CRIPSRi: a promising RNA-guided CRISPR-dCas Technology for Alzheimer's Disease and Glioma Therapy

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Abstract. The escalating prevalence and mortality rates of neurological disorders, such as Alzheimer's disease (AD) and glioma, pose significant healthcare challenges. The exploration of novel therapeutic modalities is in urgent need. Among these, CRISPR interference (CRISPRi) leveraging RNA-guided deactivated Cas proteins for the regulation of gene expression stands out for its potential in neuroepigenetic interventions. This paradigm shift from the canonical CRISPR-Cas9 system, which operates via the introduction of double-strand breaks, to CRISPRi's non-cleaving approach mitigates the risk of off-target mutagenesis, thereby presenting a more refined strategy for modulating gene activity within the intricate milieu of the central nervous system (CNS). This review highlights the shift from CRISPR-Cas9 to CRISPR-dCas technologies, focusing on the application of CRISPR interference in addressing the pathogenesis of AD and glioma. The pathological frameworks is further unpacked by revealing the pivotal roles of genes such as APP, MGMT, and TERT. Through allele-specific epigenome editing, CRISPRi has demonstrated considerable efficacy in the preclinical modulation of gene expression via targeted DNA methylation, consequently degrading the pathological manifestations. Notably, evidence suggests CRISPRi's capacity to effectuate significant downregulation of hotspot genes like APP and MGMT expression and to modulate TERT activity. Despite promising results, challenges such as off-target effects, delivery barriers, particularly across the blood-brain barrier (BBB), and potential immunogenic responses limit clinical translation. Advances in sgRNA design, novel delivery systems, and epigenetic editing tools are discussed as strategies to overcome these hurdles. Thus, CRISPRi can be positioned as one of the most promising instruments in genomic medicine, with the potential to reshape the therapeutic strategies of AD and glioma.

Keywords: CRISPR interference, Deactivated Cas protein, Alzheimer's Disease, Glioma, Gene therapy, Epigenome editing, DNA methylation, Neuroepigenetic

1. Introduction

Neurological conditions affecting the brain, spinal cord, and central nervous system (CNS), represent a significant and growing global healthcare challenge. Recent studies have shown a sharp increase in both mortality rates and disease burden, with nearly 30% of Americans affected by at least one neurological disorder [1, 2]. This trend, driven by an aging population, has led to a shift towards more research and investment in understanding and treating these conditions. Alzheimer's disease (AD) is a well-known neurodegenerative disorder that causes progressive behavioral, verbal, and cognitional decline among the elderly [3-5]. In 2019, nearly 1 in 10 American seniors suffered from some degree of AD, with the expected number projected to triple in the next 2 decades [3, 6]. Similarly, despite the rarity of CNS tumors, they represent a significant risk for the younger population, accounting for nearly one-third of the cancer-associated incidence and death rate within the children and adolescent population [7]. Glioma, derived from the neuroglial lineage, is the predominant type of primary brain tumor. Although it only constitutes a small fraction of total tumor occurrences, notorious for its lethality and severity [8-10]. However, due to the inherent heterogeneity and complexity of the CNS system. The treatments targeting these conditions remain palliative in nature since most of them are symptomatic or progression-controlled [3, 11].

The epigenomic profile regulates dynamic gene expression and function through mechanisms such as covalent modification of DNA sequence, spatial conformational changes of chromatin structure or histone modification [3, 5, 12].With the recognized importance of the methylome in neurological

conditions and reversible regulation of gene expression, current therapeutic strategies, including RNA interference (RNAi) and small molecule inhibitors, face significant limitations due to limited efficacy and specificity and insertional mutagenesis [13, 14]. In contrast, the advent of CRISPR-Cas9 technology has revolutionized genomic engineering, offering precise manipulation of genomic sequences. However, its application within the CNS is hindered by the potential for systemic off-target mutations and the complexities of *in vivo* gene editing [15-17].

