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The Applications of CRISPR Systems in Cancer Detection

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Abstract: With the increasing incidence and mortality rate of cancer, CRISPR technology needs to be applied more widely and accurately in this field. The current application of CRISPR in cancer detection is mostly aimed at cancers with clear genes as tumor markers. The detection of some cancers with unknown pathogenesis is still in the stage of development. Studying their pathogenesis through CRISPR technology may make progress and accelerate their detection and diagnosis. This article demonstrates the feasibility of CRISPR cas system and the incidence detection principles of the important disease cancer and introduces representative application cases to further demonstrate the importance and development prospects of CRISPR in this field. This article helps to gain a preliminary understanding of the application principles of CRISPR system in cancer detection, facilitating the screening of suitable related technologies and providing ideas for future study on new CRISPR technologies in cancer. In the future, CRISPR technology is highly likely to greatly accelerate the speed of cancer detection, reduce detection costs, and provide assistance for integrated cancer detection, perhaps making cancer detection as easy as nucleic acid kit testing.

Keywords: Cancer, CRISPR, detection.

1. Introduction

Based on demographic predictions, the number of new cancer cases is expected to reach 35 million by 2050 [1]. According to the latest statistical data, prostate cancer is the most common cancer among men, followed by lung cancer. Lung cancer is the most common cause of cancer death in men, followed by prostate cancer and liver cancer; For women, breast cancer and cervical cancer are the most common cancers, breast cancer and cervical cancer are the most common causes of cancer death for women, followed by lung cancer. CRISPR/Cas9 has been used to alter the genomes of many organs as a strong method. CRISPR/Cas9 and its modified versions were initially discovered in bacteria and have been widely used in adaptive immune system for engineering genomes and activating or inhibiting gene expression [2]. CRISPR technology can accelerate cancer diagnosis and improve the accuracy and specificity of its testing. At the same time, CRISPR also has important impact on cancer treatment. By regulating the expression of oncogenes, tumor suppressor genes, other tumor related genomes such as cell cycle regulatory genes, apoptosis related genes, and genes that promote tumor metastasis inhibition, as well as the immune system, gene therapy can greatly prolong the survival of cancer patients and even potentially cure cancer. Currently, CRISPR has become one of the important application tools for cancer detection, diagnosis, treatment, and prognosis. Although CRISPR has made numerous progress in this field, there are still issues such as insufficient insertion,

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being out of target activity and deletion, low efficiency of homologous guided repair. This article mainly introduces the principle of CRISPR technology represented by CRISPR Cas9 and the pathogenesis of cancer, including two specific mechanisms of EGFR and epigenetic mechanism. Its specific application cases in cancer detection include the combination with fluorescent labeling technology and gene mutation detection. It also looks forward to the possible future demands of CRISPR cas9 in cancer. Through this study, we can gain new insights and understanding into the existing and further applications of CRISPR in the field of cancer. This will help hospitals, laboratories, and other institutions choose appropriate cancer detection technologies and develop new CRCIPR technologies for cancer diagnosis, thereby reducing costs, accelerating integration and refinement, improving the accuracy of cancer testing, promoting the development of medical technology, and better solving problems for cancer patients and those with a family history of cancer.

2. The principle of CRISPR Cas9 technology

2.1. System introduction

CRISPR cas9 involves 2 parts: guide RNA and cas9 used for protein cleavage. Cas9 is actually a Cas operon composed of Cas9, Cas1, and Cas2. Guided RNA, also known as sgRNA, is composed of two parts: tracrRNA and crRNA, which bind through overlapping regions based on the principle of complementary base pairing. TracrRNA (trans activating CRISPR RNA) derive from a part of its DNA by transcribing, also known as trans activated crRNA. The source of crRNA is a precursor (pre-crRNA) composed of a leader sequence and different crRNAs. The leader sequence can initiate CRISPR transcription, while crRNA is transcribed from CRISPR. CRISPR is a cluster of regularly spaced short palindromic repeat sequences, consisting of direct repeats and different spacer sequences derived from foreign invading DNA. After precursor processing, mature crRNA is formed, and the guide sequence and partial crRNA are cleaved by RNase III. RNase III is an exogenous enzyme that does not come from the CRISPR locus, and mature crRNA does not have guide sequences or partial crRNA (Figure 1).

