



Antibody glycosylation: impact on antibody drug characteristics and quality control

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Abstract

Glycosylation is a common post-translational modification that occurs during the production of antibodies. Glycans attached to antibodies play an important role in the pharmacokinetics, efficacy, and safety of therapeutic antibodies. In the modern antibody industry, it is important to adjust and control glycosylation modifications. The formation of specific sugar structures via glycosylation engineering is constantly evolving. This review summarizes the recent progress in glycosylation modifications, as well as the major discoveries and current understanding of the mechanisms involved, to provide new ideas for the research and development of therapeutic antibodies.

Keywords Glycosylation · Antibodies · Pharmacokinetics · Analysis

Introduction

In 1986, the U.S. Food and Drug Administration approved the world's first therapeutic monoclonal antibody (mAb), the anti-CD3 monoclonal antibody OKT3, for the treatment of renal transplant rejection (Smith 1996). After more than 30 years' of rapid development, the production of antibodies has become the most profitable area of the biopharmaceutical industry. By 2017, there were 74 antibodies listed worldwide (www.biopharma.com). Currently, antibodies are extensively used to treat diseases such as cancer and autoimmune disorders, as well as to prevent organ transplant rejection.

Glycoforms of antibodies have vital importance for the maintenance of antibody structure and therapeutic effects (Borrok et al. 2012; Cymer et al. 2018; Jefferis 2009; Thomann et al. 2016; van de Bovenkamp et al. 2016). Fc (fragment crystallizable) effector functions include antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis. These functions are triggered by the formation of antigen-antibody complexes, which are subsequently

recognized by Fc γ receptors (Fc γ Rs). As one of the basic components of living organisms, glycans play an important role in the molecular recognition, adhesion, and signaling of cells (Bloem et al. 2013; Tommasone et al. 2019; Wolfert and Boons 2013; Zhang et al. 2019a). Glycans attached to proteins can be divided into three types: *N*-linked, *O*-linked, and glycosaminoglycans. Almost all mAbs have an Asn-XXX-Ser/Thr (XXX \neq Pro) sequence, which is an *N*-glycosylated sequence at the Asn²⁹⁷ residue in the heavy chain of the C_H2 domain (Eschwege et al. 2018; Zhang et al. 2019b). Some mAbs bear *O*-linked glycans at serine and threonine residues, which mainly occur in the hinge region (Plomp et al. 2015). *N*-glycosylation can influence Fc conformation, thus affecting the binding affinity of antibodies to receptors (Hayes et al. 2017; Okazaki et al. 2004; Zou et al. 2011). Therefore, in this review, we mainly discuss *N*-glycosylation, which has been thoroughly studied for many years, whereas *O*-glycosylation is still not well understood because of its complexity.

Owing to the complexity of glycan structures, antibody drugs are actually mixtures of proteins with different glycoforms (Zhou and Qiu 2019). To produce homogeneous products, methods have been developed to achieve specific *N*-glycosylation types (Wang et al. 2018). Some studies have successfully remodeled glycoforms in vitro using chemoenzymatic approaches (Giddens and Wang 2015; Hodoniczky et al. 2010; Kurogochi et al. 2015). Adding extra nutrients in the culture medium can affect Fc *N*-glycan processing (Aghamohseni et al. 2014). Moreover, altering an

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expression system has been used to generate specific glycoforms. Thus, glycoengineered *Pichia pastoris* (*P. pastoris*) has been demonstrated to be a promising expression system for mAb production, with human-like glycosylation but at a much lower cost than previous methods (Ha et al. 2011; Purcell et al. 2017). A study using a knockout and screening approach of all glycosyltransferase-related enzymes identified the key genes that control the vital steps in *N*-glycosylation of proteins in Chinese hamster ovary (CHO) cells, which led to the construction of a design matrix to produce the desired glycoforms (Yang et al. 2015b). Genome editing and protein engineering have been combined to generate IgGs with increased levels of α -2,6-sialylation in CHO cells (Chung et al. 2017).

