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The Potential and Future Perspectives of CRISPR-Cas 13 Technology in Cancer Therapy Application

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Abstract. Cancer remains a significant global health challenge due to its genetic complexity and the diverse mutations, epigenetic modifications, and dysregulated signaling pathways involved. This complexity complicates effective treatment strategies. The CRISPR system, particularly CRISPR - Cas13, presents a promising therapeutic tool, offering precise RNA targeting with high accuracy and sensitivity. This paper examines the potential of Cas 13 in cancer therapy, focusing on four key areas: the mechanism of Cas13, Cas13 applications in cancer treatment, challenges and limitations. The results of the study showed that CRISPR-Cas13 can not only be used as a potential cancer diagnostic tool, but also regulate abnormal mRNA mutations and reduce cancer risk. By knocking down oncogenic lncRNA, CRISPR-Cas13 can inhibit the proliferation and migration of cancer cells. CRISPR-Cas13 has the potential to reduce cancer incidence and reduce cancer mortality through early diagnosis and intervention. In the future, technical optimization to address the off-target effects of Cas13 will be the key to its application in cancer treatment.

Keywords: Cancer therapy, CRISPR-Cas9, CRISPR-Cas12, CRISPR-Cas13.

1. Introduction

In 2024, an estimated 2,001,140 new cases of cancer are expected to be diagnosed in the United States, with 611,720 individuals projected to succumb to the disease. Prostate, lung, and colorectal cancers are predicted to account for approximately 48% of all cancers diagnosed in men, while breast, lung, and colorectal cancers will constitute about 51% of new cancer cases in women [1]. These alarming statistics from the National Cancer Institute underscore the severity of cancer as a genetic disease. Despite the devastating impact of cancer, advances in biotechnology offer the chance to develop new treatments. One such innovation is the CRISPR system, a novel technology that has revolutionized gene editing. Initially discovered as a bacterial defense mechanism against viruses, CRISPR has been adapted for precise genome editing, showing immense potential in various biomedical applications. The CRISPR system includes CRISPR-Cas9 , which is a common use and known CRISPR, and it is the RNA-guided DNA editing tools, and Cas 9 technique widely use in the different applications such as the fluorescent imaging, base-editing, and transcriptional activation [2]. CRISPR-Cas 12 is also an RNA-guided DNA editing tool, and it shows the stronger genome-editing efficiency, so it is also used widely in base-editing and detecting transcriptional variations. CRISPR-Cas 13 is different from the previous two CRISPR systems, and it is RNA-guided RNA endonuclease activity rather than the DNA. As the Cas 13 technique has a high sensitivity and accuracy detecting ability, it can be widely used in the application of genome-editing and diagnostic fields [3]. CRISPR/Cas13 offers numerous advantages. For example, its modular construction, which consists of a single protein effector module and an RNA guide module, allows for significant scalability by enabling the production of whole libraries of various guide RNAs in addition to easy and quick design [4]. After knowing the different CRISPR proteins function , CRISPR system shows a powerful tool for genetic research and diagnostics , and its strong gene editing ability has the potential to be used in the cancer treatment area, as cancer is a genetic disease, and the CRISPR technique have the potential to edit genes to alter mutations in cancer cells, potentially preventing these cells from becoming malignant and spreading [5,6]. However, Cas 13 shows more advantages on the cancer treatment. Compared with the early CRISPR systems, the specific modular construction of Cas 13 that consists of single protein effector

module and an RNA guide module for allowing it has the high production of guide RNAs, which make cas13 is easy to use and expand, and researcher easy design the different RNA guide as Cas 13 simple structure, then to use it target the wide range of the mRNA easily and efficiently. Then, the researchers can find the multiple genes and mutation for more efficiency and save time. This ability of Cas13 is really useful in the treatment of cancer, as the cancer involves different oncogenes, and Cas13 has the ability to silence the various mutated mRNA for reducing the harmful protein that leads to cancer. Thus, this paper aims to explore the potential of CRISPR-Cas13 in cancer treatment by examining its underlying mechanisms, applications, challenges, and the current state of clinical trials.

