Original Research

Genome-scale functional genomics screening highlights genes impacting protein fucosylation in Chinese hamster ovary cells

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\begin{abstract}
N-linked glycosylation is a common post-translational modification that has various effects on multiple types of proteins. The extent to which an N-linked glycoprotein is modified and the identity of glycans species involved is of great interest to the biopharmaceutical industry, since glycosylation can impact the efficacy and safety of therapeutic monoclonal antibodies (mAbs). mAbs lacking core fucose, for example, display enhanced clinical efficacy through increased antibody-dependent cellular cytotoxicity. We performed a genome-wide CRISPR knockout screen in Chinese hamster ovary (CHO) cells, the workhorse cell culture system for industrial production of mAbs, aimed at identifying novel regulators of protein fucosylation. Using a lectin binding assay, we identified 224 gene perturbations that significantly alter protein fucosylation, including well-known glycosylation genes. This functional genomics framework could readily be extended and applied to study the genetic pathways involved in regulation of other glycoforms. We hope this resource will provide useful guidance toward the development of next generation CHO cell lines and mAb therapeutics.
\end{abstract}

\section{Introduction}

Therapeutic monoclonal antibodies (mAbs) are a fast-growing class of medicines. mAbs are less likely than small molecule compounds to interfere with normal biological processes and are often physiologically well-tolerated; thus, mAbs hold great potential as therapeutic agents against both immunological diseases as well as cancers [1,2]. A workhorse mammalian cell culture system to produce therapeutic mAbs is the Chinese hamster ovary (CHO) cell line. One key reason for the popularity of CHO in the pharmaceutical and biotechnology industries is the similarity of its resultant post-translational modifications (PTMs) to those of human cell lines, which lends to attractive pharmacokinetic properties and lower immunogenicity for the mAbs produced [3]. N-linked glycosylation is a PTM in which diverse oligosaccharides (also called glycans) are added onto conserved asparagine residues of newly-formed proteins. Manipulation of protein glycosylation during the manufacturing process can serve as a beneficial fine-tuning mechanism for mAb activity and function [4]. However, that such a vast array of glycosylation species is produced in CHO cells can also pose challenges in the production process, given the importance of various modifications to mAb function and circulatory half-life, for example [5-7]. Thus, controlling the extent and heterogeneity of mAb glycosylation is of great importance in obtaining therapeutic proteins with consistent quality and performance.

The ability to manipulate the extent of protein fucosylation, one form of glycosylation, is particularly important for the efficacy of anti-cancer mAbs [8]. A common mechanism through which these mAbs act is by recruiting and inducing immune cells to kill cancerous cells; this mechanism is known as antibody-dependent cellular cytotoxicity (ADCC) [9,10]. The fucose moiety present on asparagine (N) 297 glycans in the fragment crystallizable (Fc) region of the mAb is widely recognized as a key modulator in ADCC: afucosylation (i.e., the absence of fucose from the glycan) has been demonstrated to increase ADCC [9,10]. The fucose moiety present on asparagine (N) 297 glycans in the fragment crystallizable (Fc) region of the mAb is widely recognized as a key modulator in ADCC: afucosylation (i.e., the absence of fucose from the glycan) has been demonstrated to increase ADCC [9,10]. As a result, identifying novel methods to decrease fucosylation of mAbs is of great importance to the biopharmaceutical industry. For example, CHO cells that overexpress both Golgi α-mannosidase II (αMANII) and 4-β-N-acetylglicosaminyltransferase (GnT-III) have been successfully
used as the host to produce Obinutuzumab (GA101), a novel type II anti-CD20 mAb to treat B-Cell malignancies [15]. Additionally, several CHO models have been developed to produce afucosylated (i.e., non-fucosylated) mAbs through targeted genetic engineering efforts [14, 16–19].

