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 1,307 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.

Keywords

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

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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 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 6,164 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 8,000 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 micron, 75 micron 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 ion tolerance, variable modification M at 15.9949, and static modification C at 57.0293. The peptides' searches were performed with a species specific subset of the UniRef100 database. The identified peptides were filtered, grouped, and quantified using Scaffold (Protein Sciences, Portland, OR). The peptide and protein probabilities were set at > 90.0% and > 99.0%, respectively, while the false rate was set at lower than 1.0% to retain protein with high confidence. The identified CHO host cell proteins were described using UniRef100 ID and their expression levels were depicted using the normalized spectra count abundance between samples. The key software tools used in the statistical analysis are SEQUEST and Proteomesoftware. The average spectral count data calculated from the triplicated experiments were presented.

3. Results and discussion

3.1 Comparative proteomics

The CHO K1, CHO/*dhfr⁻* and CHO S cells have been widely used to produce cell-based biopharmaceuticals. Nevertheless, these three sublines possess quite different desired features for the production of therapeutic proteins. In this study, we applied comparative proteomic analysis and examined the expression profiling of more than 1,300 intracellular proteins of CHO K1, CHO/*dhfr⁻* and CHO S host cells. The key enzymes that regulate cell growth, metabolism of carbon and energy, and protein expression were analyzed and compared. The UniRef ID, function, names, average spectral count, and probability of these enzymes are presented in Tables 1–4. The complete datasets, including raw MS data, search parameters, search results, reference search database and summarized data with statistical analysis, were also deposited in the public repository PeptideAtlas (http://www.peptideatlas.org/, accession no. PASS00963).

3.2 Cell growth

Our experiments on cell growth showed that the cell doubling time for CHO K1, CHO/*dhfr⁻* and CHO S cells was 24±2 h, 27±2 h, and 18±2 h, respectively. It is obvious that the CHO S cells grew the fastest and the CHO/*dhfr⁻* cells grew the slowest, as previously reported [21]. The cell growth related host cell proteins that showed > 50% change of expression levels between CHO S and the other two sublines are summarized in Table 1.

As shown in Table 1, nine proteins had obviously higher expression levels in CHO S. These proteins include myosin (*myh9*), cytoskeleton-associated protein 4 (*ckap4*), myl6 protein (*myl6*), alpha-actinin-4 (*actn4*), tropomyosin alpha-3 (*tpm3*), tropomyosin alpha-4 (*tpm4*), cyclin-dependent kinase (*cdk*), tubulin alpha (*tuba*), and tubulin beta (*tubb4b*). Both myosin and cyclin-dependent kinase were expressed at > 2-fold higher levels in CHO S than in CHO K1 or CHO/*dhfr⁻* cells. Previous studies have shown that the non-muscle myosin has multiple functions, e.g., cytokinesis, cell division, cellular movement and maintenance of cell shape, via diverse isoforms, phosphorylation and/or protein binding patterns [22]. Cyclin-dependent kinase plays an important role in regulating cell cycle, transcription and mRNA processing. Our results suggest that the higher expression levels of myosin and cyclin-dependent kinase are likely positively correlated with the faster cell growth rate of CHO S cells. In contrast, seven of these nine proteins except Tpm3 and Tpm4 had the lowest expression levels in CHO/*dhfr⁻* cells (Table 1), suggesting that these proteins may account for the slow growth of CHO/*dhfr⁻* cells.

Interestingly, our data show that the expression levels of filamin (*flna*), vimentin (*vim*) and cofilin-1 (*cfl1*) in CHO K1 cells were significantly higher than CHO S and/or CHO/*dhfr⁻* (Table 1). Studies have shown that filamin α regulates cell shape and migration by remodeling the cytoskeleton [23] and enhances the surface expression of glycoprotein [24]. Protein vimentin has been reported to support the anchoring of organelles to nucleus, endoplasmic reticulum, and mitochondria [25]. The binding of filamin α to vimentin has been considered as a pivotal factor that controls cell adhesion and spreading [26]. Protein cofilin functions as a homeostatic regulator in cell biology through regulating the actin-filament dynamics [27, 28]. The higher expression indicated that these three proteins could play important role in regulating the cell growth of CHO K1.

