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MINI-REVIEW

CHO cells in biotechnology for production of recombinant proteins: current state and further potential

Jee Yon Kim · Yeon-Gu Kim · Gyun Min Lee

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Abstract Recombinant Chinese hamster ovary cells (rCHO) cells have been the most commonly used mammalian host for large-scale commercial production of therapeutic proteins. Recent advances in cell culture technology for rCHO cells have achieved significant improvement in protein production leading to titer of more than 10 g/L to meet the huge demand from market needs. This achievement is associated with progression in the establishment of high and stable producer and the optimization of culture process including media development. In this review article, we focus on current strategies and achievements in cell line development, mainly in vector engineering and cell engineering, for high and stable protein production in rCHO cells. The approaches that manipulate various DNA elements for gene targeting by site-specific integration and *cis*-acting elements to augment and stabilize gene expression are reviewed here. The genetic modulation strategy by "direct" cell engineering with growth-promoting and/or productivity-enhancing factors and omics-based approaches involved in transcriptomics, proteomics, and metabolomics to pursue cell engineering are also presented.

Keywords CHO cells · Cell line development · Vector engineering · Cell engineering · Omics-based approaches

J. Y. Kim · G. M. Lee (✉)
Department of Biological Sciences, Graduate School of Nanoscience & Technology (WCU), KAIST,
373-1 Kusong-Dong, Yuseong-Gu,
Daejeon 305-701, Korea
e-mail: gyunminlee@kaist.ac.kr

Y.-G. Kim
Biotechnology Process Engineering Center, KRIBB,
Daejeon 305-806, Korea

Introduction

The approval of human tissue plasminogen activator (Genentech, USA) as the first therapeutic protein from recombinant mammalian cells in 1986 triggered the emergence of mammalian cell culture as the industry's workhorses for biopharmaceutical production. Although a variety of alternative expression systems exists, such as microbial, insect, transgenic animal and plants, mammalian cells are the principle hosts for the commercial production of therapeutic proteins. Among the 58 biopharmaceuticals approved from 2006 to 2010, 32 are produced from mammalian cells (Walsh 2010). Despite the availability of a number of other mammalian cell lines, such as baby hamster kidney, mouse myeloma-derived NS0, human embryonic kidney (HEK)-293, and the human retina-derived PerC6, nearly 70% of all recombinant therapeutic proteins produced today are made in Chinese hamster ovary (CHO) cells (Jayapal et al. 2007).

The popularity of CHO cells can be attributed to the following reasons. Firstly, since CHO cells have been demonstrated as safe hosts for the past two decades, it may be easier to obtain approval to market the therapeutic proteins from regulatory agencies like the FDA. Secondly, low specific productivity (q), which is one of the disadvantages of using mammalian cells for protein production, can be overcome by gene amplification in CHO cells. For CHO cells, powerful gene amplification systems, such as dihydrofolate reductase (DHFR)-mediated or glutamine synthetase (GS)-mediated gene amplification, are available. Thirdly, CHO cells have the capacity for efficient post-translational modification, and they produce recombinant proteins with glycoforms that are both compatible with and bioactive in humans. Finally, CHO cells can be easily adapted to growth in the regulatory-friendly serum-free (SF) suspension

conditions, a characteristic preferred for large-scale culture in bioreactors. Currently, stirred tank bioreactors over 10,000 L scale are readily used for SF suspension cultures of recombinant CHO (rCHO) cells producing therapeutic antibody.

With an increasing biopharmaceutical market showing an average yearly growth of 35% since 2001, the popularity of CHO cells as the host for commercial production of therapeutic proteins is likely to persist, at least in the near future. Over the past two decades, a more than 100-fold yield improvement of titers in rCHO cell culture has been observed, and this improved product yield has been largely attributed to the development of serum-free medium as well as the optimization of feeding strategies (Hacker et al. 2009). Nevertheless, the demands of the ever-increasing highly competitive market still require cells to be more highly productive and to be grown in bioreactors at higher cell densities under rigorous optimization schemes. In this review, we summarize the current strategy of rCHO cell line development and discuss current achievements in vector engineering and cell engineering that make rCHO cell lines highly productive and robust for large-scale commercial production of therapeutic proteins.

Current strategy for rCHO cell line development

In many cases, a cell line is established based on clones exhibiting high productivity, and process development for manufacturing is performed using that cell line. However, every CHO clone reacts differently to different media and culture conditions, and significant effort is repeatedly required from process development teams whenever new clones are used to produce new therapeutic proteins. Accordingly, to carry out a specific cell culture process, clones suitable for that process should be selected because of clonal variability. For example, clones suitable for SF suspension fed-batch culture are selected for large-scale commercial production of therapeutic antibody. In the same context, many biotech companies have their own therapeutic protein production platform, and clones suitable for that platform are selected to facilitate and speed up the process development of new therapeutic proteins.

Figure 1 shows a representative schematic diagram of the steps involved in cell line development for therapeutic antibody production. Since development of a production cell line requires a considerable investment of time and resources, the gene of interest is first transiently expressed in HEK or CHO cells to test its efficacy and manufacturability. Once proven, the gene of interest is introduced into CHO host cell lines such as DHFR-deficient CHO (DXB11 and DG44) and CHO-K1 mostly by lipofection. The CHO host cell lines have been adapted for growth in SF suspension to save the time and effort of adapting the resulting rCHO production cell line to grow in SF suspension culture.

Once the DNA enters the host cell nucleus, it integrates into the chromosome at a random location. If necessary, high producing parental clones are subjected to gene amplification for further enhancement of q.

Increased heterogeneity, resulting from the random integration of a transfected foreign gene into the chromosome and the altered position of the integrated gene by a gene amplification system, contributes to a wide variety of individual clones regarding q. Since the isolation of clones with a high expression level clearly depends on the number of clones that have been screened, screening procedures such as the limiting dilution method are time-consuming and labor-intensive. As a result, there has been increasing use of efficient high-throughput cell screening systems, such as fluorescence-activated cell sorting (FACS), the ClonePix™ system (Genetix), the LEAP™ system (Cyntellect), and the CellCelector™ system (Aviso).

Clones are selected on the basis of their high expression levels, and their performance is further tested in SF suspension fed-batch culture, which is most widely used for large-scale commercial production of therapeutic antibody. Clones showing the best performance in SF suspension fed-batch culture in lab-scale bioreactors with pre-developed feeding cocktails are usually selected as production cell lines.

Vector engineering in cell line development

As described earlier, the establishment of a high-producing, stable rCHO cell line by gene amplification followed by an extensive screening is time-consuming and labor-intensive. Vector engineering which modulates transcriptional activity facilitates rCHO cell line development. High q can be achieved by modulating transcriptional activity via insertion of various DNA elements into a vector. In addition, the instability of q, which often occurs during long-term culture, may also be overcome by vector engineering. Although the mechanism responsible for reduced q is not fully understood, it is likely to be due to loss of gene copies, silencing of a gene by chromosomal rearrangement, and/or transcriptional inactivation by methylation (Barnes et al. 2003). In this section, the current vector engineering, which includes gene targeting by site-specific integration and a *cis*-acting element to augment gene expression, is discussed as an efficient and reliable method to establish a high-producing, stable rCHO cell line.

Gene targeting by site-specific integration

Site-specific integration of transgenes leads to predictable expression properties and circumvents extensive

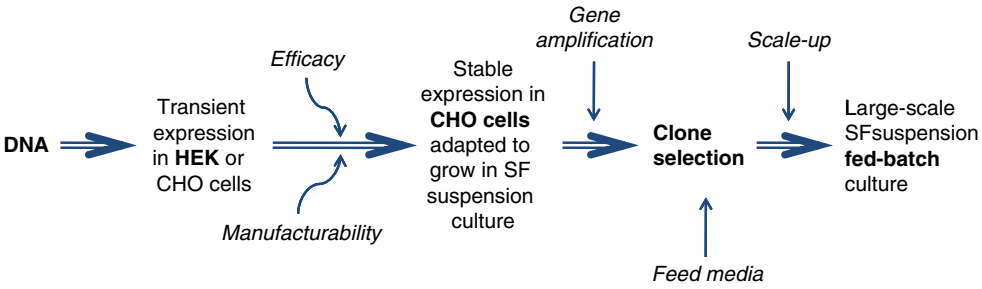

Fig. 1 Schematic diagram of steps involved in cell line development for therapeutic antibody production in rCHO cells

screening. This technique, however, requires information on the chromosomal loci at which a transgene is stably and highly expressed, a so-called hot-spot. After screening the hot-spot, a new desired transgene is introduced into this pre-defined position by vector-based site-specific integration. Successful integration of the desired transgene into the hot-spot may guarantee the fast and efficient selection of high-producing stable clones. For effective recombination and exclusion of unnecessary vector sequences of prokaryotic origin, site-specific integration is conducted using recombinase-mediated cassette exchange strategy. Among various recombinases, Cre and Flp, which respectively recognize *lox*P and *FRT* sequences, are most widely used. Until now, genome engineering by site-specific modification has been applied in various fields of biotechnology.

Kito et al. (2002) first reported the use of gene targeting based on the Cre/*lox*P system for human monoclonal antibody production in rCHO cells. Fluorescence in situ hybridization analysis of rCHO cells constructed using a *lox*P and *FRT* strategy revealed that the antibody genes were all located in the original *FRT*-tagged locus in the gene-targeted and gene-amplified cells (Huang et al. 2007).

