from the ER to Golgi and the related quality control such as glycosylation can be the next targets to increase the productivity.