Application of CRISPR Technology for Early Detection of Lung Cancer

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Abstract. Lung cancer is a disease with a high mortality rate that endangers human life and health. Due to the high late mortality rate, the early symptoms are not obvious, and the traditional diagnostic methods have misdiagnosis and missed diagnosis. However, the early diagnosis has limitations. Improving the accuracy of early screening for lung disease and reducing the harm to the human body is an important factor for improving the survival rate of lung cancer. At present, the combination of CRISPR-Cas system and cancer biomarkers has been proposed to achieve a non-invasive, convenient and accurate diagnosis. Tracing cancer biomarkers such as ctDNA and miRNA carry information from tumors, from which important information such as disease-related disease classification and disease staging can be obtained. Amplification technology amplifies the signal of tracing biomarkers and combines it with CRISPR technology. The precise cutting function of the CRISPR-Cas system can be used to present a fluorescent signal or a readable signal to enable the interpretation of disease information. In this research, the existing studies on CRISPR for lung cancer detection are summarized to help popularize relevant knowledge, and the prospect and prospect of CRISPR for lung cancer detection are discussed, hoping that better detection technology can be used for medical detection as soon as possible.

Keywords: CRISPR, lung cancer, early detection.

1. Introduction

Lung cancer is a kind of lung malignancy. Statistical data indicate that lung cancer is the main cause of male death and the second leading cause of female death [1], which seriously affects human life expectancy. However, the early symptoms of lung cancer are not obvious, and when patients are aware of the problem, it is usually in the middle and late stages, and medical technology at this time can hardly control the progression of lung cancer. Therefore, the early screening and screening accuracy of lung cancer play a crucial role in the treatment of tumors.

The introduction of gene editing technology into cancer detection provides new ideas for the accuracy and early diagnosis of cancer. Scientists have found a system of clusters of regularly spaced short palindromic repeats called CRISPR in the body of microorganisms to fight against the invasion of viruses or pathogens. A system composed of CRISPR and Cas proteins can achieve precise cutting and accurate recognition of target sequences. Based on the specific recognition of CRISPR-Cas system, this function can be applied to the detection of various diseases, helping to improve the accuracy of early diagnosis of cancer [2]. The existing cancer diagnosis techniques include imaging, biopsy and puncture. In the diagnosis process of medical images, radiation may lead to adverse consequences, and the images require doctors to make judgments based on their naked eyes and experience, which may result in misdiagnosis or missed diagnosis; while pathological biopsy requires puncture surgery, which may cause physiological harm to patients.

The application of CRISPR in cancer detection can effectively solve the problems of misdiagnosis, missed diagnosis and physiological harm. Specific identification of cancer biomarkers in body fluids is only required, and reliable case diagnosis proof can be provided efficiently, quickly, simply and noninvasively [3]. At present, there are relevant CRISPR detection technology for lung cancer, which can detect lung cancer in the early stage non-invasive and efficient and can effectively improve the early diagnosis rate of lung cancer. Current CRISPR technology mainly uses trace amounts of cancer biomarkers isolated in body fluids to amplify the signal generated by activating the cutting function of Cas proteins. Biomarkers are substances secreted or abnormally expressed in the process of cancer

disease, such as ctDNA, miRNA, sEVs and DNA methylation. The combination of biomarkers and CRISPR technology can achieve the purpose of accurate non-invasive and efficient detection. At present, the relevant research on CRISPR technology for the early detection of lung cancer is basically to extract and detect biomarkers carrying cancer pathological information. Biomarkers can be extracted in body fluids, such as exosomes or urine. However, due to the very low content of biomarkers, it is necessary to perform amplification first and then use CRISPR technology for specific cutting to obtain disease information. The CRISPR technology for lung cancer detection can effectively improve the accuracy of early detection and reduce the physiological trauma of patients, which is a very promising detection technology. For this reason, this research mainly analyzes the function of using CRISPR's specific cleavage of target sequences for lung cancer detection. The application of CRISPR in detection can achieve early accuracy and reduce trauma.

