

ORAL PRESENTATION

Open Access

Multi-omics approach for comparative studies of monoclonal antibody producing CHO cells

Camila A Orellana, Esteban Marcellin, Trent Munro, Peter P Gary, Lars K Nielsen*

From 24th European Society for Animal Cell Technology (ESACT) Meeting: C2P2: Cells, Culture, Patients, Products Barcelona, Spain. 31 May - 3 June 2015

Background

Monoclonal antibody (mAb) therapy has revolutionized the treatment of a vast range of diseases, mostly in the areas of oncology and autoimmune/inflammatory disorders [1]. With a world market exceeding 60 billion USD per year and six mAb related products in the top 10 selling drugs, the industry continues to grow at a fast rate [2]. Chinese hamster ovary (CHO) cells are the preferred production host for therapeutic production of mAbs due to their efficiency in performing post-translational modifications and their ability to produce proteins with similar properties to native human proteins [3]. Surprisingly, despite products varying only by a few amino acids in the variable region of a MAb, each production cell line is still developed by generating and screening a large strain pool, and generally the production process has to be reoptimised.

Systems biology can be used as a powerful tool for the identification of key markers of good production lines, with the aim of engineering superior host lines that more reliably produce good production clones. To date systems biology efforts have been hampered by the need to use the mouse, rat and/or human genome as a reference and has suffered from the inherent limitation in coverage of 2-dimensional gel electrophoresis or mouse or CHO cDNA microarrays. The development of new techniques such as RNA sequencing for transcriptome analysis and LC-MS/MS for proteome analysis combined with the recent release of the CHO genome has reignited interest in using quantitative proteomics and transcriptomics to study high productivity cell lines.

Materials and methods

Here we applied the latest generation of tools to two CHO cell lines that produce different levels of mAb, as described in Orellana et al [4]. The two cell lines were derived from one transfection pool using the same plasmid carrying genes for a monoclonal antibody. For each cell line, three independent vials were thawed and passaged for two weeks prior to bioreactor inoculation. Cells were cultivated in 700 ml EX-CELL[®] CD CHO Fusion Medium (Sigma Aldrich) containing 25 μ M L-Methionine sulfoximine as selection, in a 1L Mini-Bioreactor (Applikon Biotechnologies) operated at 125 rpm stirring speed, 37°C, pH 6.9 and dissolved oxygen at 50% air saturation.

RNA and protein were extracted from cells harvested in mid exponential phase. RNA samples were analysed with RNA sequencing (RNA-Seq) using the Illumina HiSeq2000 platform and 100 bp paired-end reads. TopHat and Cufflinks open-source software [5] were used with default settings for gene expression analysis, using the CHO genome as reference. Protein samples were analysed using SWATH [6]. The Paragon Algorithm from ProteinPilot v4.5 (ABSciex, Forster City CA) [7], PeakView v.1.2 software (ABSciex, Forster City CA) and the R package Limma [8] were used for data analysis. Transcripts and proteins were classified as differentially expressed if the adjusted p-value (Benjamini-Hochberg) was lower than 0.05. Gene set enrichment analysis was performed using DAVID Bioinformatics functional annotation tool [9].

Results and discussion

The high producer cell line displayed a slightly slower growth rate of 0.0310 ± 0.0002 h⁻¹ compared to the low producer cell line with 0.0340 ± 0.0004 h⁻¹. The two cell lines achieved a titre of 104.3 ± 5.5 mg/L and 52.9 ± 2.0 mg/L on day 4 respectively, with a four-fold difference in

* Correspondence: lars.nielsen@uq.edu.au
Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, QLD 4072, Australia