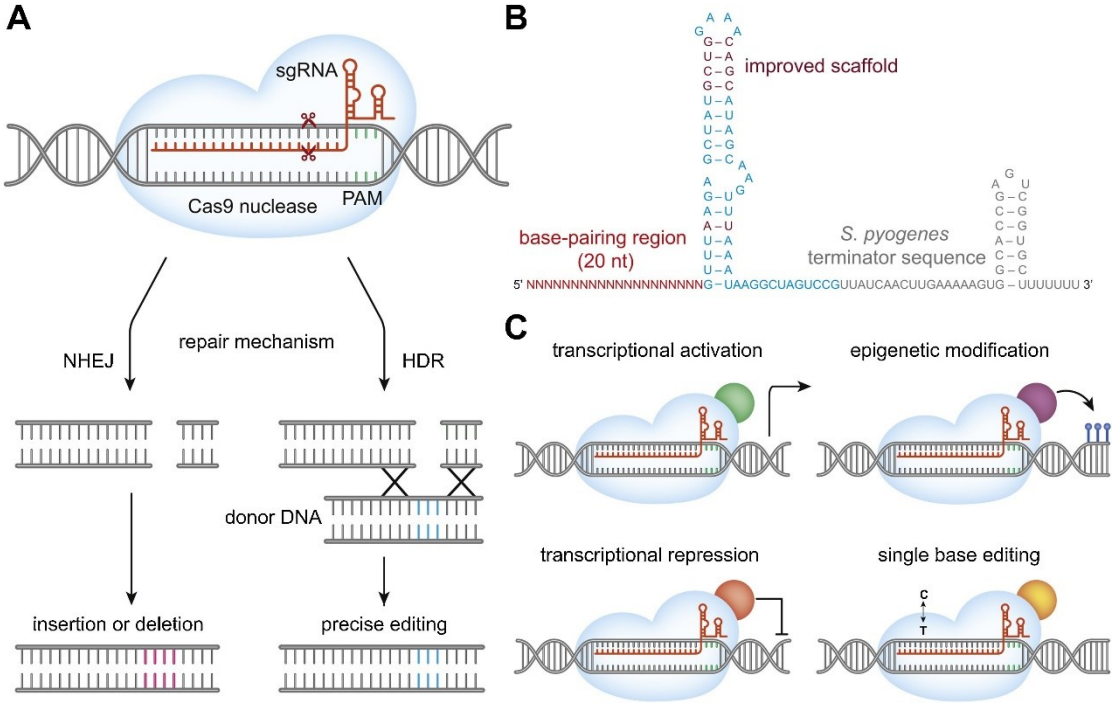

Figure 1: Mechanism of CRISPR-Cas9 gene editing [3].

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2.2. Specific process

Firstly, the DNA corresponding to tracrRNA is transcribed to obtain tracrRNA, and cas operon is transcribed and translated to obtain a cas9 protein. The guide sequence is transcribed together with the CRISPR sequence to obtain pre-crRNA, which binds to tracrRNA and Cas 9 protein. Under the action of RNase III, it undergoes cleavage to mature the RNP complex, which consists of Cas9, tracrRNA, and crRNA, with the latter two being sgRNA. The RNP complex has the function of cleaving DNA. Among them, according to PAM (pcotospacer ajecent motif), the original spacer sequence is inserted into the old spacer sequence to obtain a new spacer sequence. The recognition site of PAM is NGG, N, which is the four bases A, G, C, T, and G is the base guanine. Therefore, PAM can insert the first part of the sequence with NGG site as the original spacer sequence. In practical experiments, sgRNA is usually a single strand, which is actually a fusion of tracrRNA and crRNA into a single strand.