2. Detection of lung cancer based on CRISPR methods

2.1. Detection of miRNA as biomarkers

The miRNA is one of biomarkers that can demonstrate the progression of cancer pathologically. Abnormal expression of microRNAs is an important physiological manifestation of cancer and is involved in regulating the proliferation and metabolism of tumor cells. Complementary pairing of mRNA and miRNA can regulate the degradation of mRNA, thus affecting gene expression and regulating the proliferation, differentiation and metabolism of cells. It can lead to cell cancer [4]. Due to the small content in human cells and high miRNA family similarity, it is very difficult to detect miRNA. In order to solve the problem of accurate detection of miRNA, garland rolling ring augmentation technology is required to amplify the signal and then combine it with CRISPR-Cas9 technology to generate the signal. Therefore, a miRNA detection platform was established to achieve the purpose of high sensitivity, precision and non-invasive specific recognition. The target miRNA can hybridize with the designed two end dumbbell padlocks to form a ring padlock under the action of T4 ligase, and the chain can be extended with the help of phi29 polymerase. The amplified product is a hairpin structure containing repeated flower loops. By adding complementary sequences, a dsDNA structure that can be recognized by the CRISPR cas9 system is formed. Based on the assisted specific cutting of dsDNA structure by CRISPR-Cas9 system, the floral-ring amplification product is fissioned into the hairpin structure probe H1 probe. The generated hairpin probe can be unfolded by the target miRNA to form a complex mirNA-H1 probe. The fluorescently labeled hairpin probe H2 and the other end of the H1 hairpin probe are added to the hydride and replace the trigger, which is released and triggered to participate in the next cycle, constituting the CHA amplification process and signal to display [5].

2.2. Detection of urine synthetic biomarkers

Biomarkers in the body fluid of organisms in the microenvironment of disease state can be used as a non-invasive biopsy material. Biomarkers can provide disease diagnostic information to identify the types of diseases, monitor the degree of disease development, and also provide some treatation-related information, playing an important role in early diagnosis. However, endogenous biomarkers are affected by the abundance and stability in circulation, so the development of synthetic markers that affect the biochemically amplified analytes improves the signal-to-noise ratio of products. For immediate detection and accurate diagnosis, a high throughput, programmable in vivo nanosensor is designed for disease detection and monitoring in urine after molecular bar technology using stable DNA and sequence-specific reading using CRISPR-Cas technology. First, the DNA-encoded synthetic urine biomarker is contained in a nanocarrier, which is functionally activated by a short peptide activated by proteases. After drug administration in vivo, the DNA-encoded sensor is activated by disease-specific proteases to trigger the release of synthetic DNA barcodes. The DNA-encoded synthetic urine biomarker is designed to target the diseased site and sense dysregulated proteolysis in the tumor microenvironment, and then released in the kidney to filter and concentrate

to produce nucleic acid barcodes that can circulate in the blood. DNA barcodes in urine complement the sequence of guide RNA to activate CRISPR-Cas12a's single-stranded DNase activity after release of pathological proteolytic activity using fluorescent signals or chemically stable DNA barcodes that can be detected on strips of untreated urine. In this monitoring platform, disease classification in situ can be realized through the local protease activity in the disease microenvironment. As shown in Fig. 1, the diversity of DNA strips can open a door for the classification of complex human diseases in the context of multiple complications [6].

