World of Knowledge

Post-translational modification of glycosylation on therapeutic monoclonal antibodies – the means to the immune modulation

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Due to the advantages of safety, protein drugs (biologics) has become the mainstream for the current development of new drugs. Among them, the therapeutic monoclonal antibody is turned to become the best because of high degrees of specificity, significacy, easiness to establish platform of development and other advantages. However, the activity of antibody is deeply correlated to the glycosylation on the Fc region of antibody. This post-translational modification of glycosylation not only modulates the interplay between antibody and immune system, but also affects the efficacy and safety during the treatment.

Like a two-edged sword, drugs not only cure diseases but also poison people. Thus, safety is the utmost concern in the process of drug discovery. The unqualified safety will lead to stop drugs development in either the early stage or clinical trials. To raise the success rate and reduce the huge expenses in this process, a good strategy is essential.

Current pharmaceutical industry is shifting the focus from small molecules to biologics because of its several common strengths, such as high specificity, strong affinity, good solubility, and the low incidence of toxicity, etc. It was reported that over 9,200 new drug entities entered the stage of clinical trials and regulatory phase from 1996 to 2014, in which the biologics revealed higher cumulative success rate among these stages and exhibited up to two fold of increase in periods of the final three years – 9% and 18% for small molecules and biologics, respectively[1]. Still, side effects and immunogenicity exist among these protein drugs regardless of the developing and advancement of biotechnology. Further, variability of protein structure and alteration of post-translational modification could be elicited by the change of either cell lines or procedures in biologics expression, in which it affects the eligibility of protein drugs not only in the metabolism and activity but also in the immunogenicity.

As the fastest growing biologics, the commercial therapeutic monoclonal antibodies and the pertinent derivatives are applicable to several diseases[2], including organ transplant rejection, rheumatoid arthritis, multiple sclerosis, multiple myeloma, metastatic colorectal cancer, carcinoma of head and neck, breast cancer, and lymphoma, etc. By means of targeting the abnormal over-

expressed markers on pathogenic cells or the signal transduction molecules among pathogens, either various anti-pathogenic cytotoxicity is initiated in immune system or the pathway of pathogenic signal transduction is blocked to prevent the ferocity of pathogens.

Antibody with molecular weight of 150 KDa includes two important portions, Fab and Fc, to execute functions (Figure 1). While the Fab recognizes pathogens, the Fc either interacts with Fc gamma receptors (FcγRs) on immune cells to precisely localize immune cells to pathogens and conducts the antibody-dependent cell-mediated cytotoxicity (ADCC) (Figure 2) or attracts complements in sera to the nearby and trigger the complement-dependent cytotoxicity (CDC). The process correlates to the efficacy of antibody in treatments. Importantly, there is one pair of post-translational modification localized in the Fc region. It is glycosylation with molecular weight about 2 KDa which is able to modulate the interactions with Fc gamma receptors of immune cells as well as the subsequent antibody-mediated cytotoxicity against pathogens. By means of the engineering of glycosylation biosynthetic pathway, the Kyowa Hakko Kirin Co. Ltd. of Japan and the Roche Ltd. utilized strategies of Fc glycan's involvement in therapy to develop defucosylated monoclonal antibodies, mogamulizumab and obinutuzumab, respectively[3]. Both of them elevate the efficacy of antibody drugs and are applicable to patients of the relapsed CCR4 positive T-cell lymphoma or the chronic B-cell lymphoma.

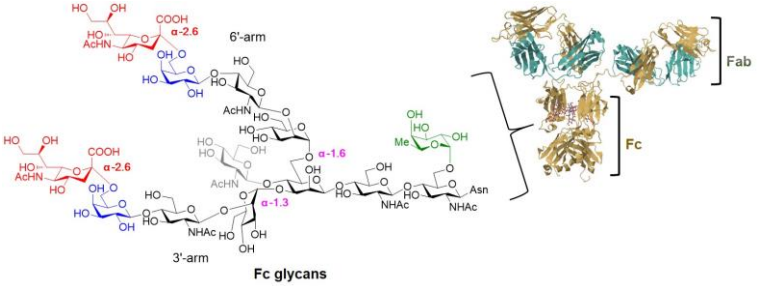

Figure 1 Structure of antibody and the Fc glycan.

