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Review

Therapeutic glycoprotein production in mammalian cells

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ABSTRACT

Over the last years, the biopharmaceutical industry has significantly turned its biologics production towards mammalian cell expression systems. The presence of glycosylation machineries within these systems, and the fact that monoclonal antibodies represent today the vast majority of new therapeutic candidates, has largely influenced this new direction. Recombinant glycoproteins, including monoclonal antibodies, have shown different biological properties based on their glycan profiles. Thus, the industry has developed cell engineering strategies not only to improve cell's specific productivity, but also to adapt their glycosylation profiles for increased therapeutic activity. Additionally, the advance of "omics" technologies has recently given rise to new possibilities in improving these expression platforms and will significantly help developing new strategies, in particular for CHO (Chinese Hamster Ovary) cells.

1. Introduction

Over the past decade, more than a hundred new biopharmaceutical products have been approved and marketed in the United States (US) and European Union (EU). Market value for these biologics was recently estimated at $140 billion US, with a total of over two hundred therapeutics (Walsh, 2014). A significant portion of these products are recombinant proteins, with an ongoing increase in the number of them produced in mammalian expression platforms (Walsh, 2014). This trend is mostly driven by the increased attention directed to post-translational modifications of these biologics, in particular towards their glycosylation state. Indeed, several efforts have been made over the last few years to understand how glycosylation can influence the biological activity of therapeutics. Studies have demonstrated that proper glycosylation profiles can improve recombinant protein properties such as increase their stability and half-life in blood circulation and decrease their immunogenicity (Ashwell and Harford, 1982; Runkel et al., 1998; Ghaderi et al., 2010; Ghaderi et al., 2012; Jefferis, 2016a; Jefferis, 2016b; Kuriakose et al., 2016).

Among the mammalian-based expression systems, CHO cells is by far, the most commonly used cell line. It is involved in the production of over 70% of recombinant biopharmaceutical proteins, most of them being monoclonal antibodies (mAbs) (Durocher and Butler, 2009; Kim et al., 2012; Butler and Spearman, 2014). This review will summarize the recent advances in production of glycoproteins in mammalian cells, with a particular emphasis on the CHO cell system. The various expression systems currently used for therapeutic glycoprotein production (Fig. 1) will be overviewed and cell engineering strategies used to improve biologics production and/or quality will be discussed. Finally, we will also describe the different "omics" approaches used lately in the field in order to improve glycoprotein production and/or glycosylation.

Abbreviations: EU, European Union; FDA, Food and Drug Administration; EMA, European Medicines agency; HEK, human embryonic kidney; shRNA, short hairpin RNA; siRNA, small interference RNA; α-gal, galactose-α1,3-galactose; Neu5Gc, *N*-glycolylneuraminic acid; IgG, Immunoglobulin G; GS, glutamine synthase; KO, knock out; DHFR, dihydrofolate reductase; MSX, methionine sulfoximine; MTX, methotrexate; SILAC, stable isotope labeling with amino acids in cell culture; iTRAQ, isobaric tags for relative and absolute quantification; Fc, fragment crystallizable; mAbs, monoclonal antibodies; cDNA, coding DNA; BHK, Baby Hamster Kidney; RMCE, recombination-mediated cassette exchange; ZFN, zinc finger nuclease; TALEN, transcription activators like effectors nucleases; NHEJ, non-homologous end joining; HDR, homologous-directed recombination; HPRT, hypoxanthine phosphoribosyltransferase; CRISPR, clustered regularly interspaced short palindromic repeats; ACE, artificial chromosome expression; S/MAR, scaffold/matrix attachment region; UCOE, ubiquitously acting chromatin opening elements; STAR, stabilizing and anti-repressor; CMV, cytomegalovirus; mTOR, mechanistic target of rapamycin; NaB, sodium butyrate; ER, endoplasmic reticulum; EPO, erythropoietin; IFNγ, interferon gamma; t-PA, tissue plasminogen activator; SNARE, *N*-ethylmaleimide-sensitive factor attachment protein receptors; ADCC, antibody-dependent-cell-mediated cytotoxicity; GnTIII, beta-1,4-mannosyl-glycoprotein 4-beta-*N*-acetylglucosaminyltransferase; ManNAc, *N*-Acetyl-D-mannosamine; NGS, next generation sequencing; NeuNAc, N-acetylneuraminic acid; TCA, tricarboxylic acid; FcγR, Fc-gamma receptor; ManII, mannosidase II

