Editorial

Pharmaceutical BIOPROCESSING

Pharm. Bioprocess.
(2014) 2(1), 1–4

The application of omics in pharmaceutical bioprocessing

The knowledge obtained in omics analysis will provide a powerful tool to further improve bioprocessing efficiency and benefit the development of both innovative biopharmaceuticals and generic biologics.

Keywords: biopharmaceuticals, bioprocessing, CHO cell culture, omics, productivity, quality

Xiaoguang Liu
Author for correspondence:
Department of Chemical & Biological Engineering, University of Alabama, 245 7th Avenue, Tuscaloosa, AL 35401, USA
Tel.: +1 205 348 0868
Fax: +1 205 348 7558
E-mail: mliu@eng.ua.edu

Shang-Tian Yang
Department of Chemical & Biomolecular Engineering, The Ohio State University, 140 West 19th Avenue, Columbus, OH, USA

Lufang Zhou
Departments of Medicine & Biomedical Engineering, University of Alabama at Birmingham, 703 19th Street South, ZRB 306, Birmingham, AL 35294, USA

The US market for mammalian cell-derived biopharmaceuticals, including monoclonal antibodies, growth factors, hormones and interferons, is estimated to exceed US$144 billion by 2016 [101]. So far, the US FDA has approved more than a hundred therapeutic proteins, and there are thousands of pipelines in the clinical trial stage. The efficient development of these pipelines requires an effective bioprocessing platform. The recent advances in CHOnomics technologies (e.g., genomics, transcriptomics, proteomics and metabolomics) can benefit the bioprocessing of CHO cell, the most popular mammalian cell in biopharmaceutical industry, based therapeutic proteins.

Genomics is the comprehensive and complete analysis of genome using new generation DNA sequencers, such as Illumina HiSeq 2000 and Life Tech SOLiD. The genome sequencing of parental CHO K1 cell was completed in 2011, which showed that the genome assembly of CHO K1 comprises 2.45-Gb sequences and 24,383 predicted genes [1]. Transcriptomics is a functional genomic analysis at the transcription level by qualifying and quantitating mRNA expression using microarray [2] or deep sequencing technology [3]. Transcriptomic analysis is a better approach to study CHO cell physiology compared with genomics due to the genome rearrangement and the complicated processing of mRNA. Proteomics is a research tool of quantifying the expression of large number of intracellular proteins at defined culture conditions using NMR, MALDI-TOF, ultra-performance LC–MS/MS or SILAC. Proteomics can detect post-translational modifications and protein interactions. Metabolomics is a qualitative and quantitative analysis of cellular metabolites using HPLC, GC–MS and/or LC–MS/MS. This article mainly focuses on the application of CHOnomics in two critical bioprocessing areas: cell and metabolic engineering, and process development including clone evaluation, feeding strategy development, medium and feed development, bioreactor process parameter optimization and quality control in manufacturing.

Protein production has been significantly enhanced with the development of cell line construction and process development. However, the host cell-regulated stability of protein production, control of protein quality, and productivity improvement of hard-to-express proteins are still big challenges in biopharmaceutical production. With the advanced CHOnomics technology, these problems can be solved by targeting physiology regulatory factors, bottlenecks and missing activities. One important strategy in cell engineering is to increase cell growth and protein quality by manipulating apoptosis regulator, such as C-Myc, Bcl-xL Fadd, Faim, Alg-2 and requiem [4]. Multiple proteins enhancing cell proliferation have also been identified using Omnis, including VAPA and VAPB/C, TOM34, RPRD1B, VCP, Bak and Bax [5,6]. Another cell-engineering strategy is to increase protein productivity by manipu-

FUTURE SCIENCE

10.4155/PBP.13.63 © 2014 Future Science Ltd ISSN 2048-9145

lating proteins involved in unfolded protein response and secretion bottleneck, such as XBP-1, ATF4, SNAREs and CERT [4]. In CHO genome analysis [1], the genes involved in protein glycosylation, fucosylation and sialylation pathways have been investigated, but the expression of approximately 50% of the human glycosylation-associated genes could not be detected in transcriptomics analysis. Therefore, the integration of proteomics and transcriptomics is important in the glycosylation regulation of therapeutic proteins. Multiple commercial vector systems are available to upregulate (or overexpress) target genes, and zinc-fingers and miRNA have been developed to knockdown (or knockout) undesired genes. miRNA regulates gene expression at the post-transcriptional level by mRNA cleavage or translational repression. Global Omics study has identified multiple transcription regulators, such as miR-7, miR-16, miR-21 and let-7b [7].