Because the *lox*P and *FRT* sequence affects the recombination efficiency of Cre and Flp recombinase, the mutant forms of these sequences, such as *lox*2272 and *m*2 in the Cre/*lox*P system, F_3 and F_5 in the Flp/*FRT* system should be evaluated. To this end, a simple and accurate analysis system was developed to estimate recombination efficiency using FACS to compare the efficiency of recombination related to various spacer mutants (Kim et al. 2007, 2008; Kim and Lee 2008). Recently, Kameyama et al. (2010) developed an accumulative site-specific gene integration system using a series of Cre-mediated integration reaction with mutated *lox*Ps sequences, allowing the repeated insertion of multiple genes into one target site.

Besides the Cre/Flp-based gene targeting, various endonucleases for genome engineering have been also applied. The endonucleases include chemical nucleases, Zinc-finger nucleases, meganucleases, and transcription activators like effectors (TALE-nucleases) (Silva et al. 2011). Because homologous gene targeting is inefficient in mammalian cells, meganucleases are applied to make DNA double-strand break (Cabaniols et al. 2010). Another recent gene targeting system is the DNA recombinase-based mammalian artificial chromosome engineering system (ACE system), which offers a clear advantage of large cloning capacity compared to other systems. An antibody-producing clone with high and stable expression was generated by utilizing this ACE system (Kennard et al. 2009).

cis-acting element for augmenting gene expression

Genetic elements may remodel chromatin to maintain the transgene in an active configuration. Generally, two components of transcription, *trans*-acting factors (transcription factor) and *cis*-acting elements (enhancer sequence and stable element for messenger RNA (mRNA)), are used to augment gene expression. Until now, a number of *cis*-acting elements have been applied to rCHO cells to enhance the expression and stability of protein production.

The most popular *cis*-acting element in rCHO cell culture is a scaffold/matrix attachment region (S/MAR), which maintains the chromatin structure in an "active" configuration through the creation of chromatin loops. This element can increase the expression of a transgene by its boundary effect, although the detailed mechanism is currently unknown. A variety of S/MARs such as chicken lysozyme MAR, human β-globin MAR, and β-interferon SAR have been tested to evaluate their use to augment expression in rCHO cells (Girod et al. 2005; Kim et al. 2004, 2005; Zahn-Zabal et al. 2001). Interestingly, Girod et al. (2005) reported that chicken lysozyme MAR acts as a *cis*-acting element on the transgene expression vector and *trans*-acting factors transfected with a separate plasmid. Furthermore, transgene expression was maximized when the chicken lysozyme MAR was simultaneously introduced with the *cis*-acting element and *trans*-acting factors.

Ubiquitous chromatin opening elements (UCOEs) are another well-known type of *cis*-acting element. Benton et al. (2002) evaluated the augmentation effect of UCOEs on viral human cytomegalovirus (hCMV) promoter-derived gene expression. A dramatic increase in the antibody production of rCHO cells was achieved because of the beneficial effect of maintaining chromatin in an "open" configuration by UCOEs.

Cell engineering in cell line development

To improve the characteristics of rCHO cells in regard to cell growth and foreign protein production, numerous cell engineering strategies have been attempted. Cell engineering has been targeted mainly to increase the time integral of viable cell concentration (IVCC) and/or q.

Figure 2 schematically depicts the effect of cell engineering for improved culture characteristics, resulting in enhanced lifespan and production. ICVV can be increased by enhanced culture longevity, improved specific growth rate (μ), and increased maximum viable cell density. This strategy targets to the reduction of cell death at the end of culture and the induction of cell proliferation at the initial stage. For the aim of enhancing q, the components in regulation of the cell cycle, folding, transport, and secretion are chosen to be key regulators. Reconstruction of the metabolic pathway also improves protein production.

Anti-apoptosis engineering

Cell death is considered an important issue to be dealt with because it affects the viable cell concentration as well as the product quantity and quality (Arden and Betenbaugh 2004). During rCHO cell culture, cell death is triggered by a variety of stresses including nutrient depletion, accumulation of toxic by-products, elevated osmolarity, and shear stress.

Cell death occurs in two forms—necrosis or two types of programmed cell death (PCD), namely, apoptosis and autophagy. While necrosis is a sudden and passive form, PCD is an active, genetically controlled process. Many researchers have recognized PCD as a target to overcome the problem mentioned above. Apoptosis has gained importance in mammalian cell culture, including that of rCHO cells for therapeutic protein production over the last two decades. The prevention of apoptosis by establishing apoptosis-resistant rCHO cells appears to be beneficial.

The apoptotic process is regulated by either activation or suppression of members of the Bcl-2 family proteins which includes three groups: anti-apoptotic Bcl-2-like proteins (Bcl-2, Bcl-xL, Bcl-w, and Mcl-1), pro-apoptotic Bax-like proteins (Bax, Bak, and Bok), and pro-apoptotic BH3-only proteins (Bim, Bad, Bid, PUMA, and Noxa) (Adams and Cory 2001). Considering their function as key regulators in apoptosis, the activation of anti-apoptotic Bcl-2-like proteins and suppression of pro-apoptotic factors is of particular interest in delaying the onset of apoptosis.

Figure 3 illustrates the genetic engineering strategies employed to overcome apoptosis in rCHO cells. Previously, it was found that apoptosis in rCHO cells was alleviated by the overexpression of anti-apoptotic proteins that include Bcl-2, Bcl-xL, and Mcl-1, or down-regulation of pro-apoptotic proteins such as Bak and Bax, resulting in increased protein production (Chiang and Sisk 2005; Cost et al. 2010; Kim and Lee 2001; Majors et al. 2009; Meents et al. 2002a; Tey et al. 2000).

Apoptotic signal is mediated by a caspase–cascade system, which is a series of proteolytic cascade in caspase by other activated cleaved caspases. Because the caspases play an essential role in apoptosis regulation including induction, transduction, and amplification of signals, the suppression of caspase activation is a promising strategy. Caspases can be divided functionally into two groups: initiator caspase and effector caspase. Inhibition of caspase-8/caspase-9,

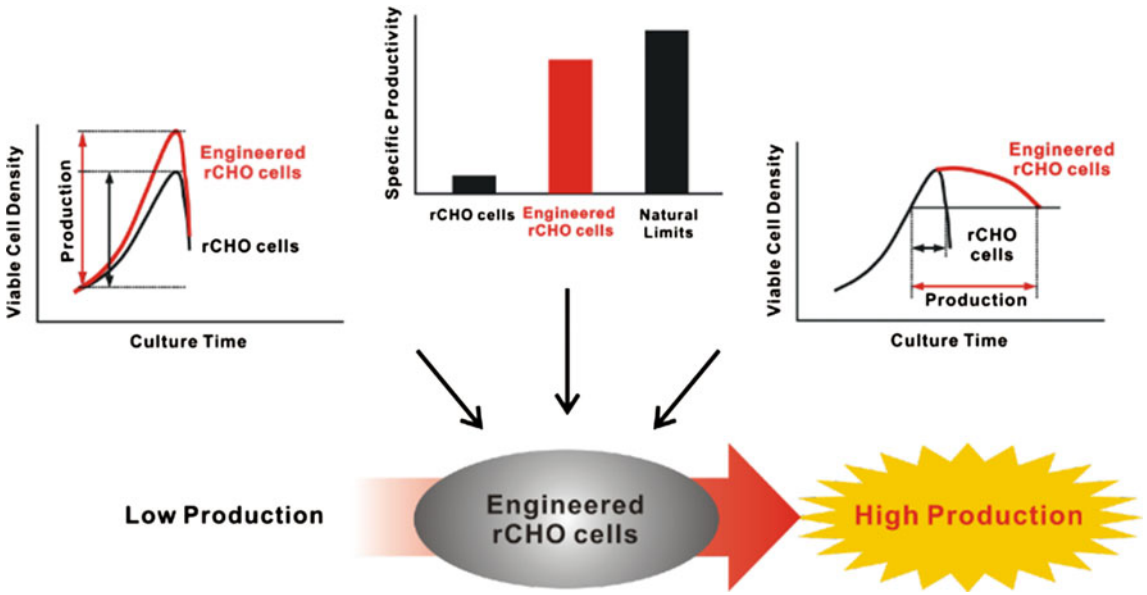

Fig. 2 Schematic depiction of the effect of cell engineering on improved culture characteristics. Cell engineering leads to enhancement of cell growth and q, which reflects on the product titer

