SUBCELLULAR FRACTIONATION COUPLED TO SHOTGUN PROTEOMICS ALLOWS THE IDENTIFICATION OF NOVEL TARGETS OF THE CLASSICAL SECRETION PATHWAY ASSOCIATED WITH INCREASED PRODUCTIVITY IN RECOMBINANT CHO CELLS

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Chinese hamster ovary (CHO) cells are the preferred eukaryotic heterologous expression system for producing recombinant glycoproteins such as antibodies. Proteomics has previously investigated different cellular processes contributing to cell protein production (CHO). However, although the classical secretory pathway (CSP) has been well documented as a bottleneck during recombinant protein (RP) production, it has not been well represented in previous proteomic studies. Therefore, we evaluated the importance of this pathway for PR production by identifying its own proteins associated with changes in PR production through subcellular fractionation coupled with shotgun proteomics. We used two CHO cell lines producing a monoclonal antibody with different specific productivities. We identified 4952 protein groups (59% coverage of the Chinese hamster proteome). Four hundred ninety-three proteins were classified as differentially expressed, of which around 80% were proposed as new targets, and one-third were assigned to the CSP. Endoplasmic reticulum (ER) stress, unfolded protein response, vesicle trafficking, glycosylation, protein synthesis and translocation in the ER lumen, and secretion of extracellular matrix components were some of the affected processes that occurred in the secretory pathway. This study provides new insights into the molecular traits of producer cells and new targets for developing new sublines with improved phenotypes for RP production.

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