CRISPR/Cas9 in Antibody Engineering: Advances in Cell Line Optimization and Therapeutic Applications

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Abstract. Derived from the adaptive immune system of prokaryotes, CRISPR-Cas9 gene editing technology has revolutionized biotechnology with its simplicity, efficiency and precision, and plays a crucial role in advancing biopharmaceuticals. This paper focuses on the application of the CRISPR/Cas9 in antibody engineering, especially its role in optimizing the CHO cell lines and advancing immunotherapy. The results of this paper show that in the CHO cell line optimization, CRISPR improves antibody yield by knocking down apoptotic genes (e.g., Bak1, Bax), modifies glycosylation patterns by editing FUT8 to improve antibody efficacy and safety, and reduces residual host cell proteins by double knockdown of C1s/MGAT1. In immunotherapy, CRISPR-modified CAR-T cells improved editing efficiency, minimized off-target effects, and mitigated GVHD and immune rejection through multi-gene editing. These findings highlight the dual value of the CRISPR in improving antibody production efficiency/quality and driving innovation in immunotherapy, laying the foundation for advances in biopharmaceuticals and personalized medicine.

Keywords: CRISPR/Cas9, Gene-editing, Antibody engineering.

1. Introduction

Monoclonal antibodies play a imperative role in the field of immunology research and hold a significant position today. Monoclonal antibodies have high specificity, can accurately bind to target antigens, have high purity and uniformity, and stable and reproducible experimental results. It is widely used in clinical trials due to its significant advantages. Once the corresponding hybridoma cells are obtained, they can be prepared in large quantities to meet the needs of scientific research, diagnosis, and treatment.

For the production of monoclonal antibodies, Chinese hamster ovarian cells (CHO) are the source of 70% of recombinant proteins and antibodies in current industrial production. Their high expression capacity, good stability and compliance with human glycosylation characteristics make them the mainstream host. Traditional monoclonal antibody production methods mainly use CHO cells. However, these cells face many significant limitations. For the variety of antibodies it produces, the expression and secretion of circulating antibodies in animal serum are restricted by the proliferation of dominant B cells in the spleen or lymph nodes, so the types of antibodies secreted into the serum are relatively limited [1]. When using CHO-K1 cell culture supernatant to evaluate the safety of CHO in vivo, scientists found that the duodenum and pancreas of the experimental group animals were significantly congested and swollen, and multiple blood routine and blood biochemical indicators were significantly different from those of the control group. It shows total host cell protein (HCP) in super netant of CHO will affect gastrointestinal digestion, reduce body immunity, disease damaging, liver and pancreatic disease and harm the health of animals [1]. And most of single-subunit vaccines have lowimmunity and it cost much to produce them, while complex glycosylation and volatile yield and quality also exist in CHO. Furthermore, the cost of CHO cell culture is high and hard to control. This has also led to the fact that CHO cell-expressed subunit vaccines have been slow to develop.

The latest CRISPR, a name derived from the term “clustered regularly interspaced short palindromic repeats”, became applicable for the production of monoclonal antibodies over the last few years. It is a kind of gene editing technology originated from the prokaryotic organism’s adaptive immunity system [2]. Compared with other genome-modifying ways, the CRISPR-Cas9 system is more simple, efficient and accurate, so it has made a lot of progress in cancer-related clinical trial

work. Apart from it is potential therapies, CRISPR - Cas9 can also be a great tool as we could use this technology to create genetically modified animals. Which could then be used to get new drugs [2]. It is necessary to increase the production efficiency of monoclonal antibodies, and engineered immune cells have great application prospects. Hence, this paper is going to look into the usage of CRISPR technology for the optimization in production cell line and the development of new methods for treatment. will focus on its role in antibody production, regulation of glycosylation, immune cell engineering etc., and will cover two sides - the role of CRISPR in making antibodies as well as in therapy.

2. A Brief Overview of CRISPR/Cas System

Before CRISPR was discovered, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were the first generation of gene-editing techniques used in gene therapy [3]. ZFN are made through the combination of zinc fingers DNA-binding proteins with non-specific cutting segments of FokI restriction endonucleases. They are present as a pair (dimeric) and cause double-strand breaks (DSB) at some points on DNA so as to make the cell repair DNA itself. In the sequence recognition part of the zinc finger domains, it makes sure the nucleases hit specific places on the genome [3]. Shamshirgaran noted that TALENs was created when FokI endonuclease domain and DNA-binding motif of TALE protein was fused. Like that of ZFNs, TALENs produce DSB at certain locus that trigger DNA repairs process and permit genetic amendments but ZFN and TALEN have a lot of problems. From the perspective of precision, it is protein-based DNA recognition as well, which will go through a complex design process, and due to its structure, there may be more off-target results. From a practical perspective, it takes lots of tiring to make and create some certain sets of DNA binding proteins. When we want to edit multiple targets, each site must have its own protein for the design and synthesis of, which is difficult to accomplish [4]. The complex and costly design process of the technologies limits the use of these technologies for basic research as well as in the clinic [3].

