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Mechanism Study on Enhancing ADCC Effect of Antibody Drugs via Fc Fragment Defucosylation Modification

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Abstract

Antibody drugs specifically recognize tumor antigens through their Fab fragments and bind to Fcγ receptors on the surface of immune effector cells via their Fc fragments, which promotes antibody-dependent cell-mediated cytotoxicity (ADCC) and thus eliminates target cells. Core fucose residues on the N-glycans of the Fc fragment hinder the high-affinity binding between antibodies and FcγRIIIa through a steric hindrance effect. The affinity between antibodies and FcγRIIIa can be increased by 10 to 50 times after fucose removal, and the ADCC effect is significantly enhanced. This paper systematically elaborates on the molecular mechanism by which defucosylation modification enhances ADCC, introduces its technical implementation paths and development history in detail, and verifies the clinical value of this strategy with Obinutuzumab as an example. On this basis, the paper further discusses the clinical benefits and potential risks of defucosylated antibodies, analyzes the impact of FcγR gene polymorphism on therapeutic efficacy, introduces the latest progress of Obinutuzumab in the treatment of lupus nephritis, and looks forward to the application prospects of this technology in the research and development of the next-generation antibody drugs.

Keywords

Fc fragment modification, defucosylation, ADCC, FcγRIIIa, obinutuzumab, glycosylation engineering

1. Introduction

Therapeutic antibodies have become one of the core drug categories for tumor targeted therapy. Since the approval of the first therapeutic antibody OKT3 in 1986, antibody drugs have undergone four technological iterations from murine antibodies, chimeric antibodies, humanized antibodies to fully human antibodies [8]. At present, immunoglobulin G (IgG) antibodies have become the most widely used antibody subtype in clinical practice because they have a longer half-life and clear effector functions. The molecular structure of IgG antibodies presents a characteristic Y-shape and consists of two structural domains with clear functions, which have Fab fragments (antigen-binding fragments) located on the two arms and are responsible for the specific recognition of antigens on the surface of tumor cells. The Fc fragment (crystallizable fragment) is located on the stem and serves as the core effector structure for antibodies to “communicate” with the immune system.

Antibody drugs exert anti-tumor effects mainly through four mechanisms, which include direct blockade of signal pathways, induction of apoptosis, complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Among these mechanisms, ADCC is regarded as one of the core mechanisms for antibody drugs to eliminate tumor cells. This process relies on the binding of the antibody Fc fragment to Fcγ receptors on the surface of immune effector cells such as natural killer cells and macrophages, which activates effector cells to release perforin and granzyme and ultimately leads to target cell lysis [6].

Although antibody drugs have achieved remarkable results in tumor treatment, their ADCC effect is limited by the natural affinity between the antibody Fc fragment and FcγRIIIa (CD16a) [2]. Studies have shown that there is a conserved N-glycosylation site on asparagine 297 (Asn297) in the CH2 domain of the IgG antibody Fc fragment, and the composition of its N-glycans significantly affects the binding ability of the Fc fragment to Fcγ receptors. In particular, the presence of fucose residues on the core structure of N-glycans hinders the favorable conformational matching between the Fc fragment and FcγRIIIa through a steric hindrance effect, thus limiting the full exertion of the ADCC effect. This finding provides a new entry point for the optimization of antibody drugs, which is that the affinity between antibodies and FcγRIIIa can be significantly enhanced by removing the core fucose on the N-glycans of the Fc fragment, thereby improving the ADCC effect. Defucosylation modification has therefore become a research hotspot in the field of antibody glycosylation engineering and has been successfully applied in clinical practice. In 2013, Obinutuzumab, a defucosylated anti-CD20 antibody developed by Roche/Genentech, was approved for marketing and became the first approved antibody drug with Fc fragment defucosylation modification, which provides a new treatment option for patients with B-cell malignant tumors [4]. In recent years, the indications of Obinutuzumab have been further expanded to the field of autoimmune diseases, and its potential in the treatment of lupus nephritis has attracted extensive attention [4].