Recent advances in CRISPR technology, notably the development of CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi), have revolutionized genetic engineering and screening in both mammalian and plant systems. These techniques, based on deactivated Cas (dCas) proteins that can bind without cleaving DNA, allow for precise regulation of gene expression. CRISPRi has shown promise in preclinical research for treating neurological disorders and various cancers by silencing oncogenic variants and pathogenic genes through targeted DNA methylation. This innovative approach offers a novel and safer modality for CRISPR-dCas9 to treat a range of neurological conditions [18-20]. This review aims to delineate the current landscape and rationale for the shift from CRISPR-Cas9 to CRISPR-dCas, following the CRISPR-dCas mechanism and recent evidence of therapeutic potential with neurological conditions, notably AD and glioma. It will critically assess the comparative advantages, limitations, and potential of these approaches, highlighting future directions to overcome challenges related to specificity, off-target effects, and clinical applicability, thereby enhancing the potential of epigenetic therapy in neurology.

2. Pathological Background

2.1. Alzheimer's Disease

The pathogenesis of AD can be considered synergistically with four biological changes occurring at different or the same time, including the over-accumulation of amyloid-beta (Aβ) peptide factors, post-translational modification of tau proteins, synaptic dysfunction, and chronic inflammation resulting from the ensuing neurotoxicity [3]. These four biological indicators are recognized as very important for monitoring the progression of AD, as well as potentially therapeutic targets or pathways for AD. However, for the curative attempt, the molecular level of pathogenesis should be investigated. First of all, the production and aggregation of Aβ peptide are attributed to the aberrant cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase. Aβ42 has the most significant aggregation tendency, leading to the accumulation of neurotoxic substances [3]. The aberrant cleavage is multifactorial, but mutations in the genes coding for APP and its corresponding secretase directly alter the APP cleavage pattern most dramatically, resulting in a skew in the ratio of Aβ peptide production and the consequent formation of toxic plaques and the pathogenesis of last onset of AD [21]. Concurrently, the metabolism of lipids, notably the role of apolipoprotein E (APOE) and its ε4 allele, significantly elevates AD risk by influencing Aβ aggregation and clearance processes. Moreover, the alteration of the intercellular signaling cascade, including those mediated by neurotrophins like brain-derived neurotrophic factor, Wnt signaling, and insulin signaling, further complicates AD's molecular landscape [21]. These molecular abnormalities can eventually lead to the variation of protein and physical dysfunction of synapses, contributing to the pathogenesis of the last onset of AD [21].

2.2. Glioma

Additionally, the manifestation of glioma, as a complex group of brain and spinal cord tumors result from a distinct but somehow similar dynamic. With a complex interplay a genetic mutation, epigenetic manipulation, and environmental factors. The central factor of gliomagenesis is genomic alternations of oncogenes and tumor suppressor genes, such as IDH1, IDH2, TP53, and mutations in the TERT promoter, which play critical roles in gliomagenesis [11]. These mutations lead to the accumulation of oncometabolites, disruption of cell differentiation, and cellular immortality, respectively. The 1p/19q codeletion, prevalent in oligodendrogliomas, denotes a distinct molecular

subclass with specific therapeutic and prognostic significance [11]. Epigenetic modifications further influence gene expression patterns, contributing to the neoplastic phenotype. The glioma microenvironment, consisting of immune cells, extracellular matrix components, and various cytokines and growth factors, is essential for tumor progression and immune evasion [11]. Additionally, ionizing radiation exposure plays an environmental factor in glioma etiology. Dysregulation of intracellular signaling pathways, including the RAS, p53, and RB pathways, drives fundamental processes such as proliferation, apoptosis resistance, and invasion [11]. The molecular heterogeneity within glioma subtypes presents challenges for developing universally effective therapies.