3.3 Carbon and energy metabolism

The metabolism analysis of glucose and lactate in host cell cultures were analyzed. The results showed that the glucose specific consumption rate was 297.4 pg/(cell•day), 243.7 pg/(cell•day) and 365.1 pg/(cell•day), and the lactate specific accumulation rate was 172.3 pg/(cell•day), 147.2 pg/(cell•day), and 233.5 pg/(cell•day), for the CHO K1, CHO/*dhfr⁻* and CHO S cells, respectively. It is clear that the CHO S has the highest glucose catabolism and the CHO/*dhfr⁻* has the lowest glucose catabolism. Intracellular metabolism of carbon and energy is crucial to cell growth, heterologous protein expression and other cellular activities. Therefore, we investigated the expression levels of the proteins involved in glycolysis and TCA cycle.

As presented in the glycolysis pathway in Fig. 2, the ATP-dependent hexokinase (*hkdc,* UniRef100_Q91W97) showed low expression in all three CHO sublines. Since the reaction of "glucose → glucose-6P" catalyzed by hexokinase is the first step in glycolysis, the up-regulation of hexokinase could improve the efficiency of glucose consumption and catabolism. Five enzymes in the glycolysis pathway of CHO cells, namely glyceraldehyde 3-phosphate dehydrogenase (*gapdh,* UniRef100_P04797), ADP-dependent phosphoglycerate kinase (*pgk,* UniRef100_P09411), enolase (*eno,* UniRef100_P04764), ADP-dependent pyruvate kinase (*pkm,* UniRef100_Q6P7S0), and lactate dehydrogenase (*ldh,* UniRef100_P04642), had relatively higher expression levels (Fig. 2). The up-regulation of Pgk and Pkm could enhance glycolysis by providing more ATP through "1,3-biphosphoglycerate → glyceraldehyde 3-phosphate" and "phosphoenolpyruvate → pyruvate". Pyruvate is a key metabolic intermediate subsequently entering into the TCA cycle within mitochondria or converting into lactate within cytosol, so the higher levels of Pkm provide TCA cycle raw materials effectively for metabolism of carbon and energy in these cells.

As shown in Fig. 3, almost all the enzymes in TCA cycle except ketoglutarate dehydrogenase (*ogdh*) had been detected and analyzed. The heat map showed that the ATP-dependent citrate lyase (*acly,* UniRef100_Q3V117) that catalyzes the formation of oxaloacetate and acetyl-CoA had significantly high expression in CHO S with high spectra count of 71, indicating a good target to up-regulate in cell engineering. In addition, six proteins showed relatively higher expression levels and played important roles in TCA cycle in CHO/*dhfr⁻*, including citrate synthase (*cs,* UniRef100_G3V936), aconitate hydratase (*aco2,* UniRef100_Q9ER34), isocitrate dehydrogenase (*idh3a,* UniRef100_F1LNF7), succinyl-CoA synthetase (*suclg1,* UniRef100_P13086), malate dehydrogenase 2 (*mdh2*, UniRef100_P04636), and fumarate hydratase (*fh,* UniRef100_P14408).

3.4 Protein expression: transcription and translation

Heterologous protein expression is a complex process including transcription, translation, post translational modifications and folding, which can significantly affect the cellular dynamics of host cells. Proteome of CHO cells reflecting the host cellular machinery will facilitate our understanding of the protein production bottlenecks.