Functional genomics screening is a powerful platform for understanding gene function in a high-throughput manner. By combining phenotypic assays with genome-wide genetic perturbation and next-generation sequencing (NGS), these screening approaches enable researchers to link biological phenomena to their corresponding genetic cause(s) [20, 21]. We wanted to demonstrate the utility of this approach in the biomanufacturing process and expand the molecular and genetic interactions involved in regulating protein fucosylation in CHO cells. We cause(s) [20, 21].

2. Materials and methods

2.1. Cell culture and LCA-binding assay

Experiments were conducted with a proprietary recombinant CHO cell line expressing a humanized IgG1 antibody. This recombinant cell line was developed using the CHOZN host cell line (MilliporeSigma) that had been previously adapted to proprietary culture medium. Cells were maintained in proprietary culture medium supplemented with 4 mg/L insulin (Gibco, A1138211), 12.5 μM methionine sulfoximine (MSX, Sigma Aldrich, M5379), and 6 mM L-glutamine (ThermoFisher, 25,030–81) in vented Erlenmeyer shake flasks (Corning, 431,144) in a shaking incubator operated at 37 °C, 5 % CO₂, and 140 rpm. For the LCA-FITC binding assay, CHO cells were incubated in the presence of 10 μg/mL LCA-FITC (Vector Labs, FL-1041) for 30 min, followed by 3 washes in phosphate buffered saline (PBS). Cells were assayed by flow cytometry on a Fortessa X-20 flow cytometer (BD Biosciences).

2.2. Expression and purification of mAb

mAbs from CHO cells were expressed via fed-batch culture process and purified using Protein A. CRISPR-knockout (KO) pools of CHO cells were cultured in GIA-1 medium supplemented with 4 mg/L human insulin (Millipore 4512–1) at a 50 mL scale in 250 mL Erlenmeyer flasks, then fed on days 2, 4, 7, 9, and 11 with proprietary feed. Cultures were incubated at 35 °C, shaken at 125 rpm, and terminated after 15 days. Supernatants were clarified by centrifugation at 1500 x g for 10 min and filtered through a 0.45 μm Stericup filter (Millipore). After 4-fold dilution of supernatant with PBS, antibodies were purified by affinity capture on a 1 mL MabSelect SuRe column (Cytiva). Each purification was performed as follows: the column was equilibrated with 10 CVs of 1X DPBS pH 7.4, loaded with 30 mL of the diluted ferment harvest, washed with 16 CVs of 1X DPBS, pH 7.4, then the antibody was eluted with 14 CVs of 50 mM glycine buffer, 50 mM NaCl, pH 3.5. The eluate was neutralized by adding 0.5 % of 0.5 M Na₂HPO₄ and filtered using a 0.2 μm Supor membrane Pall Acrodisc syringe filter. Molecular weight and fucosylation state were determined by reduced mass spectrometry.

Briefly, a sample was reduced by adding 25 mM tris (2-carboxyethyl) phosphate (TCEP, ThermoFisher, T2556) and incubating at room temperature for 20 min. 0.1 μg of sample was injected onto a 1290 Infinity II HPLC containing an Opti-Trap Protein cartridge (Optimize Technologies) and coupled to an Agilent 6230 TOF, and the results were analyzed using the Agilent MassHunter Qualitative Analysis B.07.00 software. Peaks were correlated to a particular glycoform if the observed mass was 5–9 amu below the theoretical reduced heavy chain molecular weight to account for incomplete reduction of intrachain disulfides.

