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Applications of CRISPR-Cas9 Technology in Editing Telomeres and Their Related Control Genes

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Abstract: CRISPR-Cas9, awarded the 2020 Nobel Prize in Chemistry for its discovery by Jennifer Doudna and Emmanuelle Charpentier, has revolutionized gene editing due to its versatility, precision, and efficiency. Unlike previous gene editing tools such as Zinc Finger Proteins (ZFP) and TALENs, CRISPR-Cas9 allows targeted editing of any DNA sequence with minimal off-target effects. This article explores the potential of CRISPR-Cas9 technology to edit telomeres—protective DNA structures at chromosome ends—and related genes to address telomere-associated diseases and aging mechanisms. Telomeres, consisting of repetitive DNA sequences and associated proteins, are crucial in protecting chromosomes and regulating cellular aging, with their progressive shortening linked to cancer and other age-related diseases. This article mainly discusses the molecular mechanisms of telomere maintenance, the feasibility of using CRISPR-Cas9 for telomere length control, and its implications for cancer, stem cell research, and telomere-associated diseases. While promising, further research is needed to fully understand the roles of regulatory pathways and non-genetic factors in telomere dynamics.

Keywords: CRISPR-Cas9, Telomere, Telomerase.

1. Introduction

In 2020, Jennifer Doudner and Emmanuel Carpenty were awarded the Nobel Prize in Chemistry for their discovery of the CRISPR-Cas9 system. Before the discovery of the system, the commonly used gene editing techniques were Zinc Finger Protein (ZFP) technology and Transcription activator like (TAL) effector nucleases (TALENs) technology. However, both technologies have many drawbacks, such as severe off target effects and cumbersome screening of ZFP, especially for zinc finger nucleases. As for TALENs, the protein molecules of TALENs are too large, which not only makes it more difficult for the target gene to bind to TALENs, but also the entry of large molecular substances into the body may cause an immune response, reducing their effectiveness [1]. The CRISPR-Cas9 technology overcomes the shortcomings of the aforementioned techniques and has become the most commonly used means of gene editing due to its versatility and high efficiency, which can be applied to the editing of any base sequence. At present, CRISPR-Cas9 technology has become the most mainstream gene editing technology, widely used in medical research, agriculture, biotechnology, medical diagnosis, and other fields, such as the study of diseases such as cancer, crop improvement,

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rapid detection of pathogens, etc. It has significant implications for human understanding and genetic modification [2].

Telomeres are protective structures located at the ends of chromosomes, mainly composed of repeated DNA sequences TTAGGG and related proteins, with a length of 5-20kb. Their main function is to protect chromosomes from damage and prevent chromosome loss during cell division. During each cell division, telomeres become shorter, which is closely related to cellular aging and the occurrence of certain diseases, such as cancer and cardiovascular diseases [3]. At present, there are still many problems in the research of telomeres, such as how to effectively control telomere length in some telomere related diseases such as cancer, the mechanism of telomere shortening, and the unclear relationship between telomeres, stem cells, and aging. This article mainly introduces the use of CRISPR-Cas9 technology to edit genes related to controlling telomere length, thereby achieving indirect control of telomere length, as well as the feasibility of directly gene editing telomeres to directly control telomere length. Through CRISPR-Cas9 technology, certain genes can be precisely controlled to achieve indirect regulation of telomeres, which plays an important role in the study of telomere mechanisms and telomere related diseases.

