MULTI-OMICS STRATEGY FOR CELL CULTURE MEDIUM OPTIMIZATION IN FED-BACH CHO CELL CULTIVATION

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Background and novelty:

Cell culture media development and optimization of Chinese Hamster Ovary (CHO) cells to produce biotherapeutics has been successfully accomplished using spent medium analysis of amino acids, glucose, and select water soluble vitamins. The resulting formulations have evolved into intricate combinations of metabolites, trace elements, stabilizing factors such as buffers and chelates, and lipids to allow the cells to propagate and produce more efficiently. Recently, the advent of more complex biotherapeutics, biosimilars and solubility limits has limited the effectiveness of the traditional spent medium approach. We have developed a Multi-Omics strategy for improving cell culture medium and processes utilizing proteomics and metabolomics. These approaches are focused directly on cellular functions rather than the indirect method of interrogating spent medium. Using a Multi-Omics approach to fully understand the characterization of engineered CHO cells will provide another method to further improve the titer of therapeutic proteins.

Experimental approach:

Growth performance assays of mAb producing CHO cells were conducted in a 14-day fed-batch cultivation in an Ambr® 15 microbioreactor. Cell and spent medium samples were taken to target early log phase, late log phase, and early stationary phase on days 5, 7, and 10 for untargeted proteomics and metabolomics analysis. The EasyPep™ Mini MS Sample Prep kit (Thermo Fisher Scientific) was used for the preparation of cell samples and the resulting peptide mixtures were analyzed by Orbitrap™ Fusion Lumos™ mass spectrometry with FAIMS. Cellular metabolomics and spent media metabolomics were processed using Orbitrap ID-X™ mass spectrometry. An ion current-based quantification procedure was applied in proteomics analysis. The Compound Discoverer and Proteome Discoverer suites were used for identification and quantitation of small molecules and proteins. Multi-omics data integration and interpretation was processed using Ingenuity Pathway Analysis (Qiagen). Components and pathways predicted to influence titer and growth were further screened and validated in the AMBR™15 microbioreactor system.

Results and discussion:

More than 6,500 proteins and 400 metabolites were identified and quantified across different conditions. Multiple altered signaling and metabolic pathways were revealed by comparative multi-omics analysis, such as mTOR pathway and one carbon metabolic pathway. Those pathways were significantly associated with different biological features such as cell growth, cell viability, and titer. A 5 Factor DOE consisting of sixteen basal media and two feeds/one feed strategy were designed based on those analyses and evaluated. Titer improvement up to 60% was achieved. Further experiments will be conducted to test new formulations determined from spent media and cellular analysis.