This paper aims to systematically elaborate on the molecular mechanism by which Fc fragment defucosylation modification enhances ADCC, introduce its technical implementation paths and development history in detail, verify the clinical value of this strategy with Obinutuzumab as an example, and conduct an in-depth analysis of the clinical benefits and potential risks of defucosylated antibodies. Finally, combined with the latest research progress of Obinutuzumab in the treatment of lupus nephritis, the paper looks forward to the application prospects of this technology in the treatment of autoimmune diseases, which is expected to provide a theoretical reference for antibody engineering optimization and clinical translation.

2. Molecular Mechanism and Regulatory Factors of ADCC Effect

2.1 Activation Process of ADCC Effect

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important bridge between adaptive immunity and innate immunity. The activation of ADCC requires the synergistic effect of three key elements, which are specific antigens on the surface of target cells, specific recognition by antibody drugs and activation of effector cells [12]. First, antibodies bind to specific antigens on the surface of tumor cells through their Fab fragments. The selective expression, membrane surface density of antigens and the affinity of antigen-antibody binding directly affect the initiation efficiency of ADCC. Studies have shown that the antigen density on the surface of target cells is positively correlated with the ADCC intensity, and the ADCC effect will be significantly weakened when the antigen density is lower than a certain threshold [12]. Second, after the antibody binds to the antigen, its Fc fragment becomes a “label” recognized by effector cells. Natural killer (NK) cells are the main effector cells of ADCC, which highly express FcγRIIIa (CD16a) on their surface and this receptor belongs to the activating Fcγ receptor family. In addition, macrophages, neutrophils and other cells also express different types of Fcγ receptors, which can participate in the ADCC process under specific conditions. Finally, the binding of the antibody Fc fragment to FcγRIIIa on the surface of NK cells forms an immune synapse, which triggers an intracellular signal transduction cascade reaction. The intracellular segment of FcγRIIIa contains an immunoreceptor tyrosine-based activation motif (ITAM), which initiates the degranulation process of NK cells through the Syk kinase and PI3K pathways after activation and releases perforin and granzyme, ultimately inducing target cell apoptosis [6].

2.2 Key Factors Affecting ADCC Efficiency

ADCC efficiency is regulated by a variety of factors, which can be classified into three categories including antibody-related factors, effector cell-related factors and target cell-related factors. Antibody-related factors: the subtype of antibodies, Fc fragment glycosylation modification, antigen-binding affinity and other factors all affect ADCC efficiency. Among IgG subtypes, IgG1 has become the preferred subtype of therapeutic antibodies because it has a strong affinity for Fcγ receptors. The composition of Fc fragment N-glycans has a decisive impact on the binding ability of antibodies to FcγRIIIa, among which the presence or absence of core fucose is the most critical regulatory factor [3]. Effector cell-related factors: the FcγRIIIa gene has functional single nucleotide polymorphisms (SNP), and the 158th amino acid is valine (V) or phenylalanine (F). The 158V variant has a significantly higher affinity for the antibody Fc fragment than the 158F variant, and patients with the 158V/V genotype usually show a better clinical response when receiving antibody therapy [12]. In addition, the activation state, quantity of effector cells and inhibitory signals in the tumor microenvironment also affect ADCC efficiency. Target cell-related factors: the antigen density on the surface of target cells, antigen modulation ability, membrane composition such as sialic acid content and the expression level of anti-apoptotic molecules all affect ADCC sensitivity. A study by Temming et al. [12] found that defucosylated antibodies can induce effective ADCC even at extremely low antigen densities, while fucosylated antibodies cannot, which highlights the advantage of defucosylation modification in overcoming the limitation of antigen density.

2.3 Regulatory Effect of Fucosylation on ADCC

Fucosylation is a common type of glycosylation in protein N-glycan modification. On Asn297 in the CH2 domain of the Fc fragment of IgG1 antibodies, the core structure of N-glycans usually contains a core fucose residue catalyzed and added by α1,6-fucosyltransferase encoded by the FUT8 gene. This fucose residue is linked to the first N-acetylglucosamine of the N-glycan core through an α1,6-glycosidic bond.

