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Comparative proteomic analysis of three Chinese hamster ovary (CHO) host cells

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ABSTRACT

Chinese hamster ovary (CHO)[1] cells have been widely used to express heterologous genes and produce therapeutic proteins in biopharmaceutical industry. Different CHO host cells have distinct cell growth rates and protein expression characteristics. In this study, the expression of about 1307 host proteins in three sublines, i.e. CHO K1, CHO S and CHO/dihydrofolate reductase (*dhfr*)$^-$, were investigated and compared using proteomic analysis. The proteins involved in cell growth, glycolysis, tricarboxylic acid cycle, transcription, translation and glycosylation were quantitated using Liquid chromatography tandem-mass spectrometry (LC–MS/MS). The key host cell proteins that regulate the kinetics of cell growth and the magnitude of protein expression levels were identified. Furthermore, several rational cell engineering strategies on how to combine the desired features of fast cell growth and efficient production of therapeutic proteins into one new super CHO host cell have been proposed.

1. Introduction

The Chinese hamster ovary (CHO) cells have been widely used to produce protein-based biopharmaceuticals. Compared to other mammalian cells, CHO cells have the unique advantages of robust cell growth, effective post-translational modification, and the well-established standards of good manufacturing practice (GMP). The parental CHO cell line was originally isolated from Chinese hamster by Dr. Theodore T. Puck in 1957 [1], followed by the derivation of multifarious CHO sublines, such as CHO K1, CHO/*dhfr*$^-$, and CHO S (Fig. 1). The CHO K1 subline was licensed with a glutamine synthetase (GS)-based expression system [2], and a GS negative CHO K1 subline was developed using zinc finger technology [3,4]. The CHO/*dhfr*$^-$ cells including CHO DXB11 and CHO DG44 sublines were generated using chemical mutagenesis, gamma rays or zinc finger technology to inactivate the enzyme of dihydrofolate reductase (DHFR) [5,6]. The cGMP bank of another CHO subline, CHO S with characteristics of fast cell growth, was derived from the parental CHO via adaptation [7].

The CHO sublines mentioned above exhibit noteworthy heterogeneity in their phenotypes [8]. For instance, the GS-based gene selection and amplification in CHO K1 enables high protein production, but the application of high concentration of selection reagent methionine sulfoximine MSX in production cell line construction causes unstable protein expression. The selection and amplification of heterologous genes in CHO/*dhfr*$^-$ cells is usually more effective, yet its cell growth is slower than other two sublines. CHO S cell line has relatively higher growth rate or lower doubling time, but it is laborious to develop a high protein producing cell line from this host cell due to the double selection using methotrexate MTX and puromycin. In addition, the clone stability of CHO S-based production cell line is poor, which is caused by the fact that *dhfr* is an endogenous gene and the gene amplification using high-concentration MTX is necessary. Thus, to improve the production of mmammalian cell-based biopharmaceuticals, it is highly desirable to develop an advanced CHO host cell in which fast cell growth and high protein expression will all be integrated.

The completion of the CHO K1 genome sequencing and the development of proteomics technology have provided both the genetic background and the direct measurement capability to

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[1] CHO: Chinese hamster ovary, DHFR: dihydrofolate reductase, LC–MS/MS: Liquid chromatography tandem–mass spectrometry

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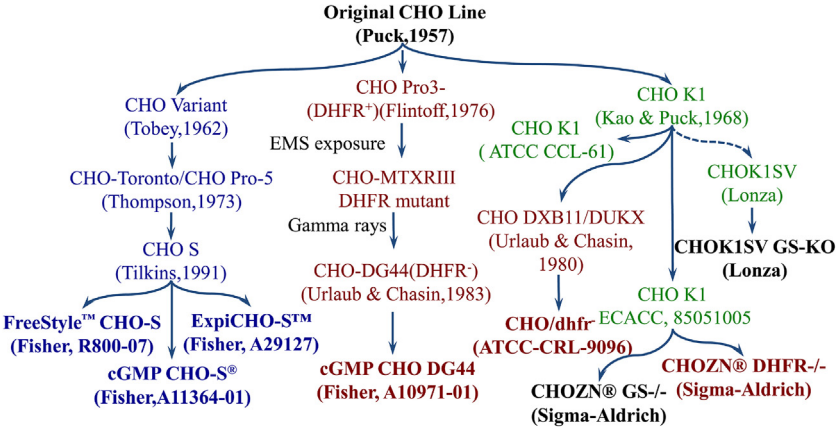

Fig. 1. Cell lineage of CHO cells.

examine the expression levels of the host cell proteins in CHO sublines [9]. Baycin-Hizal et al. have accomplished the first proteomic study of CHO K1 using 120 mass spectrometry analyzes and have identified a total of 6164 grouped proteins from cellular proteome, secretome and glycoproteome analyzes [10]. A number of other studies have analyzed the extracellular host cell proteins to evaluate the impurities in biopharmaceutical production or optimize cell culture medium [11–14]. In addition, proteomic studies have also been performed to study the effects of cell culture conditions, such as temperature, hyperosmolality, media and feeding strategy, on the expression profile of host cell proteins [15–17].