2. The Mechanism and Applications of Cas 13 for showing the potential on the Cancer Disease Therapy

2.1. CRISPR-Cas13 Introduction and Mechanism

The structure of the Cas 13 enzyme has two higher eukaryotic and prokaryotic nucleic acid-binding (HEPN) endonuclease domains that help it to cut the RNA precisely. Currently, there have been identified three Cas 13 protein families, and this family is named C2c2: “Cas 13a, Cas 13b, Cas 13C [2]. Cas 13 is naturally found in bacteria and archaea, is an RNA-guided RNA endonuclease. It uses a CRISPR RNA (crRNA) sequence to recognize and bind to complementary RNA molecules, subsequently cleaving them. This mechanism makes Cas13 particularly useful for precise gene regulation and expression at the RNA level. The process by which Cas13 operates can be broken down into three main steps: target recognition, activation upon binding, and cleavage activity [7]. The spacer region in the crRNA of the Cas13 complex first allows it to bind to a complementary target RNA sequence with a high degree of specificity. Secondly, the protein experiences a conformational shift that initiates its ribonuclease activity once the Cas13-crRNA complex attaches to the target RNA. Ultimately, Cas13 degrades the target RNA and controls gene expression by cleaving it close to the binding site.

2.2. The Functions of the CRISPR-Cas13 Show it's Possible on Cancer Therapy Research

Cas13 can be applied to a variety of genome editing and disease diagnosis, as well as virus suppression. It can even be used to track allele-specific expression of transcripts or disease-associated mutations in cells, for example, Using the SHERLOCK system, which is a system use the Cas13 enzyme RNA cutting ability for using on the diagnostics application [8]. It was diagnosed with DNA isolated from strains of E. coli and Pseudomonas aeruginosa. They also distinguished Klebsiella pneumoniae isolates with two resistance genes, such as Klebsiella pneumoniae carbapenemase (KPC) and New Delhi metal-beta-lactamase. In addition, a 2020 study found that CRISPR-Cas 13 can also suppress human immune HIV-1 infection by targeting HIV-1 RNA to reduce viral gene expression[9].There are many other similar examples of the Cas 13 related application, based on Cas13's powerful and accurate diagnostic methods and the early detection and monitoring of cancer markers from liquid biopsy samples without the need for complex instrumentation, its efficient and specific degradation and manipulation of cancer-related transcripts, and its powerful ability to program RNA[10]. These demonstrate its potential and opportunities for cancer research, identification of drug resistance mechanisms, and discovery of novel therapeutic targets.

3. CRISPR-Cas 13 Targeting miRNA, mRNA, and IncRNA On Cancer Therapy Research : Further Demonstrating Its Potential as a Future Anti-Cancer Technique

3.1. The miRNA Dysregulation and CRISPR-Cas13: a way for Cancer Diagnosis and Treatment

miRNA is a small non-coding type of RNA that can influence gene expression through transcriptional regulators or translation inhibitors of its downstream target genes. The researchers found that miRNA dysregulation actually affects some features of cancer. For example, it can sustain cancer cell

proliferative signaling, Evading cell growth suppressors, Promoting cancer cells invasion and metastasis [11]. Because of its ability to control and influence cancer cells, it also shows that miRNA can be used as a target for diagnosis and treatment of cancer. Therefore, CRISPR cas13 binding to miRNA targeting therapy has the potential to become an anticancer technology. Brunch and other researchers developed an electrochemical microfluidic biosensor based on Cas13 through research and found that some characteristics of Cas13 enzyme and miRNA activation can be used to detect miRNA in medulloblastoma (miR-19b and miR-20) [12]. Whenever a miRNA is present in the tumor, CAs13 cuts the reporting ssRNA, resulting in reduced signaling. This signal reduction allows researchers to recognize and identify cancer-related changes in MiRNA expression [13]. This also shows the possibility and potential of Cas13-mirNA coupled sensing technology as a cancer diagnostic tool.

3.2. Targeting Oncogenic mRNA Variants with Cas13 for Cancer Therapy

mRNA is the single strand molecule that carries essential messages and instructions for making the proteins. Researchers have found that mRNA may give rise to oncogenic mRNA variants due to splicing or polyadenylation [14]. They found that the cas13 system can help restore normal gene expression by manipulating abnormal mRNA variants, thereby reducing the incidence of cancer. One of the studies demonstrated this possibility. Koneemann and other researchers used nuclease-deficient dCas13 to fuse with splicing regulators, targeting the splicing regulatory elements of pre-mRNA to achieve exon exclusion and the ability of dCas13 to precisely regulate mRNA splicing (Figure 1). In the experiment, the researchers successfully achieved an exon exclusion rate of 85% in the cell model. This data further strongly proves that Cas13 technology has the potential to achieve anti-cancer function by regulating the splicing of oncogenic mRNA variants to change cancer-related gene expression [15]. Another study is that Anderson and other researchers have developed a dPspCas13b-Nudix hydrolase 21 (NUDT21) fusion protein that can target and polyadenylate reporter gene mRNA and endogenously expressed human mRNA, which can also change the stability of mRNA and enhance the sensitivity of cancer cells to anticancer drugs [16]. This also shows that NUDT21 can greatly reduce the drug resistance of cancer cells by reversing the drug resistance of cancer cells, which also shows the potential of NUDT21 to become a new technology for treating cancer [17]. In short, in these studies, it can be seen that Cas13 technology can combine mRNA to accurately express genes in cancer cells, and reverse the drug resistance of cancer cells to allow drugs to promote the death of more cancer cells. These studies reflect that the technology of combining Cas13 with mRNA has the potential to become a new editable tool for future treatment and anti-cancer.