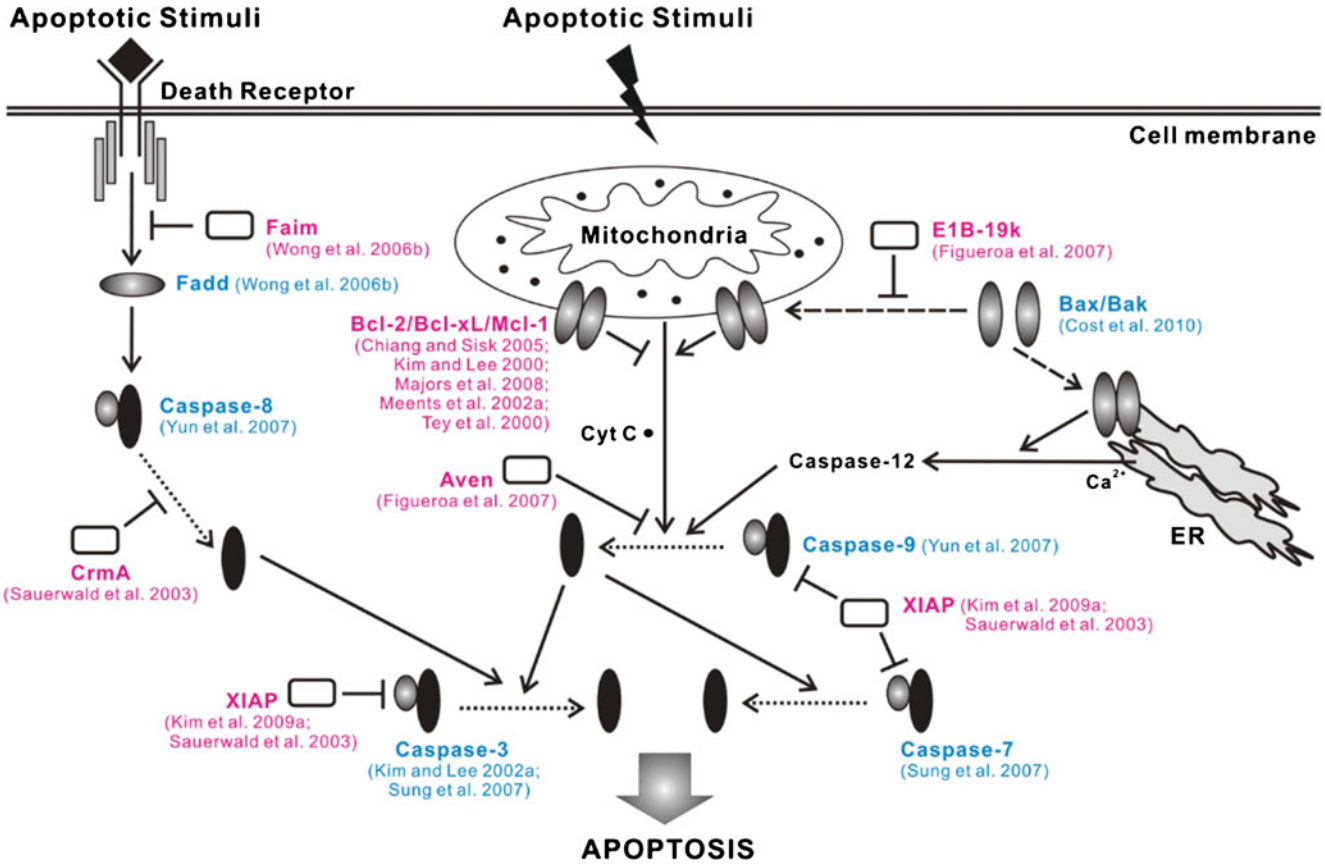

Fig. 3 Schematic diagram illustrating the genetic engineering strategies employed to overcome apoptosis in rCHO cells

initiator caspases, by overexpression of dominant negative mutants and caspase-3/caspase-7, effector caspases, by introduction of antisense RNA or small interfering RNA showed a positive effect on cell growth (Kim and Lee 2002a; Sung et al. 2007; Yun et al. 2007). Another strategy for suppressing caspases is the overexpression of intracellular caspase inhibitors, such as X-linked mammalian inhibitor of apoptosis, an inhibitor of caspase-3/caspase-7/caspase-9, and protein cytokine response modifier A, a strong inhibitor of caspase-1/caspase-8 and a weak inhibitor of caspase-3/caspase-6 (Sauerwald et al. 2002, 2003).

In addition to CHO cell engineering based on Bcl-2 family proteins and caspases, various other factors have also been applied to inhibiting apoptosis. Overexpression of adenovirus-derived anti-apoptotic E1B-19K, 30Kc6 isolated from silkworm hemolymph, and Aven, the inhibitor of apoptosome activation via interacting Apaf-1, successfully blocked apoptosis progression (Choi et al. 2006; Figueroa et al. 2007). In addition, murine double mutant-2, the inhibitor for the p53 tumor suppressor protein, was used to confer apoptosis resistance (Arden et al. 2007). Human telomerase reverse transcriptase-overexpressing rCHO cells induced the proliferation and promotion of the resistance to apoptosis (Crea et al. 2006).

Interestingly, the reports dealing with two heat shock proteins (HSPs) and taurine transporter proposed that the factors not directly involved in the apoptotic pathway can be good candidates to delay apoptosis with enhanced q (Lee et al. 2009; Tabuchi et al. 2010).

The beneficial effect of the overexpression of anti-apoptotic Bcl-2-like proteins on rCHO culture longevity is common. In contrast, there are conflicting reports on its effectiveness on q (Chiang and Sisk 2005; Lee and Lee 2003; Meents et al. 2002a; Tey et al. 2000). Some found a positive effect on q, while others found no effect on q. These conflicting results may be due to clonal variability which usually occurs in the rCHO cell line development. Inducible overexpression of Bcl-xL, which allows comparison of identical cell populations under different conditions, did not affect q of rCHO cells producing erythropoietin (Kim and Lee 2009). Nevertheless, overexpression of Bcl-2-like proteins does not have a negative effect on q.

The beneficial effect of overexpression of anti-apoptotic Bcl-2-like proteins is highlighted with q-enhancing factors, because the single use of some q-enhancing factors, such as sodium butyrate (NaBu) and hyperosmolality, has a detrimental effect on cell growth. The combined use of anti-apoptosis engineering with q-enhancing factors, e.g., NaBu

or hyperosmolality results in a dramatic increase in foreign protein production in rCHO cell culture (Kim and Lee 2001, 2002b; Kim et al. 2009a; Sung et al. 2007). We found that the maintenance of mitochondria is crucial in inhibiting apoptosis by overexpressing an anti-apoptotic protein from these studies (Sung et al. 2007).

Anti-autophagy engineering

Autophagy, also known as type II PCD, is a catabolic process that takes place through a caspase-independent lysosomal-mediated degradation pathway, which is distinguished from apoptosis, type I PCD (Levine and Klionsky 2004). Inhibition of apoptosis does not guarantee the blocking of autophagy-mediated cell death due to the independency between the two processes. Until now, only apoptosis has been the focus of studies on the alleviation of cell death in rCHO cell culture. Recently, the occurrence of autophagy at the end of batch culture of rCHO cells was observed when the nutrients were exhausted (Hwang and Lee 2008). Inversely, supplementation of nutrients could reduce cell death mediated by both apoptosis and autophagy (Han et al. 2011). In addition, the hyperosmotic condition introduced by feeding media and pH control during fed-batch culture could induce apoptosis and autophagy in rCHO cell culture (Han et al. 2010). Engineered CHO cells with overexpressed Bcl-xL or a constitutively active form of Akt could delay the autophagic cell death induced by nutrient depletion (Hwang and Lee 2009; Kim et al. 2009b).

Proliferation engineering

Successfully engineered rCHO cells with proliferation-related proteins showed an increased growth parameter, μ, and/or maximum viable cell concentration. Although μ can influence the maximum viable cell concentration, the extent of increase in one factor does not correlate with that of the other.

The rCHO cell engineering conducted with cell cycle progression by cyclin-dependent kinase like 3, cyclin E, and E2F-1 cell cycle transcription factor improved μ and/or the maximum viable cell concentration (Jaluria et al. 2007; Majors et al. 2008; Renner et al. 1995). A typical oncogenic protein, c-myc, which also has a role in cell cycle progression, was successfully applied to generate highly proliferative rCHO cells with high μ and maximum viable cell concentration (Kuystermans and Al-Rubeai 2009). Recently, mammalian target of rapamycin, which is known to have various roles in translation, vesicle traffic, cell survival, and cell proliferation, was successfully introduced to rCHO cells to improve key bioprocess-relevant characteristics of rCHO cells, including proliferation and q (Dreesen and Fussenegger 2011). The effectiveness of targets on cell proliferation discovered via omics-based approaches, such as valosin-containing protein (VCP), requiem, Alg-2, and malate dehydrogenase II, have been evaluated (Chong et al. 2010; Doolan et al. 2010; Wong et al. 2006b). The details of omics-based CHO cell engineering will be discussed in a later section.

Cell cycle engineering

A common feature of q-enhanced conditions, including physical (low temperature and hyperosmolality) and chemical (NaBu) perturbations, is cell cycle arrest (Sunley and Butler 2010). Based on this consensus, $p27^{KIP1}$ and $p21^{CIP1}$, cell cycle-regulating factors, have been used in rCHO cell engineering to obtain a similar effect. Fussenegger et al. (1997) achieved the q enhancement by transiently introducing $p27^{KIP1}$ and $p21^{CIP1}$ into secreted alkaline phosphatase (SEAP)-producing CHO cells. The use of cell cycle-arrested rCHO cells by stably overexpressing $p27^{KIP1}$ showed beneficial q-enhancing effects on the production of SEAP and soluble intracellular adhesion molecule-1 (Mazur et al. 1998; Meents et al. 2002b). Although this enhancing mechanism is not fully understood, the expanded metabolic consumption concomitant with increased consumption rates of oxygen/glutamine/glucose and an intracellular pool of AMP/ADP/ATP may partly be affected (Carvalhal et al. 2003). Similarly, $p21^{CIP1}$ overexpression can arrest the cell cycle and growth, leading to improved q accompanied by increased cell volume and biogenesis of mitochondria and ribosomes (Bi et al. 2004). The combinatorial strategy with growth-enhancing factor, Bcl-xL, and its stabilizer, C/EBPα (CCAAT/enhancer binding protein α), resulted in a further enhancement of cell growth (Astley and Al-Rubeai 2008; Fussenegger et al. 1998).