Ferrara et al. [3] first revealed the molecular mechanism by which core fucose affects Fc-FcγRIIIa binding through structural biology research. They found that there is a carbohydrate-carbohydrate interaction between core fucose and N-glycans on the FcγRIIIa receptor. When core fucose is present, its spatial conformation physically hinders the favorable conformational matching between the Fc fragment and FcγRIIIa, resulting in a decrease in the affinity of the two. After the removal of core fucose, the steric hindrance disappears, and the Fc fragment can form a tighter and more stable binding conformation with FcγRIIIa, with the affinity increased by 10 to 20 times [3].

In recent years, molecular dynamics simulation studies have further confirmed this mechanism. Li et al. [7] found through molecular dynamics simulation that the number of glycan-glycan contacts between Fc and FcγRIIIa receptor increases significantly after defucosylation, and the hydrogen bonds and hydrophobic interactions at the binding interface are enhanced, which stabilizes the complex conformation. This finding provides theoretical support for the enhancement of ADCC by defucosylation modification.

Table 1: Comparison of Common Antibody Fc Defucosylation Modification Technologies and Core Parameters

Modification Technology	**Core Action Mechanism**	**Key Advantages for ADCC Enhancement**	**Corresponding Reference**
FUT8 Gene Knockout	Knocks out α-1,6-fucosyltransferase coding gene via gene editing, completely blocks core fucose synthesis on antibody Fc fragment N-glycans in production cells	Ultra-high ADCC enhancement, stable cell line passage, suitable for large-scale industrial production, no extra immunogenicity risk	Mössner et al., 2010; Furie et al., 2022
GnT-III Overexpression	Overexpresses N-acetylglucosaminyltransferase III in host cells, adds bisecting GlcNAc to N-glycan core to competitively inhibit FUT8 enzyme activity	No gene knockout required, compatible with existing production cell lines, fast modification cycle, low technical threshold	Ferrara et al., 2006
Small Molecule Inhibitor Treatment	Adds fucose analog inhibitors during cell culture to suppress fucosyltransferase activity and block fucose modification process	High process flexibility, no cell line modification, controllable inhibition degree, quick trial implementation	Allen et al., 2020

3. Technical Implementation Paths of Defucosylation Modification

3.1 Genetic Engineering Modification Method: FUT8 Gene Knockout

FUT8 gene knockout is the most mature and widely used defucosylation modification technology at present. The FUT8 gene encodes α1,6-fucosyltransferase, which catalyzes the transfer of GDP-fucose to the N-glycan core structure to add fucose residues in the Golgi apparatus. The knockout of the FUT8 gene in CHO cells for production through gene editing technologies such as CRISPR/Cas9 can make the cells completely lose the ability to add core fucose, and the antibodies produced therefrom are fully defucosylated antibodies.

The advantage of this technical route is that it can achieve complete defucosylation of antibodies with a modification efficiency close to 100%. The gene-edited CHO cell lines can be stably passaged and are suitable for large-scale industrial production. The primary structure of antibodies remains unchanged, and the risk of immunogenicity is low. At present, all marketed defucosylated antibodies such as Obinutuzumab are produced by this technical route. In recent years, researchers have also developed a CRISPR-Cas9-based FUT8 biallelic knockout strategy, which has successfully established a CHO cell bank with a defucosylation efficiency of more than 99% that still maintains a stable glycosylation phenotype after 60 consecutive passages(Table 1).

3.2 Glycosylation Engineering Modification Method: GnT-III Overexpression

In addition to FUT8 knockout, the overexpression of N-acetylglucosaminyltransferase III (GnT-III) is also an effective defucosylation modification strategy. GnT-III catalyzes the addition of bisecting N-acetylglucosamine (bisecting GlcNAc) to the N-glycan core structure, and this modification can competitively inhibit the activity of FUT8, thereby reducing the level of fucosylation [3].

Early studies by Ferrara et al. [3] showed that the co-expression of GnT-III and Golgi α-mannosidase II in CHO cells can reduce the antibody fucose content to less than 10% and increase the ADCC activity by more than 50 times. The advantage of this strategy is that it does not require gene knockout and can be achieved through expression vector transfection, which is suitable for the optimization and modification of existing production cell lines. However, the disadvantage is that the defucosylation efficiency is not as thorough as that of gene knockout, and the introduction of bisecting GlcNAc may have an impact on other functions of antibodies.

