CRISPR/Cas gene editing and its application in the treatment of Gliomas in the brain

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Abstract. The CRISPR/Cas system has emerged as a transformative genome-editing technology, enabling precise modifications of genetic material. This paper explores the potential applications of CRISPR/Cas technology in the treatment of gliomas, particularly glioblastoma (GBM), the most aggressive and lethal brain tumor. With over 3,000 genes linked to disease-causing mutations, CRISPR/Cas offers a novel approach to address the underlying genetic alterations in cancer. Recent studies demonstrate the efficacy of CRISPR/Cas12a in knocking out miR-21, a microRNA inversely correlated with glioblastoma survival, resulting in reduced tumor growth in mouse models. Furthermore, we introduce a novel "genome shredding" approach using CRISPR/Cas9 to target repetitive DNA sequences in GBM, leading to extensive cell death, independent of the tumor's genetic profile. Despite these promising advancements, challenges remain in optimizing CRISPR's efficacy and delivery mechanisms to improve patient outcomes. This review highlights the significant potential of CRISPR/Cas systems in revolutionizing glioma therapy while addressing the need for continued research to mitigate off-target effects and enhance therapeutic delivery.

Keywords: CRISPR/Cas, Gliomas, miR-21, TMZ, Genome Shredding.

1. Introduction

1.1. Background

The CRISPR/CAS system is a genome editing technology that has revolutionized molecular biology due to its precise and site-specific gene editing capabilities. It has many parts, including crRNA, tracrRNA, Cas9, and the CRISPR sequence [1]. For CRISPR, the full name is "Clustered Regularly Interspaced Short Palindromic Repeats", which means repeating intervals of DNA with spaces in between. Cas on the other hand, is an enzyme produced by the CRISPR system, its main function being to bind to the DNA and cut it, thereby silencing the target gene [2]. Currently, cancer therapy may greatly benefit from CRISPR/Cas technology, because over 3000 genes have been associated with disease-causing mutations [3]. This means that CRISPR can potentially fix these mutations thereby fixing the root cause of cancer.

CRISPR/CAS is an adaptive system first found in the immune system of archaea and bacteria and is used to protect them from viral DNA invasions [4]. When viral DNA invades the bacterium for the first time, the bacterium recognizes a part of the viral DNA, copies it, and then stores it in the spacers, the spaces between the palindromic repeats of CRISPR. As Figure 1. shows, the CRISPR sequence is transcribed into crRNA, which binds with tracrRNA to form gRNA. This gRNA will then bind to a Cas protein, forming a complex cleaved by an enzyme known as the ribonuclease, which creates individual complexes known as effector complexes.

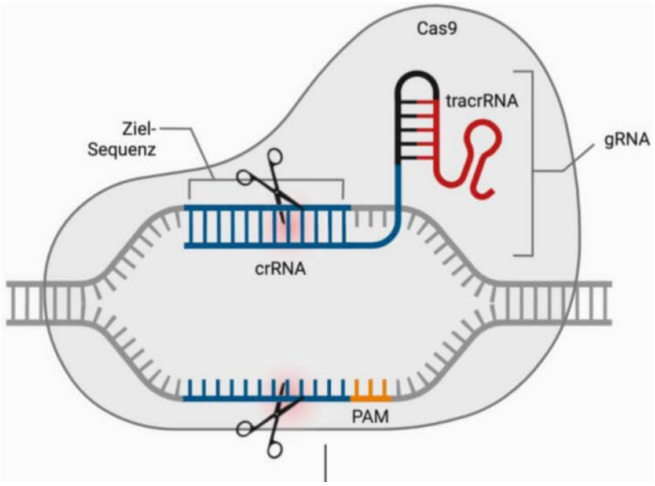

Figure 1. A diagram of the most studied CRISPR/CAS system, CRISPR/CAS 9 [5].

When the same type of viral DNA tries to enter the cell again, the gRNA within the effector complex recognizes and binds to the target DNA sequence through base-pairing interactions. Once the effector complex is bound to the target DNA, the Cas protein cleaves the DNA at the specific location, resulting in a double-strand break at the target site.

The double-strand break (DSB) induced by the Cas protein triggers DNA repair mechanisms in the cell. There are two main pathways that cells can use to repair DSBs:

Non-Homologous End Joining (NHEJ): In this pathway, the broken DNA ends are directly ligated together. NHEJ is an error-prone mechanism and can introduce small insertions or deletions (indels) at the site of the break, often leading to gene disruptions or knockouts, preventing the synthesis of a functional protein. [6]

Homology-Directed Repair (HDR): HDR relies on a template DNA sequence to repair the break. HDR can introduce precise changes, such as gene insertions or replacements, at the target site.