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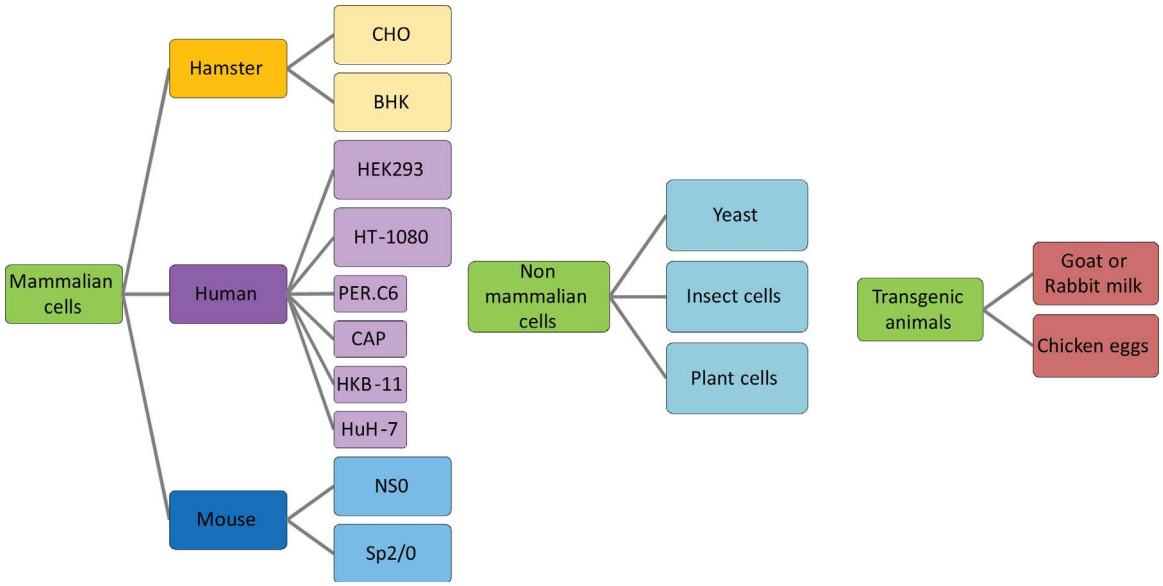

Fig. 1. Expression systems used for glycoprotein production by biopharmaceutical industries.