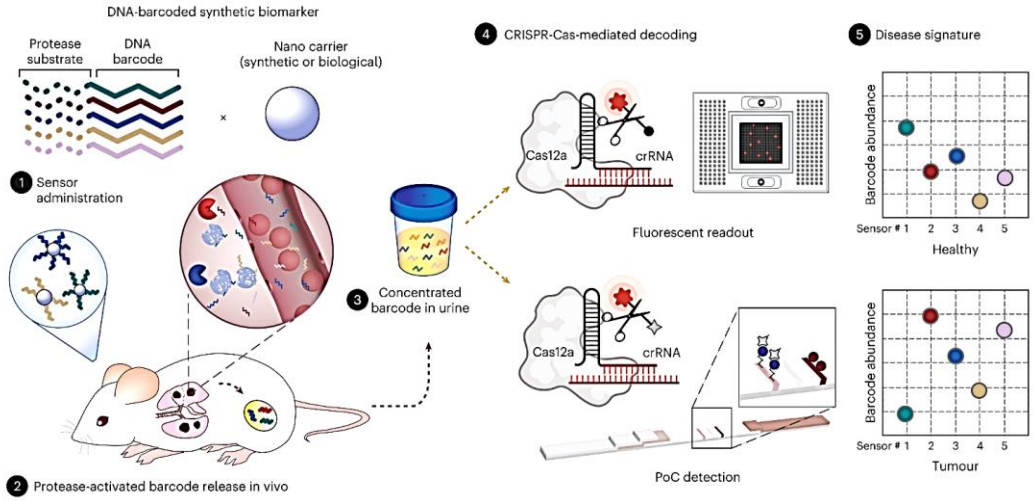

Figure 1. Application of CRISPR-Cas-amplified urinary biomarkers for cancer diagnostics [6]

2.3. Detection of lung cancer by Primer exchange reaction

In the plasma of patients with non-small cell lung cancer, there are a large number of PD-L1 positive exosomes, which are extracellular vesicles formed by oncoplasm membrane invaginations and whose components contain a variety of disease information. PD-L1 is a molecule that can help cancer cells evade immune surveillance and inhibit immune response, and the biomarkers of PD-L1 exosomes are closely related to the occurrence and development of lung cancer. PD-L1 exosome markers present in body fluids are ideal non-invasive detection materials, which have the advantages of fast and convenient, and can achieve early and dynamic real-time detection of lung cancer. Primer exchange reaction is a kind of amplification reaction that can synthesize custom single strand sequence by DNA polymerase catalyzed hair card as template under isothermal condition. It can produce a large number of amplification products in a short time to amplify the signal. After PD-L1 positive exosomes are bound by specific antibodies on immunomagnetic beads functionalized with CD63 antibodies, the cholesterol-modified DNA double strand is amplified through the hydrophobic interaction between the exosomal phospholipid bilayer and cholesterol, and the two hairpin probes are opened as templates to form DNA double strand and then shed. Finally, a large number of amplified DNA products activated the trans-cutting activity of CRISPR-Cas12a protein, and cas12 protein catalyzed the cutting of the target sequence to trigger the degradation of the methylene blue labeled DNA signal chain, resulting in a large number of short DNA fragments with electroactive methylene blue labeled ends. The molecules with fluorescence signals at the end ends were enriched to the surface of the graphite motor functionalized with hyacuramide, and the electrochemical signals of PD-L1 positive lung cancer exosome were quantitatively analyzed. The strength of the electrochemical signals is related to the early and advanced stages of lung cancer. Therefore, when evaluating the early screening and staging information of non-small cell lung cancer, it is expected to achieve the purpose of detecting whether patients have lung cancer and the progression of lung cancer in patients [7].