Chaperone engineering

The bottleneck in enhancing q is believed to be a post-translational step evidenced by the irrelevance of secreted protein level with increased gene copy number or mRNA level (Mohan et al. 2008). The endoplasmic reticulum (ER)-resident proteins have the major role in protein folding, so these proteins, mainly chaperone, were thought to be a good resource for enhancing q. Numerous reports dealing with chaperone engineering have revealed mixed results, depending on the overexpressed chaperones, target therapeutic protein, and expression system (Mohan et al. 2008). Engineered rCHO cells with overexpression of ERp57 and co-overexpression of calnexin/calreticulin exhibited q-enhancing effect on thrombopoietin (TPO) production (Chung et al. 2004; Hwang et al. 2003). Overexpression of PDI, the most studied chaperone for rCHO cell engineering,

could enhance q depending on the target protein. It appears to positively affect the antibody-producing rCHO cells, but not the rCHO cells producing TPO, interleukin-15, or a tumor necrosis factor receptor:Fc fusion protein (Borth et al. 2005; Davis et al. 2000; Mohan et al. 2007). However, in the recent report by Hayes et al. (2010), the transient expression of PDI family proteins, PDI, ERp72, or pancreatic PDI (PDIp), did not result in any improvement in the antibody productivity of rCHO cells, although this was not confirmed with stable rCHO cells. The PDI effectiveness may also depend on the q values of the rCHO cells used. The extent to which q_{Ab} was enhanced by the application of low temperature decreased as q of the antibody-producing rCHO cells used increased (Yoon et al. 2004).

Unfolded protein response-based engineering

The ER has a major role in protein processing and the transmission of signals, including signal transduction pathway for unfolded protein response (UPR) to maintain cellular homeostasis (Ron and Walter 2007). A common feature of UPR signaling pathway is elevated gene transcription encoding various ER-resident chaperones. The regulation of components in the UPR signaling pathway has drawn attention in rCHO cell engineering, considering the important roles of chaperones as described in the earlier section.

X-box binding protein 1 (XBP-1) is a protein which has been widely explored in UPR signaling pathway. Tigges and Fussenegger (2006) revealed that XBP-1s (a spliced form of XBP-1) overexpression can increase q of various therapeutic proteins, which q is not increased by XBP-1u (an unspliced form of XBP-1) in transient and stable expression studies. Similarly, it could be applied to fed-batch culture, resulting in enhanced q and titer of antibody with physicochemical properties similar to those of non-engineered cells (Becker et al. 2008). Furthermore, recent reports suggested that this strategy is specifically effective when it is applied to rCHO cells experiencing secretion bottleneck (Ku et al. 2008).

Another possible strategy is to restore the attenuation of translation in response to ER stress. Activating transcription factor (ATF4), one of the key regulators in UPR system, can return the signal of translational attenuation by phosphorylation of eukaryotic initiation factor-2α via induction of growth arrest and DNA damage inducible protein 34 (GADD34). In two reports with ATF4 and GADD34, the engineered rCHO cells with these proteins showed the enhanced q of antithrombin III (AT-III) (Ohya et al. 2008; Omasa et al. 2008). Interestingly, overexpression of XBP-1 failed to enhance production in AT-III.

Secretion engineering

In vesicle trafficking, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) have a central role in membrane fusion between transport vesicles and the target membrane (Toonen and Verhage 2003). The interaction of Sec1/Munc18 (SM) proteins with SNAREs is essential for regulating the SNARE-mediated fusion event. In an effort to increase q by relieving the bottleneck in secretion, components related to the secretary pathway have been introduced for rCHO cells. SNAREs (SNAP-23 and VAMP8) and SM protein (Sly1 and Munc18c)-based secretary engineering showed similar results (Peng et al. 2011; Peng and Fussenegger 2009).

The engineered rCHO cells by these proteins could increase the antibody titer and specific antibody productivity (q_{Ab}) by boosting the secretion, although cell growth was negatively affected. The secretion engineering strategy with ceramide-transfer protein (CERT), which efficiently transfers ceramide from the ER to the Golgi, resulted in an increase in q. Mutant CERT with increased transfer activity showed better enhancement of q than that achieved with wild-type CERT (Florin et al. 2009).

Metabolic engineering

Accumulation of ammonia and lactate during rCHO cell culture is a major concern because they adversely affect cell growth and product quality (Lao and Toth 1997; Yang and Butler 2000). These two toxic waste products are generated from energy sources, such as glutamine and glucose.

The rCHO cells genetically modified with relevant metabolic proteins have been developed for efficient utilization of metabolites. To reduce the ammonia produced from glutamine, a less ammoniagenic substrate, glutamate, was used. As a result, rCHO cells expressing GS, which catalyzes glutamate with ammonia to yield glutamine, was generated. They showed less ammonia production in the glutamate-based culture (Zhang et al. 2006). For a similar purpose, carbamoyl phosphate synthetase I and ornithine transcarbamoylase involved in urea cycle were introduced to rCHO cells, resulting in decreased ammonia accumulation (Park et al. 2000).

Wlaschin and Hu (2007) evaluated the efficiency of the fructose-specific transporter (GLUT5)-transfected rCHO cells for utilizing fructose as an alternative to glucose and its effectiveness on lactate production. Although all GLUT5-overexpressed individual clones could utilize fructose as a carbon source, only the clone with an appropriate expression level exhibited reduced lactate production. Several kinds of genetic modulation strategies to change metabolic pathways related with pyruvate, such as introducing pyruvate

carboxylase or disrupting lactate dehydrogenase A/pyruvate dehydrogenase kinases, have been investigated (Kim and Lee 2007a, b; Zhou et al. 2011).

Omics-based approach in cell line development

Omics-based approaches, such as transcriptomics, proteomics, and metabolomics, have been used in the whole process of developing rCHO cell-based production in both upstream and downstream processes. They range from clone selection, cell engineering, culture media, and culture environments in the upstream process, to protein purification and characterization in the downstream process (Gupta and Lee 2007).

A potential gene of interest has been discovered from basic research, so-called direct cell engineering. However, with the recent advances in omics tools, the detailed detection of global changes in DNA/RNA, protein, and metabolites is possible. In this section, we will review the omics-based approaches for CHO cell engineering.

Transcriptomics

Transcriptomics is the global analysis of genomic information at the transcription level by chasing mRNA expression. Among various tools for genomic analysis, DNA microarray is probably the most efficient tool for the cell culture processes, considering its massiveness and cost.

Initially, due to insufficient Chinese hamster sequence information available for probe design, non-CHO-derived DNA arrays including mouse or rat-derived DNA array were used and assessed regarding their feasibility for CHO cells (Baik et al. 2006; De Leon Gatti et al. 2007). Genomic resources have been mined through the bacterial artificial chromosome-based library and expressed sequence tag-based library (Kantardjieff et al. 2009; Omasa et al. 2009). As the use of defined sequences in proprietary and non-proprietary CHO-derived microarray has been increasing, transcriptomic analysis has become popular in rCHO cell culture. The recent public availability of the genome sequence of CHO will expedite transcriptomic study (Xu et al. 2011).

Comparative transcriptome analysis using DNA microarray in rCHO cells has been done under q-enhancing culture conditions, including low culture temperature (Baik et al. 2006; Yee et al. 2009), high osmotic condition (Shen et al. 2010), and NaBu treatment (Yee et al. 2008). In addition, similar approaches have been performed with different rCHO cell lines with high μ (Doolan et al. 2010) or high q (Nissom et al. 2006) and recombinant human bone morphogenetic protein-2 (rhBMP-2)-producing rCHO cells with PACEsol overexpression (Doolan et al. 2008). Furthermore, comparative transcriptomic analysis has been performed to find key factors regulating the apoptosis which occurs at the later stages of batch and fed-batch cultures. Four apoptosis-related genes, Fadd, Faim, Alg-2, and Requiem, were identified as a result (Wong et al. 2006a). These identified proteins were applied to anti-apoptotic CHO cell engineering by their overexpression or knockdown (Wong et al. 2006b). Nowadays, a transcriptomic result is often combined with proteome changes to consider transcriptional changes and post-transcriptional alteration (Baik et al. 2006; Doolan et al. 2010; Kantardjieff et al. 2010; Nissom et al. 2006; Yee et al. 2008).

Another fascinating resource in transcriptomics is microRNA (miRNA), which is single-stranded, non-coding RNA (18–25 nucleotides in length) and complementary to the mRNA. It can regulate global gene expression at the post-transcriptional level by mRNA cleavage or translational repression, or both. The miRNA is an attractive alternative in CHO cell engineering due to regulation of multiple targets, easy introduction into cells, and reduction in metabolic burden (Müller et al. 2008).

Gammell et al. (2007) have identified miR-21, a growth inhibitory miRNA, as being up-regulated during stationary phase growth induced either by temperature shift to low temperature or during normal batch culture. Similarly, introduction of miR-16 and let-7b, miRNAs considered as significant change in batch culture of HEK293 cells using miRNA microarray, could control the cell cycle via down-regulating of cell cycle-related proteins (Koh et al. 2009). Furthermore, miR-7 was found to be down-regulated at low culture temperature, and its overexpression could significantly increase q in CHO cells at 37°C (Barron et al. 2011). With advances in miRNA profiling technology, the study of miRNA to identify cellular targets for CHO cell engineering will increase.