2.3. Released N-glycan analysis

Glycan release and labeling was carried out using GlycoWorks RapiFluor-MS N-Glycan Kit (Waters) following manufacturer’s protocol. Briefly, 15 μg of each mAb was denatured at 90 °C for 3 min after addition of RapiGest SF Surfactant. After cooling, glycans were released using Rapid PNGase-F at 50 °C for 3 min and labeled with RapiFluor-MS labeling reagent for 5 min at room temperature. Glycans were purified using GlycoWorks HILIC elution plate (Waters) according to the manufacturer’s protocol. Eluted glycans were diluted with N,N-dimethylformamide (DMF) / acetonitrile (ACN) diluent provided with the kit. RapiFluor-labeled glycans were separated on a Waters Acquity H-class UPLC equipped with a Waters Glycan BEH Amide (2.1 mm x 150 mm, 130 A, 1.7 mm) column. Mobile phase A was 50 mM ammonium formate (pH 4.4) and mobile phase B was ACN. Column temperature was set to 60 °C. Method used to separate N-glycans included linear gradient of 30–47 % A from 0 to 24.8 min, 47 % mobile phase A from 24.8 to 25.5 min, 70 % mobile phase A from 25.5 to 26.5 min, followed by restoring to 30 % mobile phase A from 26 to 31 min [23]. Emission and excitation wavelengths used for detection were 265 nm and 425 nm, respectively.

2.4. Transfection-based CRISPR-mediated gene knockout

CHO cells were transfected with gRNA-Cas9 ribonucleoprotein (RNP) complexes using the Lonza 4D Nucleasector 96-well Shuttle System. To form RNP complexes, 30 pmol Cas9 (Alt-R Cas9 v3, Integrated DNA Technologies, IDT) and 120 pmol gRNA (duplicated crRNA and tracrRNA, IDT) were incubated together with 30 pmol Electroporation Enhancer (IDT) in a 4 mL volume at room temperature for 15 min. For each transfection, 200,000 CHO cells were washed briefly in PBS, then resuspended in 20 μL supplemented SG Cell Line buffer (Lonza) and mixed with the RNP complexes. The cell-RNP mix was transferred to Nucleofette strips and transfected using program FF-137. After transfection, 80 μL medium was immediately added, cells were transferred to microplates, and further cultured at 37 °C without shaking. Cells were passaged and expanded every 3–5 days.

2.5. FACS-based genome-wide CRISPR screening

FACS-based CRISPR screens were performed following protocols previously described [24]. SpCas9 lentiviral particles and a genome-wide gRNA lentivirus library were both obtained from the Broad Institute (https://www.broadinstitute.org/genetic-perturbation-platform). The genome-wide gRNA library comprised 84,289 gRNA targeting 21,758 annotated protein-coding CHO genes, as well as 1000 non-targeting gRNA as negative controls. To generate CHO cells stably expressing Cas9, cells were transduced with SpCas9 lentivirus, then selected with 6 μg/mL blasticidin (Gibco) for 10–14 days. 200 million Cas9-expressing CHO cells were then transduced with a pooled lentiviral gRNA library, targeting a multiplicity of infection (MOI) of ~0.3 (see Fig. S1). Cells were selected with 6 μg/mL puromycin (Gibco) for 10–14 days and expanded to 250 million cells total. On the day of sorting, doubly-transduced cells were stained with LCA-FITC (10 μg/mL) for 30 min at 37 °C. Before sorting, 10 million cells were collected to serve as a
reference (presort). FACS was performed on a FACS Aria Fusion sorter (BD Biosciences), where the top (LCA-high) and bottom (LCA-low) 10% of the fluorescein histogram was used for gating; 8 million cells from each population were collected into separate tubes. Three independent screening replicates were performed.

2.6. Amplification and NGS of gRNA cassettes

Genomic DNA was isolated from cell pellets using Zymo Genomic DNA Isolation Kits. To amplify gRNA cassettes of interest, we followed a protocol from the Broad Institute’s Genetic Perturbation Platform (PCR of sgRNAs, shRNAs, and ORFs from genomic DNA for Illumina sequencing (https://www.broadinstitute.org/genetic-perturbation-platform)). 28 cycles of PCR were performed for each of three cell populations (LCA-high, LCA-low, and presort) as well as a plasmid-only control sample. After PCR, each Illumina-adapted ampiclon product was purified using SPRI select beads (Beckman Coulter) and brought to 4 nM prior to being pooled for sequencing on an Illumina NextSeq500 instrument using an Illumina high-output v2 75 cycle kit.