2.4. HOGG1 human 8-pruriguine DNA glycosylase and flap endonuclease

HOGG1 human 8-prurioguanine DNA glycoxylase and flap endonucrenase 1 (FEN1) are considered potential biomarkers for lung cancer, and the accumulation and mutagenic effects of oxidative DNA damage may be carcinogenic, in which 8-oxo-7, 8-dihydroguanine (8-oxoG) is a mutation that permanently reverses DNA replication due to base damage. Human 8-OXo-guanine DNA glycosylase (hOGG1) is a cancer evaluation marker for 8-oxoG. The hOGG1 can eliminate 8-oxoG to repair the base, so abnormal expression of hOGG1 may lead to cancer. FEN1 is involved in the development of cancer course, and its expression level is closely related to the survival, function, reproduction, and apoptosis of cancer cells. Moreover, low differentiation in FEN1 non-small cell lung cancer leads to diversity of FEN1 levels. The above two substances can be used as biomarkers for lung cancer diagnosis and prognosis. The development of a highly selective, sensitive, programmable, and versatile targeting platform for the analysis of HOGG1 human 8-pruriguine DNA glycosylase and flap endonuclease is of great clinical importance. To build an efficient diagnostic platform using Crispr-Cas12a system, first identify HOGG and FEN1 with a DNA-flapping dumbbell probe (FDP). The hOGG1 and FEN1 can identify and cut the FDP with a dual-function dumbbell probe. The post-cut notch can be identified by the formation of a specific closed DNA dumbbell probe CDP with no free ends by T4DNA ligase, then T7 RNA polymerase recognizes the special region of CDP as a template for initiating RCT, and then, generates an ultra-long single stranded RNA (ssRNA) with repeated crRNA. The cas12a-crRNA complex recognizes and activates ssDNA activators that enable cas12 to transcut. After cutting, a large number of TAMRA or FAM fluorophore signals can be generated. The green fluorescence of FAM is FEN1 positive, and the red fluorescence of TAMRA is Hogg1 positive. Different from the traditional cas12 detection method, the traditional cas12a system activates signal generation by recognizing amplification products, while the FDP-RCT-Cas12A-mediated biosensor generates a large number of crRNA repeats by Cas12a through RCT. Activate CRISPR-Cas12a crRNA trans-cleavage activity. In this study, RCT and CRISPR-Cas12a signal amplification techniques were used to simultaneously detect biomarkers hOGG1 and FEN1 in lung cancer cells. The detection method is highly specific and sensitive, and provides a new tool for the diagnosis of lung cancer [8].

2.5. Detection of ctDNA

Circulating tumor DNA (ctDNA) is the DNA fragment of tumor genome free in the blood inside and outside the human body. Because it carries tumor information, it can be used as a biomarker of cancer, and has the advantages of immediate, dynamic monitoring and minimally invasive in the diagnosis of lung cancer. However, the amount of ctDNA in body fluids is very low, usually only 0.01% of cell free DNA (cfDNA) [9], so the detection of CTDNA requires very precise techniques to assist. At present, the method of detecting ctDNA is costly and difficult to operate, so the CRISPR-Cas combined detection method is introduced. The absence of pam on some sequences limits the scope of use of this technology and off-target effect may lead to the absence of wild type rather than mutant type in cutting, resulting in false positive results and reduced accuracy. Therefore, a new method is proposed: A new method consisting of RPA and CRISPR/Cas12a monitors ctDNA. First, RPA technology was used for recombinant polymerase amplification, and a large number of products of ctDNA were rapidly amplified at 37℃ to achieve exponential amplification of target DNA. There may be no 4-ntPAM sequence in the upstream sequence of targeted mutations. In order to solve this problem, a specific RPA primer containing TTTN sequence should be designed to systematically introduce PAM sites, or secondary PAM can be used to replace regular PAM on sequences without PAM, thus improving the universality of this method. In addition, an MT-specific crRNA was designed to distinguish the MT mutant sequence from the WT wild-type sequence. To improve the specific recognition of SNV with single nucleotide variants, crRNAs are introduced with one or two mismatched bases to cover all possible nucleotide spacing sequences. When the crRNA binds to the targeted MT sequence, cas12a quickly transcuts the FAM-labeled ssDNA to produce a strong fluorescent signal, which can be fluorescently corrected or visually read. However, when crRNA is

mismatched with non-targeted WY sequence, the activity of cas12a will be reduced and the fluorescence signal generated will be weakened [10].

3. Conclusion

The CRISPR system is a gene-editing tool that can precisely target cut DNA sequences, which can be used to detect cancer. As mentioned above, because all kinds of cancer biomarkers have the characteristics of small amounts that are difficult to extract and the extraction work is cumbersome, the amplification of the signal using CRISPR technology alone is still not enough to obtain accurate results. The combination of CRISPR with other techniques, such as isothermal amplification and primer exchange reactions, enables accurate early detection of lung cancer. However, the CRISPR-Cas12a system also has a certain probability of off-target effects. And CRISPR-Cas12a can only bind to one crRNA, so CRISPR-Cas12a gene editing efficiency is low. In the future, it can try to use CRISPR technology in combination with other new technologies to complement each other for more accurate detection and improve gene editing efficiency by improving CRISPR off-target effects and CRISPR-Cas12a affinity with crRNA.

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