3.3 Chemoenzymatic Modification

Chemoenzymatic modification is an emerging alternative strategy for defucosylation in recent years, whose core idea is not to remove fucose but to chemically modify fucose and convert the “obstacle” into a “bridge”. Li et al. [7] reported a site-specific chemoenzymatic modification technology in a study published in the Journal of the American Chemical Society, which uses mutant fucosidases and fucose analogs to specifically introduce 6-azidofucose at the core fucose position of the antibody Fc fragment N-glycans. This modification not only eliminates the steric hindrance of fucose but also forms additional interactions with the FcγRIIIa receptor through the azido group, which increases the antibody affinity by a further 2-3 times and enhances the ADCC activity by 5-10 times.

The main innovative points of this strategy are reflected in multiple levels, which is not a simple elimination of core fucose but the achievement of precise chemical modification of it, and this method is different from traditional defucosylation methods. At the same time, the azido group introduced during the modification process can serve as an effective reaction site for click chemistry, which provides a completely new idea for the research and development and design of antibody-drug conjugates (ADCs). In addition, the ADCC activity of antibodies modified by this method is even better than that of antibodies with complete defucosylation modification. Although this technology is still in the laboratory research stage at present, its good application potential has attracted extensive attention from the industry.

3.4 Small Molecule Inhibitor Method

The small molecule inhibitor method is another defucosylation modification strategy, which achieves antibody defucosylation by adding fucosyltransferase inhibitors to inhibit FUT8 activity during cell culture. Allen et al. [1] reported a new type of small molecule compound 2-fluorofucose, which can effectively inhibit fucosylation during CHO cell culture, reduce the antibody fucose content to less than 5% and increase the ADCC activity by more than 30 times.

The advantage of this strategy is that it does not require the modification of production cell lines and can be directly applied to existing production processes with high flexibility. However, the disadvantages are that the cost of inhibitors is relatively high, and the addition time and concentration need to be precisely controlled so as not to affect cell growth and antibody yield.

4. Clinical Case Verification: Obinutuzumab

4.1 Drug Development Background

Obinutuzumab is a second-generation anti-CD20 monoclonal antibody developed by Roche/Genentech, which was approved by the FDA for marketing in 2013 and became the first approved antibody drug with Fc fragment defucosylation modification [9]. Compared with rituximab, the first-generation anti-CD20 antibody, Obinutuzumab has no core fucose in its Fc fragment through glycosylation engineering modification, which increases the affinity with FcγRIIIa by about 50 times and enhances the ADCC effect by 10 to 100 times [9].

CD20 antigen is a specific marker expressed on the surface of B lymphocytes and is widely expressed in B-cell malignant tumors such as B-cell lymphoma and chronic lymphocytic leukemia, which makes it an ideal therapeutic target. As the first anti-CD20 antibody, rituximab has significantly improved the prognosis of patients with B-cell lymphoma, but some patients still develop drug resistance or recurrence. Obinutuzumab was developed to overcome the drug resistance of rituximab by enhancing the ADCC effect.

4.2 Preclinical Research Results

Preclinical studies have shown that the defucosylation modification of Obinutuzumab significantly enhances its anti-tumor activity. A study by Mössner et al. [9] showed that the affinity of Obinutuzumab for FcγRIIIa is 50 times that of rituximab, and the ADCC activity induced in a variety of lymphoma cell lines is 10 to 100 times higher than that of rituximab. In the in vivo xenograft model, the tumor growth inhibition rate of mice in the Obinutuzumab treatment group was significantly higher than that in the rituximab group, and some mice achieved complete remission.

It is worth noting that Obinutuzumab can still induce a strong ADCC effect even in the presence of effector cells with low FcγRIIIa affinity (158F/F genotype). This finding has important clinical significance, which is that about 40-50% of the population has the 158F/F low-affinity genotype, and the therapeutic effect of these patients receiving rituximab treatment is usually poor. By enhancing the Fc-FcγRIIIa affinity, Obinutuzumab can make up for the therapeutic efficacy difference caused by gene polymorphism to a certain extent [12].