The proteins associated with transcription that had obviously different expression levels among the three CHO sublines are summarized in Table 2. The UniRef ID, protein name, gene name, MS counts and probability were reported. Seven of the transcription correlated proteins showed higher expression levels in CHO K1 than in CHO S or CHO/*dhfr⁻*. These seven proteins include ribonucleoside-diphosphate reductase (*rrm1*), protein Ascc3l1 (*snrnp200*), nucleolin (*ncl*), DEAH (Asp-Glu-Ala-His) box polypeptide 15 (*dhx15*), nucleolin-related protein NRP (*nrp*), pre-mRNA-processing-splicing factor 8 (*prpf8*), and nuclear ribonucleoprotein F (*hnrnpf*). CHO K1 subline is capable to produce heterologous proteins at commercial scale, and the reported high titer (e.g. > 5 g/L) of therapeutic proteins was produced by using CHO K1 cells [29]. These seven host cell proteins in transcription could contribute to the high production of biopharmaceuticals by CHO K1 cells.

The proteins in translation process that showed distinct expression levels among three CHO sublines are listed in Table 3. The six enzymes that correlate with high capability of heterologous protein expression in CHO K1 cells, including serine/arginine-rich-splicing factor 1 (*srsf1*), elongation factor 1-gamma (*eef1g*), 40S ribosomal protein S12 (*rps12*), 40S ribosomal protein S4, X isoform (*rps4x*), 40S ribosomal protein S9 (*rps9*), and 60S ribosomal protein L3 (*rpl3*), were identified (Table 3). Another six proteins, namely translation initiation factor 3 (*eif3l*), heat shock protein HSP 90-alpha (*hsp90aa1*), rps16 protein (*rps16*), 60S ribosomal protein L18 (*rpl18*), 60S ribosomal protein L9 (*rpl9*), and regulator of nonsense transcripts 1 (*upf1*), were found to be expressed at the highest levels in CHO S cells (Table 3). All three CHO sublines expressed significantly high levels of elongation factor 2 (*eef2*) with MS counts of 118–130 and elongation factor 1-alpha 1 (*eef1a1*) with MS counts of 65–74. Elongation factors have been cloned to optimize expression vector and improved biopharmaceutical production [30]. The high levels of elongation factors detected in this study also confirmed that these three CHO sublines are good host cells to produce heterologous proteins.

3.5 Protein expression: post-translational modification and refolding

CHO cells enable glycosylation and sialylation of polypeptides in endoplasmic reticulum and Golgi, which is important for the refolding, stability and bioactivity of synthesized proteins. N-glycosylation begins with the addition of core oligosaccharide ($Glc_3Man_9GlcNAC_2$) to the specific asparagine residues of the nascent polypeptides, which is catalyzed by oligosaccharyltransferases [31]. We found that all four glycosyltransferase subunits, i.e. subunit 1 (*rpn1*), STT3A (*stt3a*), STT3B (*stt3b*) and 48 kDa subunit (*ddost*), were expressed at low levels in CHO/*dhfr⁻* cells (Table 4), suggesting that the glycosylation of heterologous therapeutic proteins could be improved by up-regulating the expression of glycosyltransferase in CHO/*dhfr⁻* cells. Multiple enzymes are involved in the reactions post glycosylation, such as the glucosidase (*ganab*) that trims glucose, the lectin chaperones (*calr*, *canx*) that recognize and bind to monoglucosylate glycoforms ($Glc_1Man_9GlcNAC_2$ protein) as a folding acceleration signal, and the UDP-glucose:glycoprotein glucosyltransferase (*uggt1*) that senses misfolded glycoprotein and properly folded glycoprotein [32]. All these enzymes were expressed at high levels in CHO S cells (Table 4), led us to speculate that the glycoprotein folding cycle, also called calnexin/calreticulin cycle, is very efficient in the CHO S subline.

We also investigated the enzymes facilitating protein folding. As shown in Table 4, we found that both CHO S and CHO/*dhfr⁻* cells had high expression levels of the 78 kDa glucose-regulated protein (*hspa5*, a.k.a. immunoglobulin heavy chain binding proteins/"BiP/Grp78") that binds the nascent nonglycosylated proteins and/or supports protein refolding [33], and the protein disulphide isomerases (*pdia3*) and the peptidyl-prolyl cis-trans isomerase (*ppib*) that directly catalyze the rate-limiting steps in protein folding [34, 35].

