Elucidation of the CHO Super-Ome (CHO-SO) by Proteoinformatics

Chinese hamster ovary (CHO) cells are the preferred host cell line for manufacturing a variety of complex biotherapeutic drugs including monoclonal antibodies. We performed a proteomics and bioinformatics analysis on the spent medium from adherent CHO cells. Supernatant from CHO-K1 culture was collected and subjected to in-solution digestion followed by LC/MS/MS analysis, which allowed the identification of 3281 different host cell proteins (HCPs). To functionally categorize them, we applied multiple bioinformatics tools to the proteins identified in our study including SignalP, TargetP, SecretomeP, TMHMM, WoLF PSORT, and Phobius. This analysis provided information on the presence of signal peptides, transmembrane domains, and cellular localization and showed that both secreted and intracellular proteins were constituents of the supernatant. Identified proteins were shown to be localized to the secretory pathway including ones playing roles in cell growth, proliferation, and folding as well as those involved in protein degradation and removal. After combining proteins predicted to be secreted or having a signal peptide, we identified 1015 proteins, which we termed as CHO supernatant-ome (CHO-SO), or superome. As a part of this effort, we created a publically accessible web-based tool called GO-CHO to functionally categorize proteins found in CHO-SO and identify enriched molecular functions, biological processes, and cellular components. We also used a tool to evaluate the immunogenicity potential of high-abundance HCPs. Among enriched functions were catalytic activity and structural constituents of the cytoskeleton. Various transport related biological processes, such as vesicle mediated transport, were found to be highly enriched. Extracellular space and vesicular exosome associated proteins were found to be the most enriched cellular components. The superome also contained proteins secreted from both classical and nonclassical secretory pathways. The work and database described in our study will enable the CHO community to rapidly identify high-abundance HCPs in their cultures and therefore help assess process and purification methods used in the production of biologic drugs.