2. The composition and molecular mechanism of telomeres

2.1. The composition of telomeres

Telomeres are mainly composed of repeated DNA sequences TTAGGG and protective proteins, forming a three-dimensional spatial structure. Protective proteins mainly include TRF1, TRF2, TIN2, POT1, TPP1, Rap1, etc. [4]. TRF1 (Telomeric Repeat binding Factor 1) protein is the main binding protein of telomeres, responsible for identifying and binding to telomere repeat sequences. It plays a key role in regulating telomere length and preventing telomere fusion. The TRF2 (Telomeric Repeat Binding Factor 2) protein also binds to telomere repeat sequences and participates in the protection of telomere structure. It plays an important role in forming telomere loops (T-loops) and inhibiting DNA damage responses. TIN2 (TRF1 interacting Nuclear Protein 2) protein is an adapter protein that binds to TRF1 and TRF2 to help stabilize telomere complexes. It also plays a key role in regulating telomere length and maintaining telomere structure. TPP1 (Ten Elle Protein 1) protein binds to telomeres and participates in maintaining telomere integrity. It forms a complex with POT1 to help protect telomeres from the effects of DNA damage repair mechanisms. POT1 (Protection of Telomeres 1) protein is mainly responsible for recognizing and binding to telomere single stranded DNA, protecting telomeres from damage, and playing an important role in regulating telomere length and maintaining telomere stability. Rap1 (Repressor/Activating Protein 1) protein binds to telomeres and participates in regulating the structure and function of telomeres. It not only plays a role in telomere protection, but also participates in regulating gene expression. Different protective proteins have different structures and functions. By interacting and regulating the structure and function of telomeres, they ensure the stability of chromosomes, thereby affecting cell growth and aging processes. There are specific structural domains on the protective protein that can specifically recognize the TTAGGG sequence on telomeres, thereby interacting with telomere DNA and enabling it to effectively and stably bind to telomere DNA. Protective proteins usually exist in the form of complexes to enhance their binding ability, and they bind to telomere DNA in a three-dimensional manner. Among them, the six protective proteins mentioned above bind to form telomeric shelterin complexes, which protect the structure of telomeres and regulate their length.

There is a transition region between telomeres and normal DNA, typically composed of shorter DNA sequences that may contain gene regulatory elements such as enhancers, promoters, or gene sequences that can be expressed. At the same time, this region also binds to various protective proteins, such as TRF1 and TRF2, which play a stabilizing and protective role at the junction of telomeres and

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chromosomes. The chromatin state in the transition region may differ from adjacent normal DNA, and the telomere region is usually rich in heterochromatin, exhibiting different structural and functional characteristics. Transition regions play an important role in cell division and telomere maintenance, ensuring effective connections between telomeres and chromosomes, thereby maintaining genome integrity.

2.2. The composition and molecular mechanism of telomerase

Telomerase is responsible for regulating the length of telomeres. It is an enzyme complex mainly composed of two parts: telomerase reverse transcriptase (TERT), which is responsible for converting RNA templates into DNA, and telomerase RNA component (TERC), which provides template sequences and guides telomere synthesis. Its encoded genes are TERT gene and TERC gene, respectively. The activity of telomerase is regulated by various factors, including cell type, signaling pathways, and some transcription factors. After activation of TERT, telomerase can specifically recognize the TTAGGG sequence of telomeres and bind to the DNA at the end of telomeres, forming an active enzyme complex. Then, using TERC as a template, short DNA repeat sequences (such as TTAGGG) are added to the 3' end of telomeres, increasing their length.

In addition, the length of telomeres is also regulated by many other genes and factors, which interact to form a precise regulatory network [5].

3. Mechanism of CRISPR-Cas9 to edit telomere-related genes

The use of sections to divide the text of the paper is optional and left as a decision for the author. Where the author wishes to divide the paper into sections the formatting shown in table 2 should be used.

3.1. The composition of CRISPR-Cas9

The CRISPR-Cas9 system is mainly composed of CRISPR loci, Cas proteins, and gRNA components, and functions by identifying neighboring sequences of exogenous prototype spacer sequences. Among them, the CRISPR system consists of 25-50bp palindromic sequences with intermediate repeats in the genome. There are also leading sequences upstream of the repeats and spacer sequences, which are generally considered the promoter sequences of the CRISPR cluster. The CRISPR Cas system identifies the target gene through these spacer sequences. Cas protein is a type of enzyme involved in foreign DNA recognition and cleavage, mostly located near CRISPR sites. The functional modules of Cas protein can be divided into four types, namely adaptation, expression, interference, and assistance [6]. gRNA is composed of trans activating CRISPR RNA (tracrRNA) and CRISPR RNA (crRNA), where tracrRNA is an auxiliary RNA responsible for assisting crRNA in binding to Cas9 protein. crRNA is a part of the CRISPR system that contains sequences complementary to the target DNA sequence, responsible for recognizing and binding to specific DNA targets. Its recognition process requires the localization of PAM sequences with NGG sequences [7].