The CRISPR/Cas system was first discovered by scientists in 1987 [7] and was first utilized for gene editing when the individuals George Church, Jennifer Duodena, Emmanuelle Charpentier, and Feng Zhang used it to modify targeted regions of genomes. [8] Researchers later discovered ways to manipulate genes, such as designing gRNAs complementary to the DNA sequences they want to edit and delivering plasmids, viral vectors, or ribonucleoprotein complexes to introduce different Cas proteins and gRNAs. Additionally, scientists can also influence the repair system in the cell of interest by either introducing donor DNA templates to induce HDR or just creating DSB to induce NHEG. This allows scientists to manipulate genetic material to potentially cure diseases linked to an individual's DNA.

1.2. Introduction to Gliomas in the Brain

Gliomas are the most malignant and aggressive form of brain tumors and are responsible for many brains tumor-related deaths. They account for 30% to 40 % of all intracranial tumors. They are typical tumors of middle age, with a peak incidence between 40 and 65 years [9]. Gliomas are classified into four different grades, which are a measure of how aggressive the tumor appears under the microscope and its molecular profile.

1) Grade 1 gliomas are juvenile pilocytic astrocytomas or JPAs. This grade of glioma is most common in children and can be cured by surgery alone.

2) Grade 2 or low-grade gliomas are usually treated with surgery but may require further treatment afterward. Grade 2 gliomas can recur over time and become more aggressive (they can turn into grade 3 or 4 gliomas).

3) Grade 3 gliomas are called high-grade gliomas and are usually treated surgically. However, it is almost always followed by treatments such as chemotherapy and radiation.

4) Grade 4 gliomas are known as GBM(Glioblastoma), the most common type of glioma, with an incidence of 3–4 per 100,000 persons per year [10].

Out of the four grades, the most relevant grade of gliomas are GBMs, since they are the most common and deadliest. The primary treatment for Glioblastoma involves surgically removing as much of the tumor as possible. However, due to the invasive nature of Glioblastoma, complete removal of the cancer is often not achievable, leading to residual tumor cells. This usually causes recurrence and would require postsurgical treatments such as Temozolomide and Radiation Therapy. Temozolomide is a DNA-alkylating chemotherapeutic agent that works by damaging the DNA of tumor cells, impeding their ability to replicate. Although this treatment is effective, temozolomide can cause unwanted toxicity and does not eliminate the disease [11]. Radiation involves targeting the tumor site with high-energy radiation to kill remaining cancer cells and shrink residual tumor tissue. However, side effects of the therapy include fatigue, memory or concentration problems, hair loss, nausea, and skin changes [12]. CRISPR/cas9-related treatment, on the other hand, can potentially avoid these side effects.

2. CRISPR applications to treat Glioma in the brain

Currently, there are many articles out there with supporting research and experimentation proving that CRISPR/Cas-based therapeutics have great potential in the realm of cancer therapy.

One of the potential treatment methods utilizing CRISPR/Cas is the regulation of miR-21 because it has been shown that expression levels of miR-21 in tumors are inversely correlated with the length of survival of glioblastoma patients [13]. MiR-21 is a small, non-coding transcript involved in the regulation of gene expression in several cellular metabolic pathways, including the regulation of proliferation [14]. In an experiment regarding levels of miR-21 in correlation to glioma cells, researchers designed plasmids to introduce the specific crRNA "JIR327" as well as cas12a to three different glioma cell types, CT2A, U87, and GL261 to remove alleles encoding miR-21. These cells are known as miR-21 KO cells, and CT2A and GL261 are mouse glioma cell lines while U87 is a human glioma cell line. There are two experiments present in this study, one in vitro and one in vivo. For the in vitro experiment, the researchers compared the functional effect of miR-21 KO using proliferation, migration, and invasion assays. They found that all three KO cells displayed reduced proliferation, migration, and invasion as compared to wild type cells (Figure 2).