3.6 Strategies to engineer host cell

In this study, the intracellular proteins involved in the regulation of cell growth, glycolysis, TCA cycle, transcription, translation and glycosylation were studied and compared among three different CHO hosts. The proteomic analysis indicated some possible strategies of

rational host cell engineering for fast cell growth, high protein expression and preferable protein quality, which warrants further evaluation and studies.

Cell growth regulation—Over-expression of anti-apoptotic genes, e.g., *bcl-2* or *bcl-xL*, has been tried to increase the viability of CHO cells, thereby the protein production [36, 37]. Meanwhile, gene regulation and culture condition optimization has also been used to induce G1 phase arrest during cell cycle to improve cellular metabolism [38, 39]. Different from those previous studies, the comparative proteomics in our study has identified some cytoskeletal proteins (i.e. filamin and vimentin) with significantly different expression levels among the three CHO sublines tested. These cytoskeletal proteins can maintain cell shape, keep intracellular transport and affect the formation of mitotic spindles for cell division in addition to the regulation of protein translation process [40]. Thus, we suggest that by manipulating the expression levels of these cytoskeleton-associated proteins, the cell growth and protein production in CHO cells may be improved.

Protein expression regulation—Several strategies, including host cell engineering, expression vector optimization, high producing cell line development, and production process parameters optimization, can be used to improve protein production. Cell engineering is a powerful tool to enhance protein production but gene manipulation requires a comprehensive understanding of host cell regulation of transcription, translation and post-translational modification. Studies have shown that the introduction of an artificial zinc finger transcription factor (ZFP-TF) in CHO cells has improved antibody production by 10-fold [41], and the overexpression of E2F-1 transcription factor has led to 20% increase of cell viability [42]. In our current study, we have identified a few transcription regulators in CHO S (*snrnp200* and *prpf8*) and CHO/*dhfr⁻* (*rrm1*) cells, of which the up-regulation could be used to improve transcription efficiency. Translation elongation factor has been used in vector optimization, but the translation initiation is a key rate-limiting step that is more desirable to control the translation [43]. Our proteomics results suggest that the translation initiation factors, 40S ribosomal protein and 60S ribosomal protein, can be used to enhance the translation in CHO/*dhfr⁻* cells. We have also found that the elongation factors are expressed at high levels in all three CHO sublines, so to further up-regulate the expression of translation elongation factors is not a good choice for host cell engineering.

Protein quality regulation—Reinhart *et al.* reported that the choice of CHO subline would affect the glycan structure of the antibodies produced besides cell culture conditions [44]. O'Callaghan *et al.* also reported that CHO clonal hosts had different glycosylation capability [45]. Therefore, the genetic engineering of the post-translational modification of CHO host cells can be an effective approach to enhance clinical efficacy of therapeutic proteins [46]. The proteomic analysis in our study suggests that the overexpression of the glycosyltransferase may be able to improve protein quality in CHO/*dhfr⁻* cells. And at the same time, the post-glycosylation modification could also be improved by up-regulating the expression of the correlated enzymes. Since the enzymes that catalyze protein refolding have high expression levels in all three CHO hosts, the results suggest that it may not be practical to manipulate the refolding process.

4. Conclusions

In this study, comparative proteomics was applied to systematically characterize and compare the expression levels of intracellular proteins in three different CHO host cell sublines (CHO K1, CHO S, and CHO/$dhfr^-$). The study provides new insight into the host cell regulation of cell growth, metabolism as well as protein production and protein quality, and the potentially associated host regulators were analyzed. Finally, the strategies to engineer different CHO host cells were also proposed, which may also provide a guideline for the design of a novel CHO host. The work could be considered as a reference for CHO subline selection, rational cell engineering and even bioprocess optimization to effectively produce biopharmaceuticals with high titer and better quality.