Even though the development of antibodies helps deal with many diseases, the immunogenic reactions they cause should not be ignored. Although in some cases, glycosylation can attenuate immunological responses, many allergic reactions caused by glycans have been reported, and some glycans have the potential to react with other epitopes, leading to immunogenic reactions (Macher and Galili 2008).

Overall, appropriate glycosylation is critical for the pharmacokinetics, Fc effector functions, and safety of antibodies; thus, it is important to test for glycans to control the quality of antibodies. Therefore, the exploration of analytical methods to evaluate the properties of glycans plays an important role in the laboratory and in industry. Many analytical strategies that investigate glycans have been developed over the years (Cao et al. 2018; Purohit et al. 2018; Zhang et al. 2016a).

Impact of glycosylation on antibody half-lives

The half-lives of full-length IgG antibodies are usually longer than those of small-molecular cytokines and can be weeks (Luo et al. 2017). One reason is that IgGs can be bound by the neonatal Fc receptor (FcRn), which is expressed by vascular endothelial cells and macrophages, etc. FcRn binds IgGs via pH-dependent interactions in the endosomal compartment, thereby rescuing antibodies from lysosomal degradation, recycling antibodies to the cell surface, and modulating antibody clearance. Additionally, the type of *N*-glycosylation modification contributes to the half-life of an antibody (Higel et al. 2016; Li et al. 2006; Zhang et al. 2016a). Antibodies with a terminal sialic acid have a significantly longer half-life than non-sialylated antibodies. The mechanism involves the asialoglycoprotein receptor, which can bind the terminal galactose and *N*-acetylglucosamine (GlcNAc) of the glycoprotein, leading to the endocytosis-mediated degradation of the glycoprotein and a decrease in its half-life. The terminal sialic acid masks the galactose, thus protecting antibodies from rapid clearance (Lee et al. 2002). Liu et al. generated recombinant TNFR2:Fc fusion proteins, with varying degrees of the sialic acid content, in glycoengineered

P. pastoris strains and discovered a positive correlation between the content of sialic acid and the pharmacokinetic properties of the proteins in rats (Liu et al. 2013). Sialic acid (+) pertuzumab showed a significantly longer half-life than sialic acid (–) pertuzumab (Luo et al. 2017).

Antibodies with a high content of terminal mannose show faster clearance from the blood. The mannose receptor, also called the macrophage mannose receptor, is an endocytic receptor for glycans and is expressed in many tissues. It has 10 extracellular domains: 1 NH₂-terminal cysteine-rich domain, 8 tandem carbohydrate recognition domains, and 1 fibronectin type II repeat domain. The receptor binds the monosaccharides mannose, fucose, and GlcNAc (Garcia-Aguilar et al. 2016). A study that explored how Fc glycoforms influence the clearance of four human IgGs, demonstrated that therapeutic IgGs with a high mannose content in the Fc region have a shorter half-life than other glycoforms (Goetze et al. 2011). Wright et al. produced an IgG with a high mannose content using the Lec1 cell line and observed a slightly reduced affinity for Fc γ RI, as well as a reduced half-life (Wright and Morrison 1998). Yu et al. obtained antibodies with a high content of mannose using a mannosidase inhibitor, kifunensine, which was then purified and cut by mannosidase to Man5 glycans. The authors discovered that antibodies bearing a high-mannose glycoform clear faster than those bearing a fucosylated-complex glycoform, whereas the pharmacokinetic properties of antibodies with the Man8/9 glycoform are similar to those of Man5 antibodies. The mannosidase present in mouse serum converts most Man8/9 to Man6 after 24 h (Yu et al. 2012). However, Yang et al. demonstrated that antibodies that are highly decorated with mannose do not clear faster than undecorated antibodies, whereas the same IgGs decorated with 2-imino-2-methoxyethyl-1-thiomannoside to maintain the normal glycosidic bond do clear faster. Therefore, the authors have come to a contradictory conclusion that glycosylation is not an obvious concern for the clearance of therapeutic proteins (Yang et al. 2015a). Thus, whether mannose influences the pharmacokinetics of antibodies and, if so, how this occurs must be elucidated in the future.

