TAILORING ANTIBODY GLYCOSYLATION VIA INTEGRATING GENOME AND PROTEIN ENGINEERING TO GENERATE PREFERRED GLYCOFORMS ON THE FC REGION

Mike Betenbaugh, Johns Hopkins University, USA
beten@jhu.edu

One critical quality attribute of therapeutic antibodies is the glycosylation pattern at the Fc region.We combined genome editing of CHO cells and protein engineering of the IgG Fc region to allow antibodies presenting high level of galactosylation or exclusively α-2,6 sialylation. To generate IgG with high α-2,6 sialylation, we combined amino acid mutations in the Fc region of IgG and introduction of α-2,6 sialyltransferase in CHO to produce IgGs with significant levels of both α-2,6 and α-2,3 sialylation. Furthermore, to produce exclusively α-2,6 sialylation IgG in CHO, CRISPR/Cas9 was implemented to disrupt two dominant α-2,3 sialyltransferase genes (ST3GAL4 and ST3GAL6), then α-2,6 sialyltransferewas introduced in a α-2,3 sialylation knockout cell line. Notably, no α-2,3 linked sialic acids of IgG produced from the α-2,3 sialyltransferase knockout-α-2,6 sialyltransferase overexpression pools were detected by HPLC sialic acid quantification after the α-2,3 linkage specific sialidase cleavage. Finally, glycosylation analysis of IgG with four amino acid mutations generated by an α-2,3 sialyltransferase knockout-α-2,6 sialyltransferase overexpression stable CHO clone rendered >75% of sialylated glycans, among which 62.5 % was **biantennary disialylated** glycans.

Interestingly, the disruption of two α-2,3 sialyltransferases (ST3GAL4 and ST3GAL6) from CHO cells in conjunction with protein engineering of the Fc region produced IgGs with a great majority of bigalactosylated and fucosylated (G2F) glycoforms. Expression of the IgG with engineered Fc region (F241A) in triple gene knockout (FuT8-/-, ST3GAL4-/- and ST3GAL6-/-) CHO cells lowered the galactosylation content to 65% bigalactosylated glycoform (G2). However, overexpression of IgGs with four amino acid substitutions from the α-2,3 sialyltransferases knocked out CHO cells reconstituted the fraction of G2 glycoform back up to approximately 80%. Collectively, this study, to our knowledge, is the first attempt for generating highly galactosylated or solely α-2,6 sialylated N-glycans on antibodies *in vivo*, allowing researchers in both academia and industry to evaluate the significance of tailoring glycosylation on IgGs in biomedicine and biotechnology applications.

References:
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