Acknowledgments

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Highlights

- Comparative proteomic analysis of three CHO sublines was performed

- Host cell proteins regulating cell growth and protein expression were identified
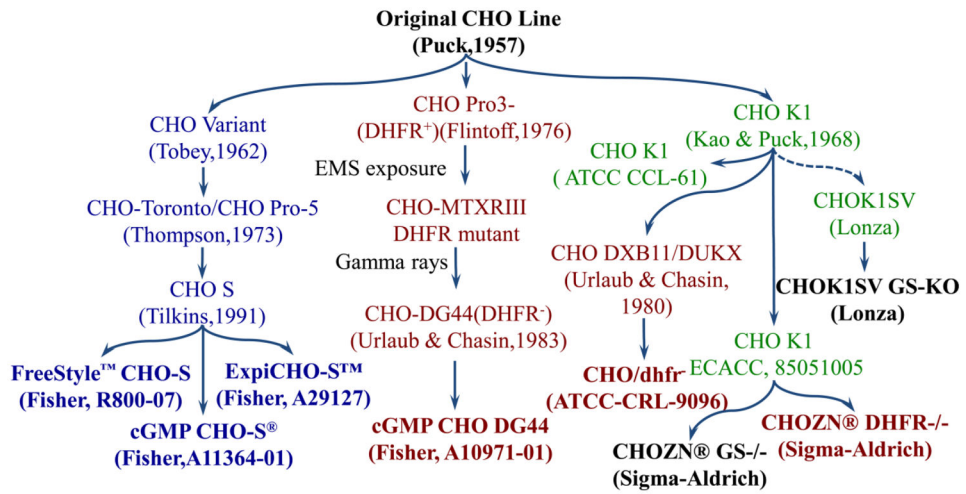
- Rational cell engineering strategies were proposed
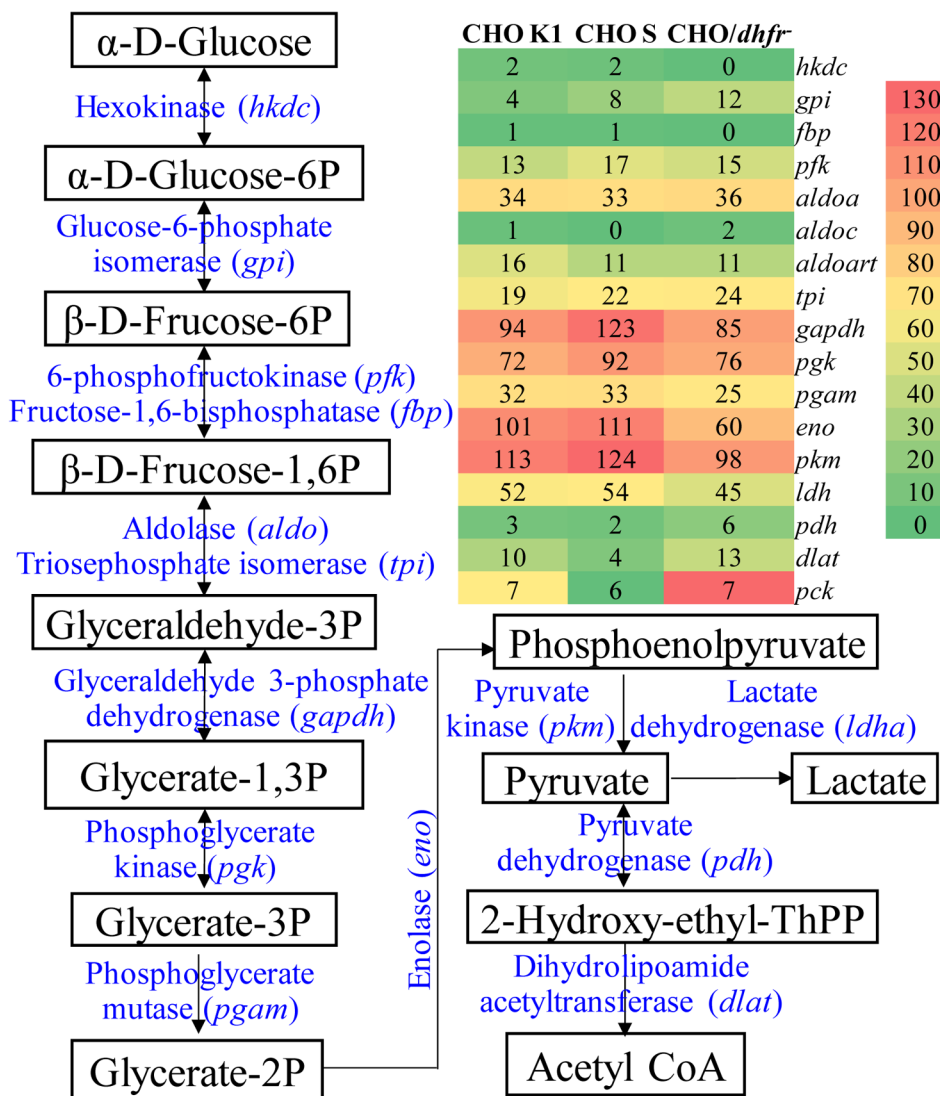
Figure 1.
Cell lineage of CHO cells.