Impact of glycosylation on ADCC and CDC of antibodies

ADCC and CDC are the most important therapeutic functions of mAbs. The influence of various glycoforms on ADCC has been widely discussed (Dekkers et al. 2017; Treffers et al. 2018). Many antibodies bear a core fucose in the Fc region, which is attached to the core GlcNAc. Alpha (1,6)-fucosyltransferase (FUT8) is responsible for the transfer of fucose to the core GlcNAc.

The absence of the core fucose can enhance the affinity between Fc and Fc γ RIIIa, thus increasing the ADCC. However, more than 90% of antibodies produced in CHO

cells are fucosylated (Raju et al. 2000). Several strategies have been implemented to improve the binding affinity of Fc γ RIIIa and ADCC by designing afucosylated antibodies. A typical strategy is to disrupt FUT8 to obtain antibodies without a core fucose (Nimmerjahn et al. 2007). Scientists at Kyowa Hakko Kirin Pharma knocked out *FUT8* in CHO cells and created the patented POTEILLIGENT® technology to produce antibodies that lack the core fucose. An mAb that targets C-C chemokine receptor 4 (CCR4), mogamulizumab (Poteligeo®), was created using this technology and was the first approved biological agent that targets CCR4 (Subramaniam et al. 2012). The removal of fucose from the oligosaccharides of human IgG1 antibodies significantly reduces the number of antigens required for ADCC and activation of natural killer cells (Treffers et al. 2018). In a human IgG4 isotype antibody, which has reduced ADCC activity compared to that of a human IgG1 isotype antibody, its efficacy is increased by afucosylation both in vivo and in vitro (Gong et al. 2016). Quilizumab is an afucosylated humanized anti-M1 prime therapeutic antibody, and its binding affinity for Fc γ RIII and bioactivity is enhanced (Brightbill et al. 2014). Targeting the de novo GDP-fucose biosynthesis pathway using an overexpressed bacterial GDP-6-deoxy-d-talose synthetase results in > 80% fucose-free IgG1, with a 13-fold enhanced binding affinity and 11-fold enhanced ADCC bioactivity (Kelly et al. 2018). In addition, the overexpression of beta 1,4-*N*-acetylglucosaminyltransferase III leads to the enrichment of antibodies in bisected oligosaccharides and an increase in non-fucosylated antibodies (Ferrara et al. 2006).

Terminal sialic acid in the Fc region reduces the ADCC of antibodies, which may be due to a decrease in the binding affinity between Fc and Fc γ RIIIa at a high content of sialic acid. In addition, terminal sialic acid in the Fc can decrease the binding of antibodies to cell surface antigens, possibly due to the reduction of hinge region flexibility, but not due to differences in intrinsic affinity between the antigen binding (Fab) arm and its target (Scallan et al. 2007). Sialic acid at Asn²⁹⁷ is important for the anti-inflammatory effect of antibodies. The inflammatory modulating effect of sialic acid modification is mediated by sialic acid binding to immunoglobulin type lectins (Siglecs) expressed on mononuclear phagocytes. Siglecs can trigger strong inhibitory signaling pathways via an intracellular immunoreceptor tyrosine-based inhibition motif when bound to sialic acid (Lubbers et al. 2018). The lack of sialic acid significantly reduces the affinity between Fc γ Rs and intravenous gamma globulin (IVIg), but increases the anti-inflammatory efficacy of IVIg, suggesting that a pathway not related to Fc γ Rs exists (Nimmerjahn and Ravetch 2008). Accordingly, Luo et al. generated desialylated pertuzumab by enzymatic hydrolysis and observed increased ADCC and CDC with improved Fc γ RIIIa binding affinity (Luo et al. 2017).