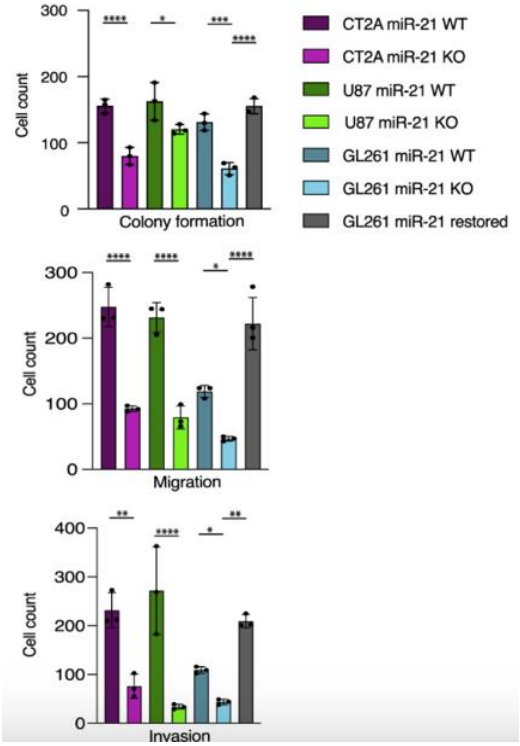

Figure 2. Comparative Analysis of Proliferation, Migration, and Invasion Capabilities Between miR-21 Knockout (KO) and Wild-Type Glioma Cells In Vitro [15]

For the experiment, the researchers injected miR-21 KO and wild-type glioma cells into the brains of mice. Subsequently, imaging revealed reduced tumor sizes and slower growth in mice injected with miR-21 kick-off cells as compared to mice that weren't injected with miR-21 KO cells [15]. Figures B, E, and G show a decrease in Luminescence in all three KO cells over time, which represents the reduction of tumors in the mice. Figures C, F, and H are Kaplan-Meier survival curves that display higher survivability of mice with injected MiR-21 KO cells.

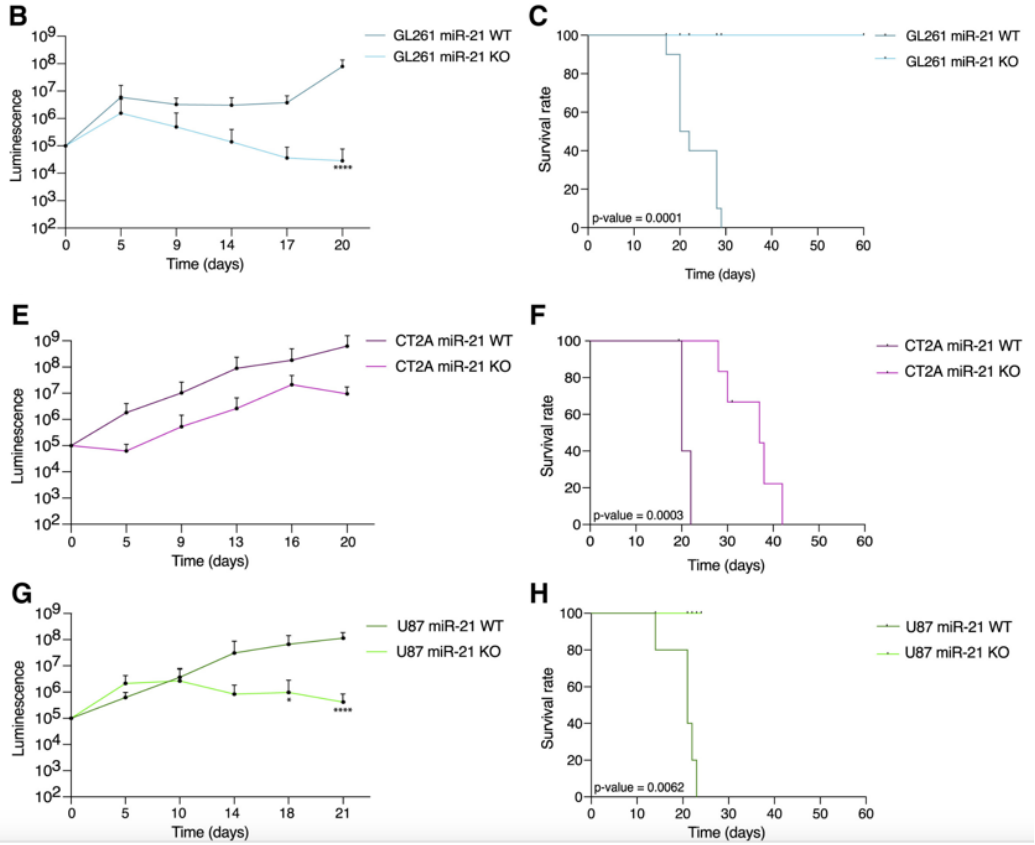

Figure 3. Comparative Analysis of Tumor Growth and Survival Rates Between miR-21 Knockout (KO) and Wild-Type Glioma Cells in Mice Models [15]

These experiments provide direct evidence proving that CRISPR can be utilized in human glioma therapy since the MiR-21 KO cells incorporating CRISPR-cas12a can weaken and reduce glioma cells in vivo(mice).

