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Research review paper

Improving the secretory capacity of Chinese hamster ovary cells by ectopic expression of effector genes: Lessons learned and future directions

Henning Gram Hansen [a,*], Nuša Pristovšek [a], Helene Faustrup Kildegaard [a], Gyun Min Lee [a,b,**]

[a] The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark
[b] Department of Biological Sciences, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

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ABSTRACT

Chinese hamster ovary (CHO) cells are the preferred cell factory for the production of therapeutic glycoproteins. Although efforts primarily within bioprocess optimization have led to increased product titers of recombinant proteins (r-proteins) expressed in CHO cells, post-transcriptional bottlenecks in the biosynthetic pathway of r-proteins remain to be solved. To this end, the ectopic expression of transgenes (effector genes) offers great engineering potential. However, studies on effector genes have in some cases led to inconsistent results. Whereas this can in part be attributed to product specificity, other experimental and cellular factors are likely important contributors to these conflicting results. Here, these factors are reviewed and discussed with the objective of guiding future studies on effector genes.

Contents

1. Introduction . . . 65
2. CHO host cell lines . . . 65
3. Clonal variation . . . 67
4. Effector gene origin . . . 68
5. Secretion bottleneck and ER stress . . . 68
6. Effector gene dosage . . . 69
7. Expression platforms . . . 70
   7.1. Duration – from transfection to answer . . . 70
   7.2. Gene dosage and combining genes . . . 70
   7.3. Transfection stress and variability . . . 70
   7.4. ER stress . . . 71
   7.5. Secretion bottleneck . . . 71
   7.6. Clonality and clonal variation . . . 71
   7.7. Validation . . . 71

Abbreviations: ATF6c, activating transcription factor 6; CHO, Chinese hamster ovary; DHFR, dihydrofolate reductase; effector gene, a transgene ectopically expressed with the purpose of obtaining a phenotypic change; IE, inducible expression; EPO, erythropoietin; ER, endoplasmic reticulum; HEK, human embryonic kidney; MAb, monoclonal antibody; pcd, pg per cell per day; PDI, protein disulphide isomerase; PTM, post-translational modification; q_p, specific protein productivity; r-protein, recombinant protein; SCS, single-cell sorting; SEAP, secreted alkaline phosphatase; SEE, stable episomal expression; SGE, stable gene expression; TGE, transient gene expression; YY1, transcription factor Yin Yang 1; VEGF, vascular endothelial growth factor; XBP-1S, spliced form of X-box-binding protein 1.

* Correspondence to: H. G. Hansen, DTU Biosustain, Building 220, Kemitorvet, 2800 Kgs. Lyngby, Denmark.
** Correspondence to: G.M. Lee, Department of Biological Sciences, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea.
E-mail addresses: hgra@biosustain.dtu.dk (H.G. Hansen), gyunminlee@kaist.ac.kr (G.M. Lee).

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8. Product quality . . . 72
9. Concluding remarks and future directions . . . 72
Acknowledgements . . . 73
References . . . 73

1. Introduction

Chinese hamster ovary (CHO) cells are the most frequently used cell host for biopharmaceutical production of glycoproteins (Walsh, 2014). Besides being the host cell used for the first approval of a recombinant biopharmaceutical produced in mammalian cells in 1986 (Wurm, 2004), CHO cells are the preferred choice for a number of reasons. First, CHO cells can easily be adapted for high-density suspension growth in a chemically defined, serum-free medium in large-volume cultures (Kim et al., 2012; Sinacore et al., 2000). Second, gene amplification methods have been established for CHO cells, leading to high specific productivity (q_p) of recombinant protein (r-protein) in stable cell lines (Durocher and Butler, 2009). Third, CHO cells are less prone to virus infection than other mammalian production cell lines and are therefore regarded as a safe host for the production of human therapeutics (Berting et al., 2010). Last, CHO cells and other mammalian cells are the platform of choice for the production of human recombinant glycoproteins because of their ability to correctly make human-like post-translational modifications (PTMs), in particular glycosylation (Butler and Spearman, 2014). Human-like PTMs turn r-protein products into functional drug molecules with reduced immunogenicity, prolonged serum half-life and high pharmacological efficacy in the human body (Walsh and Jefferis, 2006).

The production of r-proteins in CHO cells in optimized bioprocesses can reach q_p of 50–90 pg per cell per day (pcd) (Hacker et al., 2009). As previously pointed out by Khan and Schröder (2008), professional secretory plasma cells are capable of secreting IgM at a rate of 200–400 pcd (Fazekas et al., 1980; Randall et al., 1992). This clearly indicates that nature's physiological limit not yet has been reached and thus, intracellular rate-limiting steps in protein production remain to be resolved. Indeed, post-transcriptional rate-limiting steps in the biosynthetic pathway of r-proteins have been reported multiple times in CHO cells (Johari et al., 2015; Kallehauge et al., 2016; S.J. Kim et al., 1998; Ku et al., 2008; Schröder et al., 1999) as well as in other mammalian cells (Barnes et al., 2004; Fann et al., 1999). The presence of a post-transcriptional bottleneck suggests that there are many opportunities to improve the secretory pathway machinery in CHO cells. Moreover, artificial protein scaffolds such as fusion proteins are becoming more popular in the biopharmaceutical industry with increasing market shares (Aggarwal, 2014). These non-native scaffolds are in general more prone to misfolding (Lee et al., 2007). Thus, the cost-efficient production of these difficult-to-express fusion proteins will most likely require substantial engineering of the folding machinery in the secretory pathway.

