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Modulating carbohydrate–protein interactions through glycoengineering of monoclonal antibodies to impact cancer physiology

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Diverse glycans on proteins impact cell and organism physiology, along with drug activity. Since many protein-based biotherapeutics are glycosylated and these glycans have biological activity, there is a desire to engineer glycosylation for recombinant protein-based biotherapeutics. Engineered glycosylation can impact the recombinant protein efficacy and also influence many cell pathways by first changing glycan–protein interactions and consequently modulating disease physiologies. However, its complexity is enormous. Recent advances in glycoengineering now make it easier to modulate protein-glycan interactions. Here, we discuss how engineered glycans contribute to therapeutic monoclonal antibodies (mAbs) in the treatment of cancers, how these glycoengineered therapeutic mAbs affect the transformed phenotypes and downstream cell pathways. Furthermore, we suggest how systems biology can help in the next generation mAb glycoengineering process by aiding in data analysis and guiding engineering efforts to tailor mAb glycan and ultimately drug efficacy, safety and affordability.

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Introduction

Monoclonal antibodies (mAbs) are the major category of glycoprotein-based therapeutic drugs, approved by the US Food and Drug Administration (FDA) [1]. Furthermore, they have attained considerable success in many therapies, including cancer, over the past three decades [2]. Despite advances in contemporary biopharmaceutical technologies, many challenges remain in efficiently manufacturing effective and affordable antibody-based drugs. Since glycosylation essentially impacts the therapeutic efficacies of mAbs [3], it is desirable to control glycoforms on therapeutic mAbs for the next generation mAb development. With a fast growing repertoire of innovative technologies, glycoengineering promises to allow us to further tune and control the activities of therapeutic mAbs [4••,5]. An effective glycoengineered mAb usually modulates specific interactions between the designed glycans and target proteins, thereby impacting the activity of downstream pathways that control cancer physiology. Conversely, a wrong glycan can induce unwanted side effects and even adverse immunogenic response [6]. For example, some colorectal cancer patients have developed hypersensitivity to the FDA approved mAb cetuximab [7].

Several intriguing and unsolved questions in mAb glycoengineering remain, including the following. Which glycan structures will provide the optimal mAb? How can we efficiently and reliably engineer a consistent glycoform on mAbs? Challenges in answering these questions stem from our limited understanding regarding the intricate relationships between glycans, proteins, and host cell physiology. Furthermore, even when desired glycoforms are known, it has been difficult to unravel all of the factors that influence glycosylation and to control the complex system. Systems biology provides a powerful toolbox for integrating heterogeneous omics data and for deciphering the mechanisms and interactions between molecules and pathways, using network analysis, mathematical modeling, and simulation [8,9]. An abundance of omics technologies have been developed to aid in studying expression systems (e.g., [10]), but the application of omics data and systems biology in glycoengineering is still in its infancy. Here we review the state-of-art knowledge of glycan–protein interactions in the context of FDA-approved therapeutic mAbs and then summarize several innovative technologies that can help control the glycoforms on mAbs. Finally, systems biology-based glycoengineering approaches are explored with an emphasis on how systems biology can be used to advance

anti-tumor mAb development toward a predictable glycoengineering era.