Proteomics

Two-dimensional gel electrophoresis (2-DE) combined with mass spectrometric analysis is a widely used proteomic tool for identifying proteins changed under specific conditions in rCHO cells, as summarized in Table 1.

To enhance q, several strategies have been attempted in rCHO cell culture through physical or chemical manipulation of environmental perturbations (O'Callaghan and James 2008). Temperature and osmolarity are the key factors in the cell culture process. It is well-known that rCHO cells under hypothermia and hyperosmolarity show enhanced q. To understand the intracellular events, proteomic studies have been investigated under these conditions (Baik et al. 2006; Kantardjieff et al. 2010; Kaufmann et al. 1999; Kumar et al. 2008; Lee et al. 2003). Similarly, the effects of various chemical components, such as NaBu, DMSO, zinc sulfate,

Table 1 Comparative proteomics under specific conditions in rCHO cells

Comparison	Product	Culture condition	Reference
Temperature shift (30°C)	SEAP	Serum/adherent/T-flask/batch	Kaufmann et al. (1999)
Temperature shift (33°C)	EPO	Serum/adherent/T-flask/batch	Baik et al. (2006)
Temperature shift (31°C)	–	Serum/suspension/spinner flask/batch	Kumar et al. (2008)
Temperature shift (33°C) 2 mM Butyrate	Antibody	SFM/suspension/shaking flask/batch	Kantardjieff et al. (2010)
Hyperosmotic (450 mOsm/kg)	Antibody	Serum/adherent/T-flask/batch	Lee et al. (2003)
0.5 mM Butyrate 80 μM Zinc sulfate 1.5 μg/mL Tunicamycin	hGH	SFM/adherent/T-flask/batch	Van Dyk et al. (2003)
3 mM Butyrate	hTPO	SFM/suspension/shaker flask/batch	Baik et al. (2008)
1 mM Butyrate	IFN-γ	Serum/adherent/T-flask/batch	Yee et al. (2008)
1.5% DMSO	HBsAg	Serum/adherent/T-flask/batch	Li et al. (2006)
Cytochalasin D	SEAP	Serum/adherent/T-flask/batch	Hayduk and Lee (2005)
Insulin or bFGF	–	Serum/adherent/T-flask/batch	Lee et al. (1996a)
Hydrolysate	Antibody	SFM/suspension/shaking flask/batch	Kim et al. (2011)
3 mM Butyrate Bcl-xL overexpression	EPO	SFM/suspension/bioreactor/batch	Baik and Lee (2010)
Bcl-xL overexpression	–	SFM/suspension/bioreactor/fed-batch	Carlage et al. (2009)
E2F-1 overexpression	–	Serum/adherent/T-flask/batch	Lee et al. (1996b)
PACEsol overexpression	rhBMP-2	CDM/suspension/shaking flask/batch	Meleady et al. (2008)
c-myc overexpression	–	Serum/adherent/T-flask/batch	Kuystermans et al. (2010)
With different metabolic profiles	Antibody	SFM/suspension/bioreactor/fed-batch	Pascoe et al. (2007)
With different growth rates	Antibody	CDM/suspension/shaking flask/batch	Doolan et al. (2010)
With different media compositions	EPO	SFM/suspension/shaker flask/batch	Baik et al. (2011)

tunicamycin, cytochalasin D, and growth factors, which increase μ and/or q, on the changes of rCHO proteome also have also been studied (Baik et al. 2008; Hayduk and Lee 2005; Kantardjieff et al. 2010; Lee et al. 1996a; Li et al. 2006; Van Dyk et al. 2003; Yee et al. 2008). Recently, differently expressed proteins of rCHO cells cultivated in SF medium supplemented with optimized hydrolysates mixtures, yielding the highest μ or the highest q_{Ab}, were identified by comparative proteomics (Kim et al. 2011).

The proteome in rCHO cells engineered by overexpressing and/or down-regulating an effecter protein may be altered. Proteomic approaches to chase global protein changes by Bcl-xL overexpression have been performed to determine the key components (Baik and Lee 2010; Carlage et al. 2009). Influences on rCHO proteome by overexpression of E2F-1, which has a positive function in the cell proliferation, have been evaluated (Lee et al. 1996b). In addition, influences on rCHO proteome by the overexpression of soluble paired basic amino acid cleaving enzyme (PACEsol), which affects q of rhBMP-2, have also been evaluated (Meleady et al. 2008). Recently, the differentially expressed intracellular proteins by overexpressing c-myc, known to be a typical oncogenic protein and enhanced integral viable cell density, were investigated (Kuystermans et al. 2010).

Proteomic results have been successfully applied to rCHO cell engineering. Pascoe et al. (2007) analyzed the protein changes by comparing two different antibody-producing rCHO cells with different lactate profiles in the same fed-batch culture condition. Similarly, the proteome of rCHO cells with high μ was compared with that of cells with low μ. The proteomic result along with the transcriptomic result could provide a potential candidate for the enhancement of cell growth (Doolan et al. 2010). The overexpression of the identified protein, VCP, could improve cell concentration without affecting cell viability, although the effectiveness depended on the clone. Recently, Baik et al. (2011) evaluated the intracellular

responses in rCHO cells adapted to grow in serum-free suspension culture. The overexpression of the identified protein, heat shock protein 70 kDa and/or 60 kDa (HSC70, HSP60), showed 10% to 15% enhanced cell concentration during serum-free adaptation and 15% to 33% reduction of adaptation time. In conventional 2-DE, a quantitative comparison of the proteins on different gels is challenging due to the high degree of gel-to-gel variations, arising from the heterogeneity of polyacrylamide gel in preparation and fluctuations due to changes in electrical current, pH, and temperature during the procedure.

In addition, staining methods, especially silver staining, also contribute to this variation. To overcome the gel-to-gel variation in conventional 2-DE, two-dimensional differential in-gel electrophoresis (2-D DIGE) was developed (Timms and Cramer 2008). Generally, the most widely used labeling reagents in the 2-D DIGE are synthetic *N*-hydroxysuccinimidyl (NHS) ester derivatives of cyanine dyes, Cy2, Cy3, and Cy5 (NHS-Cy2, NHS-Cy3, and NHS-Cy5). The mixture of Cy3 and Cy5-labeled test samples and Cy2-labeled internal standard are resolved on a single 2-D gel and three images of the gel are compared. The 2-D DIGE technique has recently been applied to rCHO cells to evaluate the altered protein expression level under various culture conditions (Baik and Lee 2010; Doolan et al. 2010; Kumar et al. 2008; Meleady et al. 2008).

Metabolomics

Another omics-based approach attracting attention in rCHO cell culture is metabolomics, which is coupled with nuclear magnetic resonance (NMR) or mass spectrometry (MS) to chase low molecular endogenous metabolites. In general, the extent of changes in metabolite is significant compared with the extent of changes in DNA, RNA, and protein. However, unlike the DNA/RNA and protein, the qualitative analysis system for metabolite has not been fully established yet.

Easy amplification of a sample for DNA/RNA and the existence of specific probe, antibody, for protein are benefits for qualitative analysis. In contrast, there is a limitation to analysis of metabolites due to non-amplicable characteristics and the lack of public probes for them. Despite the technical difficulty, there are reports to identify crucial extracellular metabolites or to chase intracellular metabolite flux for media development and apoptosis reduction using liquid chromatography–MS-based or NMR spectroscopy-based metabolomics in rCHO cell culture (Bradley et al. 2010; Chong et al. 2009, 2010, 2011; Goudar et al. 2010). Among them, Chong et al. (2010) demonstrated the typical metabolomics-based CHO cell engineering. They found that malate accumulation was most significant in the medium of rCHO fed-batch culture. Subsequent cell engineering to overexpress malate dehydrogenase II resulted in significant growth improvement in IVCC.

Concluding remarks

Significant improvement in therapeutic protein production of rCHO cells has been achieved with the development of cell line and culture processes involving media development. In particular, the establishment of a highly and stably producing cell line is of utmost importance considering the heterogeneity and different responses to the various environments of rCHO cells. This review focuses on current achievements in vector engineering and cell engineering in cell line development.

The combinatorial cell engineering strategy with regulation of multiple targets is becoming popular. In the same context, the use of miRNA in cell engineering will increase in the future because miRNA can regulate the global gene expression and can easily be introduced into cells. In addition, combinatorial analysis of transcriptomics with proteomics will enter into general use to compensate the transcriptional changes as well as post-transcriptional alteration.

Although the application of vector engineering and cell engineering to rCHO cell culture has various benefits, the limited success, to date, in its application may be due to the insufficient genomic information of rCHO cells. In this regard, a recent report dealing with CHO-K1 genomic sequence can lead to the greatest progression in the current strategy of cell line development (Xu et al. 2011). The finding of hot-spots and better understanding of its neighboring sequence may help to generate high producers, as described in the previous bioinformatics analysis for identifying it in the human genome using a computational method (Girod et al. 2007). In addition, the complete genomic information of CHO cells will be helpful in the identification step in omics-based approaches.