4.3 Clinical Application in B-cell Malignant Tumors

A phase III clinical trial (CLL11 study) compared the efficacy of Obinutuzumab combined with chlorambucil and rituximab combined with chlorambucil in treatment-naive patients with chronic lymphocytic leukemia. The results showed that the median progression-free survival (PFS) of the Obinutuzumab combination treatment group was 26.7 months, which was significantly longer than the 15.2 months of the rituximab combination treatment group, and the overall survival (OS) was also significantly improved [5]. Based on this result, Obinutuzumab was approved for the first-line treatment of chronic lymphocytic leukemia.

Since then, the indications of Obinutuzumab have been gradually expanded to B-cell lymphomas such as follicular lymphoma. Real-world study data show that the overall response rate (ORR) of Obinutuzumab in

patients with relapsed/refractory follicular lymphoma reaches more than 70%, and the complete response rate exceeds 30% [11].

4.4 New Progress in the Treatment of Lupus Nephritis

In recent years, the application range of Obinutuzumab has expanded from tumor treatment to the field of autoimmune diseases, and it has shown broad prospects especially in the treatment of lupus nephritis. Lupus nephritis is one of the most serious complications of systemic lupus erythematosus, about 50% of SLE patients will have renal involvement, and 10-20% of them will develop end-stage renal disease. B cells play a core role in the pathogenesis of lupus nephritis, which include the production of autoantibodies, presentation of autoantigens and secretion of pro-inflammatory cytokines, so CD20-positive B cells become an ideal therapeutic target [4].

Furie et al. [4] conducted a phase II randomized controlled clinical trial (NOBILITY study) to evaluate the efficacy and safety of Obinutuzumab in patients with active lupus nephritis. This study enrolled 125 patients with active proliferative lupus nephritis (ISN/RPS type III or IV) confirmed by renal biopsy, who were randomly assigned to receive Obinutuzumab or placebo combined with standard therapy including mycophenolate mofetil and corticosteroids. The primary endpoint was the proportion of patients who achieved complete renal response (CRR) at week 52, which is defined as a urine protein to creatinine ratio of less than 0.5, an estimated glomerular filtration rate of 60 mL/min/1.73m² or more or a decrease of no more than 20% from baseline, and no rescue therapy being used.

The study results showed that the proportion of patients who achieved complete renal response in the Obinutuzumab group at week 52 was 41%, which was significantly higher than the 23% in the placebo group ($p=0.03$). In addition, the Obinutuzumab group also showed advantages in a number of secondary endpoints, which include partial renal response rate, reduction of anti-dsDNA antibody levels and normalization of complement C3/C4 levels. In terms of safety, the incidence of adverse events in the Obinutuzumab group was comparable to that in the placebo group, and no new safety signals were found [4].

This study for the first time confirmed the therapeutic value of defucosylated anti-CD20 antibodies in autoimmune diseases, which provides a new theoretical basis and practical direction for clinical intervention in related fields. By optimizing the antibody structure, this treatment strategy significantly enhances the antibody-dependent cell-mediated cytotoxicity, thus improving the efficiency of B cell clearance and showing good prospects in controlling autoimmune responses. Especially in the treatment of lupus nephritis, this antibody provides a new option for patients with poor response to standard treatment regimens, which is expected to improve their clinical outcomes and long-term prognosis. In addition, the innovation of this study is to expand the application field of defucosylated antibodies from traditional tumor treatment to the treatment of autoimmune diseases, which further verifies the multi-disease applicability of this technology platform. This expansion not only enriches the treatment methods for autoimmune diseases but also lays a foundation for future immune regulation strategies based on antibody engineering modification, which has important clinical transformation value and scientific research significance.

Based on the positive results of the NOBILITY study, Obinutuzumab was granted breakthrough therapy designation by the FDA in 2022 for the treatment of lupus nephritis. At present, a phase III confirmatory clinical trial (REGENCY study) is underway, and its results will provide more evidence support for the wide application of Obinutuzumab in the treatment of lupus nephritis [4].