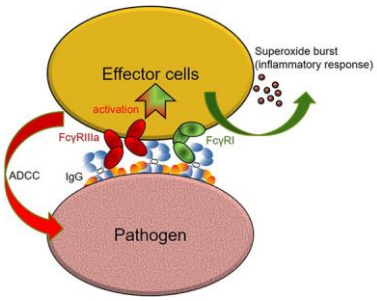

Figure 2 Antibody-dependent cell-mediated cytotoxicity (ADCC).

However, accompanied by the shift of cell lines or procedures in antibody expression, Fc glycosylations change among different lots of production and show uncertainty in efficacy and safety[4]. For instance, it is common to find N-glycolylneuraminic acid in antibodies expressed in the mouse myeloma cell lines, such as NS0 or SP2/0. Though this carbohydrate is a derivative of sialic acid, it is reported to be a potential immunogen which could elicit immunogenicity. Thus, before a new antibody drug enter the clinical trials, characterization of glycosylation is extremely important in the stage of " Chemistry, Manufacturing and Controls (CMC) ".

To overcome the heterogeneity of Fc glycans in therapeutic monoclonal antibodies, in collaboration with Prof. Chi-Huey Wong, our lab focuses on the discovery of homogeneously Fc glycosylated antibodies[5]. By means of chemo-enzymatic methods, Fc glycans of commercial antibodies, including transtuzumab, rituximab, and others, were “cut” and “glued” to constitute single forms of Fc glycosylation on antibodies. It is indicated in the analysis of surface plasma resonance (SPR) that several factors correlate to the affinity towards FcγRIIIa, receptors which dominate the ADCC. Those include the presence of core-fucosylation, the linkage of sialylation and the length in glycan branches. Meanwhile, similar tendency is also observed in the transtuzumab mediated ADCC against SKBR3 carcinoma. Moreover, the set of various homogeneously glycosylated transtuzumab reveal effects of glycan length in the binding towards FcγRI, crucial receptors pertinent to cytotoxicity and inflammation.

Based on the structure-activity relationship (SAR) of our studies between Fc glycan and antibody, one Fc glycan concomitant with de-core fucosylation and 2,6-sialylation (called SCT) shows potential and was selected for further study. We found that the SCT structure not only elevates the cytotoxicity of transtuzumab against breast carcinoma but also intensity the killing activity of rituximab against B lymphoma cells. Besides, it is accessible to treat the resistant strain of B lymphoma cells and maintain quite good efficacy. Further, in those cells infected by H1N1 influenza, this SCT structure also grants the neutralizing FI6 antibody upgraded ADCC activity and show increased survival rates to protect those infected mice. In short, it implied a universal Fc glycan (SCT) which is suitable to intensify the cytotoxicity of antibodies against various targets with diverse pathogenic properties.

Lastly but not least, besides the cytotoxicity against pathogens, the Fc glycan of antibody is also reported to play a role in half-life of antibodies, inflammatory responses, autoimmune, etc. Still, it was too difficult to prepare antibody with single glycosylation and thus unable to clarify the correlation between the glycan structure and the various modulation of antibodies in the immune system. Hope our current progress and efforts in glycan related research can benefit the advancement of this field and prompt a new generation of therapeutic antibodies with greater efficacy and less side effects.

Extended readings:

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2. Ecker, D.M., Jones, S.D. & Levine, H.L. The therapeutic monoclonal antibody market. *MAbs* **7**,

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3. Beck, A. & Reichert, J.M. Marketing approval of mogamulizumab: A triumph for glyco-engineering. *mAbs* **4**, 419-425 (2012).
4. Hansel, T.T., Kropshofer, H., Singer, T., Mitchell, J.A. & George, A.J.T. The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov* **9**, 325-338 (2010).
5. Lin, C.-W. *et al.* A common glycan structure on immunoglobulin G for enhancement of effector functions. *Proc Natl Acad Sci USA* **112**, 10611-10616 (2015).