3.2. Disign of CRISPR-Cas9

Taking the KBTBD6 and POP5 genes discovered in research as examples, gRNA design is mainly divided into four steps. The first step is to screen the target genes. After obtaining the base sequences of KBTBD6 and POP5 genes, a 20 bp target sequence is selected, usually located in the promoter or coding region of the gene, and a PAM of NGG is ensured after the sequence. This ensures the specificity of binding, ensuring that Cas protein can accurately bind to the target gene, which is also the guarantee of the accuracy of CRISPR-Cas9 technology. Next is specificity assessment, using

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bioinformatics tools such as BLAST to examine the specificity of the selected target sequence in the genome, ensuring that it does not undergo non-specific binding with other genes or regions. Next is functional testing, where gRNA is synthesized based on the screened sequence, and its effectiveness and specificity are tested in a cell model. The efficiency of gene editing is evaluated by transfecting gRNA and Cas9 protein. The two intermediate steps are the verification of gRNA design, confirming the correctness of the gRNA design sequence to ensure that it can correctly guide the binding of Cas protein to the target gene. Finally, the optimization of gRNA involves adjusting its sequence based on experimental results, conducting multiple rounds of design and testing to improve editing efficiency. The CRSIPR-Cas9 technology still has off target issues, and adjusting the gRNA sequence can reduce the off-target rate to some extent [8].

4. Progress and application of CRISP-Cas9 in editing telomere related genes

4.1. Research in the field of cancer

The research on telomere editing can be applied to the treatment of cancer. During normal cell division, telomeres gradually shorten, ultimately leading to cellular aging. However, the vast majority of cancer cells often prolong their telomeres and evade aging by activating telomerase (such as TERT). Therefore, the activity of telomerase in cancer cells is significantly higher than that in normal cells. How to control and interfere with telomerase activity is a direction for controlling cancer cells and treating cancer [9]. For example, in the treatment research of small cell lung cancer (SCLC), researchers have developed a telomere targeted drug to deplete the cancer initiating cells (CIC) of small cell lung cancer and promote anti-tumor immunity. The activity of telomerase is crucial for the proliferation of SCLC. Researchers utilized a nucleoside analogue, 6-thio-2 '- deoxyguanosine (6TdG), as an inhibitory drug to transfer it to telomeres via telomerase. 6TdG has a strong affinity for telomerase. After administration, 6TdG is converted into 6-thio-2 '- deoxyguanosine-5' - triphosphate and immediately incorporated into telomeres, causing rapid dysfunction and decapitation of telomeres, leading to DNA damage in tumor cells [10]. Using CRISPR-Cas9 technology, functional fragments can be inserted into telomeres to disrupt their function and control cancer cell proliferation.

4.2. Research on telomere mechanisms

There is still a gap in the mechanism of action of telomeres and telomerase, including the regulation of telomerase activity and the molecular mechanism of telomeres themselves. However, using CRISPR-Cas9 technology, it is possible to study a specific factor in a targeted manner.The telomere protective structure, shelterin, is formed by the proteins TRF1, TRF2, TIN2, POT1, TPP1, and Rap1. Researchers used CRISPR-Cas9 technology to knock out genes expressing the corresponding proteins, resulting in conditional knockout (KO) cells that lacked the relevant subunit proteins that makeup shelterin. Researchers obtained a large number of KO cells through CRISPR technology to study the interactions and functions between different subunits that make up shelterin [11]. In research, the CRSIPR-Cas9 technology can more effectively and accurately target and knock out a specific gene in cells, and explore the consequences of its deletion. This is of great significance for the study of the mechanism of telomere action and related factors controlling telomeres.

4.3. Research on telomere related diseases

The use of CRISPR-Cas9 technology can provide new ideas for the treatment of telomere related diseases. Diseases related to telomeres are generally associated with dysfunction or uncontrolled length of telomeres. For example, Hutchinson-Gilford Progeria (HGPS) is a premature aging disorder that is associated with mutations in the LMNA gene that controls telomere length. The mutation

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results in the expression of a lamin A protein mutant, Progein, which is missing 50 amino acids and accumulates in cells, leading to telomere shortening and dysfunction, ultimately resulting in cellular aging. Researchers have developed a CPRISPR-Cas9 strategy targeting HGPS, aimed at blocking the accumulation of lamin A and progein in vivo. LMNA genes encode laminC (encoded by exons 1-10) and lamin A (encoded by exons 1-12) through alternative splicing and polyadenylation. Due to the optional nature of laminA, this strategy involves disrupting the final part of the LMNA gene and using CRISPR-Cas9 technology to edit the gene encoding laminA, thereby inhibiting the production of laminA/progerin without affecting laminC [12]. The treatment plan for this technology is still under research, but it can be seen that CRISPR-Cas9 technology has brought new research ideas for the treatment of telomere related diseases.