The Cas9 protein exert its cleavage function mainly by depending on the 2 domains :HNH and RuvC domain . The RuvC domain complete the process of recognition and fission of single stranded DNA which is not integral to sgRNA bases, while the HNH domain finish the process of recognition and fission of exogenous DNA complementary to sgRNA bases. The fission area is on the position of the outer side of the 5 'end of the third amino acid upstream of PAM, and is simultaneously cleaved by the HNH domain and RuvC domain.

3. The pathogenesis of cancer

3.1. Overview

Cancer is a complex and multifaceted disease. Radically, it is DNA mutations that stimulate cancer gene and quiescent tumor inhibition factors cause this genetic disease , besides epigenetic abnormal regulation that coordinates the expression of normal gene [3]. In normal cells, oncogenes refer to a special gene type that has a wide occurrence in the genomes of both humans and other creatures, and remains highly stable during biological evolution, harmless to cells. However, when oncogenes are abnormally activated in cancer cells, they continue to function, promoting unlimited division and proliferation of cancer cells. The common activation mechanisms currently include point mutations, translocation activation, amplification of oncogenes, and decreased methylation of oncogenes. When tumor suppressor genes are normal, they play a role in inhibiting cell proliferation, tumorigenesis, and promoting cell differentiation. Many tumors have the deletion or inactivation of two alleles of tumor suppressor genes, and tumor suppressor genes lose their anti-cancer function after mutation or deletion. However, mutations in a single allele cannot suppress the function of the gene. Only when two alleles mutate simultaneously, the gene loses its normal anti-cancer function. The inactivation of tumor suppressor genes can be roughly divided into two categories: point mutations or deletions in DNA, and epigenetic mechanisms such as DNA methylation and histone deacetylation that inhibit allele expression. Most cancers are recognized and attacked by the immune system, but due to tumor mediated immune suppression and evasion mechanisms, cancer can progress[4]. At the same time, tumor cells in the body can escape the inspection and monitoring of immune cells: in a strong body, there are possibilities to produce a small number of cells with abnormal functions and potential of cancer as well, but if the immune system can recognize the abnormal surface features of these cells, it can kill them and prevent cancer from occurring. However, when the body's immune system weakens due to various reasons, abnormal cells cannot be cleared in a timely manner by immune cells or has a balanced state with cells of immunity system . As abnormal cells evolve, they will hide their surface features, making cells in immunity system less possible to detect and kill them. They may also use innumerable defending mechanisms to intervene the function of those functional cells.

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Technology advancements, involving the whole sequences of many subtypes of cancers, have elucidated how the change of genes and epigenes are in reference to particular markers of cancer nowadays. CRISPR technology has played an important role in it[5]. Therefore, molecular biology tests for cancer usually include tumor related genes, tumor related viral genes, tumor marker genes, or mRNA.

3.2. EGFR gene mutations and detection mechanism

Elevated levels of epidermal growth factor receptor (EGFR), a growth factor receptor tyrosine kinase, and/or its homologous ligands have been identified as common components of various cancer types and appear to promote solid tumor growth [6]. The cancer gene surface growth factor receptor (EGFR) is expressed over the regular level in most patients with the disease named small cell lung cancers (NSCLC). EGFR is a member of the EGF (epidermal growth factor) - related tyrosine receptor family. By binding to ligands, the receptor undergoes homodimerization and heterodimerization, activating endogenous tyrosine kinases and triggering the cascading process of signals in the downstream , mainly including 3main pathways. These signaling pathways have effective stimulating effects on cell growth, fission, migration, and generation of blood vessel. Currently, it is known that most NSCLC have EGFR overexpression, with a squamous cell carcinoma expression rate of 85%, adenocarcinoma and large cell carcinoma expression rates of 65%, and small cell carcinoma expression rates being relatively low. Studies have shown that EGFR TKIs have poor efficacy when mutations are detected at the T790M and 20 Ins sites of the EGFR gene. Expression beyond normal level of EGFR protein is very normal(40%-80%) in patients diagnosed with NSCLC and is associated with invasiveness and poor prognosis.