MiR-21 knockout-based therapy offers a more targeted and potentially less toxic approach than conventional therapies such as temozolomide and radiation. By focusing on specific genetic alterations associated with cancer progression and resistance, miR-21 knockout therapy could provide a more personalized and effective treatment option while minimizing off-target effects. In addition, CRISPR can induce permanent changes to the cancer cell's DNA, resulting in a lasting impact on cancer cells and potentially long-term remission. Conventional therapies typically provide only temporary control of the cancer, which can lead to tumor recurrence and further treatments.

In conventional treatment of GBM, temozolomide leads to hypermutation in a significant portion of patients, resulting in tumors that become resistant to further TMZ treatment. [16-17] This is because the Methylation status of the O-6-methylguanine-DNA methyltransferase (MGMT) promoter determines sensitivity to the alkylating agent temozolomide (TMZ), meaning that a portion of patients are insensitive to TMZ. [18-19] In addition, these hypermutated GBMs currently do not have any effective treatment. Therefore, there exists a need for treatment strategies that can effectively eliminate GBM cells irrespective of their mutational and epigenetic profile. Researchers have devised a potential treatment that could tackle this problem, known as "genome shredding".

Genome shredding work using CRISPR-Cas9 to target highly repetitive sequences in the non-coding genome of cancer cells, which induces multiple DNA double-strand breaks and leads to rapid and extensive DNA fragmentation and cell death. Upon quantifying the mutational landscape progression in patient primary GBM, researchers found Cas9 targetable repeat sequences in the DNA of those GBM cells. Additionally, the researchers also found that TMZ-induced hypermutation in recurrent GBM can lead to unique cancer-specific repeat sequences. This can allow CRISPR-Cas9 to target these sequences, and then perform genome shredding.

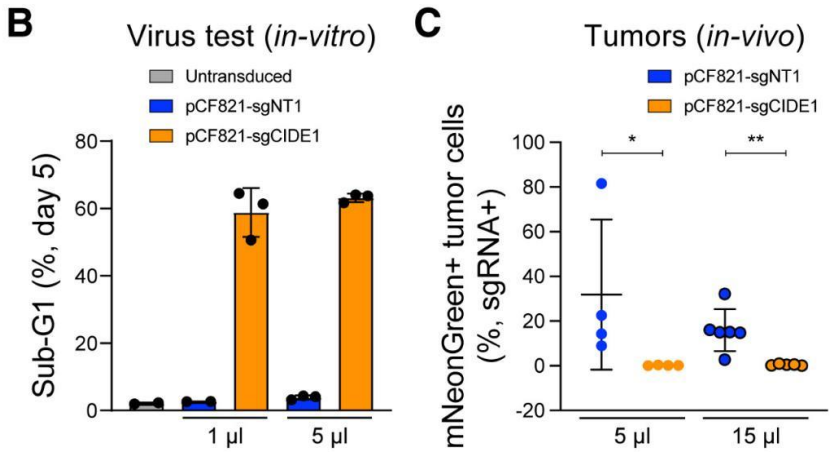

Figure 4. Figure 4B Shows efficient cell transduction and destruction for sgCIDE-1, and Figure 4C shows most in vivo sgCIDE-1 transduced cells were effectively eliminated. [20]

To support this theory, the researchers performed both in vitro and in vivo experiments. In one of the in vitro experiments the researchers conducted, they found that targeting the repetitive sequences in primary and recurrent GBM led to rapid and robust depletion of GBM cell lines, including patient-derived recurrent GBM cells and glioblastoma stem-like cells, regardless of their genetic or epigenetic profile (Fig 4B). Another experiment was performed in vivo on mice, and they found that the experimental group that was injected with cells expressing cancer-specific sgCIDE-1 (sgRNA) had a vast number of their GBM cells eliminated (Fig 4C).