In addition to the above three aspects, the technology of editing telomeres and related genes through CRSIPR-Cas9 also has important applications in many other fields, such as stem cell research, human aging research, and so on.

5. Research prospects and challenges

Due to its convenience, accuracy, and other advantages, CRISPR-Cas9 technology has broad prospects. Its specific applications in the study of telomeres and related genes can be mainly divided into three aspects: firstly, CRISPR-Cas9 technology can facilitate the editing of telomere related genes. For example, in the study of telomere mechanisms mentioned earlier, it is possible to efficiently obtain a large number of KO cells that have knocked out a specific gene, which is of great significance for the research of telomere molecular mechanisms and telomere control networks. Secondly, the CRSIPR-Cas9 technology has provided new ideas for the treatment of some telomere related diseases, such as Hodgkin's syndrome. Currently, there is no effective treatment method, but CRISPR-Cas9 technology has made gene therapy possible for this disease. Not only Hodgkin's syndrome, but many other telomere related diseases can also be treated with CRISPR-Cas9 technology. Thirdly, CRISPR-Cas9 technology can directly edit telomeres. Although there are currently no relevant cases, as a part of chromosomes, telomeres themselves are also composed of DNA and proteins. Therefore, theoretically, it is possible to use CRISPR-Cas9 technology to directly edit telomeres and control telomere length.

At the same time, CRISPR-Cas9 technology also has certain defects and limitations. The first issue is the off-target problem. Although CRISPR-Cas9 technology has a lower off target rate compared to traditional gene editing methods such as ZFP and TALENs, it is still inevitable that off target problems will occur, which has a certain impact on the success rate of research and will also have corresponding effects in subsequent application stages. Secondly, during the experiment, it is difficult to measure the length of telomeres. Currently, it is not possible to directly detect the length of telomeres through simple physical means or instruments. Therefore, it is challenging to efficiently and continuously record changes in telomere length during the experiment. This can lead to complex and cumbersome research processes related to telomeres, and increase the cost and probability of errors [13]. Finally, if one wishes to directly cleave telomere DNA to alter telomere length, the TTAGGG repeat sequence of telomere DNA may result in misidentification or misidentification and cleavage, and protective proteins and shelterin on telomeres may also protect telomeres from being cleaved [14]. This requires CRISPR-Cas9 technology to be able to specifically recognize and cleave a specific number of sequences located at specific positions on telomeres, ensuring that the editing process is not affected by protective proteins.

In summary, although the use of CRISPR-Cas9 technology for editing telomeres and their related genes has broad prospects in both research and application fields, there are still some urgent issues that need to be addressed, both in terms of telomeres and gene editing technology itself and its application process.

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6. Conclusion

CRISPR-Cas9 technology, as the most widely used gene editing technology, utilizes gRNA guidance and Cas protein cleavage to complete gene cutting and insertion and can cleave DNA fragments of any base sequence. Telomeres, as important controllers of DNA division and cellular aging processes, have significant research significance. This article comprehensively elaborates on the molecular mechanism of telomeres, how to use CRISPR-Cas9 to edit telomeres and related genes, the application progress of CRISPR-Cas9 in telomere research, and the prospects and shortcomings of this research. It comprehensively explains the feasibility, research direction, and ideas of applying CRISPR-Cas9 technology to telomere research, editing telomeres and controlling telomere length related genes. Moreover, this technology has made certain progress in cancer, telomere related diseases, and telomere mechanism research. This is of great significance for exploring the molecular mechanisms of telomere regulation and some studies related to telomeres, such as stem cells and cancer. However, this article only analyzes from the perspective of telomeres and their related control genes, and does not address some non genetic factors involved in telomere regulation, such as small molecules and regulatory factors. To thoroughly reveal the network of telomere regulation, more in-depth research is needed on the roles of regulatory genes, regulatory factors, and various regulatory pathways.

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