3.3. Epigenetic mechanisms

Epigenetic variation refers to heritable changes in gene function that ultimately result in phenotypic changes, without altering the DNA sequence of the gene[7]. Initially, epigenetics referred to the study of how genes and their products form phenotypes. Today, it mainly focuses on the mechanisms by which cells commit to specific forms or functions, and then transmit these functional or structural states through the cell lineage [8]. In the past few years, there has been a improved knowledge of the mechanisms underlying epigenetic diseases, which are associated with four epigenetic processes: chromatin remodeling, genomic imprinting, X chromosome inactivation, and noncoding RNA regulation [9]. Numerous research results have shown that tumor formation is the result of genetic mutations, including epigenetic changes. Epigenetic changes during the process of carcinogenesis can also activate oncogenes and inactivate tumor suppressor genes, playing an important role in the occurrence, development, and metastasis of tumors.

The molecular mechanisms of epigenetic abnormalities in tumor cells mainly include:

1) Genomic imprinting loss: In certain tissues and cells of mammals, a pair of alleles that control a certain phenotype are differentially expressed due to genetic differences, that is, the body only expresses alleles from one parent, regardless of its own gender. This phenomenon is called genomic imprinting. This is a self-monitoring mechanism formed by mammals during long-term evolution, and the loss of genomic imprints is believed to be associated with susceptibility to tumors. For example, under normal circumstances, the IGF-2 (insulin-like growth factor-2) gene only expresses alleles derived from the father, while maternal alleles are imprinted. Research has found that the loss of IGF-2 imprints increases the risk of developing colorectal cancer DNA methylation. Changes in DNA methylation in tumor cells are another form of genetic material alteration in tumor cells. It includes low methylation of the entire genome and high methylation of the promoter. The current

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focus is on the transcriptional silencing of tumor suppressor genes caused by high methylation of CpG islands in the promoter region, which is likely the initial manifestation of tumor proliferation.

2) Histone modification and chromatin remodeling: During selective gene silencing or gene expression in cellular life activities, the genomic DNA sequence in chromatin generally does not change, but chromatin decoupling in the nucleus can undergo highly dynamic changes, resulting in corresponding changes in transcriptional activity in certain genomic regions. This chromatin remodeling can activate or silence genes. Core histones are highly conserved alkaline proteins with spherical domains, and DNA is enveloped by relatively unstructured flexible 'tails' protruding from nucleosomes [10]. The modification of histones and their methylation, acetylation, and phosphorylation in nucleosomes plays an important role in maintaining gene expression patterns and normal chromosome structure and function. Any small external changes can have a significant impact on cell phenotype and transcription patterns.

4. Specific applications of CRISPR in cancer detection

4.1. Combining fluorescence labeling technology

Detection in early times and timely therapy of cancer can effectively reduce the possibility of death, make treatment outcomes better, and enhance patients' standard of life after operations. Although cancer detection also use several other technologies widely , such as immune technology and imaging technology, they need improvement in sensitivity, specificity, and speed, while molecular biotechnology is far superior to most other cancer detection technologies in terms of accuracy and specificity. Therefore, identifying genes related with cancer through examination of genes is vital to preventing cancer. Gootenberg et al. developed a system based on this principle named specific high-sensitivity enzyme reporter gene(UNLOCKSHERLOCK) that combines CRISPR with fluorescent labeling technology. The system is composed of 32different parts: RNA guided RNaseand,signal to report . SHERLOCK is a single-molecule detection method for nucleic acid targets. The specific operation process is as follows: Firstly, blood, saliva, urine, throat swabs and other specimens are collected. As the cas system used cuts RNA, it needs to be amplified to form a large amount of target RNA, guiding RNA to activate the activity of the cas enzyme system through target recognition. Enzymes bind to Cas enzymes to form CRISPR Cas RNA complexes, which are activated by binding to complementary target RNA. This activation triggers incidental cleavage of non-specific RNA reporter. When the reporter molecule (fluorophore) labeled with fluorescence is intact, it is "locked" and does not emit light due to the inhibition of luminescent molecules. Once the activated CRISPR-Cas13 complex is cleaved, the "lock" is opened and fluorescence is emitted.