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References

Adams JM, Cory S (2001) Life-or-death decisions by the Bcl-2 protein family. Trends Biochem Sci 26:61–66

Arden N, Betenbaugh MJ (2004) Life and death in mammalian cell culture: strategies for apoptosis inhibition. Trends Biotechnol 22:174–180

Arden N, Majors BS, Ahn SH, Oyler G, Betenbaugh MJ (2007) Inhibiting the apoptosis pathway using MDM2 in mammalian cell cultures. Biotechnol Bioeng 97:601–614

Astley K, Al-Rubeai M (2008) The role of Bcl-2 and its combined effect with p21CIP1 in adaptation of CHO cells to suspension and protein-free culture. Appl Microbiol Biotechnol 78:391–399

Baik JY, Lee GM (2010) A DIGE approach for the assessment of differential expression of the CHO proteome under sodium

butyrate addition: Effect of Bcl-x_L overexpression. Biotechnol Bioeng 105:358–367

Baik JY, Lee MS, An SR, Yoon SK, Joo EJ, Kim YH, Park HW, Lee GM (2006) Initial transcriptome and proteome analyses of low culture temperature-induced expression in CHO cells producing erythropoietin. Biotechnol Bioeng 93:361–371

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

Baik JY, Ha TK, Kim YH, Lee GM (2011) Proteomic understanding of intracellular responses of recombinant Chinese hamster ovary cells adapted to grow in serum-free suspension culture. Biotechnol Prog. doi:10.1002/btpr.685

Barnes LM, Bentley CM, Dickson AJ (2003) Stability of protein production from recombinant mammalian cells. Biotechnol Bioeng 81:631–639

Barron N, Kumar N, Sanchez N, Doolan P, Clarke C, Meleady P, O'Sullivan F, Clynes M (2011) Engineering CHO cell growth and recombinant protein productivity by overexpression of miR-7. J Biotechnol 151:204–211

Becker E, Florin L, Pfizenmaier K, Kaufmann H (2008) An XBP-1 dependent bottle-neck in production of IgG subtype antibodies in chemically defined serum-free Chinese hamster ovary (CHO) fed-batch processes. J Biotechnol 135:217–223

Benton T, Chen T, McEntee M, Fox B, King D, Crombie R, Thomas TC, Bebbington C (2002) The use of UCOE vectors in combination with a preadapted serum free, suspension cell line allows for rapid production of large quantities of protein. Cytotechnology 38:43–46

Bi JX, Shuttleworth J, Al-Rubeai M (2004) Uncoupling of cell growth and proliferation results in enhancement of productivity in p21CIP1-arrested CHO cells. Biotechnol Bioeng 85:741–749

Borth N, Mattanovich D, Kunert R, Katinger H (2005) Effect of increased expression of protein disulfide isomerase and heavy chain binding protein on antibody secretion in a recombinant CHO cell line. Biotechnol Prog 21:106–111

Bradley SA, Ouyang A, Purdie J, Smitka TA, Wang T, Kaerner A (2010) Fermentanomics: monitoring mammalian cell cultures with NMR spectroscopy. J Am Chem Soc 132:9531–9533

Cabaniols JP, Ouvry C, Lamamy V, Fery I, Craplet ML, Moulharat N, Guenin SP, Bedut S, Nosjean O, Ferry G, Devavry S, Jacqmarcq C, Lebuhotel C, Mathis L, Delenda C, Boutin JA, Duchâteau P, Cogé F, Pâques F (2010) Meganuclease-driven targeted integration in CHO-K1 cells for the fast generation of HTS-compatible cell-based assays. J Biomol Screen 15:956–967

Carlage T, Hincapie M, Zang L, Lyubarskaya Y, Madden H, Mhatre R, Hancock WS (2009) Proteomic profiling of a high-producing Chinese hamster ovary cell culture. Anal Chem 81:7357–7362

Carvalhal AV, Marcelino I, Carrondo MJ (2003) Metabolic changes during cell growth inhibition by p27 overexpression. Appl Microbiol Biotechnol 63:164–173

Chiang GG, Sisk WP (2005) Bcl-x_L mediates increased production of humanized monoclonal antibodies in Chinese hamster ovary cells. Biotechnol Bioeng 91:779–792

Choi SS, Rhee WJ, Kim EJ, Park TH (2006) Enhancement of recombinant protein production in Chinese hamster ovary cells through anti-apoptosis engineering using 30Kc6 gene. Biotechnol Bioeng 95:459–467

Chong WP, Goh LT, Reddy SG, Yusufi FN, Lee DY, Wong NS, Heng CK, Yap MG, Ho YS (2009) Metabolomics profiling of extracellular metabolites in recombinant Chinese hamster ovary fed-batch culture. Rapid Commun Mass Spectrom 23:3763–3771

Chong WP, Reddy SG, Yusufi FN, Lee DY, Wong NS, Heng CK, Yap MG, Ho YS (2010) Metabolomics-driven approach for the improvement of Chinese hamster ovary cell growth: overexpression of malate dehydrogenase II. J Biotechnol 147:116–121

Chong WP, Yusufi FN, Lee DY, Reddy SG, Wong NS, Heng CK, Yap MG, Ho YS (2011) Metabolomics-based identification of apoptosis-inducing metabolites in recombinant fed-batch CHO culture media. J Biotechnol 151:218–224

Chung JY, Lim SW, Hong YJ, Hwang SO, Lee GM (2004) Effect of doxycycline-regulated calnexin and calreticulin expression on specific thrombopoietin productivity of recombinant Chinese hamster ovary cells. Biotechnol Bioeng 85:539–546

Cost GJ, Freyvert Y, Vafiadis A, Santiago Y, Miller JC, Rebar E, Collingwood TN, Snowden A, Gregory PD (2010) BAK and BAX deletion using zinc-finger nucleases yields apoptosis-resistant CHO cells. Biotechnol Bioeng 105:330–340

Crea F, Sarti D, Falciani F, Al-Rubeai M (2006) Over-expression of hTERT in CHO K1 results in decreased apoptosis and reduced serum dependency. J Biotechnol 121:109–123

Davis R, Schooley K, Rasmussen B, Thomas J, Reddy P (2000) Effect of PDI overexpression on recombinant protein secretion in CHO cells. Biotechnol Prog 16:736–743

De Leon Gatti M, Wlaschin KF, Nissom PM, Yap M, Hu WS (2007) Comparative transcriptional analysis of mouse hybridoma and recombinant Chinese hamster ovary cells undergoing butyrate treatment. J Biosci Bioeng 103:82–91

Doolan P, Melville M, Gammell P, Sinacore M, Meleady P, McCarthy K, Francullo L, Leonard M, Charlebois T, Clynes M (2008) Transcriptional profiling of gene expression changes in a PACE-transfected CHO DUKX cell line secreting high levels of rhBMP-2. Mol Biotechnol 39:187–199

Doolan P, Meleady P, Barron N, Henry M, Gallagher R, Gammell P, Melville M, Sinacore M, McCarthy K, Leonard M, Charlebois T, Clynes M (2010) Microarray and proteomics expression profiling identifies several candidates, including the valosin-containing protein (VCP), involved in regulating high cellular growth rate in production CHO cell lines. Biotechnol Bioeng 106:42–56

Dreesen IA, Fussenegger M (2011) Ectopic expression of human mTOR increases viability, robustness, cell size, proliferation, and antibody production of Chinese hamster ovary cells. Biotechnol Bioeng 108:853–866

Figueroa B Jr, Ailor E, Osborne D, Hardwick JM, Reff M, Betenbaugh MJ (2007) Enhanced cell culture performance using inducible anti-apoptotic genes E1B-19K and Aven in the production of a monoclonal antibody with Chinese hamster ovary cells. Biotechnol Bioeng 97:877–892

Florin L, Pegel A, Becker E, Hausser A, Olayioye MA, Kaufmann H (2009) Heterologous expression of the lipid transfer protein CERT increases therapeutic protein productivity of mammalian cells. J Biotechnol 141:84–90

Fussenegger M, Mazur X, Bailey JE (1997) A novel cytostatic process enhances the productivity of Chinese hamster ovary cells. Biotechnol Bioeng 55:927–939

Fussenegger M, Schlatter S, Dätwyler D, Mazur X, Bailey JE (1998) Controlled proliferation by multigene metabolic engineering enhances the productivity of Chinese hamster ovary cells. Nat Biotechnol 16:468–472

Gammell P, Barron N, Kumar N, Clynes M (2007) Initial identification of low temperature and culture stage induction of miRNA expression in suspension CHO-K1 cells. J Biotechnol 130:213–218

Girod PA, Zahn-Zabal M, Mermod N (2005) Use of the chicken lysozyme 5′ matrix attachment region to generate high producer CHO cell lines. Biotechnol Bioeng 91:1–11

Girod PA, Nguyen DQ, Calabrese D, Puttini S, Grandjean M, Martinet D, Regamey A, Saugy D, Beckmann JS, Bucher P, Mermod N (2007) Genome-wide prediction of matrix attachment regions that increase gene expression in mammalian cells. Nat Methods 4:747–753

Goudar C, Biener R, Boisart C, Heidemann R, Piret J, de Graaf A, Konstantinov K (2010) Metabolic flux analysis of CHO cells in perfusion culture by metabolite balancing and 2D [13C, 1H] COSY NMR spectroscopy. Metab Eng 12:138–149

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

Hacker DL, De Jesus M, Wurm FM (2009) 25 years of recombinant proteins from reactor-grown cells—where do we go from here? Biotechnol Adv 27:1023–1027