Compared to traditional gene editing techniques, CRISPR does have an advantage. CRISPR has 3 steps in its mechanism: adaption, crRNA biogenesis, and interference. Exogenous DNA fragment inserted as spacer into CRISPR site. CRISPR RNA biogenesis step after that the CRISPR array gets transcribed into small crRNAs through this process and later they're used for recognizing targets with complimentary base-pairing. Lastly in the interference phase, the Cas9 endonuclease forms a ribonucleoprotein along with the crRNA, recognizes the target sequence near the PAM sequence, and introduces a DSB at a specific site in order to make precise gene knockouts, insertions or repairs. CRISPR can achieve recognition of target DNA sequences by using RNA for hybridization, thus improving accuracy and reducing off-target effects [4]. In terms of operation, only one guide RNA needs to be designed, so there is no need to engineer specific proteins for each DNA sequence [5]. Therefore, this simplifies the procedure and significantly shortens the experimental schedule. Moreover, CRISPR achieves efficient multi-path genome editing by introducing multiple guide RNAs, providing greater flexibility [4-5]. From a cost perspective, guide RNA synthesis is inexpensive, Cas proteins can be reused, and it significantly reduces experimental costs. These advantages make CRISPR widely applicable and have shown great potential in plant and animal gene editing, gene therapy research, and microbial engineering.

In the CRISPR system, a multitude of CRISPR - associated proteins (Cas) exist, and CRISPR - Cas9 stands out as the most frequently utilized and latest iteration of the CRISPR system. Researchers merge a single - guide RNA (sgRNA) with the Cas9 protein to create a complex, and this complex allows accurate cleavage at particular DNA locations. When the sgRNA binds to the Cas9 protein, the former ribonucleoprotein complex searches the genome for a corresponding DNA sequence. Recognition happens solely when the target site is next to a PAM, and after successful binding, the complex causes a precise DSB at that specific genomic position [6].

3. CRISPR-Engineered Cells in Monoclonal Antibody Production

3.1. Optimization of Host Cell Lines

Although the technology for producing antibodies using CHO cells is relatively mature, CHO cells have some limitations due to the complex process and high costs of production. Therefore, we can use the efficient and flexible CRISPR technology to generate more productive and stable CHO cell lines through gene editing. Moreover, antibody production can be increased by knocking out endogenous genes and/or integrating transgenes.

One promising strategy to enhance the productivity and robustness of CHO cells in biomanufacturing involves knocking out pro-apoptotic genes. Apoptosis typically constrains the longevity and viability of producer cell lines especially as stressed by large scale culture conditions. The latest work reported in ACS Synthetic Biology, Orlova et al. used a Bak1, Bax knockout CHO cell line as a model system to study the performance and stability of the cells under different stress settings. From these results, it can be seen that it was effective to knockout Bak1 and Bax to increase the survival rate and production capacity [7]. these double-knockout cell lines are of great value for understanding how apoptosis control impacts cell line development and protein expression. Finally, gene knockout of apoptosis related genes in the pathway helps to increase production, and also makes this CHO expression platform more robust and consistent.

3.2. Glycosylation Engineering

When making treatment proteins by using CHO cells, the most serious problem is the difference between its normal glycosylation method and that of human cells. This difference leads to the existence of non-human glycan structure, like core fuctos, α-Gal, Neu5Gc and so on and that would trigger immune response of patients and decrease the efficiency and security of bio-pharmaceuticals. To deal with this problem, the glycosylated pattern of CHO cells need to be more human like.

A few years back, scientists employed old gene editing techniques to engineer glycosylation. Yang et al. edited the CHO cell line by focusing on and deleting the FUT8 gene, which codes for the α1,6 fucosyltransferase – this is the enzyme that adds the basic fucose residue to the N-linked glycan chain [8]. Yang et al. used large-scale glycoproteomics research to demonstrate that removing FUT8 considerably cut down on the core fucosylation [8]. So the recombined CHO cells made antibodies without fucose, and these antibodies had more ADCC, this is an important way lots of monoclonal antibody medicines work. This kind of change, the CHO-derived protein's glycan spectrum was very similar to the human antibodies, and its clinical safety and treatment results were enhanced [8].

