INVESTIGATION OF THE ROLE OF UBIQUITINATION IN ER STRESS MECHANISMS IN RECOMBINANT CHO CELLS

Dr. Paula Meleady, School of Biotechnology & National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland.
Paula.meleady@dcu.ie
Dr. Esen Efeoglu, National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland.
David Ryan, National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland.
Christiana Sideri, National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland.
Dr. Michael Henry, National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland.

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The Chinese hamster ovary (CHO) cell line is the dominant mammalian expression system for biopharmaceutical production. Improving the efficiency of production of complex biotherapeutics will be critical in controlling costs to healthcare systems as more of these drugs come to market. In CHO production cell cultures, the expression of high levels of recombinant biopharmaceuticals is linked to inducing endoplasmic reticulum (ER) stress, causing enhanced unfolded protein response (UPR) levels to maintain cell viability and productivity. ER stress mechanisms are poorly understood in CHO cells, and are a major bottleneck in improving the efficiency of production of high cost recombinant biopharmaceuticals. In particular, cellular processes such as folding and degradation in the ER have attracted significant attention in the field of recombinant protein production. To counteract against misfolded proteins, the ER has in place quality control mechanisms, including the UPR and ER-associated degradation (ERAD), and these interact in a coordinated manner with the ubiquitin proteasome system (UPS). There is now increasing evidence from a number of CHO cell line engineering studies that the UPS pathway itself plays an important role in regulating growth, productivity and product quality of rCHO cells.[1,2]

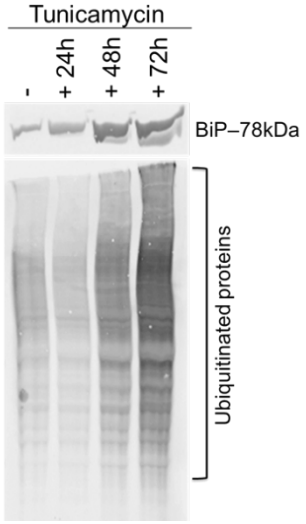

Figure 1: Western blot analysis of CHO DP12 cells after treatment with tunicamycin, using BiP and pan-ubiquitin antibodies, shows an increase of ubiquitination at 24, 48, and 72h.

In this study, we have used artificial inducers of the UPR and ER-stress in recombinant CHO cell lines to characterize the proteome and the ubiquitinated proteome of CHO cells following exposure to thapsigargin and tunicamycin. Recombinant CHO cells were exposed to both inducers for 24, 48 and 72 hours and samples were collected for proteomic analysis. We used a label free LC-MS/MS proteomic approach using a high resolution Orbitrap Fusion Tribrid mass spectrometer and identified ~ 800 differentially expressed proteins. Pathways such as ERAD, ubiquitin ligase complex at ER membrane and protein folding were significantly enriched following Gene Ontology analysis. Figure 1 shows induction of BiP, a key regulator of ER stress, and increased ubiquitination following treatment with tunicamycin. We then enriched the ubiquitinated proteome of these treated cells and identified > 100 differentially expressed ubiquitinated peptides, the first time that this has been reported in recombinant CHO cells. A Lys-ε-diglycine (diGly) mark on peptides after tryptic digestion of proteins indicates a site of ubiquitination. Immunoaffinity-based enrichment of diGly remnant-containing peptides (using an anti-K-ε-GG antibody) was used for enrichment of ubiquitinated peptides for LC-MS/MS. Sites of ubiquitination were also identified on relevant peptide sequences from proteins of interest.

Integration of the proteomic and ubiquitinated proteomic data has revealed new insights into the potential roles of ubiquitination, and associated UPS pathway proteins (e.g. ligases and deubiquitinating enzymes), in regulating productivity of recombinant CHO cells. This will enhance our understanding of the UPR and ER stress mechanisms induced during the production of recombinant biotherapeutics in recombinant CHO cells.

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