Han YK, Kim YG, Kim JY, Lee GM (2010) Hyperosmotic stress induces autophagy and apoptosis in recombinant Chinese hamster ovary cell culture. Biotechnol Bioeng 105:1187–1192

Han YK, Ha TK, Lee SJ, Lee JS, Lee GM (2011) Autophagy and apoptosis of recombinant Chinese hamster ovary cells during fed-batch culture: effect of nutrient supplementation. Biotechnol Bioeng 108:2182–2192

Hayduk EJ, Lee KH (2005) Cytochalasin D can improve heterologous protein productivity in adherent Chinese hamster ovary cells. Biotechnol Bioeng 90:354–364

Hayes NV, Smales CM, Klappa P (2010) Protein disulfide isomerase does not control recombinant IgG4 productivity in mammalian cell lines. Biotechnol Bioeng 105:770–779

Huang Y, Li Y, Wang YG, Gu X, Wang Y, Shen BF (2007) An efficient and targeted gene integration system for high-level antibody expression. J Immunol Methods 322:28–39

Hwang SO, Lee GM (2008) Nutrient deprivation induces autophagy as well as apoptosis in Chinese hamster ovary cell culture. Biotechnol Bioeng 99:678–685

Hwang SO, Lee GM (2009) Effect of Akt overexpression on programmed cell death in antibody-producing Chinese hamster ovary cells. J Biotechnol 139:89–94

Hwang SO, Chung JY, Lee GM (2003) Effect of doxycycline-regulated ERp57 expression on specific thrombopoietin productivity of recombinant CHO cells. Biotechnol Prog 19:179–184

Jaluria P, Betenbaugh M, Konstantopoulos K, Shiloach J (2007) Enhancement of cell proliferation in various mammalian cell lines by gene insertion of a cyclin-dependent kinase homolog. BMC Biotechnol 7:e71

Jayapal KP, Wlaschin KF, Hu WS, Yap MG (2007) Recombinant protein therapeutics from CHO cells—20 years and counting. Chem Eng Prog 103:40–47

Kameyama Y, Kawabe Y, Ito A, Kamihira M (2010) An accumulative site-specific gene integration system using Cre recombinase-mediated cassette exchange. Biotechnol Bioeng 105:1106–1114

Kantardjieff A, Nissom PM, Chuah SH, Yusufi F, Jacob NM, Mulukutla BC, Yap M, Hu WS (2009) Developing genomic platforms for Chinese hamster ovary cells. Biotechnol Adv 27:1028–1035

Kantardjieff A, Jacob NM, Yee JC, Epstein E, Kok YJ, Philp R, Betenbaugh M, Hu WS (2010) Transcriptome and proteome analysis of Chinese hamster ovary cells under low temperature and butyrate treatment. J Biotechnol 145:143–159

Kaufmann H, Mazur X, Fussenegger M, Bailey JE (1999) Influence of low temperature on productivity, proteome and protein phosphorylation of CHO cells. Biotechnol Bioeng 63:573–582

Kennard ML, Goosney DL, Monteith D, Zhang L, Moffat M, Fischer D, Mott J (2009) The generation of stable, high MAb expressing CHO cell lines based on the artificial chromosome expression (ACE) technology. Biotechnol Bioeng 104:540–553

Kim NS, Lee GM (2001) Overexpression of bcl-2 inhibits sodium butyrate-induced apoptosis in Chinese hamster ovary cells resulting in enhanced humanized antibody production. Biotechnol Bioeng 71:184–193

Kim NS, Lee GM (2002a) Inhibition of sodium butyrate-induced apoptosis in recombinant Chinese hamster ovary cells by constitutively expressing antisense RNA of caspase-3. Biotechnol Bioeng 78:217–228

Kim NS, Lee GM (2002b) Response of recombinant Chinese hamster ovary cells to hyperosmotic pressure: effect of Bcl-2 overexpression. J Biotechnol 95:237–248

Kim SH, Lee GM (2007a) Down-regulation of lactate dehydrogenase-A by siRNAs for reduced lactic acid formation of Chinese hamster ovary cells producing thrombopoietin. Appl Microbiol Biotechnol 74:152–159

Kim SH, Lee GM (2007b) Functional expression of human pyruvate carboxylase for reduced lactic acid formation of Chinese hamster ovary cells (DG44). Appl Microbiol Biotechnol 76:659–665

Kim MS, Lee GM (2008) Use of Flp-mediated cassette exchange in the development of a CHO cell line stably producing erythropoietin. J Microbiol Biotechnol 18:1342–1351

Kim YG, Lee GM (2009) Bcl-xL overexpression does not enhance specific erythropoietin productivity of recombinant CHO cells grown at 33 °C and 37 °C. Biotechnol Prog 25:252–256

Kim JM, Kim JS, Park DH, Kang HS, Yoon J, Baek K, Yoon Y (2004) Improved recombinant gene expression in CHO cells using matrix attachment regions. J Biotechnol 107:95–105

Kim JD, Yoon Y, Hwang HY, Park JS, Yu S, Lee J, Baek K, Yoon J (2005) Efficient selection of stable chinese hamster ovary (CHO) cell lines for expression of recombinant proteins by using human interferon beta SAR element. Biotechnol Prog 21:933–937

Kim MS, Kim WH, Lee GM (2007) A simple analysis system for the estimation of recombination efficiency using fluorescence-activated cell sorting. J Biotechnol 127:373–384

Kim MS, Kim WH, Lee GM (2008) Characterization of site-specific recombination mediated by Cre recombinase during the development of erythropoietin producing CHO cell lines. Biotechnol Bioprocess Eng 13:418–423

Kim YG, Kim JY, Lee GM (2009a) Effect of XIAP overexpression on sodium butyrate-induced apoptosis in recombinant Chinese hamster ovary cells producing erythropoietin. J Biotechnol 144:299–303

Kim YG, Kim JY, Mohan C, Lee GM (2009b) Effect of Bcl-xL overexpression on apoptosis and autophagy in recombinant Chinese hamster ovary cells under nutrient-deprived condition. Biotechnol Bioeng 103:757–766

Kim JY, Kim YG, Han YK, Choi HS, Kim YH, Lee GM (2011) Proteomic understanding of intracellular responses of recombinant Chinese hamster ovary cells cultivated in serum-free medium supplemented with hydrolysates. Appl Microbiol Biotechnol 89:1917–1928

Kito M, Itami S, Fukano Y, Yamana K, Shibui T (2002) Construction of engineered CHO strains for high-level production of recombinant proteins. Appl Microbiol Biotechnol 60:442–448

Koh TC, Lee YY, Chang SQ, Nissom PM (2009) Identification and expression analysis of miRNAs during batch culture of HEK-293 cells. J Biotechnol 140:149–155

Ku SC, Ng DT, Yap MG, Chao SH (2008) Effects of overexpression of X-box binding protein 1 on recombinant protein production in Chinese hamster ovary and NS0 myeloma cells. Biotechnol Bioeng 99:155–164

Kumar N, Gammell P, Meleady P, Henry M, Clynes M (2008) Differential protein expression following low temperature culture of suspension CHO-K1 cells. BMC Biotechnol 8:e42

Kuystermans D, Al-Rubeai M (2009) cMyc increases cell number through uncoupling of cell division from cell size in CHO cells. BMC Biotechnol 9:e76

Kuystermans D, Dunn MJ, Al-Rubeai M (2010) A proteomic study of cMyc improvement of CHO culture. BMC Biotechnol 10:e25

Lao MS, Toth D (1997) Effects of ammonium and lactate on growth and metabolism of a recombinant Chinese hamster ovary cell culture. Biotechnol Prog 13:688–691

Lee SK, Lee GM (2003) Development of apoptosis-resistant dihydrofolate reductase efficient Chinese hamster ovary cell line. Biotechnol Bioeng 82:872–876

Lee KH, Harrington MG, Bailey JE (1996a) Two-dimensional electrophoresis of proteins as a tool in the metabolic engineering of cell cycle regulation. Biotechnol Bioeng 50:336–340

Lee KH, Sburlati A, Renner WA, Bailey JE (1996b) Deregulated expression of cloned transcription factor E2F-1 in Chinese hamster ovary cells shifts protein patterns and activates growth in protein-free medium. Biotechnol Bioeng 50:273–279

Lee MS, Kim KW, Kim YH, Lee GM (2003) Proteome analysis of antibody-expressing CHO cells in response to hyperosmotic pressure. Biotechnol Prog 19:1734–1741

Lee YY, Wong KT, Tan J, Toh PC, Mao Y, Brusic V, Yap MG (2009) Overexpression of heat shock proteins (HSPs) in CHO cells for extended culture viability and improved recombinant protein production. J Biotechnol 143:34–43

Levine B, Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 6:463–477

Li J, Huang Z, Sun X, Yang P, Zhang Y (2006) Understanding the enhanced effect of dimethyl sulfoxide on hepatitis B surface antigen expression in the culture of Chinese hamster ovary cells on the basis of proteome analysis. Enzyme Microb Technol 38:372–380

Majors BS, Arden N, Oyler GA, Chiang GG, Pederson NE, Betenbaugh MJ (2008) E2F-1 overexpression increases viable cell density in batch cultures of Chinese hamster ovary cells. J Biotechnol 138:103–106