Figure 2.
Heat map of protein expression involved in glycolysis. Red: high expression, Yellow: medium expression, and Green: low expression.
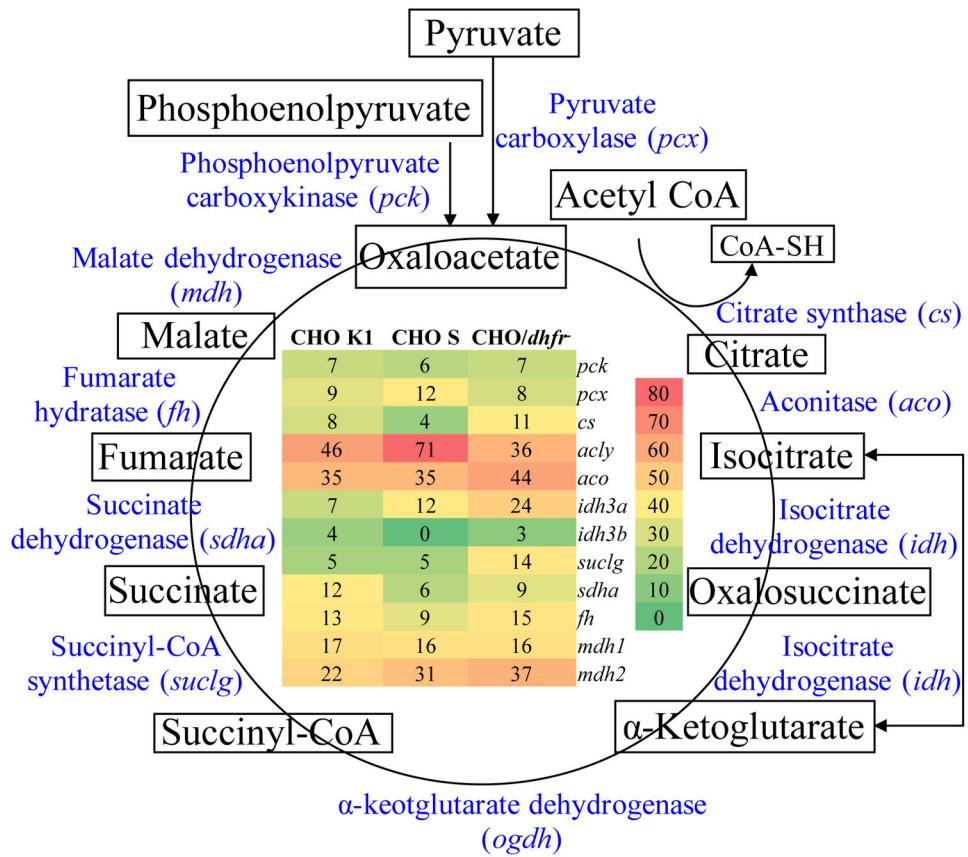
Figure 3.
Heat map of protein expression involved in TCA cycle. Red: high expression, Yellow: medium expression, and Green: low expression.