2.7. Genome-wide CRISPR screening data deconvolution

Following NGS deconvolution, data were analyzed to understand global characteristics of screening results. After the demultiplexing of reads (bcl2fastq, Illumina), quantification of gRNA across all samples was done with a custom Perl script. Briefly, users either specify the position in the read containing the gRNA or the flanking primer sequence (if gRNA position is not constant in all reads). Sequences between the flanking sequences (or by location) were extracted and compared to a database of gRNA for each library. Only perfectly-matched gRNA sequences were used in the generation of a count matrix. Normalization between all samples was done using the TMM method [25] implemented in the edgeR R Bioconductor package. Hit selection (i.e., differential analysis) at the gRNA level was carried out using limma R Bioconductor package [26] and statistical tests at the gene level were carried out using Robust Rank Aggregation (RRA) [27].

3. Results

3.1. LCA binding to CHO cells correlates with fucosylation levels of purified mAb products

As a first step in developing a phenotypic CRISPR screen, we evaluated a cell surface binding assay for its potential use as a FACS-based screening assay. The lectin Lens culinaris agglutinin (LCA) is a widely-used probe in glycobiology. LCA was our probe of choice because it specifically binds to core fucose on the Fc N-glycan, whereas other species of fucose binding lectins, such as Aurelia aurita lectin (AAL) and Aspergillus oryzae lectin (AOL), exhibit a broader specificity to fucosylated glycans [22]. Core fucosylation of proteins is executed by Fut8, which transfers fucose from GDP-fucose to N-glycans [28,29], and disruption of Fut8 in CHO cells has been shown to decrease LCA-binding and result in the production of afucosylated mAbs [30].

To assess the compatibility of this assay with a CRISPR-mediated gene KO approach, we targeted either Fut8 or Slc35c1, a solute carrier gene required for core fucosylation, for KO in CHO cells. We used a CHO cell line that expresses a humanized IgG1 mAb to enable direct measurement of mAb fucosylation in addition to cell surface LCA-binding (see Methods). After KO by transfection-based CRISPR (see Methods), mAb from each CRISPR-KO pool was produced via fed batch culture and antibodies were purified using Protein A affinity chromatography. Purified mAbs were then characterized by reduced mass spectrometry and released glycan analysis. While control cells produced mAbs that have normal fucosylation of the Fc (predominantly G0 glycans), both Fut8- and Slc35c1-KO cells produced mAbs that are largely afucosylated (predominantly G0 glycans) (Fig. 1A,B and Table 1). We also observed that fluorescently labeled LCA (LCA-FITC) binding to CRISPR-KO cells was dramatically decreased; measurement by flow cytometry showed a nearly 10-fold reduction of LCA-FITC fluorescence of Fut8- or Slc35c1-KO cells compared to control (Fig. 1C). Together, these results confirm that LCA-cell surface binding can be used as a proximal assay to detect perturbations that impact mAb core fucosylation in CHO cells.

3.2. A FACS-based, genome-wide CRISPR knockout screen identifies top regulators of fucosylation in CHO cells

Pooled CRISPR screening enables high-throughput evaluation of gene function related to a phenotype via the introduction of thousands of unique guide RNA (gRNA) in a single lentiviral pool [21]. To target each gene in the CHO genome for CRISPR-KO, we generated a library of lentiviruses comprising >84,000 unique gRNA and ~1000 negative control gRNA, which are not predicted to target any region of the CHO genome. We transduced CHO cells stably expressing Cas9 with this lentiviral pool at low multiplicity of infection (MOI) (Fig. S1) to ensure the integration of only a single lentiviral construct per cell. Transduced cells were selected via puromycin resistance, then cells were stained with LCA-FITC and sorted by FACS (Fig. 2A). Samples from three populations were collected for subsequent genetic analysis: cells with high LCA-binding (LCA-high), cells with low LCA-binding (LCA-low), and LCA-FITC-labeled cells prior to FACS (pre-sort). From each cell population, genomic DNA was isolated and the gRNA sequences (i.e., barcodes) amplified, sequenced, and quantified (Fig. S2A). The technical robustness of pooled CRISPR screens is often assessed by the correlation of NGS-recovered gRNA populations between replicate screens. For FACS-based screens, correlations between pre-sort (reference) samples and post-sort populations are examined. The correlation coefficients between each subpopulation for our screen ranged from 0.73 to 0.98 (Fig. S2B), indicating good technical reproducibility between screening replicates.