3. CRISPR and its challenges

CRISPR/Cas is a relatively new technique in the medical realm, and many articles with experimentation on the potential medical applications found that as of right now, it is difficult for CRISPR to replace conventional glioma therapies. The most studied CRISPR/Cas system, CRISPR/Cas 9, is influenced in its efficacy by many factors. These factors include target DNA site selection, sgRNA design, off-target cutting, frequency/efficiency of HDR versus NHEJ, Cas9 activity, and mode of delivery. [21]

For example, the article on genome shredding found significant decreases in GBM cells after their experimentation, but using the Kaplan-Meier analysis, it is revealed that there is no survival benefit of sgCIDE-1-treated groups, suggesting that the therapeutic delivery was insufficient and

that elimination of 15%-20% of tumor cells does not prolong overall survival (Fig 5). [20]

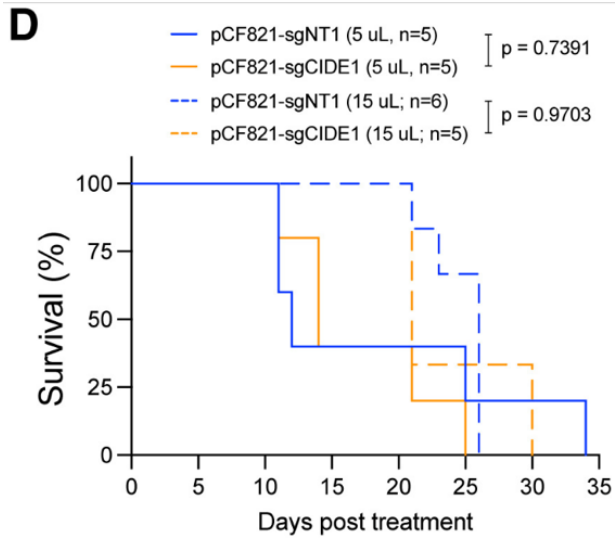

Figure 5. Kaplan-Meier shows no statistically significant survival benefit.[21]

This is due to the poor overall efficacy of glioma lysis with direct sgRNA injections, meaning that it is difficult to deliver effective in vivo CRISPR genome shredding as of currently.

Therefore, researchers need further development on developing CRISPR/cas derived techniques to eliminate or avoid factors that influence CRISPR/Cas efficacy for CRISPR to be able to be utilized in modern-day Glioma therapy.

4. Conclusion

The advancement of CRISPR technology marks a significant milestone in the field of medical genetics, particularly in the treatment of aggressive cancers such as gliomas. As demonstrated in recent studies, CRISPR/Cas systems, including both Cas12a and Cas9, exhibit tremendous potential for innovative therapeutic applications. The ability to precisely edit genetic material opens new avenues for targeting the underlying genetic alterations associated with glioblastoma (GBM), the most lethal form of brain tumor.

Utilizing the CRISPR-Cas12a genome-editing system, researchers have successfully removed alleles encoding miR-21, a microRNA that negatively correlates with glioblastoma survival. This approach has shown promising results in preclinical models, leading to a significant reduction in glioma cell proliferation and tumor growth in mice. The potential to translate these findings into human applications offers hope for more effective and personalized treatment strategies for patients suffering from GBM. Such targeted therapies could minimize the adverse effects commonly associated with conventional treatments like temozolomide and radiation therapy, thereby improving the quality of life for patients.

Furthermore, the CRISPR-Cas9 system's capability to target highly repetitive sequences in the non-coding genome presents another innovative approach to combat GBM. By inducing multiple DNA double-strand breaks, this method can lead to rapid and extensive DNA fragmentation, ultimately resulting in cancer cell death. This strategy holds promise for patients who have developed resistance to standard treatments, offering a potential avenue for long-term remission.

Despite these advancements, several challenges remain in the application of CRISPR technology in clinical settings. Issues such as off-target effects, which may lead to unintended genetic modifications, and the efficacy of delivery mechanisms are critical hurdles that need to be addressed. The current delivery methods for CRISPR components often fall short of achieving the desired therapeutic outcomes, necessitating further research and development. Enhancing the precision and efficiency of CRISPR delivery systems is essential for maximizing the therapeutic potential of this technology and ensuring patient safety.

In conclusion, while the current development of CRISPR technology showcases its transformative potential in glioma therapy, continued research is imperative to refine these techniques. By overcoming existing challenges related to off-target effects and delivery mechanisms, CRISPR-based therapies could significantly improve patient outcomes and extend survival rates for individuals battling GBM. The ongoing exploration of CRISPR's capabilities not only underscores its promise in cancer treatment but also reflects the broader potential of gene editing technologies in revolutionizing modern medicine. The future of glioma therapy may very well hinge on the successful integration of CRISPR technology into clinical practice, paving the way for more effective and less toxic treatment options.

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