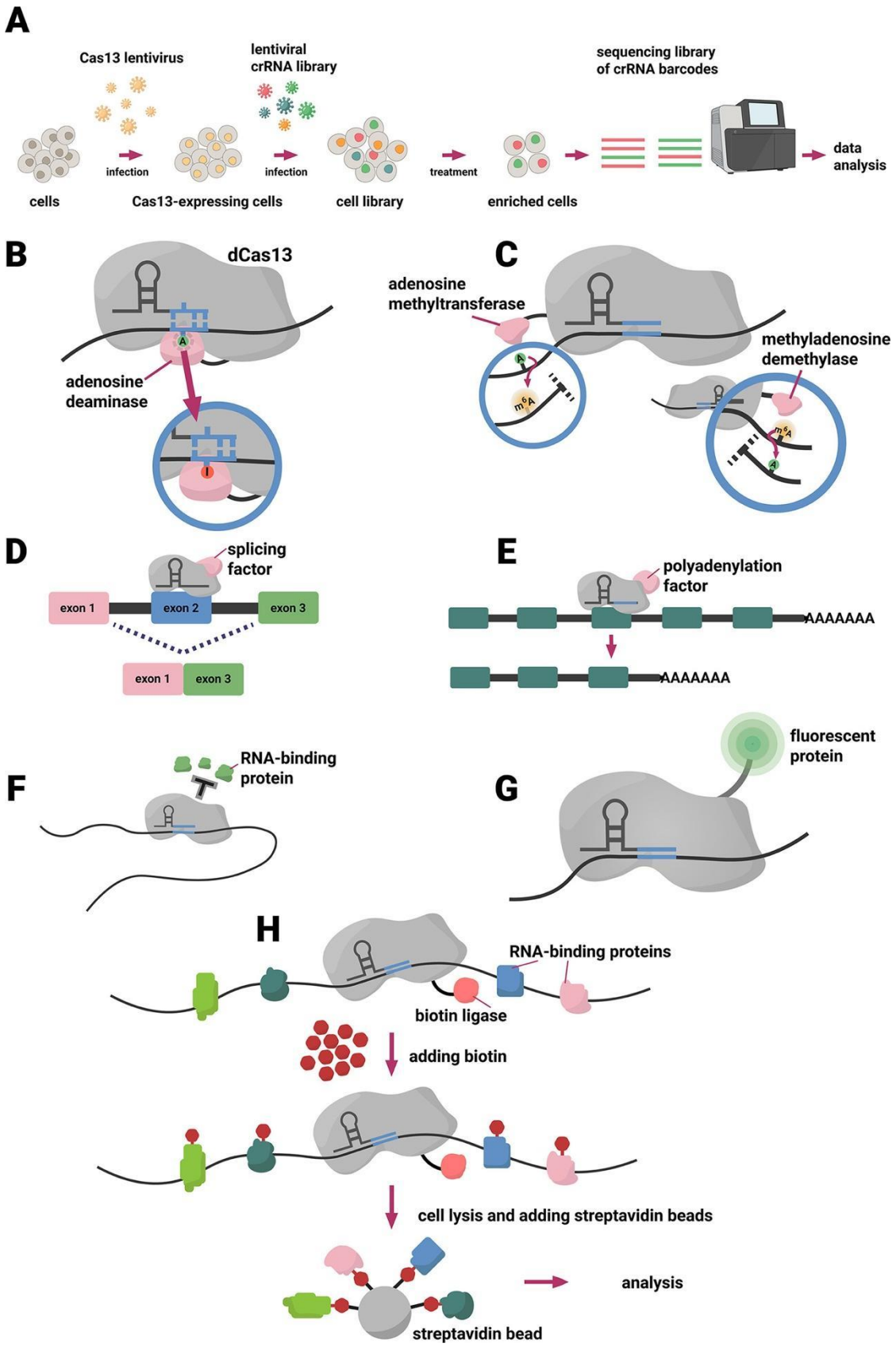

Figure 1. Applications of Cas13-based tools in Cancer Research [18].