2.3. DNMT Family

By adding methyl groups to the cytosine residue of CpG dinucleotide, the DNA DNMT family serves as the crucial epigenetic toolkit during cell differentiation and development, resulting in the disruption of transcription factor binding or heterochromatin transition, which in turn represses gene expression [22, 23]. Among the major enzymes in this family, DNMT1 is tasked with the maintenance of methylation patterns post-DNA replication, safeguarding the hereditary continuity of the gene regulatory network [23, 24]. Similarly, DNMT3A and DNMT3B specialize in de novo methylation, creating new methylation marks essential for developmental processes and adaptive responses to external changes [22]. Research underscored the profound impact that the knockout of DNMT1, DNMT3A, or DNMT3B has on cellular fate, significantly influencing the pluripotent potential and even causing catastrophic demethylation of embryonic stem cells [24, 25]. Additionally, DNMT3A and DNMT3B are also involved in the methylation of non-CpG islands, a process crucial for the development of neurons and synaptic plasticity, thereby facilitating long-term memory and learning abilities [24]. Thus, DNMT3A and DNMT3B are thought to be a viable enzyme to be utilized for epigenetic editing in neurodegenerative conditions and brain tumorgenesis.

3. CRISPR Technologies

3.1. CRISPR-Cas9 to CRISPR-dCas9

CRISPR-Cas systems originated from the prokaryotic immune defence system against viruses. Among three major types of CRISPR-Cas systems, CRISPR-Cas9 was repurposed as the potential tool for gene editing due to its simplicity [17]. The system is considered to consist of a binary architecture: a guide RNA (gRNA) complex and the Cas9 effector protein. Within the gRNA complex, crRNA and tracrRNA were chimerically combined as a singular entity known as the single guide RNA (sgRNA) with maintained base-pairing fidelity and high specificity, akin to its natural antecedent [17]. Moreover, Streptococcus pyogenes Cas9 (SpCas9), as the most widely adopted effector protein, encapsulated with a target recognition (REC) lobe and a nuclease (NUC) lobe. This configuration enables the complex formation with sgRNA, facilitating the locus-specific enzymatic cleavage of DNA [15, 17]. REC lobe is responsible for reading the protospacer adjacent motif (PAM), the unique DNA barcode that is next to the target editing site [26]. This localization then further synergistically collaborated with the NUC compartment, which comprises the RUVC and HNH nuclease domains, allowing site-specific cleavage to the target DNA sequence [15, 17]. The double-strand break (DSB) created by endonuclease activity is repaired by non-homologous end joining or homology-directed repair whereupon [27]. CRISPR-Cas9 has been employed in multiple genomic and epigenomic applications, including gene knock-outs and knock-ins, high-throughput screening, and the development of model organisms [27, 28]. However, the heavy reliance on endogenous cellular repair pathways presents substantial risks during in vivo applications since minor but error-prone off-target mutagenesis can lead to systemic disruption and cumulative damage across the genome, along with inflammation and neurotoxicity. Consequently, despite the technique's precise targeting and cutting efficiency, its biocompatibility for treating the nervous system, which is complex and fragile, remains low [15, 16, 17].

Thereupon, by introducing missense mutations in the Cas9 nuclease's active sites, one of the studies demonstrated that Cas proteins can enzymatically inactivate RUVC and HNH domains, nullifying their cutting ability while preserving their recognition and DNA-binding capability [29]. This refinement makes the repurposed CRISPR-Cas9, named dCas9-sgRNA, into a reversible and graduated gene activation or repression system by inducing temporary alternation to the genome [30, 31]. Epigenetic editing can be further applied to deactivated Cas(dCas) proteins when activator or deterrent structural domains are attached to the dCas9 complex enabling activation of transcription or repression based on the two different methodologies. The dCas9- Transcriptional Modulator(TM) employs fusion constructs with transcriptional modulators for gene expression regulation, utilizing activatory or inhibitory domains such as the Kruppel-associated box (KRAB) domain for repression and VP16 oligomers for activation. For instance, dCas9-KRAB has demonstrated substantial repression of target genes in HeLa cells, while VP64 and its variants have shown remarkable efficacy in transcriptional activation [17].

