Progress and prospect of protein detection methods based on CRISPR-Cas system

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Abstract. This is a review article on protein detection methods based on CRISPR-Cas system. This article introduces the application of CRISPR-Cas system in the field of gene editing, and then focuses on the protein detection technology based on CRISPR-Cas system. These technologies take advantage of the targeting and high efficiency of the CRISPR-Cas system, combined with detection methods such as fluorescence or mass spectrometry, to detect a variety of proteins with high sensitivity. This article also compares the advantages and disadvantages of these techniques and traditional protein detection methods, and discusses the application prospects of these techniques in clinical diagnosis and biological research.

Keywords: CRISPR-Cas, protein detection, fluorescence, clinical diagnosis, bioresearch.

1. Introduction

Protein is one of the important components of life activities. Therefore, in many research fields, it is very important to detect the existence and content of protein accurately and quickly. For example, in the medical field, rapid and sensitive detection of the presence of specific proteins can be used for cancer screening and diagnosis. [1, 2]In the field of food safety, detecting the content of certain proteins in food can be used to detect whether food is contaminated.[3] In addition, the detection of proteins also plays an important part in drug development. Accurate detection of the content of specific proteins in drugs can be used to optimize drug efficacy.[4]

At present, the commonly used protein detection techniques include ELISA, Western blot and mass spectrometry.[5] ELISA is a common protein quantification and detection method, but its sensitivity is not high and requires a large number of protein-specific antibodies, so its cost is high. The improved MOFLISA based on it can obtain better sensitivity and reduce false positives and false negatives to a certain extent.[6, 7] Western blot is a common protein separation and detection method, but it requires a complex operation process, and the sensitivity of this detection method for some proteins is limited.[8, 9] Mass spectrometry is a high-throughput, high-sensitivity protein detection method, but it is more complex, requires high equipment costs, and requires strict operational skills.[10, 11]

CRISPR-Cas technology is an efficient and accurate gene editing and regulation technology that has emerged in recent years. However, CRISPR-Cas technology can also be applied to protein detection in addition to gene editing. A series of efficient and specific protein detection platforms can be constructed by using the nucleic acid recognition and cleavage ability of CRISPR-Cas system. [3, 12, 13] Compared with traditional protein detection methods, CRISPR-Cas technology has the advantages of high sensitivity, strong specificity and easy operation, so it has broad application prospects in the field of protein detection.

Table 1. A comparison of different strategies for protein detection

Method	Target	LOD	Detection range	Refs.
ELISA	AFB1	0.18 ng·mL−1	0.5-20 ng·mL−1	[7]
MOFLISA	AFB1	0.009 ng·mL−1	0.01-20 ng·mL−1	[7]
Western blot	ACE-536	1.0 ng·mL−1	-	[9]
ICP-MS	BNPP	0.09 ug·L−1	-	[11]
CRISPR-Cas12a	antibodies	1 pM	-	[12]

2. The principle and development of CRISPR-Cas system

CRISPR-Cas technology is a gene editing technology based on bacterial and bacterial-like immune system, which has the characteristics of high efficiency, precision and easy operation. The basic principle is that bacteria preserve the genetic material fragments in exogenous viruses and plasmids in some regions of their own genome (CRISPR sequence), and form an activated 'CRISPR-Cas immune system ' together with Cas protease and nucleic acid. When the exogenous genetic material invades the cell again, the CRISPR sequence will be transcribed into a specific CRISPR RNA, which will bind to the Cas protease and guide the enzyme to cleave the invading DNA molecule, thereby degrading it.[14]

With the continuous improvement and promotion of technology, the research field of CRISPR-Cas is also expanding. At present, it has been applied to genetics, biomedicine, agriculture and other fields, and has brought unprecedented breakthroughs. For example, CRISPR-Cas technology has been applied to cancer gene mutation research[15], treatment and prevention of monogenic hereditary diseases[16], biomedical research[17], and has made significant progress in grain crops gene editing in the field of agriculture.[18]

Zhang 's team at MIT has made great contributions to expanding the application of CRISPR-CAS system to the field of molecular diagnosis. [19, 20]They found and reported two proteins, Cas12a and Cas13a, which expanded the CRISPR-CAS system. In the subsequent studies, Zhang 's team and Doudna 's team proposed and tested the feasibility of CRISPR-CAS system for molecular diagnosis, and successively proposed and improved molecular detection methods including SHERLOCK and DETECTR.[21-23] These studies have matured the technology of CRISPR-CAS for detection and opened up a new and highly sensitive detection method. The unique binding and enzyme activity of the CRISPR-Cas mechanism have stimulated the development of many bioanalytical strategies for the detection and imaging of nucleic acids, proteins and small molecules.

In summary, CRISPR-Cas technology is widely used in academia and industry. In the future, it will continue to receive extensive attention and research, which will have a profound impact on the field of life sciences.

3. Application of CRISPR-Cas system in protein detection

3.1.CRISPR-based biosensors for protein detection

CRISPR-Cas system has been proved to have excellent performance in nucleic acid detection. Many researchers have begun to study the cascade of CRISPR-Cas system with other protein detection technologies to develop CRISPR-based protein detection technology and expand the application field of CRISPR. At present, the application of CRISPR in the field of protein detection has made some achievements. The sensitivity and accuracy of protein detection have been greatly improved because of the characteristics of CRISPR.