High-mannose-content antibodies (5–9 mannose residues) have an enhanced binding affinity for Fc γ RIIIa and an

enhanced ADCC (Yu et al. 2012), with the latter likely due to afucosylation because these antibodies have a high mannose but low fucose content (Reusch and Tejada 2015). The presence of bisecting GlcNAc at Asn¹²⁵ and Asn²⁹⁷ has been shown to improve ADCC efficacy. By remodeling Rituxan® and Herceptin® with a soluble recombinant rat β -1,4-*N*-acetylglucosaminyltransferase III, Hodoniczky et al. obtained therapeutic antibodies with a high (> 80%) bisecting GlcNAc content and observed an approximately tenfold increase in ADCC (Hodoniczky et al. 2010). However, Shinkawa et al. demonstrated that the most crucial factor for increasing ADCC is the lack of fucose (Shinkawa et al. 2003).

Galactose plays a small role in the ADCC of an antibody; however, the terminal content of galactose in the Fc region is strongly related to CDC. For rituximab, adding a terminal galactose increases the antibody binding with the complement component 1q (C1q), thus increasing CDC, but terminal galactose has little influence on the ADCC (Peschke et al. 2017). Compared with antibodies produced in mouse myeloma cells, those produced in CHO cells are less galactosylated but have a more degalactosylated (G0) glycoform (Raju and Jordan 2012). Antibodies with the G0 glycoform appear to have a low affinity for both C1q and Fc γ Rs (Dashivets et al. 2015; Nimmerjahn et al. 2007). Gramer et al. explored a practical strategy to control antibody galactosylation by feeding uridine, manganese chloride, and galactose to the cell culture medium, which increases the content of terminal galactose (Gramer et al. 2011).

Impact of glycosylation on antibody immunogenicity

The glycosylation of antibodies influences on their structure, function, and immunogenicity (Zhou and Qiu 2019). There are two ways that glycosylation modulates the immunogenic properties of antibodies: the oligosaccharide chains attached to the surface of antibodies can trigger specific immune responses; and the glycan moieties can cover some epitopes on the surface of antibodies to reduce its immunity (Gao et al. 2017; Jennewein and Alter 2017; Lubbers et al. 2018). Here we only discuss the influence of glycosylation on immunogenicity.

Therapeutic antibodies are likely to be immunogenic which reduces their safety and efficacy (Wolf et al. 2015). The formation of anti-drug antibodies (ADAs) is a marker for immunogenicity. Once ADAs develop in patients, therapeutic antibodies are cleared rapidly, which results in the loss of efficacy. According to studies of patients with Crohn's disease treated with anti-TNF- α antibodies infliximab and adalimumab, the annual risk for loss of response is 13% and 24%, respectively (Billioud et al. 2011; Gisbert and Panes 2009). In 2016, Pfizer withdrew bococizumab, the humanized antibody binding proprotein convertase subtilisin/kexin type 9, from development in late phase III, due to its high immunogenicity

characterized by the generation of ADAs and attenuation of efficacy (Wang et al. 2019). Non-human glycosylation and glycosylation-related immunogenic structures have a direct impact on antibody immunogenicity. Moreover, glycosylation influences antibody stability, which can lead to protein aggregation, another major source of immunogenicity (Hristodorov et al. 2013).

Antibodies produced in other eukaryotic systems (plants, *P. pastoris*, insects), and non-human mammalian cells, may bear glycoforms that are antigenic for humans, such as alpha-galactose (alpha-gal) and *N*-glycolylneuraminic acid (Neu5Gc) (Liu et al. 2018b; Macher and Galili 2008; Steinke et al. 2015; Strasser et al. 2008). Because of the deletion of the alpha-gal gene and a mutation in the Neu5Gc gene during human evolution, antibodies against alpha-gal and Neu5Gc exist, and immunoreactions that involve these two antibodies have been reported (Varki 2001). Cetuximab is expressed by SP2/0 murine myeloma cells, and in addition to the *N*-glycosylation site in its Fc region, the Fab region contains *N*-glycans with terminal alpha-gal, which can cause a strong allergic reaction (Janinbussat et al. 2013). Neu5Gc is a non-human sialic acid derived from red meat, which is immunogenic to humans (Alisson-Silva et al. 2016). Padler-Karavani et al. suggested that a continuous Neu5Gc antigen antibody reaction may lead to an inflammatory reaction, which may contribute to some diseases in humans (Padler-Karavani et al. 2008). Antibodies against Neu5Gc alone are not associated with an increased risk of colorectal cancer (CRC). However, when total antibody responses against all Neu5Gc-glycans were combined, a positive correlation between Neu5Gc antibodies and CRC risk was observed using a sialoglycan microarray (Samraj et al. 2018). Therefore, antibodies containing Neu5Gc have the potential to cause allergic reactions in human bodies.