Table 1

Representative proteins regulating cell growth in CHO K1, CHO S, and CHO/*dhfr*⁻.

Protein		Spectral Count[a]			
UniRef ID[b]	Name[c]	CHO K1	CHO S	CHO/*dhfr*⁻	Probability
G3V6P7	Myosin, heavy polypeptide 9, non-muscle (*myh9*)	52±9.9	175±7.1	36±11	1
Q8BMK4	Cytoskeleton-associated protein 4 (*ckap4*)	19.5±0.7	25±7.1	7.5±0.7	1
B2GV99	Myl6 protein (*myl6*)	15.5±3.5	23±4.2	10±2.8	1
P57780	Alpha-actinin-4 (*actn4*)	60±4.2	86±8.5	41.5±4.9	1
Q58E70	Tpm3 protein (*tpm3*)	17.5±3.5	36.5±3.5	25.5±3.5	1
P09495	Tropomyosin alpha-4 chain (*tpm4*)	23±5.6	45±5.7	35.5±2.1	1
P11440	Cyclin-dependent kinase 1 (*cdk1*)	6.5±2.1	13±0	4±4	1
P68373	Tubulin alpha-1C chain (*tuba1c*)	89.5±0.7	122±12.7	82±4.2	1
Q6P9T8	Tubulin beta-4B chain (*tubb4b*)	93±5.6	127±26.8	87±4.2	1
B7FAU9	Filamin, alpha (*flna*)	134±16.9	127.5±4.9	0±0	1
P20152	Vimentin (*vim*)	216.5±30.4	3±0	55±0.7	1
P45592	Cofilin-1 (*cfl1*)	46.5±2.1	37±0	20.5±0.7	1

Note:

[a]The average spectral count data with standard deviation that were calculated from triplicated experiments were presented.

[b]The annotation was performed using Mouse UniRef100 database. The identified proteins were presented with UniRef100 ID.

[c]All raw MS data, search parameters, search database and summarized data with statistic analysis were deposited to a public repository PeptideAtlas (http://www.peptideatlas.org/, accession no. PASS00963).

Table 2

Key enzymes involved in transcription that showed different expression in CHO K1, CHO S, and CHO/*dhfr⁻*.

Protein		Spectral Count			
UniRef ID	Name	CHO K1	CHO S	CHO/*dhfr⁻*	Probability
P07742	Ribonucleoside-diphosphate reductase (*rrm1*)	41.5±0.7	8±0	1±1.4	1
F1LNJ2	Protein Ascc3l1 (*snrnp200*)	35.5±6.3	2±1.4	23±2.8	1
P13383	Nucleolin (*ncl*)	72.5±6.3	21±1.4	39.5±2.1	1
D3ZD97	DEAH (Asp-Glu-Ala-His) box polypeptide 15 (*dhx15*)	21.5±6.3	8±7.0	18±1.4	1
Q9QZX1	Nucleolin-related protein NRP (*nrp*)	48.5±6.3	19±1.4	34±0	1
Q99PV0	Pre-mRNA-processing-splicing factor 8 (*prpf8*)	30±7.0	0±0	17.5±6.3	1
Q9Z2X1	Nuclear ribonucleoprotein F (*hnrnpf*)	22.5±4.9	21.5±0.7	8.5±2.1	1
Q4JG03	Mcl-1 ubiquitin ligase (*huwe1*)	4±1.4	21.5±9.1	11.5±2.1	1
B7ZWF1	Ddx3x protein (*ddx3x*)	22.5±3.5	36.5±2.1	10±2.8	1
O08629	Transcription intermediary factor 1-beta (*trim28*)	16.5±0.7	21±0	10±1.4	1
A1L333	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 (*ddx5*)	21±1.4	24.5±12.0	12±2.8	1
P63017	Heat shock cognate 71 kDa protein (*hspa8*)	145±24.0	135.5±31.8	123.5±4.9	1

Table 3

Host cell proteins involved in translation regulation in CHO K1, CHO S, and CHO/*dhfr⁻*.