Researchers used ZFN technology to knock out FUT8 in CHO cells. The resulting FUT8-deficient cell lines were capable of consistently producing fully afucosylated antibodies that retained correct folding and full biological activity. This research provided early experimental support for the idea that modifying glycosylation enzymes, particularly FUT8, can lead to meaningful improvements in product quality. Collectively, these studies emphasize the value of glycoengineering in CHO cells, not only to reduce immunogenicity risks but also to enhance the functional attributes of recombinant therapeutic proteins.

CRISPR technology can delete endogenous genes and/or integrate transgenes with high precision and efficiency, and this advantageous function can be applied to the glycosylation engineering. The study demonstrated the efficient use of CRISPR/Cas9 technology to disrupt the FUT8 gene in CHO cells, leading to the generation of afucosylated antibodies with enhanced therapeutic properties. Not only that, CRISPR/Cas9 enabled rapid and high-frequency gene knockout within just three weeks, and this is far faster than the traditional methods such as homologous recombination and ZFNs. The editing efficiency ranged from 9% to 25%, and the proportion of knockout clones increased to over 50% as lentil lectin (LCA) selection was applied. Importantly, the FUT8-deficient CHO cells exhibited normal growth behavior and viability, ensuring their suitability for large-scale biomanufacturing. This strategy not only improved the antibody-dependent cellular cytotoxicity

(ADCC) of the produced antibodies but also demonstrated the scalability and practicality of CRISPR-mediated glycoengineering in industrial settings [9].

3.3. Reducing Immunogenicity and Host Cell Proteins

Impurities in drugs can also affect the quality of drugs. Therefore, we need to eliminate the problem proteins in proteins, also known as impurity proteins. Host cell protein (HCP) is an impurity generated in the process, and this protein may cause adverse immune reactions in the recipient [10]. Li et al. used CRISPR-Cas9 to double-knockout C1s (protease) and MGAT1 in CHO cells, eliminating the non-specific protein cleavage problem during the expression process of HIV vaccine antigen (gp120), which significantly reduced the residual HCP level in CHO cells and improved the quality and stability of recombinant proteins [10].

4. CRISPR-Modified Immune Cells in Therapeutic Applications

4.1. CAR-T and CRISPR: CRISPR/Cas-based CAR-T cells

Chimeric antigen receptor T-cell (CAR-T) therapy represents a personalized form of immunotherapy. In this therapy, T cells from the patient are collected and modified to produce CARs. These engineered receptors enable T cells to detect and bind directly to tumor-specific antigens independently of MHC presentation, triggering targeted cytotoxic activity [11]. Moreover, CARs are most often introduced into T cells using retroviral or lentiviral delivery systems. Nevertheless, their uncontrolled genomic insertion may result in problems such as abnormal clonal proliferation, potential oncogenic conversion, inconsistent transgene expression, and eventual transcriptional repression [11]. These problems have largely restricted the clinical application of CAR-T. Therefore, when producing CAR-T, the modification is very important, using CRISPR Cas technology can ensure the safety and efficacy of CAR-T better.

At present, CAR-T cells can be produced through three main CRISPR-based strategies. Among them, CRISPR/Cas9 is the most extensively utilized gene-editing technology, CRISPR/Cas12a and CRISPR/Cas13d serve as alternative methods [12]. Scientists have explored three main methods to introduce CRISPR constructs into human primary T cells: (i) Transferring CRISPR components via viral vectors such as lentivirus or adeno-associated virus; (ii) Co-delivering Cas9 mRNA and sgRNA; (iii) Directly delivering the RNP complex formed by Cas9 protein and sgRNA [12]. Because the gene knockout efficiency of CRISPR/Cas9 is relatively high, it can exceed 75% at the PD-1 locus. Additionally, some studies have integrated sgRNA and CAR sequences into the same vector and achieved gene editing of CAR-T cells by electroporating Cas9 mRNA. Since the knockout efficiency is affected by the target and sgRNA design, to enhance the gene editing effect, researchers introduced 2’-O-methyl 3’-thio-phosphorothioate or its PACE variant (MSP) modifications into sgRNA and co-electroporated it with Cas9 protein into T cells and CD34+ hematopoietic stem cells. The results showed that MS modification could increase the insertion/deletion rate by approximately 2.4 times (30.7% vs 12.8%), significantly enhancing the editing efficiency [13].

4.2. CRISPR in Allogeneic Cell Therapy

Furthermore, when producing allogeneic "universal" CAR-T cell therapies, CRISPR can precisely modify donor-derived T cells at multiple sites. Firstly, by knocking out the endogenous T cell receptor (TCRα chain encoding genes TRAC or TRBC), the binding of these exogenous T cells to the antigens recognized by the recipient themselves can be blocked, avoiding their attack on the recipient's normal tissues and attenuating the occurrence of graft-versus-host disease. Secondly, the B2M subunit of class I HLA of human is targeted by knock-out, then by this way, the probability that donor T cell is cleared or destroyed by the recipient is reduced, so the risk of immune rejection response. And thus these multi gem editing techniques allow for the CAR T to be prepared so that all the patients can make use of it on an as used basis [14].