During the development of antibodies, which has involved generating mouse antibodies, chimeric antibodies, humanized antibodies, and the latest fully human antibodies, the immunogenicity of antibodies has been constantly reduced. However, there are emerging novel strategies for better productivity, activity, and pharmacokinetic and pharmacodynamic performances (Bas et al. 2019; Mimura et al. 2016; Zhao et al. 2018). These strategies such as sequence mutations, hybrid structures (bi-specific or antibody-protein hybrid), and even cell culture processes can subsequently change glycosylation modifications. An ideal strategy is to produce antibodies with desirable homogeneous non-immunogenic glycans. A milestone was reached when a group of scientists successfully produced trastuzumab with homogeneous remodeled glycans. They expressed trastuzumab in *P. pastoris* and used endoglycosidases to cleave the high-mannose glycans from the host species, then used trans-glycosylase and a stable glycan donor α 2,6-linked sialylglycopeptide to produce the homogeneous trastuzumab bearing α 2,6-SCT (named Her-SCT)

(Liu et al. 2018a). This shed light on how to optimize the glycosylation of antibodies to avoid immunogenicity as well as improve therapeutic safety and efficacy.

Advances in *N*-glycosylation analysis

Given the significant impact of *N*-glycans on the half-lives of antibodies in serum and their bio-activity, there is an urgent need to develop robust methods for *N*-glycosylation analysis. Several strategies have been conceived to identify the *N*-glycans attached to the Fc region of antibodies. Glycan modification of antibodies can be determined via three different stages: at an intact glycoprotein level, at a glycopeptide stage, or by analyzing the released glycan (Azadi and Heiss 2009; Behnken et al. 2014). In complex situations, these three different analytical strategies are usually combined to analyze glycosylation. *N*-glycans can be released by amidases such as peptide *N*-glycosidase F, resulting in a deaminated protein or peptide and a free glycan (Tarentino and Plummer 1994). However, the removal of *N*-glycans by this method is not complete, and the glycans released need to be determined. When there are *N*- and *O*-glycans to release, chemical release, especially hydrazinolysis is the most common approach (Geyer and Geyer 2006).

After their removal, the subsequent derivatization of glycans with a fluorophore or chromophore is usually carried out to facilitate chromatographic detection. 2- Aminobenzamide (2-AB), 2-aminobenzoic acid (2-AA), and 8-aminopyrene-1,3,6-trisulfonate are widely used to label glycans. Recently, some studies have indicated that the use of a procainamide label yields a fluorescence intensity approximately 30-fold higher than that obtained with the 2-AB label (Klapoetke et al. 2010; Kozak et al. 2015). The labeling reagent RapiFluor-MSTM shows a greater measurement sensitivity in both fluorescence (10 \times over 2-AB) and mass spectrometry (MS; 150 \times over 2-AB) detection (Lauber et al. 2015). As a last step, glycans usually need to be purified to meet the requirements of detection instruments and improve the analyte properties by the enrichment, sample desalting, and removal of impurities and potentially interfering substances (Cao et al. 2014; Kussmann et al. 1997). The purification methods include reversed-phase high-performance liquid chromatography (RP-HPLC), hydrophilic interaction liquid chromatography (HILIC), solid-phase extraction (Yu et al. 2005), gravity-fed chromatography on an S cartridge column (Yang et al. 2016), liquid–liquid extraction, and gel filtration. Generally, different methods of purification are adapted to different labeling methods (Ruhaak et al. 2010).