We next identified screening hits by comparing the read counts of gRNAs present in each FACS-sorted cell population (Supplemental Tables 1 and 2). As expected, gRNA targeting Fut8 and Slc35c1 were enriched in the LCA-low population (Fig. 2B,C), confirming that KO of these genes reduces LCA-binding in our screening assay.

We also recovered gRNA targeting the GDP-mannose 4,6 dehydrogenase (Gmds) and GDP-\(\alpha\)-fucose synthase (Tsta3) in the LCA-low population (Fig. 2). These genes are part of the de novo fucosylation pathway [31-33], which produces the majority of GDP-fucose through conversion of GDP-mannose. GDP-fucose synthesized in the cytosol is then transported into the lumen of the Golgi apparatus by SLC35C1 where it serves as the substrate for fucosylation reactions [34,35].

To more broadly characterize screening hits, we selected genes whose CRISPR perturbation resulted in either increased or decreased LCA binding with \(q\)-values of \(<0.3\) \((n = 112 from both directions) and submitted them to the DAVID Bioinformatics tool [36,37]. This analysis identified gene sets significantly enriched in N-Glycan biosynthesis, protein processing in endoplasmic reticulum (ER), and amino sugar and nucleotide sugar metabolism (Fig. 3).

To experimentally validate our top screening hits, we performed individual, array-based CRISPR gene KO followed by the LCA-binding FACS assay. We selected the top 12 screening hits by effect size from both LCA-high and LCA-low populations and targeted each gene using transfection-based CRISPR (see Methods). Transfected cells were cultured for 9 days to allow for protein turnover before being subjected to LCA-FITC staining and FACS analysis. Of 24 gene KO cell pools, 21 (87.5%) demonstrated a statistically significant \((p<0.05)\) difference in LCA-binding compared to control (Fig. 4). These assay results show good agreement with NGS-based gene enrichment scores obtained in the genome-wide pooled screen, further validating our phenotypic screening strategy.
4. Discussion

Fucosylation is a form of glycosylation in which fucose is transferred to glycans. Fucose is a common building block for N- and O-linked glycans produced in mammalian cells. A total of 14 fucosyltransferases (FUT family members) have been identified in the CHO genome; these gene products transfer a fucose residue from GDP-fucose to an acceptor substrate. Of these, FUT8 is the only \( \alpha_1,6 \)-fucosyltransferase that transfers fucose via an \( \alpha_1,6 \) linkage to the innermost N-acetylglucosamine on N-glycans for core fucosylation [29]. As anticipated, KO of Fut8 was shown as one of the strongest screening hits; the remaining Fut genes did not have noticeable impact on altering the cell-surface LCA reactivity (Fig. S3A; Supplemental Table 2).

Asparagine-linked glycosylation (Alg) pathway genes are responsible for assembly of the lipid-linked oligosaccharide structure in the ER membrane during nascent N-glycosylation [38]. Alg6, Alg8, and Alg10b were all recovered as LCA-low (afucosylation) hits. Other afucosylation hits recovered include STT3 oligosaccharyltransferase complex catalytic subunit A (Stt3a), mannosyl-oligosaccharide glucosidase (Mogs), and glucosidase II alpha subunit (Ganab). Taken together, our data suggest that depletion of ALG and oligosaccharyltransferase complexes may disrupt intracellular trafficking, resulting in depletion the substrate for core fucosylation in CHO cells (Fig. 3).