Cell engineering via gene manipulation could be a powerful tool to construct an innovative host cell. However, the lack of the fundamental understanding of the regulation of cell growth and protein expression has hindered the rational host cell engineering. To our best knowledge, the comparison of the intracellular proteins' expression among different CHO sublines has not been performed so far. In this study, we aimed to establish a comprehensive understanding of the different phenotypes of three CHO sublines (CHO K1, CHO/*dhfr*− and CHO S) by comparing their intracellular proteomics profiling. The expression levels of the key enzymes (or proteins) involved in cell growth, glycolysis, tricarboxylic acid (TCA) cycle, transcription, translation and glycosylation were analyzed and compared. The enzymes with different expression levels that correlate to cell growth and protein expression were presented. Finally, the strategies to rationally construct next generation of CHO host cells were also discussed.

2. Materials and methods

2.1. CHO cells and cell culture

Three suspension CHO sublines, including CHO K1, CHO/*dhfr*− and CHO S, were analyzed in this study. The CHO K1 and CHO S were purchased from Thermo Fisher Scientific (Waltham, MA), and CHO/*dhfr*− was purchased from ATCC (Manassas, VA). The seed culture of CHO K1, CHO S and CHO/*dhfr*− were maintained in the three basal media of HyClone CDM4CHO (Hyclone Laboratories, Logan, UT), Gibco CD CHO (Life Technologies, Grand Island, NY) and Sigma EX-CELL CHO DHFR− (Sigma-Aldrich, St. Louis, MO), respectively. All the cell culture media were supplemented with 8 mM l-glutamine (final concentration). The sodium hypoxanthine and thymidine supplements were added to the EX-CELL CHO DHFR- medium. The batch cultures were seeded with viable cell density of 0.3×10^6 cells/mL. The cells were cultivated with triplication in suspension cultures in 125-mL disposable shaker flasks at 37 °C, 5% CO_2 and 120 rpm in a humidified incubator (Caron, Marietta, OH).

2.2. Extraction and digestion of host proteins

To prepare proteomics samples, the cell cultures were sampled between early and mid-log phases, i.e. day 3 (CHO K1 and CHO S) and day 4 (CHO/*dhfr*−). At sampling points, the average viable cell densities were 2.2×10^6 cells/mL and the viabilities were maintained at >99%. Three flasks of each cell were carried out to collect cell samples for the extraction of host cell proteins. The CHO cells collected from batch cultures were centrifuged at 8000 rpm for 5 mins at 4 °C, washed for three times using PBS buffer, and stored at −80 °C for further proteomic analysis. All reagents and supplements used in this study were purchased from Thermo Fisher Scientific unless otherwise specified.

The detailed procedure of host cell protein extraction and digestion was described in previous publications [18,19]. In brief, the host cell proteins were first extracted from cell pellets using M-PER, denatured and run into a 10% SDS Bis-Tris PAGE. Then the sliced protein gel was equilibrated in 100 mM ammonium bicarbonate, reduced, carbidomethylated, dehydrated and digested with Trypsin Gold (Promega, Madison, WI). Finally, the digested peptide was extracted, concentrated and resolubilized in 20 μL of 5% CAN/0.1% formic acid prior to analysis by 1D reverse phase LC-nESI-MS2.

2.3. LC–MS/MS analysis

LC–MS/MS was applied to acquire the high-quality peptide precursor and fragment ion data as described in literature [19]. Each proteomics sample was injected to LC–MS/MS with triplication. A 1260 Infinity nHPLC stack (Agilent, Santa Clara, CA) equipped with a Jupiter C-18 column (300 Å, 5 μm, 75 μm I.D. × 15 cm, Phenomenex) was run to separate the digested peptides. The peptides were eluted using 0%–30% acetonitrile in D.I. H_2O containing 0.1% formic acid with a flow rate of 0.3 μL/min. The peptide fractions were sprayed into a hybrid mass spectrometer (MS, Thermo Orbitrap Velos Pro) equipped with a nano-electrospray source to gain proteomics data. All data were collected in collision-induced dissociation mode. The instrument configuration during data collection followed previous publication [18–20].

2.4. Protein identification

The collected XCalibur RAW files were centroided and converted to MzXML format using ReAdW and converted to mgf files using MzXML2Search. The data were searched with SEQUEST against UniProt-derived proteome databases of both mouse and rat. The searching parameters include trypsin digestion, two missed cleavages sites, 20 ppm of precursor mass tolerance, 0.36 Da fragment