Alternatively, the dCas9-Epigenetic Effector (EE) leverages the targeted binding capability of dCas9, combined with the functional diversity of various epigenetic enzymes, to precisely edit epigenetic marks across the genome. For instance, when dCas9 is fused with DNA methyltransferases such as DNMT3A or DNMT3B, it can direct the addition of methyl groups to CpG sites, effectively silencing gene expression through the methylation process [15]. Similarly, histone modification is achieved by coupling dCas9 with enzymes like histone acetyltransferases, exemplified by dCas9-p300, which promotes gene expression through acetylation, or histone deacetylases (HDACs) like dCas9-HDAC3, which suppress gene activity via deacetylation. Beyond methylation and acetylation, the integration of domains from enzymes like TET1 for DNA demethylation or LSD1 for histone demethylation also offers new possibilities [17]. Despite the similarities, the modification done by dCas-TM may occur concurrently and induce widespread genomic alternation, whereas dCas-EE is designed to focus on loci-specific modulation [17].

3.2. CRISPRi

Therefore, a new terminology, CRISPRi, as a promising allele-specific epigenome editing (ASEE) tool, is categorized under both the dCas-EE and dCas-TM systems for various therapeutic needs. By tethering transcriptional repressor domains or epigenetic enzymes with the dCas9-sgRNA complex, the CRISPRi complex can recruit the desired co-repressors and stimulate the signaling cascade, leading to the methylation or histone modification which robustly decreases the gene expression [30]. Nevertheless, unlike the CRISPR-Cas9 system, the reversibility of CRISPRi system through its versatile addition of epigenetic marks provides huge potential for extending the genetic engineering toolbox when treating neurological disorders. Its effectiveness in inhibiting multiple oncogenic variants has been evident in various preclinical cancer research, including glioma [27, 28, 32]. Moreover, CRISPRi-induced hypermethylation of the CpG/non-CpG site within the promotor region facilitates DNA methylation-dependent silencing of pathogenic genes, attenuating AD pathogenesis [19, 33]. KRAB domain, HDAC complexes, DNMT3 and other protein domains are actively investigated as the “star” molecules of CRISPRi for their effective transcriptional repression or chromatin regulation ability within diverse target sites. The de novo methyltransferases DNMT3A or DNMT3B, in conjunction with the DNMT3L chaperone, can be fused with the dCas protein to create the dCas9-DNMT3 complex [34]. It has presented the ability to effectively induce site-specific hypermethylation of CpG site in MGMT and TRET-derived promoter region, the key regulatory region leading to the manifestation of AD and glioma, potentially projecting a novel direction for CRISPRi to treat a broader spectrum of neurological conditions [18, 19, 20].

4. Recent Findings

4.1. CRISPRi in Alzheimer's Disease

The evidence started by demonstrating the significance of APP protein in maintaining the neurons and synapse functionality. In one of the recent studies, the researchers aimed to investigate the developmental and neurological consequences of full deletion of APP proteins, including APP, APLP1, and APLP2 in mice, which can potentially elucidate the role of the APP protein family participated among neurodegenerative diseases such as AD [35]. By crossbreeding the genetically engineered mouse models carried the single gene deletion of APP family proteins, the triple knock-out (TKO) mice were eventually reproduced in the following generations of offspring. After the examination of the neuron status and survival rate of the TKO model, the study underscores the detrimental impact of APP absence on neuronal integrity, along with the complexity of APP proteins that contribute to various brain developments [35]. Thus, the conventional CRISPR-Cas9 therapy is not feasible for treating AD due to its direct cut-and-repair mechanism.