3.3. CRISPR-Cas13 Targeting lncRNA-GACAT3: A Novel Approach to Bladder Cancer (BC) Therapy

Long non-coding RNAs (lncRNAs) are RNA molecules with a length of more than 200 nucleotides and do not code for proteins. They play an essential role in the physiological and pathological

processes of cells, especially closely related to the occurrence of cancer. A study on BC found that Cas13 system technology can inhibit cell proliferation, induce apoptosis, and inhibit cell migration by knocking down lncRNA-GACAT3. GACAT3 is a lncRNA that plays a key role in tumor formation, and researchers have shown that it plays an important role in cell proliferation, migration, and apoptosis, and has shown significant carcinogenic activity in experiments. To investigate the effect of GACAT3 on BC, the researchers collected 32 groups of urothelial malignancies and used CRISPR-Cas13 biotechnology to detect the role of GACAT3 in BC. The results showed that when GACAT3 was knocked down, the proliferation of BC cells was effectively inhibited, and the cell migration ability was significantly decreased. Based on this, the researchers believe that CRISPR-Cas13 targeting GACAT3 has the potential to inhibit cancer cell proliferation and migration. Experimental data showed that BC cell lines T24 and 5637 were transfected with GACAT3-related CRISPR-Cas13 and negative control CRISPR-Cas13, respectively 48h after transfection, ELISA results showed that cell migration was significantly inhibited, and apoptosis increased after GACAT3 knockdown. At the same time, CCK-8 detection results showed that the cell proliferation rate was significantly reduced after GACAT3 knockdown [19]. In conclusion, Cas13 technology can effectively inhibit the proliferation and migration of BC cells and induce cell apoptosis by targeting GACAT3 knockdown. This further confirms the potential of Cas13 in anti-cancer therapy, in particular the possibility of achieving anti-cancer effects by targeting specific lncRNAs.

4. Discussion

Although experiments and data have shown that Cas13 can effectively bind miRNA, mRNA and lncRNA, and show potential in anti-cancer technology, the limitations of Cas13 in cancer treatment are still significant, preventing it from becoming a fully reliable therapeutic tool. As an emerging gene-editing tool, Cas13 is promising, but its off-target effects are one of the major challenges currently faced. Off-target effects are caused by mis binding of gRNA to non-target sequences, which can cause Cas13 to mistarget non-target mRNA, thus disrupting normal cell function. Such inaccurate editing raises a number of issues, starting with the possibility of unwanted interference with gene expression or even the creation of new mutations or diseases. Especially in cancer treatment, off-target effects can lead to serious consequences, not only can not effectively inhibit oncogenes, but also can promote the spread of cancer cells or cause other healthy cells to mutate. In addition, the lack of precision of Cas13 targeting also means that it is prone to accidentally injuring healthy tissue in the process of selectively attacking cancer cells, further reducing the safety and effectiveness of the treatment. Therefore, the off-target effect directly affects the application prospect of Cas13 in cancer therapy [20]. Because it cannot accurately target and inhibit cancer cells, the treatment will be less effective and may lead to more side effects. To address these issues, much future research is needed to optimize the design and application of Cas13, especially in terms of improving gRNA specificity and reducing off-target effects, to ensure that it can be a safe and effective anti-cancer tool [21].With further research and technical improvements, Cas13 is expected to become a reliable cancer treatment tool in the future, but there are still significant challenges to its application, especially until the off-target effect problem is resolved.

5. Conclusion

CRISPR-Cas13, as a new gene editing technology, has the potential to be applied in the field of anti-cancer and cancer treatment. Not only can it bind to miRNAs as a potential cancer diagnostic tool, but it can also regulate abnormal mRNA variants and reduce cancer risk. In addition, by knocking down cancer-causing lncRNA, CRISPR-Cas13 can inhibit the proliferation and migration of cancer cells, thereby improving anti-cancer effects and reducing the incidence of cancer. However, the high off-target effect of Cas13 technology is still the main limiting factor for its application in cancer therapy, making it unable to be fully applied in clinical anticancer therapy. Nevertheless, Cas13 shows great potential to reduce the incidence of cancer, enabling early diagnosis of cancer before it becomes serious, and timely intervention and prevention, thereby reducing mortality due to cancer. Therefore,

further experimental research and technical optimization, especially in addressing the off-target effects of Cas13, will be key to the future application of this technology in cancer therapy. However, the high frequency of off-target effects of Cas13 is also a limitation of the current use of this technology for cancer treatment, so that it cannot be fully implemented as a tool for anti-cancer. However, this technology also brings the possibility of reducing the incidence of cancer, so that more people can be diagnosed before the cancer is about to become serious and timely cancer prevention and prevention, thereby reducing the death rate caused by cancer. Therefore, more experiments and in-depth research are needed to solve the off-target effects of Cas13, which will also give this technology more possibilities and opportunities to be truly practiced in cancer treatment.

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