Engineering CHO cells by the ectopic expression of transgenes (hereafter referred to as effector genes) is an attractive solution to improve the secretory capacity of CHO cells. In many cases, such engineering efforts have led to positive effects on q_p on a variety of r-proteins (see recent reviews (Fischer et al., 2015; Hussain et al., 2014; Nishimiya, 2013)). This multitude of studies showing positive effects clearly underpins the potential of expressing effector genes. However, as previously pointed out (Hussain et al., 2014; Kim et al., 2012; Mohan et al., 2008), some effector genes are flawed by inconsistent effects. To exemplify this, all published studies on r-protein productivity (volumetric productivity or q_p) in CHO cells with the ectopic expression of the widely studied protein disulphide isomerase (PDI) are listed in Table 1. PDI is an endoplasmic reticulum (ER)-resident enzyme conferring disulphide isomerase activity (Hatahet and Ruddock, 2009). Moreover, PDI forms and reduces disulphide bonds in nascent polypeptides in the lumen of the ER and in parallel inhibits the aggregation of folding intermediates through its function as a chaperone (Appenzeller-Herzog and Ellgaard, 2008). The reported effects of overexpressing PDI on volumetric productivity and q_p vary from a two-fold decrease through no effect to a 1.4-fold increase. This inconsistency, can to some extent, be explained by product specificity, as several different r-proteins have been used as model proteins. In fact, PDI overexpression only increased q_p for one of four monoclonal antibody (MAb) variants in a parallel experimental setup (Pybus et al., 2014). However, many cellular and experimental factors are at play when examining how an effector gene affects volumetric productivity and q_p (Fig. 1). Thus, it is likely that factors other than product specificity are involved in the inconsistency of PDI's effect on volumetric productivity and q_p of r-proteins.

In contrast to PDI, the effect of many effector genes on volumetric productivity or q_p has only been reported once (Hussain et al., 2014; Nishimiya, 2013). Notwithstanding product specificity, it is likely that a considerable number of these effects are conditional – for example, specific to the monoclonal cell line or the expression platform being used. The applicability of such conditional effects is often limited to the research group in question and not to the CHO engineering field in general. Here, cellular and experimental factors that potentially affect the outcome when studying effector genes will be described and discussed. If these factors are appreciated, the risk of unintentionally investigating conditional effects can be minimized and the chance of finding true positive effects can be increased.

2. CHO host cell lines

In 1957, the immortalized, original CHO cell line (the common ancestor for all CHO cell lines) was established from the ovaries of an outbred female Chinese hamster (Puck, 1957; Wurm, 2013). This original cell line has led to a multitude of commercially available and proprietary CHO cell lines (Wurm, 2013). Being an immortalized cell line, the genome of CHO cells is inherently unstable (Frye et al., 2016). Moreover, dihydrofylate reductase deficiency (DHFR) in the widely used DXB11 and DG44 cell lines was achieved by subjecting cells to radiation- and chemical-mediated mutagenesis, respectively (Urlaub et al., 1983; Urlaub and Chasin, 1980). Thus, host CHO cell lines constitute a genomically diverse family in terms of single nucleotide polymorphisms (Lewis et al., 2013), copy-number variations (Kaas et al., 2015) and karyotypes (Wurm and Hacker, 2011). Moreover, it has recently been suggested to regard CHO host cell lines as 'quasispecies', emphasizing the extensive genetic heterogeneity residing in the CHO host cell family (Wurm, 2013).

When CHO host cell lines are compared, they are found to be not only genetically divergent but also phenotypically diverse. For example, it has been shown that the ER size in the CHO-K1 host cell line is larger compared to a DXB11-derived host cell line, and the mitochondrial mass was also found to be higher in CHO-K1 cells (Hu et al., 2013). These phenotypic differences might explain the approximately 10-fold lower q_p observed for the DXB11-derived host cell line compared to CHO-K1 cells, which was obtained for two different MAbs from stable gene-amplified clones (Hu et al., 2013). In a recent CHO bibliome study by Golabgir et al. (2016), a meta-analysis of bioprocess studies showed that the specific growth rate and q_p of DXB11- compared to DG44-derived cell lines were significantly higher and lower, respectively. Although the bibliome data consist of a range of process conditions and experimental setups, both DG44 and DXB11 are DHFR-deficient cell lines. Consequently, the gene-amplification process and clone selection are therefore comparable, warranting the comparison of q_p.