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Exploiting the proteomics revolution in biotechnology: from disease and antibody targets to optimizing bioprocess development

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Recent advancements in proteomics have enabled the generation of high-quality data sets useful for applications ranging from target and monoclonal antibody (mAB) discovery to bioprocess optimization. Comparative proteomics approaches have recently been used to identify novel disease targets in oncology and other disease conditions. Proteomics has also been applied as a new avenue for mAb discovery. Finally, CHO and *Escherichia coli* cells represent the dominant production hosts for biopharmaceutical development, yet the physiology of these cells types has yet to be fully established. Proteomics approaches can provide new insights into these cell types, aiding in recombinant protein production, cell growth regulation, and medium formulation. Optimization of sample preparations and protein database developments are enhancing the quantity and accuracy of proteomic results. In these ways, innovations in proteomics are enriching biotechnology and bioprocessing research across a wide spectrum of applications.

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Introduction

There has been a transformation in biotechnology in the way that it now relies extensively on vast data sets generated using proteomics techniques. Both label free and comparative proteomics can now identify and quantify thousands of cellular proteins for therapeutic discovery purposes and to increase understanding of production hosts such as the Chinese hamster ovary (CHO) and *Escherichia coli* cells. At the earliest research stages, proteomics can identify new targets for infectious diseases, inflammatory diseases, chronic conditions, and cancer. Comparative proteomics between normal and diseased cells has the potential to identify differentially expressed proteins, which may represent novel targets and biomarkers. At the next stage in drug discovery, proteomics can be used to identify mAbs that might have therapeutic utility directly from the serum of patients (e.g., infectious disease). Finally, proteomics can be used to reveal differentially expressed host proteins between high and low biotherapeutic producers in order to aid cell line engineering efforts for increased recombinant protein productivity by manufacturing cells.

Proteomics aids disease target identification

Applying 'omics tools to study the changes as cells progress from healthy to a diseased state is a valuable strategy for identifying potential therapeutic targets. In this role, comparative proteomics has been used for novel target identification in cancer, inflammatory, neurological and other diseases. For example, a comparison between human pulmonary adenocarcinoma and surrounding healthy tissue revealed over 30 differentially expressed proteins [1]. Two of the proteins significantly up-regulated in the cancer tissue were PKM2 and cofilin-1 [1]. On the basis of the proteomics results, PKM2 was targeted by RNA interference knockdown *in vitro*, which led to decreased cell growth and apoptosis induction in a cancer model cell line [1].

Protein interactions involved in disease progression can sometimes occur at the cell surface. Human cancer cell lines and osteoblast control cells were biotinylated to isolate surface proteins and then compared to identify over 150 significantly up-regulated proteins [2]. A surface proteomics experiment revealed that ephrin type-A receptor 2 (EphA2) is the most widely expressed surface protein on the cancer cells, suggesting it as a novel target for osteosarcoma biotherapeutics [2]. These cell surface receptors can then serve as targets for therapeutic intervention [2,3]. Proteomics analysis is useful for identification of biomarkers and novel drug targets as well as for evaluation of the physiological effects of drug candidates.