A number of literature have proven the hypomethylation of APP protein is the pathogenesis origin of AD [21, 36]. In a 2022 study, the fusion of CRIPSR-dCas9 and DMNT3A domain to effectively methylate the APP promoter is proposed. sgRNA construct was first been designed and cloned for precise targeting of the APP promoter. For the comprehensive evaluation, the research was separated into in vivo studies on mouse models and cell culture experiments [37]. APP knock-in (APP-KI) mice, engineered to express mutant forms of the APP gene linked to AD, were used to closely mimic the disease's pathology. The dCas9-Dnmt3a system was delivered into the brains of these mice through stereotaxic injection into the dentate gyrus region. Parallel to that, mouse primary fibroblasts and NIH/3T3 cells were also used in an in vitro study to validate the dCas9-DNMT3A system's efficiency. Behavioral tests and molecular techniques were conducted to assess cognitive functions and evaluate the methylation status of the APP gene, changes in APP expression, and Aβ peptide levels respectively. Some promising results were revealed. First, they demonstrated targeted DNA methylation of the APP promoter locus using dCas9-DNMT3A with high specificity, which led to a notable reduction in APP mRNA expression in both cultured mouse cells and in the mouse brain without significant off-target effect [37]. Notably, the application of dCas9-DNMT3A in APP-KI mouse primary neurons resulted in an altered DNA methylation landscape at the APP promoter, significantly reduced levels of APP mRNA, and a subsequent decrease in Aβ21 levels compared to control neurons [37]. These results were mirrored by in vivo findings where dCas9-DNMT3A-mediated APP methylation in the brain altered Aβ plaque formation and attenuated neurological-related impairments in the APP-KI mouse model, providing evidence of the efficacy of CRIPSRi in mitigating AD-related pathologies.

4.2. CRISPRi in Glioma

4.2.1. MGMT gene

The CRIPSRi technology was also proposed as a promising tool in treating glioma. The recent efforts are focusing on hypermethylation of the promoter region in two of the most investigated prognostic biomarkers, MGMT and TERT gene. First of all, evidence showed that the MGMT gene methylation serves as a critical biomarker in glioblastoma (GBM), where its methylation status can significantly affect patient response to temozolomide (TMZ), a type of chemotherapy drug [18]. The enzyme encoded by the MGMT gene is responsible for repairing alkylated DNA by removing alkyl groups from guanine, thereby counteracting the therapeutic effects of alkylating agents. When the MGMT gene promoter is methylated, the gene's expression is silenced, reducing the level of repair enzyme, and making tumor cells more susceptible to alkylating chemotherapy [18].

Following this mechanism basis, multiple cell line experiments were conducted recently to observe the therapeutic efficiency of the dCas9-DNMT3A system for MGMT gene methylation. The researchers cultured LN18 glioma cells and treated them with TMZ after introducing a dCas9-DNMT3A catalytic domain (CD) via lentiviral transduction [38]. After validating the methylation

patterns by multiple sequencing and transcriptomic/proteomic analyses, RT-qPCR analysis confirmed that the successful targeted methylation of MGMT at specific CpG clusters in the promoter and enhancer regions using the dCas9-DNMT3A system, leading to significant downregulation of MGMT expression and enhanced chemosensitivity in vitro with minimal off-target effects [38]. Meanwhile, a significant reduction in cell viability with TMZ treatment in methylated cells was identified, with a marked decrease in survival rates at both 25 μM and 100 μM TMZ concentrations compared to control groups [38]. A similar study also validated the result. By introducing the dCas9-DNMT3A-3L complex through the sgRNA's specific localization of the MGMT gene [39]. The transfected human GBM cells like LN18 and T98G demonstrated a substantial decrease in viability by nearly 400μM, attributed to the almost complete MGMT repression for LN18 by the dCas9-DNMT3A-3L-mediated methylation [39]. Moreover, the increased TMZ-induced cytotoxicity indicated an increased level of chemosensitivity, showcasing not only the efficacy of the CRISPRi system in reducing MGMT expression but also its potential to reverse TMZ resistance [39].

