Optimization of IgG production in CHO cells under T-REx™ Overexpressing XBP-1(s) System

Gulis, G.¹; Mello-de-Sousa, T.M.²; Maranhao, A.Q.¹; Brigido, M.M.¹

¹University of Brasilia, Institute of Biological Sciences, Department of Cell Biology, DF, Brazil, ²Institute of Chemical Engineering, Vienna University of Technology, Austria.

INTRODUCTION: Optimization of the protein production is still a challenging problem in biotechnology. Different techniques of transcription and translation engineering have been used to improve protein secretion, but there still remain many open problems in post-translation modifications of the secreted protein and cell line stability. Overexpression of X-box binding protein (spliced form XBP-1(s)) has been shown to increase production of several proteins by the expansion of size of endoplasmic reticulum (ER) and enhancing of the total protein synthesis. However, there is still no complete understanding about the relation between production and apoptosis. The stress of our work is on the simultaneous study of the regulation of the production of secreted protein (on the example of immunoglobulin G (IgG)) and the regulation of cell viability by overexpression of XBP-1(s) in Chinese hamster ovary cells (CHO) using T-REx™ system at different conditions.

MATERIALS AND METHODS: To analyze the production of IgG, we used enzyme-linked immunosorbent assay (ELISA). During all experiments cells were monitored by viability assay. mRNA levels were analyzed by real-time PCR (qRT-PCR). Isolated and purified monoclonal antibody was subjected to FACS analysis using FITC labeling.

RESULTS AND DISCUSSION: The obtained results show two times increase of production of IgG under induction of 1µg/mL of doxycycline during 6 days of incubation of CHO cells compared to the control. Also, viability tests and real-time PCR demonstrate the enforcing effect of overexpression of apoptotic XBP-1(s) and high IgG production by toxicity of higher concentration doxycycline on cell viability during shorter period time. Furthermore, obtained monoclonal antibody was tested by FACS on antigen ligation and showed comparable results with commercial IgG.

CONCLUSION: The obtained data demonstrates the potential of T-REx™ overexpressing XBP-1(s) system to improve CHO cell culture protein production and to delay cell death.

Keywords: CHO cells, protein production, X-box binding protein (spliced form (XBP-1(s))), T-REx™ system, tetracycline, doxycycline

The work was supported by CAPES/PNPD and UnB