2. Cell hosts

2.1. Chinese hamster ovary cells

CHO cells are widely used for glycoprotein production because of their numerous advantages. These cells can achieve substantial production rate, are suitable for large-scale industrial suspension culture and can be adapted to grow in various serum-free and chemically defined culture media (Kim et al., 2012; Lai et al., 2013). Since CHO cells produce recombinant glycoproteins with human-like glycans, the generated products are to more likely be compatible and bioactive within human hosts (Kim et al., 2012; Lai et al., 2013). Furthermore, these cells are refractory to infection by human viruses, which minimizes biosafety risks for commercial production purpose (Boeger et al., 2005). This decreased susceptibility could be attributed to the fact that many viral entry genes are not expressed in CHO cells (Xu et al., 2011). Moreover, different gene amplification systems have been developed and used in CHO cells, which allow for high titer yields and good specific productivity (Durocher and Butler, 2009; Kim et al., 2012; Lai et al., 2013). There are many examples of biotherapeutic glycoproteins approved by the Food and Drug Agency (FDA) and the European Medicines Agency (EMA) currently produced in these cells. Several monoclonal antibodies such as Siltuximab (SYLVANT®), Pertuzumab (PERJETA®) and Rituximab (RITUXAN®), as well as other proteins such as tissue plasminogen activator (tPa, ACTILYSE®, ACTIVASE®) and Human DNAse (PULMOZYME®) are just some of the many examples of biotherapeutics generated in CHO cells (for a recent list see (Dumont et al., 2015)). In 2015, more than half of the thirteen new biologics approved were recombinant proteins produced in CHO cells (Sellick et al., 2011a). Among these products, four monoclonal antibodies, Daratumumab (DARZALEX®), Mepolizumab (NUCALA®) and Evolocumab/Alirocumab (REPATHA®/PRALUENT®) are used to treat multiple myeloma, asthma and hypercholesterolemia, respectively. The same trend is currently observed in 2016, where again more than half of the approved biotherapeutics are produced in CHO cells (FDA, 2016). Although CHO cells possess many advantages for glycoprotein productions, they are unable to produce some types of human glycosylation, such as α-2,6-sialylation and α-1,3/4-fucosylation (Patnaik and Stanley, 2006). Moreover, CHO cells produce glycans that do not occur in human cells, namely *N*-glycolylneuraminic acid (Neu5Gc) and galactose-α1,3-galactose (α-gal), even though these occurring at very low levels (e.g < 2% and < 0.2% respectively) (Bosques et al., 2010; Ghaderi et al., 2010; Dietmair et al., 2012b; Ghaderi et al., 2012). The human immune system can produce antibodies against these *N*-glycans that could contribute to immunogenicity/neutralization of the corresponding biotherapeutics (Galili et al., 1984; Noguchi et al., 1995; Tangvoranuntakul et al., 2003; Chung et al., 2008; Macher and Galili 2008; Padler-Karavani et al., 2008; Ghaderi et al., 2010; Padler-Karavani and Varki, 2011). CHO cells also have limited ability to gamma-carboxylate recombinant proteins such as clotting factors (Kumar 2015), even though some improvements have been achieved through metabolic engineering work (Rehemtulla et al., 1993; Liu et al., 2014). Proteins requiring proteolytic processing for maturation may not always be fully cleaved and active when expressed in CHO. For example, co-expression of furin was shown to allow the production of fully cleaved and active von Willebrand factor in an industrial-scale CHO perfusion system (Fischer et al., 1995) and of the coagulation factor VIII B-domain (Demasi et al., 2016). Similarly, co-expression of proprotein convertases allowed for the efficient maturation of human bone morphogenetic protein-7 (Sathyamurthy et al., 2015).

2.2. Human cell lines

One way to favor human-like glycosylation would be to use human cell lines for recombinant protein production. This strategy would warrant that proteins harbor, if not the ideal glycosylation pattern, at least a non-immunogenic glycans (Swiech et al., 2012). The most commonly used human cell lines to manufacture glycoprotein therapeutics are the HEK293 cells and the HT-1080, respectively from human embryo kidney and fibrosarcoma origin (Rasheed et al., 1974; Graham et al., 1977). Drotrecogin alfa (Xigris®), the first therapeutic glycoprotein produced in human cells (HEK293) approved by FDA and EMA, was accepted by both agencies in 2001 and 2002 respectively. However, it was removed from the market in 2011, since it failed to show significant beneficial effects. Yet, only four biological glycoproteins were approved in the following decade by FDA and/or EMA. These four therapeutics, namely Agalsidase alfa, Epoetin delta (DYNEPO®), Idursulfase (ELAPRASE®) and Velaglucerase alfa (VPRIV®), are produced using a gene activation technology (proprietary of Shire) in HT-1080 cells (Moran, 2010). Epoeitin delta produced in HT-1080 was found to have better homogeneity of its tetra-antennary glycans, higher sialic acid content and no Neu5Gc, compared to CHO-produced erythropoietin (Llop et al., 2008). However, this product was voluntarily withdrawn for commercial reasons (Dumont et al., 2015). As for