Metabolic engineering can directly manipulate the carbon, energy, glycosylation, folding and secretion pathways. Recent transcriptomics and genomics studies have identified 1.84 million reads, 29,000 transcripts and key enzymes [8], and several metabolomics studies have investigated both intracellular and extracellular metabolites of CHO cell culture [9–11]. The developed CHO metabolism knowledge can be used to guide direct metabolic engineering and create a novel host cell. The most popular metabolically engineered CHO cell is CHO DG44, in which the dihydrofolate reductase gene (*dhfr*; Life Tech, CA, USA) has been deleted so that the cell line can be metabolically selected with proper heterogenous gene expression. The overexpression of glutamine synthesis (GS; Lonza, Basel, Switzerland) can reduce the accumulation of ammonia. Other applications of metabolic engineering include reduction of lactate accumulation by downregulating LDH-A [12] and increase of glycosylation by overexpressing glycotransferase or sialylase.

The efforts to investigate CHO cell profiling under various cell culture conditions enable rational process development in clone evaluation, fed-batch development, medium development, bioreactor parameter optimization and quality control.

Process development (PD) is critical to improve productivity and control the quality of biopharmaceuticals. The small-scale high-throughput screening has been applied to optimize process parameters and evaluate production clones, but the high capital investment and complicated operation of high-throughput hamper its application. Moreover, the focus of biopharmaceutical bioprocessing has recently shifted from productivity to product quality control and process regulation. Rational PD is a promising strategy to meet these PD goals. The principle of rational PD is to design a specific production process for different production cell lines and biologics products. The development work is based on cell response to process parameters and extracellular metabolites (or nutrient supplements). The efforts to investigate CHO cell profiling under various cell culture conditions enable rational process development in clone evaluation, fed-batch development, medium development, bioreactor parameter optimization and quality control.

The lead production clone used in manufacturing needs be defined from top clones in process development. Because the construction of high-producing cell lines of most biopharmaceuticals relies on gene amplification, clone-to-clone variation of protein production performance is evident. The application of omics analysis to early-stage clone evaluation could define an efficient lead clone by taking into account both clone variation and process effect. Moreover, the defined lead clone could have excellent protein productivity and quality performance in manufacturing due to the rational screening and evaluation.

Fed-batch process has been widely used in the production of many biologics as it can increase protein productivity by two- to ten-fold while controlling glycosylation and sialylation. The optimization of nutrient feeding strategy is the key to develop a successful fed-batch process. The nutrient depletion induces apoptosis while over feeding increases accumulation of toxic metabolites, therefore it is very important to develop a suitable feeding schedule based on metabolomics analysis. The knowledge of cell growth, protein productivity, glycosylation and sialylation, host cell proteins and enzymes obtained in metabolomic and proteomic analysis benefits fed-batch development. For example, the finding of a correlation between ammonia and branching reactions in glycosylation associated with Golgi has enabled protein glycosylation regulation via fed-batch process development [13]. In addition, the extracellular metabolite analysis of spent medium can guide the formulation development of feeding nutrients.

Medium development is another important consideration in cell culture process development. Omics analysis and metabolic flux analysis of cell culture are valuable for optimizing production medium. For example, the development of new-generation chemical defined medium at Gibco® is based on intracellular metabolism analysis (fingerprinting) of CHO production cell line and spent medium analysis (footprinting). In addition, the omics studies of CHO cell culture supplied with the key components (e.g., growth factor, NaBu, zinc sulphate, and hydrolysate) have revealed the regulation mechanism of protein production [14–16], which could further direct medium optimization.

The effects of bioreactor conditions, such as temperature shift, pH shift, and osmolarity stress on protein production and host cell protein expression have been evaluated using omics technology [17]. The application of metabolic flux analysis with intracellular and extracellular metabolite profiling could identify the critical process operation parameter [18]. Comparative transcriptomic analysis has shown a positive correlation between antibody productivity and regulating proteins under different culture conditions, such as DDB1, AP-2 and AP-3, and SPPR [6]. The reduction of lactate can also be regulated via metabolic shift and nutrients supplement with proteomics analysis [19].

In additon, Omics analysis of bioprocessing is a valuable tool to guide process scale-up and provide troubleshooting in the manufacturing of biopharmaceuticals. For example, transcriptomic analysis has been used to direct mammalian cell culture scale-up by maintaining consistent transcriptomics fingerprints [20]. The reduced productivity and the incomplete protein expression or post-translational modifications in biopharmaceutical manufacturing can be diagnosed using omics.