Its working principle is to program a special CRISPR Cas enzyme and detect the occurrence of particular nuclear acid features, i.e. corresponding fluorescent signals, in the sample through the intelligent amplicon detection. When detecting the corresponding feature , the CRISPR Cas enzyme system is stimulated to generate a strong and obvious signal. This signal can be adapted for simple paper strip testing in laboratory equipment or provide electrochemical readings that can be read in simple digital products such as a phone. The characteristic of SHERLOCK diagnostic tool is that its sensitivity and specificity are comparable to traditional PCR based methods, but it does not require complex equipment and the estimated cost is very low [11].

4.2. Detection of gene mutations

The California University, Berkeley and the Keck Institute at Claremont College has made up a collaborative team and combined gene editing technology with nanoelectronics to create a new handheld device called CRISPR Chip that can find particular gene changes in short times. Diagnosing

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diseases related with genes or evaluating the accurateness of gene editing technology can realize fast by this portable and fast device .

The team connected the Cas9 protein variant - a Cas9 variant that is without vitality and can detect a particular location in DNA but does not make it split - to a transistor including a special material-graphene. When this CRISPR system detects a specific site in its targeted DNA, it combines it and accelerate a vairation in graphene conductivity, altering the properties in electricity of the transistor and enabling the detection of mutated genes. These changes can be easily found by using a simple handheld device invented by the cooperating team.

This device is a combination of nanoelectronics and modern biology, integrating the precise characteristics of CRISPR technology with the ultra sensitivity of graphene transistors, making it both precise and fast. This device can be used for rapid diagnosis of diseases, helping workers in hospital or laboratory to study improved personalized therapy plans for different patients through rapid genetic testing, and can also be used to find out whether CRISPR combines particular DNA sequences.

5. Advantages and development prospects of CRISPR Cas9 applications

The CRISPR Cas genome editing system derived from prokaryotes has improve our ability to control, diagnose, picture, and explicate specific DNA and RNA sequences in live cells of different creatures. The targets of CRISPR have a high frequency of distribution in the genome, high targeting accuracy, and can perform gene manipulation on multiple targets simultaneously. The system is simple and easy to prepare, thus saving a lot of time and economic costs.

At present, technology can activate the CRISPR Cas system to generate a strong and obvious signal when a marker is detected. This signal can be applied to laboratory equipment for simple paper strip testing or to provide electrochemical readings that can be read with a simple single digital machines like a phone. Moreover, the temperature control of CRISPR technology is not as strict as PCR, and the temperature control system does not require a large space. In the future, it can greatly improve its miniaturization, portability, automation, and integration, thereby reducing economic and labor costs such as consumables and electricity. In the future, designing and manufacturing portable small machines with functions such as sampling, amplification, and CRISPR detection can reduce the difficulty of cancer detection and perhaps achieve self-service cancer detection. Small integrated machines may have the potential to be used in community hospitals, small clinics, and even purchased at home, which is of great significance for patients with a family history of cancer inheritance and long-term exposure to carcinogenic environments who may require multiple continuous tests for cancer occurrence.

6. Conclusion

This article first introduces the principles of CRISPR technology and cancer, and then details the practical applications of CRISPR in detection technology. Through this article, we can briefly understand the current applications of CRISPR in the field of cancer, which is beneficial for healthcare workers to choose and develop related technologies. However, this article has limited elaboration on the specific applications of CRISPR, and many applications of CRISPR in cancer detection have not been mentioned in this article. SHERLOCK, as a representative of CRISPR in detection technology, although extremely representative, cannot represent the latest progress and existing technology of CRISPR. We can conclude that since CRISPR developed into a gene editing tool, CRISPR technology has achieved gene amplification, splicing, and editing by providing a simpler and more universal method to manipulate the genomes, transcriptomes, and epigenomes of various organisms. Meanwhile, the application of CRISPR Cas in therapeutic methods is becoming increasingly important in different fields of medicine. The role of CRISPR in cancer research,

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detection, and treatment is just beginning to emerge. Future studies are needed to CRISPR technology achieving greater integration, simple operation, convenient transportation and storage, making cancer detection as easy as nucleic acid kit testing. In summary, the development of CRISPR/Cas9 technology has greatly accelerated cancer research in many fields.

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