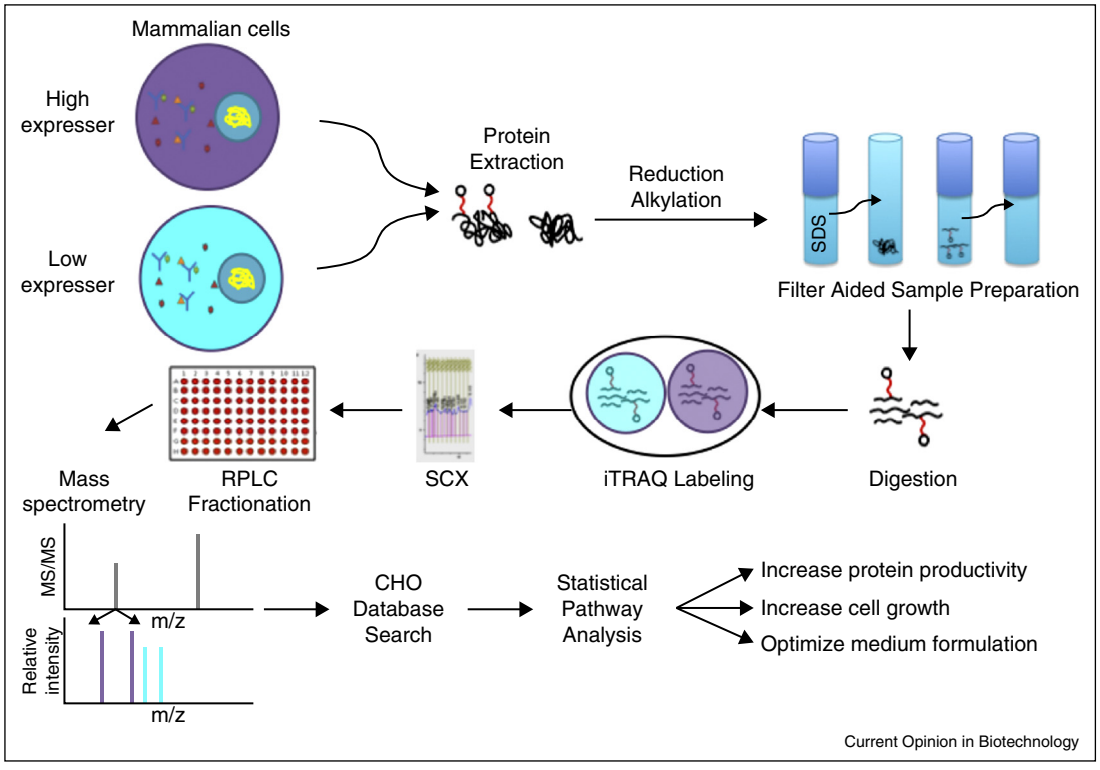
Proteomics discovers novel mAbs

MAb therapy has revolutionized the biotechnology industry and transformed the treatment of a range of different diseases. Proteomic methods are now being incorporated into the mAb discovery process as a complement to traditional hybridoma and phage display technologies. Both Cheung *et al.* [4] and Wine *et al.* [5•] applied proteomics to discover mAbs from the serum of immunized rabbit and mice. They employed affinity purification to the antibody mixture [4] and digested antibody fractions were then analyzed by nano-flow liquid chromatography coupled to tandem mass spectrometry (MS/MS). The spectra were then mapped to next generation sequencing (NGS) derived transcript sequences of the immunoglobulin heavy chain variable region to elucidate the antibody composition of a polyclonal serum response following immunization [5•]. In this way, proteomics along with other 'omics tools, is now being used for the identification of potentially valuable mAbs directly from serum of animals.

Proteomics supports bioprocess development

Optimizing the manufacturing processes of biotherapeutics represents one avenue for making drug costs more affordable. Thus there is a desire to develop and utilize stable cell lines with high yields and even higher product quality. Proteomics can serve an important role in this effort by identifying those factors that enhance the cell's capacity to produce high yields of protein therapeutics. CHO cell lines have been the dominant biotherapeutic production hosts due to their adaptability to bioprocessing and the presence of post-translational modifications compatible with humans. The recently published proteomic analysis of CHO cells [6•] has increased knowledge of CHO host cell proteins and pathways and will likely form the basis for future cell engineering efforts aimed at improving cell line characteristics. This large-scale analysis focused on intracellular proteins, the secretome and the glycoproteome, based on the original draft CHO genome [6•,7,8]. Pathway analysis revealed that protein processing and apoptosis related genes were enriched in expression, whereas steroid hormone and glycosphingolipid metabolic pathways were depleted [6•]. Comparative proteomics, such as isobaric tags for relative and absolute quantitation (iTRAQ), can be used to identify differentially expressed proteins associated with key cellular properties including protein production, cell growth, reduced apoptosis, favorable glycosylation, and optimized medium formulations (Figure 1).

Figure 1



Overview of an optimized proteomics experiment for mammalian cell culture protein quantification. Proteins are extracted and subjected to reduction, alkylation, filter aided sample preparation, and digestion. Digested peptides are labeled with iTRAQ reagents, fractionated by bRPLC, and injected into LC/MS/MS. The resultant peaks are analyzed by mapping to CHO genome databases. Differences in peak levels represent relative protein expression levels between cell lines. Identification and quantification of proteins aids bioprocess development efforts to increase protein yields or other applications.