Viktoria et al. have summarized common methods used for the analysis of glycosylation (Dotz et al. 2015). Different analytical methods have different advantages and disadvantages, which are complementary in some aspects, especially for the detection of complex and variable glycans attached to

antibodies (Adamczyk et al. 2014; Reusch et al. 2015). HPLC can identify glycans by their retention times and quantify glycans by their peak areas, but standards are required to compare the retention time to the data in a database (GlycoBase (Lisacek et al. 2017)). Capillary electrophoresis with laser-induced fluorescent detection is also widely used to characterize released glycans (Szabo et al. 2011). HILIC is popular for the analysis of compounds with high polarity and is highly selective for antibodies with a typical glycan pattern (Melmer et al. 2010). HILIC has a higher resolution than other HPLC methods for analyzing free glycans (Reusch et al. 2015). However, all non-MS-based techniques have a drawback in that they are unable to identify unknown compounds, and MS has its drawback in that it is unable to distinguish between stereoisomers.

MS techniques are currently the most advanced methods of analysis of antibody glycosylation, owing to their high sensitivity and efficient combination with other separation techniques. Some low-abundant glycan species, such as bi-antennary complex-type, high-mannose, hybrid, and hybrid bisected structures, can be detected and quantified by high-performance anion-exchange chromatography coupled with MS (Maier et al. 2016). Glycans that are usually analyzed by LC-MS are listed in Table 1. For glycopeptide analysis, the therapeutic antibody can be digested with a protease. Then, the glycan is enriched by RP-HPLC and the glycopeptide is detected by MS after separation (Nilsson 2016; Stadlmann et al. 2008). Site specificity of the glycan can be determined by MS analysis of the glycopeptide fusing protease digestion. Capillary zone electrophoresis-electrospray ionization-MS (CZE-ESI-MS) may resolve intact glycopeptides generated from standard proteins, within 9 min (Qu et al. 2018b). Qu et al. combined RP-HPLC and CZE-ESI-MS

and achieved superior *N*-glycopeptide analysis. RP-ultra HPLC (UHPLC), coupled with CZE-ESI-MS, generated ~35% more *N*-glycopeptides than direct RP-UHPLC-ESI-MS analysis and ~70% more *N*-glycopeptides than direct CZE-ESI-MS analysis (Qu et al. 2018a). Our group has used UHPLC coupled with quadrupole time-of-flight MS to analyze the glycosylation profiles of anti-PD-1 antibodies, and demonstrated the product consistency among different passages of stable CHO cell lines (Zhao et al. 2018).

For the analysis of intact glycoproteins, ESI-MS is a powerful tool to resolve structure and determine the position of the linkage of various monosaccharides. MS analysis of intact glycoproteins can provide their molecular weights and almost all glycan patterns (Barallobre-Barreiro et al. 2017). The comparison of these data for biosimilars and origin drugs, can rapidly determine their consistency. Other separation methods, coupled with MS, are also promising tools for the analysis of intact glycoproteins. Lectin microarrays, coupled with a sophisticated detection system, provide a high-throughput platform to characterize specific glycan variants by testing intact glycoproteins (Zhang et al. 2016a; Zhang et al. 2016b). Despite all this progress, the analysis of *N*-glycopeptides remains challenging because of their microheterogeneity (different glycoforms attached to one glycosylation site as well as different site occupancy) of the glycoprotein.

Control of antibody glycosylation

The glycosylation of antibodies is strongly related to the process of posttranslational modification of proteins in host cells (Hurtado-Guerrero and Davies 2012). Therapeutic glycoproteins can be produced using many different hosts, such as