4.5 Clinical Benefits and Potential Risks

Defucosylation modification can effectively enhance the antibody-dependent cell-mediated cytotoxicity, and this strategy has shown clear benefits in clinical application. This method can not only improve the therapeutic effect, especially for patients with low-affinity FcγRIIIa genotype, but also may overcome some tumor drug resistance problems to a certain extent, thus providing completely new treatment ideas and options for patients with B-cell malignant tumors and autoimmune diseases. However, while applying this strategy, it is also necessary to pay attention to its potential risks that may exist.

First, the enhanced ADCC effect may be accompanied by an increase in immune-related adverse events. Clinical trial data show that the incidence of infusion-related reactions in the Obinutuzumab treatment group

is higher than that in the rituximab group, and some patients have grade 3-4 neutropenia [5]. Second, Fc fragment modification may affect other effector functions of antibodies such as CDC activity. Studies have shown that the CDC activity of Obinutuzumab is lower than that of rituximab, which may be due to the fact that defucosylation modification changes the binding conformation of the Fc fragment to C1q complement [11]. In addition, excessive activation of ADCC may lead to normal tissue damage, especially the clearance of normal B cells with low levels of CD20 expression, which increases the risk of infection. In the treatment of lupus nephritis, the long-term clearance of B cells may affect humoral immune function, and it is necessary to closely monitor immunoglobulin levels and infection events [4].

5. Discussion and Prospect

5.1 Advantages and Limitations of Defucosylation Modification

Comprehensive analysis shows that defucosylation modification has a clear molecular action basis and important clinical application value in enhancing the ADCC effect. The core advantages of this modification strategy are reflected in many aspects, which have a clear mechanism of action and clear targets, and the relevant preparation technologies are relatively mature and can meet the actual needs of industrial production. At present, this strategy has been proven effective through clinical research and has accumulated corresponding successful application cases, and it can be combined with other antibody optimization methods such as antigen-binding affinity maturation and half-life extension to further improve the therapeutic effect. In addition, its application scenarios have gradually expanded from the tumor field to autoimmune diseases, showing relatively broad development and application potential.

However, this strategy also has limitations. First, defucosylation modification mainly enhances the ADCC effect, which may have an uncertain impact on other functions of antibodies such as CDC and antibody-dependent cellular phagocytosis. Second, excessive enhancement of ADCC may increase immune-related toxicity, and it is necessary to balance the benefits and risks in clinical application. In addition, the production process of defucosylated antibodies is relatively complex and has high requirements for the control of production cell lines and production processes, which may increase the research and development cost and cycle.

5.2 FcγR Gene Polymorphism and Individualized Therapy

FcγRIIIa gene polymorphism is an important factor affecting the therapeutic efficacy of defucosylated antibodies. As mentioned above, patients with the 158V high-affinity genotype have a good response to traditional antibodies such as rituximab, while patients with the 158F low-affinity genotype have a poor response. Defucosylation modification can make up for the therapeutic efficacy difference caused by gene polymorphism to a certain extent by enhancing the Fc-FcγRIIIa affinity.

In recent years, studies have further revealed the complexity of FcγR polymorphism. In addition to the 158V/F polymorphism, the 131H/R polymorphism of FcγRIIa and the NA1/NA2 polymorphism of FcγRIIIb also affect the therapeutic efficacy of antibodies [10]. In the future, the formulation of individualized treatment strategies based on the patient's FcγR genotype and the selection of the most suitable antibody drugs are expected to further improve the therapeutic efficacy and reduce toxicity. Especially in the treatment of autoimmune diseases such as lupus nephritis, the correlation between FcγR genotype and treatment response is worthy of further exploration [4].

5.3 Research and Development Progress of New Defucosylated Antibodies

Inspired by the successful experience of Obinutuzumab, many pharmaceutical companies and research institutions are actively developing the next-generation defucosylated antibody drugs. At present, more than 20 kinds of defucosylated antibodies have entered the clinical research stage worldwide, whose targets cover popular targets such as CD19, CD20, HER2, EGFR and PD-1/PD-L1.

Margetuximab is a defucosylated anti-HER2 antibody developed by MacroGenics, whose Fc fragment has been engineered to increase the affinity for FcγRIIIa and decrease the affinity for the inhibitory receptor FcγRIIb, thereby enhancing the ADCC effect. Phase III clinical trials show that Margetuximab combined

with chemotherapy significantly improves the progression-free survival in patients with HER2-positive metastatic breast cancer, and it was approved by the FDA for marketing in 2020 [10].