Majors BS, Betenbaugh MJ, Pederson NE, Chiang GG (2009) Mcl-1 overexpression leads to higher viabilities and increased production of humanized monoclonal antibody in Chinese hamster ovary cells. Biotechnol Prog 25:1161–1168

Mazur X, Fussenegger M, Renner WA, Bailey JE (1998) Higher productivity of growth-arrested Chinese hamster ovary cells expressing the cyclin-dependent kinase inhibitor p27. Biotechnol Prog 14:705–713

Meents H, Enenkel B, Eppenberger HM, Werner RG, Fussenegger M (2002a) Impact of coexpression and coamplification of sICAM and antiapoptosis determinants bcl-2/bcl-x(L) on productivity, cell survival, and mitochondria number in CHO-DG44 grown in suspension and serum-free media. Biotechnol Bioeng 80:706–716

Meents H, Enenkel B, Werner RG, Fussenegger M (2002b) p27Kip1-mediated controlled proliferation technology increases constitutive sICAM production in CHO-DUKX adapted for growth in suspension and serum-free media. Biotechnol Bioeng 79:619–627

Meleady P, Henry M, Gammell P, Doolan P, Sinacore M, Melville M, Francullo L, Leonard M, Charlebois T, Clynes M (2008) Proteomic profiling of CHO cells with enhanced rhBMP-2 productivity following co-expression of PACEsol. Proteomics 8:2611–2624

Mohan C, Park SH, Chung JY, Lee GM (2007) Effect of doxycycline-regulated protein disulfide isomerase expression on the specific productivity of recombinant CHO cells: thrombopoietin and antibody. Biotechnol Bioeng 98:611–615

Mohan C, Kim YG, Koo J, Lee GM (2008) Assessment of cell engineering strategies for improved therapeutic protein production in CHO cells. Biotechnol J 3:624–630

Müller D, Katinger H, Grillari J (2008) MicroRNAs as targets for engineering of CHO cell factories. Trends Biotechnol 26:359–365

Nissom PM, Sanny A, Kok YJ, Hiang YT, Chuah SH, Shing TK, Lee YY, Wong KT, Hu WS, Sim MY, Philp R (2006) Transcriptome and proteome profiling to understanding the biology of high productivity CHO cells. Mol Biotechnol 34:125–140

O'Callaghan PM, James DC (2008) Systems biotechnology of mammalian cell factories. Brief Funct Genomic Proteomic 7:95–110

Ohya T, Hayashi T, Kiyama E, Nishii H, Miki H, Kobayashi K, Honda K, Omasa T, Ohtake H (2008) Improved production of recombinant human antithrombin III in Chinese hamster ovary cells by ATF4 overexpression. Biotechnol Bioeng 100:317–324

Omasa T, Takami T, Ohya T, Kiyama E, Hayashi T, Nishii H, Miki H, Kobayashi K, Honda K, Ohtake H (2008) Overexpression of GADD34 enhances production of recombinant human antithrombin III in Chinese hamster ovary cells. J Biosci Bioeng 106:568–573

Omasa T, Cao Y, Park JY, Takagi Y, Kimura S, Yano H, Honda K, Asakawa S, Shimizu N, Ohtake H (2009) Bacterial artificial chromosome library for genome-wide analysis of Chinese hamster ovary cells. Biotechnol Bioeng 104:986–994

Park H, Kim IH, Kim IY, Kim KH, Kim HJ (2000) Expression of carbamoyl phosphate synthetase I and ornithine transcarbamoylase genes in Chinese hamster ovary dhfr-cells decreases accumulation of ammonium ion in culture media. J Biotechnol 81:129–140

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

Peng RW, Fussenegger M (2009) Molecular engineering of exocytic vesicle traffic enhances the productivity of Chinese hamster ovary cells. Biotechnol Bioeng 102:1170–1181

Peng RW, Abellan E, Fussenegger M (2011) Differential effect of exocytic SNAREs on the production of recombinant proteins in mammalian cells. Biotechnol Bioeng 108:611–620

Renner WA, Lee KH, Hatzimanikatis V, Bailey JE, Eppenberger HM (1995) Recombinant cyclin E expression activates proliferation and obviates surface attachment of Chinese hamster ovary (CHO) cells in protein-free medium. Biotechnol Bioeng 47:476–482

Ron D, Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 8:519–529

Sauerwald TM, Betenbaugh MJ, Oyler GA (2002) Inhibiting apoptosis in mammalian cell culture using the caspase inhibitor XIAP and deletion mutants. Biotechnol Bioeng 77:704–716

Sauerwald TM, Oyler GA, Betenbaugh MJ (2003) Study of caspase inhibitors for limiting death in mammalian cell culture. Biotechnol Bioeng 81:329–340

Shen D, Kiehl TR, Khattak SF, Li ZJ, He A, Kayne PS, Patel V, Neuhaus IM, Sharfstein ST (2010) Transcriptomic responses to sodium chloride-induced osmotic stress: a study of industrial fed-batch CHO cell cultures. Biotechnol Prog 26:1104–1115

Silva G, Poirot L, Galetto R, Smith J, Montoya G, Duchateau P, Pâques F (2011) Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Curr Gene Ther 11:11–27

Sung YH, Lee JS, Park SH, Koo J, Lee GM (2007) Influence of co-down-regulation of caspase-3 and caspase-7 by siRNAs on sodium butyrate-induced apoptotic cell death of Chinese hamster ovary cells producing thrombopoietin. Metab Eng 9:452–464

Sunley K, Butler M (2010) Strategies for the enhancement of recombinant protein production from mammalian cells by growth arrest. Biotechnol Adv 28:385–394

Tabuchi H, Sugiyama T, Tanaka S, Tainaka S (2010) Overexpression of taurine transporter in Chinese hamster ovary cells can enhance cell viability and product yield, while promoting glutamine consumption. Biotechnol Bioeng 107:998–1003

Tey BT, Singh RP, Piredda L, Piacentini M, Al-Rubeai M (2000) Influence of bcl-2 on cell death during the cultivation of a Chinese hamster ovary cell line expressing a chimeric antibody. Biotechnol Bioeng 68:31–43

Tigges M, Fussenegger M (2006) Xbp1-based engineering of secretory capacity enhances the productivity of Chinese hamster ovary cells. Metab Eng 8:264–272

Timms JF, Cramer R (2008) Difference gel electrophoresis. Proteomics 8:4886–4897

Toonen RF, Verhage M (2003) Vesicle trafficking: pleasure and pain from SM genes. Trends Cell Biol 13:177–186

Van Dyk DD, Misztal DR, Wilkins MR, Mackintosh JA, Poljak A, Varnai JC, Teber E, Walsh BJ, Gray PP (2003) Identification of cellular changes associated with increased production of human growth hormone in a recombinant Chinese hamster ovary cell line. Proteomics 3:147–156

Walsh G (2010) Biopharmaceutical benchmarks 2010. Nat Biotechnol 28:917–924

Wlaschin KF, Hu WS (2007) Engineering cell metabolism for high-density cell culture via manipulation of sugar transport. J Biotechnol 131:168–176

Wong DC, Wong KT, Lee YY, Morin PN, Heng CK, Yap MG (2006a) Transcriptional profiling of apoptotic pathways in batch and fed-batch CHO cell cultures. Biotechnol Bioeng 94:373–382

Wong DC, Wong KT, Nissom PM, Heng CK, Yap MG (2006b) Targeting early apoptotic genes in batch and fed-batch CHO cell cultures. Biotechnol Bioeng 95:350–361

Xu X, Nagarajan H, Lewis NE, Pan S, Cai Z, Liu X, Chen W, Xie M, Wang W, Hammond S, Andersen MR, Neff N, Passarelli B, Koh W, Fan HC, Wang J, Gui Y, Lee KH, Betenbaugh MJ, Quake SR, Famili I, Palsson BO, Wang J (2011) The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. Nat Biotechnol 29:735–741. doi:10.1038/nbt.1932

Yang M, Butler M (2000) Effects of ammonia on CHO cell growth, erythropoietin production, and glycosylation. Biotechnol Bioeng 68:370–380

Yee JC, de Leon Gatti M, Philp RJ, Yap M, Hu WS (2008) Genomic and proteomic exploration of CHO and hybridoma cells under sodium butyrate treatment. Biotechnol Bioeng 99:1186–1204

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

Yoon SK, Hwang SO, Lee GM (2004) Enhancing effect of low culture temperature on specific antibody productivity of recombinant Chinese hamster ovary cells: clonal variation. Biotechnol Prog 20:1683–1688

Yun CY, Liu S, Lim SF, Wang T, Chung BY, Jiat Teo J, Chuan KH, Soon AS, Goh KS, Song Z (2007) Specific inhibition of caspase-8 and −9 in CHO cells enhances cell viability in batch and fed-batch cultures. Metab Eng 9:406–418

Zahn-Zabal M, Kobr M, Girod PA, Imhof M, Chatellard P, de Jesus M, Wurm F, Mermod N (2001) Development of stable cell lines for production or regulated expression using matrix attachment regions. J Biotechnol 87:29–42

Zhang F, Sun X, Yi X, Zhang Y (2006) Metabolic characteristics of recombinant Chinese hamster ovary cells expressing glutamine synthetase in presence and absence of glutamine. Cytotechnology 51:21–28

Zhou M, Crawford Y, Ng D, Tung J, Pynn AF, Meier A, Yuk IH, Vijayasankaran N, Leach K, Joly J, Snedecor B, Shen A (2011) 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 153:27–34