SLC35C1 is a member of the solute carrier 35 (SLC35) family of nucleotide sugar transporters and is critical to protein fucosylation via its function in translocating nucleotide sugar substrates, such as GDP-

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**Table 1**

<table>
<thead>
<tr>
<th>RNP transfection</th>
<th>Methods</th>
<th>Man5</th>
<th>Man6</th>
<th>Man6 + Lys</th>
<th>Man7</th>
<th>Man8 + Lys</th>
<th>G0-GlcNAc</th>
<th>G0F-GlcNAc</th>
<th>G0</th>
<th>G0F</th>
<th>G1</th>
<th>G1F</th>
<th>G2</th>
<th>G2F</th>
<th>% afucosylated</th>
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<tr>
<td>Parental (WT)</td>
<td>Reduced MS</td>
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<td>0.1</td>
<td>2.6</td>
<td>2.0</td>
<td>0.1</td>
<td>0.0</td>
<td>5.8</td>
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<td>83.1</td>
<td>0.0</td>
<td>5.4</td>
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<td></td>
<td>Released glycan MS</td>
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<td>0.7</td>
<td>0.12</td>
<td>5.4</td>
<td>0.4</td>
<td>86</td>
<td>0.0</td>
<td>3</td>
<td>0.0</td>
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<td>2.2</td>
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<td>0.0</td>
<td>5.4</td>
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<td>0.0</td>
<td>5.8</td>
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<tr>
<td></td>
<td>Released glycan MS</td>
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<td>0.6</td>
<td>0.7</td>
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<td>5.1</td>
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<td>86</td>
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* High mannose structures are not included in the calculation for % afucosylated
fucose, from the cytosol to ER or Golgi [33]. As expected, Slc35c1 showed the single strongest loss-of-fucose (afucosylation) phenotype recovered in our screen (Fig. 2B). Although we did not observe that loss of the related gene, Slc35c2, was involved in CHO protein fucosylation (Fig. S3B), overexpression of Slc35c2 has been shown to reduce expression of the fucosylated epitopes LewisX and sialylated LewisX in CHO cells, suggesting that exogenously expressing Slc35c2 may interfere with fucosylation activity [39, 40]. Further, while our screen is, in principle, compatible with discovery of both positive and negative regulators of fucosylation, standard CHO cell culture conditions generally produce high levels of core fucosylation of cell surface glycoproteins (see LCA-FITC staining of control cells in Fig. 1C). Therefore, our screen is likely to be less sensitive for the discovery of factors that increase fucosylation due to limited dynamic range. Nevertheless, our approach was powerful enough to identify genes Slc35a1 (CMP-sialic acid (Sia) transporter), Slc35a2 (UDP-Galactose (Gal) transporter), and Slc35a3 (UDP-N-acetylglucosamine (GlcNAc) transporter) enriched in the LCA-high fraction, providing a systems approach to interrogate the interplay of protein fucosylation among transport of selected CMP-Sia, UDP-Gal, and UDP-GlcNAc (Fig. S3B; Supplemental Table 2).

We found that several mannose-glycoprotein N-acetylgalactosaminyltransferase genes – Mga1, Mga2, Mga4b and Mga5 – were top hits impacting protein core N-linked fucosylation (Fig. 2 and Fig. S3C). These branching GlcNAc transfers enzymes incorporate GlcNAc into N-glycan antennae: MGAT1 and MGAT2 form mono- and biantennary N-glycans, and MGAT4 and MGAT5 are responsible for further branching [40–42]. Loss of Mga2, Mga4b, and Mga5 was associated with increased LCA-binding. The addition of a GlcNAc residue by MGAT4 and MGAT5 to core-fucosylated, biantennary N-glycans has been shown to abrogate the binding affinity of LCA [43]. Perhaps in the absence of these enzymes, and thus the absence of these antennary GlcNAc residues, increased LCA-binding could be expected. Loss of Mga1 resulted in low LCA-binding; this is consistent with the idea that glycosylation patterns in Mga1-deficient CHO cells are limited to only early intermediates in the N-linked glycosylation pathway, which may result in blocked core fucosylation [44]. We did not observe a functional impact of Mga3 on fucosylation, likely explained by Mga3 gene inactivation in CHO cells [45].