Table 1 *N*-Glycan structures of mAbs analyzed by LC-MS

Glycans	Structures	Glycans	Structures
G0F-GN		hM3F	
G0		G0F	
M5		M6	
G1		G1F	
G2F		G2FS1	
G2FS2		▲=Fucose ■=N-acetylglucosamine ●=Mannose ●=Galactose ◆=N-acetylneuraminic acid †=IgG	

human cells (Backliwal et al. 2008; Ravindranath et al. 2018; Sengupta et al. 2018), mammalian cells of other species, yeast strains (Li et al. 2008; Scholler et al. 2006; Shaheen et al. 2013), and genetically edited animals which have their own unique glycosylation mechanisms to produce distinct glycan patterns (Chen and Murawsky 2018). Different cell lines can produce different glycan patterns. For example, mouse SP2/0 and NS0 cells produce antibodies with alpha-1,3-gal and Neu5Gc, respectively (Chung et al. 2008). Yeasts can be used to produce antibodies without fucose because they are not able to synthesize GDP-fucose (Zhang et al. 2011). Certain cell lines are more likely to produce immunogenic glycoforms, whereas others are more likely to produce glycoforms that improve the clinical therapeutic characteristics of antibodies; thus, modifying cell lines to obtain desired glycoforms is a promising approach. At present, CHO cells are predominantly used to express therapeutic antibodies, and more than 70% of recombinant biopharmaceutical proteins are produced in this cell line (Buettner et al. 2018). In a recent study, CHO cells were successfully modified by CRISPR/Cas9 technology to minimize alpha-2,3-sialylation and over-express alpha-2,6-sialylated glycans to improve the ADCC and anti-inflammatory efficacies of antibodies (Chung et al. 2017). *P. pastoris* has been engineered with a humanized glycosylation pathway, and an anti-human epidermal growth factor receptor 2 mAb produced using this platform shows an improved ADCC bioactivity compared to that of trastuzumab (Zhang et al. 2011). Several research groups, including our group, disrupted both *FUT8* alleles in the CHO-DG44 cell line by sequential homologous recombination and demonstrated that *FUT8*^{-/-} cells are an ideal host to stably produce completely defucosylated, high-ADCC antibodies with a constant quality and efficacy for therapeutic use (Sun et al. 2015; Yamane-Ohnuki et al. 2004; Zong et al. 2017).

In addition, various culture conditions, such as the temperature, pH, dissolved oxygen, and nutrient level, have been demonstrated to affect the glycosylation of antibodies (Aghamohseni et al. 2014; Seo et al. 2013). For example, the human cell line rF2N78 produces aglycosylated antibodies when glucose is depleted and feeding glucose can prevent this phenomenon (Seo et al. 2014). The results of this study demonstrate that glucose is a necessary nutrient for the production of glycosylated antibodies. Variations in culture pH increase the chance of altering the glycan pattern (Muthing et al. 2003). Factors that influence glycan patterns are listed in Table 2. To assure the biochemical identity and bioactivity of antibodies, it is crucial to control the production batch.

Conclusion

Glycosylation is closely related to the pharmacokinetics properties, Fc effector functions, and safety of antibodies; and

Table 2 Factors influencing glycan patterns of antibodies

Categories	Factors	References
Host cell line	Human cells, mammalian cells, yeast strains, genetically edited animals, etc.	(Backliwal et al. 2008; Scholler et al. 2006; Sun et al. 2015)
Culture conditions	Temperature, pH, dissolved oxygen, and nutrient level, etc.	(Muthing et al. 2003; Seo et al. 2013)

glycan patterns can be affected by the expression systems and culture conditions. Glycoforms of antibodies produced in mammalian host cells are different from human serum IgG glycoforms and some are immunogenic to humans. Consequently, there is a need to develop appropriate analytical methods for analyzing the glycan patterns of antibodies. Because glycans can influence the half-lives of antibodies and enhance their ADCC and CDC, the glycoform design can be used to modulate some therapeutic properties of antibodies. Therefore, glycoengineering is a promising approach to promote the development of therapeutic antibodies. Because of the complexity of *N*-glycans and glycosylation sites, this application still faces some difficulties. The effects and mechanisms associated with the glycosylation of therapeutic antibodies need further exploration. It is also necessary to apply the results of laboratory studies to the pharmaceutical industry. The potential of glycoengineering to improve the therapeutic properties of antibodies deserves more attention.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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