With the continuing growth of mammalian cell-based biopharmaceutical market, it is of great interest for biopharmaceutical industry to rationally design and develop effective bioprocessing to further improve protein productivity and quality. The advanced omics technologies, including genomics, transcriptomics, proteomics, metabolomics, and other omics analysis, have been developed to investigate whole cell profiling, provide genome-scale understanding of host cells, and identify the targets to engineer in cell engineering or regulators to manipulate in process development. The knowledge obtained in omics analysis will provide a powerful tool to further improve bioprocessing efficiency and benefit the development of both innovative biopharmaceuticals and generic biologics.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Xu X, Nagarajan H, Lewis NE *et al.* The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. *Nat. Biotechnol.* 29(8), 735–741 (2011).

2 Schaub J, Clemens C, Schorn P *et al.* CHO gene expression profiling in biopharmaceutical process analysis and design. *Biotechnol. Bioeng.* 105(2), 431–438 (2010).

3 Morozova O, Marra MA. Applications of next-generation sequencing technologies in functional genomics. *Genomics* 92(5), 255–264 (2008).

4 Datta P, Linhardt RJ, Sharfstein ST. An 'omics approach towards CHO cell engineering. *Biotechnol. Bioeng.* 110(5), 1255–1271 (2013).

5 Lim SF, Chuan KH, Liu S *et al.* RNAi suppression of Bax and Bak enhances viability in fed-batch cultures of CHO cells. *Metab. Eng.* 8(6), 509–522 (2006).

6 Kang S, Ren D, Xiao G *et al.* Cell line profiling to improve monoclonal antibody production. *Biotechnol. Bioeng.* doi:10.1002/bit.25141 (2013) (Epub ahead of print).

7 Barron N, Kumar N, Sanchez N *et al.* Engineering CHO cell growth and recombinant protein productivity by overexpression of miR-7. *J. Biotechnol.* 151(2), 204–211 (2011).

8 Becker J, Timmermann C, Jakobi T *et al.* Next-generation sequencing of the CHO cell transcriptome. *BMC Proc.* 5(Suppl. 8), P6 (2011).

9 Chong WP, Goh LT, Reddy SG *et al.* Metabolomics profiling of extracellular metabolites in recombinant Chinese hamster ovary fed-batch culture. *Rapid Comunm. Mass Spectrom. RCM* 23(23), 3763–3771 (2009).

10 Dietmair S, Hodson MP, Quek LE *et al.* Metabolite profiling of CHO cells with different growth characteristics. *Biotechnol. Bioeng.* 109(6), 1404–1414 (2012).

11 Sellick CA, Hansen R, Maqsood AR *et al.* Effective quenching processes for physiologically valid metabolite profiling of suspension cultured mammalian cells. *Anal. Chem.* 81(1), 174–183 (2009).

12 Zhou M, Crawford Y, Ng D *et al.* Decreasing lactate level and increasing antibody production in Chinese hamster ovary cells (CHO) by reducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases. *J. Biotechnol.* 153(1–2), 27–34 (2011).

13 Cuperlovic-Culf M, Barnett DA, Culf AS, Chute I. Cell culture metabolomics: applications and future directions. *Drug Discov. Today* 15(15–16), 610–621 (2010).

14 Gupta P, Lee KH. Genomics and proteomics in process development: opportunities and challenges. *Trends Biotechnol.* 25(7), 324–330 (2007).

15 Baik JY, Joo EJ, Kim YH, Lee GM. Limitations to the comparative proteomic analysis of thrombopoietin producing Chinese hamster ovary cells treated with sodium butyrate. *J. Biotechnol.* 133(4), 461–468 (2008).

16 Kantardjieff A, Jacob NM, Yee JC *et al.* Transcriptome and proteome analysis of Chinese hamster ovary cells under low temperature and butyrate treatment. *J. Biotechnol.* 145(2), 143–159 (2010).

17 Yee JC, Gerdtzen ZP, Hu WS. Comparative transcriptome analysis to unveil genes affecting recombinant protein productivity in mammalian cells. *Biotechnol. Bioeng.* 102(1), 246–263 (2009).

18 Boghigian BA, Seth G, Kiss R, Pfeifer BA. Metabolic flux analysis and pharmaceutical production. *Metabolic Eng.* 12(2), 81–95 (2010).

19 Pascoe DE, Arnott D, Papoutsakis ET, Miller WM, Andersen DC. Proteome analysis of antibody-producing CHO cell lines with different metabolic profiles. *Biotechnol. Bioeng.* 98(2), 391–410 (2007).

20 Jayapal Kp, Goudar C. Transcriptomics as a tool for assessing the scalability of mammalian cell perfusion systems. *Adv. Biochem. Eng. Biotechnol.* doi:10.1007/10_2013_239 (2013) (Epub ahead of print).

» Website

101 Markets and markets. www.marketsandmarkets.com