Protein		Spectral Count			
UniRef ID	Name	CHO K1	CHO S	CHO/*dhfr⁻*	Probability
H7BX95	Serine/arginine-rich-splicing factor 1 (*srsf1*)	21.5±0.7	14±1.4	8±1.4	1
Q68FR6	Elongation factor 1-gamma (*eef1g*)	59±2.8	40.5±6.3	27±0	0.99
P63323	40S ribosomal protein S12 (*rps12*)	28±2.8	21.5±2.1	14±1.4	0.93
Q545F8	40S ribosomal protein S4, X isoform (*rps4x*)	28.5±0.7	26±1.4	14.5±2.1	1
D3YWH9	40S ribosomal protein S9 (*rps9*)	27.5±12.0	20.5±7.7	13.5±0.7	1
P21531	60S ribosomal protein L3 (*rpl3*)	24±1.4	16.5±2.1	12±2.8	0.99
G3V7G9	Translation initiation factor 3, subunit L (*eif3l*)	16±2.8	23.5±4.9	6.5±2.1	1
P82995	Heat shock protein HSP 90-alpha (*hsp90aa1*)	95.5±2.1	166±22.6	48.5±3.5	1
Q5CZY9	Rps16 protein (*rps16*)	24±7.0	31±4.2	14.5±2.1	1
P35980	60S ribosomal protein L18 (*rpl18*)	20±2.8	23.5±3.5	11±0	1
P17077	60S ribosomal protein L9 (*rpl9*)	27.5±4.9	28.5±3.5	13.5±3.5	1
Q9EPU0	Regulator of nonsense transcripts 1 (*upf1*)	17.5±4.9	24±4.2	4±1.4	1
F1M9V7	Protein Npepps (*npepps*)	8.5±0.7	14.5±0.7	24±2.8	1
P50475	Alanine--tRNA ligase, cytoplasmic (*aars*)	28.5±6.3	15.5±0.7	32.5±17.6	0.99
P05197	Elongation factor 2 (*eef2*)	126±8.4	130±14.1	118±0	1
P10126	Elongation factor 1-alpha 1 (*eef1a1*)	74±9.9	71±11.3	65±4.2	1

Table 4

Post-translation modification proteins with different expressions in CHO K1, CHO S, and CHO/*dhfr⁻*.

Protein		Spectral Count			
UniRef ID	Name	CHO K1	CHO S	CHO/*dhfr⁻*	Probability
P46978	Glycosyltransferase subunit STT3A (*stt3a*)	13±8.4	6±0	2±0	1
Q3TDQ1	Glycosyltransferase subunit STT3B (*stt3b*)	4±0	2.5±0.7	0.5±0.7	0.99
O54734	Glycosyltransferase 48 kDa subunit (*ddost*)	13±0	13.5±2.1	6.5±3.5	1
Q91YQ5	Glycosyltransferase subunit 1 (*rpn1*)	26.5±2.1	26±5.6	24±0	0.99
P06761	78 kDa glucose-regulated protein (*hspa5*)	113.5±6.3	126.5±5.6	190.5±47.3	1
D3ZAN3	Alpha glucosidase 2 alpha neutral subunit (*ganab*)	23±2.8	33±0	17±7.0	0.99
Q9JLA3	UDP-glucose:glycoprotein glucosyltransferase 1 (*uggt1*)	19.5±0.7	22.5±0.7	13±4.2	1
P18418	Calreticulin (*calr*)	30.5±7.7	51.5±7.7	27.5±2.1	1
P35564	Calnexin (*canx*)	11±1.4	11.5±6.3	8.5±3.5	0.99
P08113	Endoplasmin (*hsp90b1*)	129.5±9.1	170±24.1	142.5±28.9	1
P27773	Protein disulfide-isomerase A3 (*pdia3*)	75±2.8	101.5±7.7	117.5±6.3	1
P24368	Peptidyl-prolyl cis-trans isomerase B (*ppib*)	9±1.4	20±1.4	24.5±2.1	1