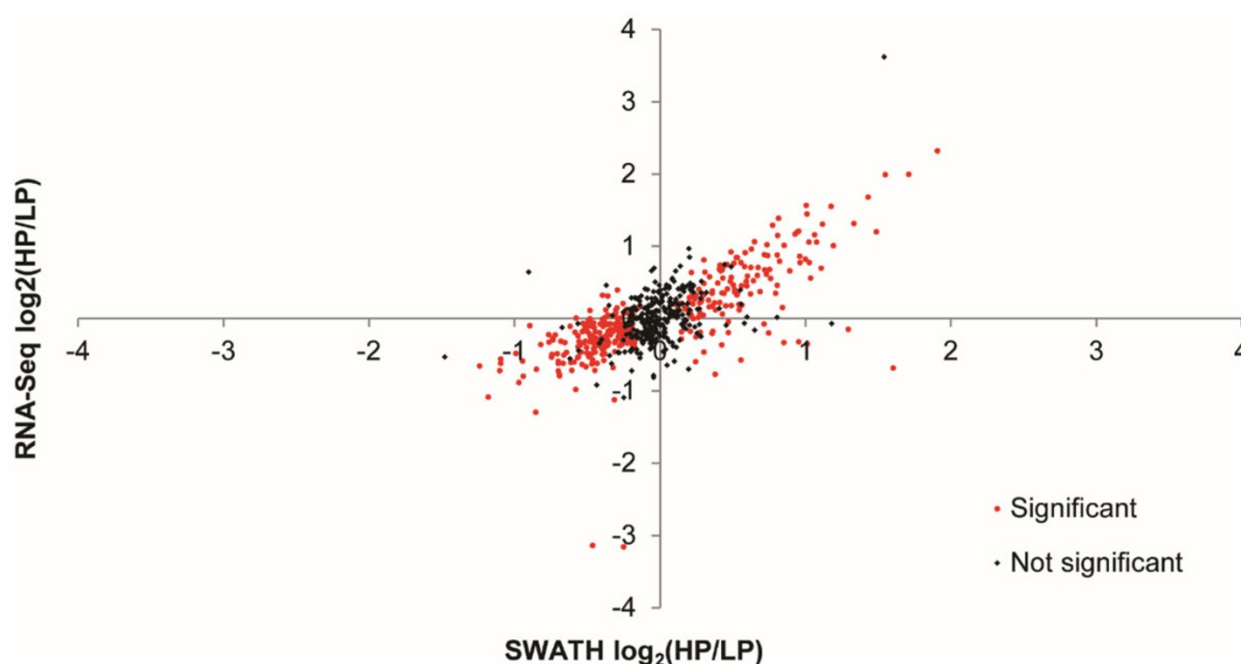


Figure 1 RNA-Seq and SWATH log₂-transformed fold change between high producer (HP) and low producer (LP) cell lines of common transcripts/proteins. Protein with a minimum of 2 peptides with 95% confidence were used. Highlighted in red are the proteins classified as differentially expressed using SWATH technique.

mAb specific productivity (19.5 ± 1.0 pg/cell/day compared to 4.6 ± 0.2 pg/cell/day).

More than 100 million reads were obtained for each sample by RNA-Seq, with more than 83% of the reads mapping to the CHO reference genome. 14,300 transcripts and 714 proteins were quantified and tested for differential expression. Despite the fact that both clones come from the same transfection pool, 58% of the quantified transcripts and 56% of the quantified proteins varied significantly. Figure 1 shows the log₂-transformed fold change between the high producer and the low producer cell lines for common transcripts/proteins between RNA-Seq and SWATH techniques. For most of the genes (80%), the direction of regulation, i.e. up- or down-, agreed between transcripts and proteins.

Three key biological processes were identified by proteomics as up-regulated in the high producer cell line: glutathione biosynthesis, actin filament processes and intracellular transport, while several growth-related processes were down-regulated as expected from the lower growth rate. Metabolomic analysis confirmed that the high producing cell line displayed higher intracellular levels of glutathione. These processes may be important for conferring high mAb production, and as such gene candidates have been nominated for targeted engineering of high-expression cell lines.

Acknowledgments

The authors would like to thank Robin Palfreyman, the Queensland node of Metabolomics Australia, the proteomics facilities at SCMB, and SAFC - Sigma Aldrich.

Published: 14 December 2015

References

- Aggarwal RS: What's fueling the biotech engine-2012 to 2013. *Nat Biotechnol* 2014, **32**:32-39.
- EvaluatePharma: *World preview 2014, outlook to 2020* Evaluate Ltd; 2014 [http://info.evaluategroup.com/rs/evaluatepharmaltd/images/EP240614.pdf].
- Wurm FM: Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol* 2004, **22**:1393-1398.
- Orellana CA, Marcellin E, Schulz BL, Nouwens AS, Gray PP, Nielsen LK: High-antibody-producing Chinese hamster ovary cells up-regulate intracellular protein transport and glutathione synthesis. *J Proteome Res* 2015, **14**:609-618.
- Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L: Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 2012, **7**:562-578.
- Gillet LC, Navarro P, Tate S, Rost H, Selevsek N, Reiter L, Bonner R, Aebersold R: Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics* 2012, **11**:O111 016717.
- Shilov IV, Seymour SL, Patel AA, Loboda A, Tang WH, Keating SP, Hunter CL, Nuwaysir LM, Schaeffer DA: The paragon algorithm, a next generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. *Molecular & Cellular Proteomics* 2007, **6**:1638-1655.
- Smyth GK: *limma: Linear Models for Microarray Data*. In *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. Springer New York; Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S 2005:397-420, Statistics for Biology and Health.

9. Huang da W, Sherman BT, Lempicki RA: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.** *Nat Protoc* 2009, **4**:44-57.

doi:10.1186/1753-6561-9-S9-O8

Cite this article as: Orellana *et al.*: Multi-omics approach for comparative studies of monoclonal antibody producing CHO cells. *BMC Proceedings* 2015 **9**(Suppl 9):O8.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