Two enzymes that are part of the salvage fucosylation pathway, fucose kinase (FCSK) and fucose-1-phosphate guanylyltransferase...
unique gRNA, 3 biological replicates) is shown for each sample. Error bar, median absolute deviation. Student’s t-test comparing targets listed to NTC. ** P < 0.005; *** P < 0.0005; * P < 0.05; ns, not significant.

Fig. 4. Screening hits are well-validated in arrayed, LCA-binding assay. Quantification of LCA-cell surface binding to KO cell pools. For each KO cell pool indicated, median fluorescence intensity (MFI) of cell-bound LCA-FITC signal was measured by FACS, then normalized to the MFI of non-targeting control (NTC) cells. Results are shown as Percent Activity compared to NTC. Dotted lines indicate 100 % activity and location of NTC bar on plot. Median from 12 data points (4 unique gRNA, 3 biological replicates) is shown for each sample. Error bar, median absolute deviation. Student’s t-test comparing targets listed to NTC.

It is important to note that while we identified multiple genes whose KO affects the fucosylation process in CHO cells, we did not specifically assess their effect on cell proliferation, protein secretion, or mAb titer. During our short-term validation studies, cells were cultured in a static multi-well plate, and we did not observe marked differences in cell density by eye. However, CHO cells used for biologics production at scale are maintained in culture for multiple weeks and under highly controlled conditions. Thus, any long-term effect on cell viability or proliferation should not be excluded from our studies. Fully understanding a gene’s role in each of these processes – effect on fucosylation, cell proliferation, protein expression, and mAb titer – while outside the scope of this work, would be a critical part of any strategy for generating genetically engineered cells for commercial mAb production.

Cas9-based methods are very effective in single-gene knockout screening; they greatly underperform in combinatorial genetic screening due to the complexities of gRNA library construction and challenges in introducing the dual gRNA expression system into target cells [47]. Cas12a, an RNA-programmable DNA endonuclease similar to Cas9, has emerged as a promising tool for multiplexed genetic perturbations; this enzyme can process multiple guide RNAs expressed as a single transcript, simplifying efforts toward genetic interaction screening at scale [48]. We anticipate that Cas12a combinatorial gene perturbation approaches might discover novel cell engineering targets that represent promising glycoengineering strategy to generate the next generation of afucosylated mAbs.

Glycosylation profiles of mAbs play a vital role in drug product quality as well as therapeutic safety and efficacy, and the ability to fine-tune the extent or type of mAb glycosylation is a common challenge faced in the biopharmaceutical industry. An elegant solution is to generate genetically modified, ‘platform’ CHO cell lines designed to produce mAbs with specific glycosylation profiles [15,22,23,31]. Toward this end, we performed a genome-wide CRISPR screen in CHO cells to identify novel genes involved in regulating the fucose content on mAbs, as fucosylation levels play a critical role in the therapeutic efficacy of anti-cancer mAbs. We combined this unbiased, high-throughput approach with targeted single gene-KO, demonstrating a broad functional genomics screening platform that is widely applicable to studying the biological regulation of other mAb modifications. Other forms of mAb glycosylation – for example, mannosylation or sialylation – could be particularly well-suited to FACS-based, pooled CRISPR screening, given the wide array of glycobiology reagents available commercially. Finally, we envision that CRISPR-based functional glycomics screening can add new knowledge of glycosylation processes and translate into rationally designed glycoengineering strategies that provide improved properties to glycoprotein therapeutics, as exemplified by afucosylated IgG therapeutic antibodies.

AbbVie disclosure

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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