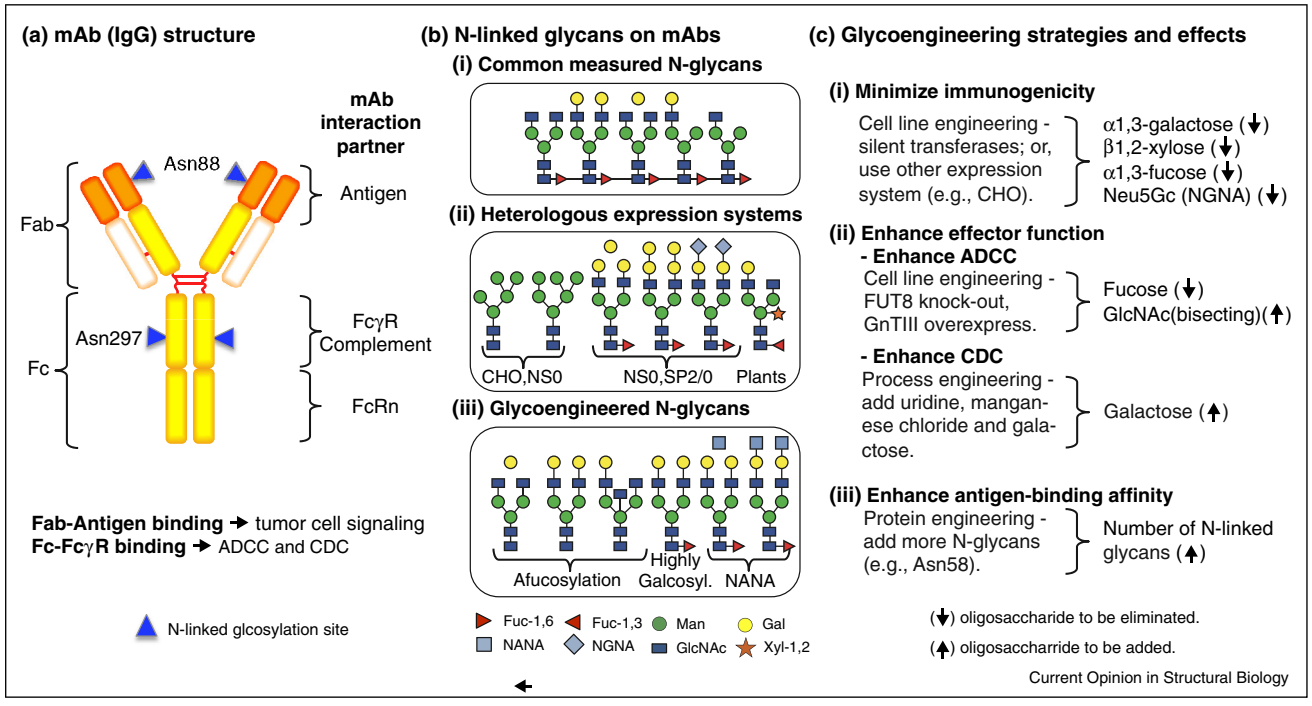
Glycan–protein interactions involving therapeutic antibodies impact cancer physiology

Glycosylation helps to modulate interactions between mAbs and antigens or Fcγ receptors (Figure 1a), and impact the efficacy and safety of a biotherapeutic drug. The glycan–protein interactions of FDA-approved therapeutic mAbs in various cancer settings and their subsequent effects reported in the literature are summarized in Table 1.

Fab–antigen interaction. Fab glycans have several roles in modulating interactions with receptors and glycoengineering can help reduce negative interactions leading to immunogenicity. Early research demonstrated that engineering N-linked oligosaccharides on the Fab region can enhance the antigen-binding affinity of mAbs [11]. However, a comprehensive understanding of the glycans on Fab region is still lacking. One example of their role is the allergenic responses to therapeutic antibody cetuximab [7]. It was produced by murine myeloma cells (SP2/0), which adds an additional α1,3-galactose (the α-Gal epitope) on the N-linked oligosaccharide at Asn88 of the Fab region (Figure 1a). Unfortunately, the human IgE recognizes the non-human α-Gal epitope and leads to downstream immune responses, such as hypersensitivity reactions (anaphylaxis) after drug treatments. Therefore, extra efforts must be made to glycoengineer the drug to reduce α-Gal content and solve the immunogenicity problem (e.g., [12]). Moreover, the Fab–antigen binding affinity could be affected by targeting receptors. Recent mutagenesis studies showed that the potential glycosylation sites (Asn16, Ans25, Asn41, and Asn83) on Programmed death 1 (PD1) could impact antigen-binding affinity (e.g., nivolumab and pembrolizumab) by influencing its local structure [13]. Physiologically, PD1 is an inhibitory receptor that suppresses T cell responses to avoid auto-immunity. Indeed, many factors can affect Fab–antigen binding in cancer treatment, and glycoengineering can be applied in mAb design either to optimize Fab–antigen binding affinity or to eliminate immunogenicity problem.

Beyond immunogenicity, Fab-binding can target cancer by modulating glycan–protein interactions. This occurs by either directly targeting glycans (Fab–glycan interaction) or indirectly modulating downstream protein–glycan

Figure 1



Therapeutic mAb structure, glycoforms, and glycoengineering strategies for generating desired glycoforms. **(a)** The structure of an IgG with interaction-partner binding regions and N-linked glycosylation sites (highlighted in blue triangles) are annotated. **(b)** The dominant N-linked glycans on mAbs can vary depending on the host and product. However, (i) common glycans on therapeutic mAbs have been measured. (ii) MAbs expressed in heterologous expression systems introduce non-human compatible sugars and linkages, leading to immunogenicity and low serum half-life. (iii) Glycoengineering aims to make mAbs with N-glycans that are human compatible and exhibit enhanced mAb efficacy and safety. **(c)** Many glycoengineering efforts aim to enhance the drugs and achieve any of the three effects (i–iii) by modifying glycans on mAbs. NANA: N-glycolylneuraminic acid (hyper-sialylation). Data of **(b)** in this figure was adapted from [34].