5. Challenges and Limitations

The nucleus enzyme protein is the most critical element of CRISPR-mediated genome editing. The nucleus enzyme protein actually comes from bacteria. But this protein could also sometimes make our bodies try to fight it off. That makes it more likely we'll have a bad reaction, so it's less effective. To address this issue, Raghavan and his colleagues investigated which regions of the Cas9 and Cas12 proteins are specifically recognized by the immune system[15]. They employed advanced mass spectrometry technology to identify the immunogenic fragments and discovered three short peptide sequences of approximately 8 amino acids in length, which might trigger an immune response. Among them, Cas9 is derived from Streptococcus pyogenes, and Cas12 is derived from Staphylococcus aureus. Based on these findings, they collaborated with CYUS Biotechnology Company to redesign the nucleases - removing the identified immunogenic fragments while retaining their enzymatic activity. Subsequently, computational prediction tools confirmed that the possibility of the modified protein activating an immune response was significantly reduced [15].

A major obstacle to the broad use of CRISPR technology is the presence of off-target effects. How to accurately assess and detect off-target effects and propose corresponding strategies to reduce them is an important research direction in the current field of gene editing. In gene editing, generally, only a 20-nucleotide sequence is needed to locate and precisely knock out a gene. However, at the same time, there are many base fragments in the genome sequence that are very similar to a specific 20-nucleotide sequence. Therefore, during the gene editing process, fragments very close to the target of the guide RNA will also bind and be knocked out, which is what is called 'off-target'. Nowadays, to reduce the harm caused by off-target effects, it is necessary to develop and rationally utilize effective tools for predicting off-target effects, develop new gene editing systems, design targets using high-quality reference genomes, and also require good gene editing tool delivery systems [16].

6. Future Perspectives

Looking ahead, the application of CRISPR in cell and gene therapy will continue to deepen and be combined with emerging technologies such as machine learning, live cell imaging, and faster, more economical DNA sequencing, significantly enhancing the precision and safety of editing. At the clinical level, more types and scales of clinical trials will be launched, providing key data for the next generation of CAR-T, NK cell therapy, and other gene therapies. As safety and efficacy are verified, CRISPR may not only be used to treat diseases but also extend to preventing major health threats, such as editing specific genes in high-risk populations in advance to reduce the incidence of neurodegenerative diseases or cardiovascular diseases. In allogeneic cell therapy, CRISPR will further optimize the knockout strategies of HLA genes and immune-related genes, promoting the commercialization of "universal" cell products. In broader biological applications, CRISPR may also be used to regulate the microbiome, enhance the immune compatibility of transplanted organs, or produce customizable antibody-secreting cells, forming a true "in vivo factory". These advancements will greatly change the landscape of personalized medicine and precision treatment. Additionally, products generated by CRISPR technology are expected to become routine choices, from pig organs suitable for transplantation to drought-resistant and high-yield rice varieties, to customizable microbiomes for health fine-tuning, all of which may enter daily life in the future. These developments will not only advance the scientific frontier but also reshape the landscape of healthcare, agriculture, and environmental sustainability.

7. Conclusion

CRISPR technology is redefining modern biotechnology, demonstrating a landmark breakthrough in industrial antibody production and therapeutic development. By enabling highly precise gene knockout, optimization of glycosylation pathways, and removal of host cell proteins, CRISPR has effectively enhanced the quality, safety, and production efficiency of the CHO cell

platform, thereby overcoming the long-standing limitations of traditional biopharmaceuticals. These improvements not only optimize the yield and functional activity of antibodies but also expand the possibilities of therapeutic protein design, providing a new direction for the next generation of biologics. Meanwhile, CRISPR also shows great potential in the field of immunotherapy, indicating its ability to achieve safer, more efficient, and more universal personalized treatment plans, especially in the engineering of CAR-T and universal allogeneic immune cells. However, there are still many challenges in the process of moving towards clinical and industrial applications. The off-target effects and immunogenicity risks of CRISPR technology remain key obstacles that limit its wide application. But with the rapid progress of computational tools, innovative nucleases, and integrated delivery strategies, these problems are gradually being solved. With the improvement of regulatory systems and the accumulation of clinical validation, CRISPR-engineered cells are expected to move from laboratory research to mainstream medical and biotechnological platforms and be applied to people's daily lives. Ultimately, the dual value of CRISPR in promoting antibody production and therapeutic breakthroughs will have great potential for sustainable development in the fields of precision medicine, biopharmaceuticals, and broader agriculture and the environment.

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