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Anti-tumor IgE antibody: enhancing strength by stripping sugars

Antibody based therapeutics have emerged as a key player in the management of diseases including cancer with majority of monoclonal antibodies (mAbs) belonging to the IgG class. Recently, increasing efforts have been made to develop non-IgG class immunoglobulins for immunotherapy. IgE-based therapeutics are an emerging field of research. Notably an IgE molecule targeting folate receptor alpha has completed phase 1 of clinical trials for ovarian cancer therapy and progressed to phase 1b trial. Compared with IgG, IgE antibodies have multiple useful properties such as higher affinity for their cognate receptors, longer half-life in tissues, absence of inhibitory receptors, and enhanced antigen uptake as well as presentation by antigen presenting cells to cognate T cells. Therefore, the development of engineered IgE molecules for antibody-based treatment of human diseases is desirable.

Antibodies are glycoprotein and the types of glycan and its composition are dependent on the choice of the expression host and culture conditions. The glycosylation of therapeutic antibodies raises safety concerns such as immunogenicity, can impact product consistency, and influence solubility, stability, pharmacokinetics and effector functions. Therefore, regulatory agencies such as the World Health Organisation (WHO) and the European Medicines Agency (EMA) adhere to stringent guidelines requiring thorough characterization and identification of glycans before a drug is submitted for approval.

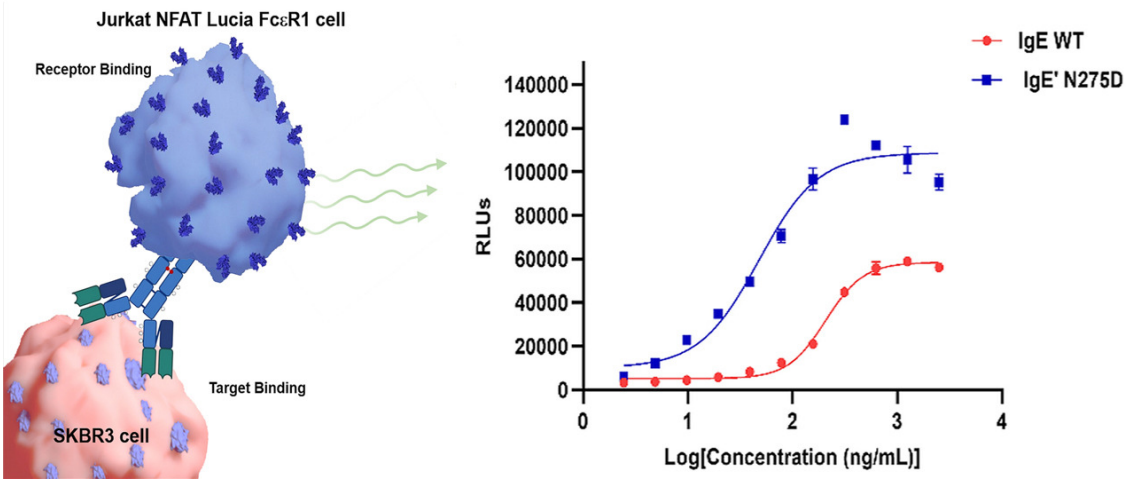

Fig. 1. Analysis of the effector function of the deglycosylated IgE (IgE'N275D) compared with IgE WT using Jurkat NFAT Lucia stably expressing FceR1 reporter cell line.

IgE antibodies are heavily glycosylated and their expression in mammalian cell lines generates a diverse pool of glycosylated antibodies (glycoforms) leading to antibody heterogeneity which might impact efficacy, dosage, safety, and biological function. In addition, the functional role of IgE glycosylation remains poorly understood.

We have performed a systematic mutational analysis of the amino acid residues involved in the N-glycosylation of IgE and characterized its functional role using multidisciplinary approaches. Our study

revealed that glycosylation of the conserved asparagine residue at the 275th position is critical for the overall yield and stability of the molecule. However, deglycosylated IgE (IgE' N275D) exhibited superior biological function, contradicting the notion that glycans at the 275th position are crucial for the biological function of IgE (Fig. 1). To the best of our knowledge, the study provides first example of a customized Jurkat Lucia NFAT cell line stably expressing the FceRI receptor to facilitate functional validation of engineered IgE antibodies (Fig. 1).

For bioproduction, an IgE molecule with low glycosylation complexity is desirable to minimize batch-to-batch variability and enhance therapeutic efficacy. Our analysis has established two functional antibodies, one which lacks glycosylation (IgE' N275D) and the other with reduced glycans complexity at the Asn 275th position (IgE' N275), as candidate molecules for further development as IgE biotherapeutics (Fig. 2).

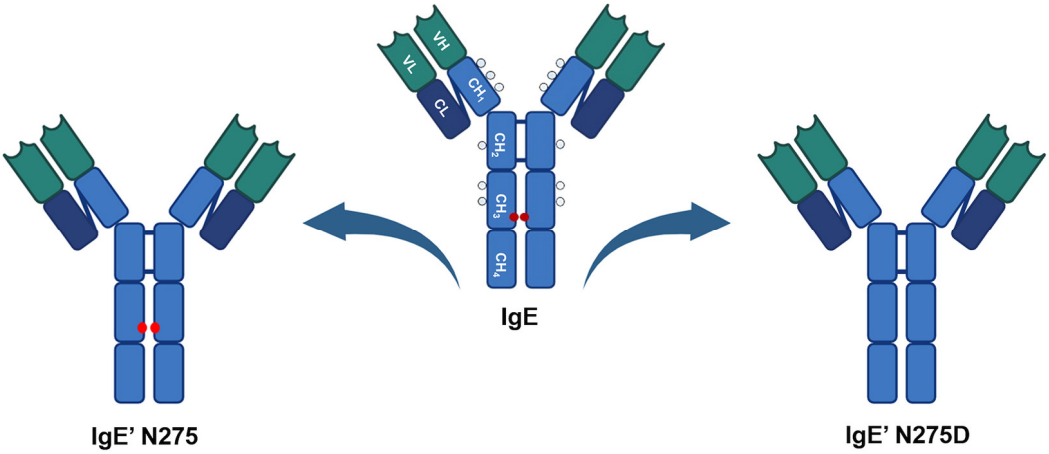

Fig. 2. Development of glycovariants with low or no glycosylation complexity. Glycosylation sites are represented by open circle and conserved glycan site is represented by red circle. (VH: variable region of heavy chain; CH: constant domain of heavy chain; CL: constant domain of light chain)

In summary, our study unveils an intricate relationship between N-glycosylation sites and the structural–functional dynamics of IgE antibodies. Furthermore, it offers novel insights into the IgE scaffold, paving the way for the development of more effective and stable IgE-based therapeutics.

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Publication

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