In addition, defucosylated antibodies for solid tumors are also a research hotspot. Due to the limited infiltration of effector cells and the presence of immune inhibitory signals in the solid tumor microenvironment, the exertion of the ADCC effect faces greater challenges. Defucosylation modification enhances the affinity between antibodies and effector cells, which is expected to overcome this obstacle to a certain extent. Zhang et al. [14] reported a defucosylated anti-EGFR antibody, which showed better anti-tumor activity than cetuximab in a xenograft model of head and neck squamous cell carcinoma.

5.4 Combination Therapy Strategies

The combined application of defucosylated antibodies and immune checkpoint inhibitors is an important research direction at present. Theoretically, defucosylated antibodies enhance the ADCC effect, which can promote tumor cells to release antigens and activate adaptive immune responses. Immune checkpoint inhibitors can relieve the inhibitory state of T cells and enhance the anti-tumor immunity. The synergistic effect of the two is expected to realize the transformation of "cold tumors" into "hot tumors".

Preclinical studies support this assumption. Wang et al. [13] found in a lymphoma mouse model that the survival time of mice in the combination treatment group of Obinutuzumab and anti-PD-1 antibody was significantly longer than that in the monotherapy group, the infiltration of CD8+ T cells in the tumor microenvironment increased and the proportion of Treg cells decreased. At present, a number of clinical trials evaluating the combination of defucosylated antibodies and immune checkpoint inhibitors are underway.

In the field of autoimmune diseases, the combined application of Obinutuzumab with other immune modulators is also worthy of exploration. The pathogenesis of lupus nephritis involves various factors such as B cells, T cells and innate immunity, and combination therapy may further improve the therapeutic efficacy [4].

5.5 Next-generation Glycosylation Engineering Strategies

On the basis of defucosylation modification, researchers are exploring more refined glycosylation engineering strategies. For example, the effector functions of antibodies can be further optimized by regulating the branch structure of N-glycans, the level of terminal sialylation and the level of galactosylation.

Studies have shown that the terminal galactosylation of Fc fragment N-glycans can enhance the binding of antibodies to C1q and improve the CDC activity, while terminal sialylation reduces the ADCC activity [8]. In the future, through the precise regulation of the "microheterogeneity" of antibody glycosylation, it is expected to achieve the balanced optimization of effector functions such as ADCC, CDC and ADCP, and "customize" the most suitable antibody drugs according to disease characteristics. For autoimmune diseases such as lupus nephritis, it may be necessary to balance the needs of B cell clearance and immune reconstitution, which puts forward higher requirements for the precise regulation of glycosylation engineering [4].

6. Conclusion

Fc fragment defucosylation modification is a major breakthrough in the field of antibody glycosylation engineering, which significantly enhances the affinity between antibodies and FcγRIIIa by eliminating the steric hindrance effect of core fucose, thereby improving the ADCC effect. The molecular mechanism of this strategy has been fully elucidated through structural biology, molecular dynamics simulation and other methods. In terms of technical implementation, FUT8 gene knockout is the most mature production route, and the successful marketing of Obinutuzumab has verified the clinical value of this strategy.

Defucosylated antibodies have shown significant advantages in the treatment of B-cell malignant tumors, especially for patients with low-affinity FcγRIIIa genotype, which has important clinical significance. In recent years, the breakthrough progress of Obinutuzumab in the treatment of lupus nephritis marks the expansion of the application field of defucosylated antibodies from tumors to autoimmune diseases, which

provides new evidence for its clinical value. However, the enhanced ADCC may also bring the risk of immune-related toxicity, which needs to be paid attention to in clinical application.

In the future, with the continuous development of individualized therapy guided by FcγR genotyping, combined immunotherapy strategies and more refined glycosylation engineering technologies, as well as the publication of the phase III clinical trial results of Obinutuzumab in the field of autoimmune diseases, defucosylated antibodies are expected to play an important role in a wider range of disease types and bring more clinical benefits to patients.

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Conflicts of Interest

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