3.2. CRISPR-based peptide library display

With the innovation and development of biotechnology, the ability of large-scale protein research has been greatly improved. The study of custom protein and peptide libraries has been shown to be important for biological applications including disease diagnosis, therapeutic antibody development, and directed evolution. Based on the in vitro peptide display platform ' PICASSO ' of CRISPR-Cas system, a peptide sequence library marked by sgRNA sequence was constructed by fusing the recombinant peptide into the Cas9 (dCas9) protein with loss of cleavage activity. PICASSO is a new high-throughput protein detection technology. Its emergence has opened up the way for the development of protein detection research and diagnostic tools.[24]

3.3. CRISPR-based protein interaction detection

The study of protein-protein interaction (PPI) and RNA-protein interaction plays an important role in revealing the mechanism of disease and the discovery of targeted drugs. The CARPID（CRISPR-Assisted RNA-Protein Interaction Detection Method) technology developed by CRISPR / CasRx system and the PPIs detection technology based on CRISPR-Cas12a sensing system provide new ideas and methods for protein interaction detection[25, 26], and expand the application of CRISPR in the field of biological research.

3.4. Identification of post-translational modification of proteins based on CRISPR-Cas system

The development of gene editing technology provides a new tool for the study of protein post-translational modification. For example, CRISPR / Cas9 technology can accurately edit genes in cells, including modifying related genes. By editing genes related to modifying enzymes or substrates, the effects of modification on protein function can be studied. Gene editing technology, together with mass spectrometry, antibody technology and proteomics technology, provides help for the identification of protein post-translational modification.[27] At present, the technology of protein post-translational modification using CRISPR-Cas has the advantages of high throughput, high specificity and high efficiency. However, the analysis of the technology is more complicated, and there exist some errors.

4. Analysis of the advantages and disadvantages of CRISPR-Cas system

CRISPR-Cas system has the advantages of high sensitivity, high selectivity, real-time and low cost in the field of biological detection and analysis. Compared with traditional protein detection technology, the use of CRISPR-Cas technology has been greatly improved in terms of detection sensitivity. The selectivity and detection time also have great advantages due to the characteristics of the CRISPR-Cas system. Some applications combine CRISPR-Cas system with POCT (point-of-care testing) technology to achieve portable detection.[28] The combination of these technologies makes protein detection based on CRISPR-Cas system more advantageous than traditional methods.

However, due to the imperfect research of CRISPR-Cas system in the field of protein detection, there are still some problems such as high complexity and false positive and false negative. The side effects caused by the effect of CRISPR-Cas technology on the normal physiological function of cells also need to be further evaluated.

In summary, although CRISPR-Cas technology has many advantages in the field of protein detection, there are still some deficiencies in its practical application. Continuous optimization and improvement are needed to further improve its accuracy and reliability to meet the needs of various fields.

5. The prospect and prospect of CRISPR-Cas technology

5.1. Development direction

At present, the research of CRISPR-Cas technology in the field of protein detection mainly focuses on improving its detection sensitivity, selectivity and specificity. By combining other detection techniques, such as POCT, electrochemical analysis, customized peptide library, etc., we can further improve the related research of CEISPR-Cas and explore its potential. In addition, more molecular targets need to be further expanded to achieve more target detection and expand the application of CRISPR-Cas system.[13]

5.2. Potential market demand

Protein detection has a wide range of applications in food safety, medical testing and life sciences. With the continuous development and improvement of CRISPR-Cas technology, it will have a wide

market demand in the fields of medical diagnosis, medicine development, disease mechanism exploration and food safety detection.

5.3. The future research and application

The future research and application of CRISPR-Cas technology in the field of protein detection requires more in-depth research on the basic principles and mechanisms of CRISPR-Cas, based on which more excellent detection routes can be designed. It is also necessary to expand new targeted proteins and research methods, and systematically evaluate the safety of CRISPR-Cas for protein detection. In addition, CRISPR-Cas technology can be combined with traditional biological analysis methods to explore more applications of CRISPR-Cas in the field of protein detection.

6. Summary

This article reviews the application of CRISPR-Cas technology in the field of protein detection, including CRISPR-Cas-based customized protein and peptide library research, CRISPR-Cas-based interaction detection, and CRISPR-Cas-based protein post-translational modification identification. The detection principle, method flow, advantages and disadvantages of these applications are introduced. In this paper, the application prospect of CRISPR-Cas technology in protein detection is analyzed from the aspects of technology development direction and potential market demand. At the same time, some suggestions and prospects for the future research direction in this field are put forward.

In general, CRISPR-Cas technology has broad application prospects in the field of protein detection. It has the advantages of high sensitivity, high selectivity, real-time, versatility and low cost, and is suitable for medical, life sciences, food safety and other fields. However, there are also some shortcomings in the practical application of this technology, such as high complexity, false positive and false negative still exist, safety has yet to be evaluated, etc., which need to be continuously optimized and improved.

In terms of future research directions, it is necessary to continuously strengthen the research on the basic principles and mechanisms of CRISPR-Cas technology, develop more excellent detection methods, and strengthen safety research. At the same time, it is also necessary to explore cooperation with other technologies to further promote its wide application in the field of protein detection. Overall, CRISPR-Cas technology has good research prospects and application prospects in the field of protein detection, which is worthy of further research and exploration.

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