4.2.2. TERT gene

Furthermore, TERT is the catalytic subunit of telomerase, an enzyme crucial for telomere elongation and thus, for enabling replicative immortality, a hallmark of cancer cells [40]. The association between epigenetic modification of TERT gene and its expression level is somehow counterintuitively to the typical genes. Commonly, DNA methylation in specified loci is correlated with transcriptional repression, effectively inhibiting gene expression. Nonetheless, the TERT gene presents an exception. The methylation status of its promoter region exhibits a sophisticated correlation with its regulatory expression mechanisms. Due to the amplified telomerase activity, the more elevated expression of TERT is significantly correlated with the enhanced replicative capacity of neoplastic cells [41]. However, by manipulating methylation specificity and intensity, CRISPRi technology has revealed a unique case in which augmenting the methylation status of the TERT promoter leads to TERT under-expression, contradicting the expected promotion of its expression [41].

Thus, recent efforts have been conducted to realize the CRISPRi potential in targeting TERT promoters. First, One of the studies employed the CRISPR-dCas9-DNMT3A system to target and modify the hTERT gene in U87 glioma cells [41]. The study involved designing sgRNA specific to the hTERT gene using CRISPR software and constructing a plasmid vector, which facilitates the delivery of sgRNA and Cas9 hybrids into the cells. Flow cytometry and PCR were used to monitor the dCas9 entry and methylation status [41]. After the successful plasmid transfection, a significant reduction in hTERT expression was observed at a transfection concentration of 80 μg/mL of gRNA, compared to both the control group and basal cells, consequently suggesting a successful reduction of telomerase activity at the protein level [41]. Moreover, the results from flow cytometry analysis also indicate a higher rate of apoptosis in glioma cells transfected with gRNA, with early and late apoptosis rates significantly increased compared to gRNA-absent cells [41]. These results presented compelling evidence that the dCas9-DNMT3A system effectively modifies the methylation condition of the hTERT gene promoter, region leading to altered hTERT expression and reduced telomerase activity in U87 glioma cells.

Additionally, in a 2023 study, the fusion of CRISPR-dCas9 system with the DNMT3A-3L R887E mutant domain was leveraged for the methylation of the TERT promoter across various cancer cell lines [42]. By specifically localizing the TERT promoter variants with sgRNA within cancer cell lines, such as Hep-G2 and A-549, the study aimed to use a series of in vitro studies to evaluate the impact of dCas9-DNMT3A-3L complex on TERT expression and cell viability [42]. The result showcased a remarkable specificity. In Hep-G2 cells, targeting the C228T mutation resulted in an average methylation upregulation of 16% on the targeted allele [42]. Similarly, a significant increase of 32% methylation was observed in A-549 cells, Moreover, Hep-G2 cells exhibited a 20-fold decrease in TERT RNA expression when targeted with the sgRNA for the C228T mutation, compared to a sixfold decrease when compared to cells treated with a scrambled sgRNA. Additionally, the study observed a slight increase in cell mortality in Hep-G2 cells post-treatment, suggesting CRISPRi's potential for

targeted TERT suppression in enhancing chemosensitivity [42]. Therefore, these studies collectively highlight the efficacy of CRISPRi in modulating gene expression via targeted methylation of pivotal genes such as APP, MGMT, and TERT, offering promising avenues for therapeutic interventions in Alzheimer's disease and glioma.

5. Limitation

Despite the successful stories that occurred in the in vivo and in vitro studies, the implementation of the dCas9-sgRNA or CRISPRi system in the clinical setting remains in the premature stage, warranting more studies. Several significant challenges must be acknowledged and addressed.

The off-target effects, ubiquitous concern across CRISPR technologies, pose a particularly acute risk where unwanted gene modulation could lead to irreversible effects like permanent mutagenesis on neural function and development [11, 15, 17, 27, 28]. Thus, the necessity for precise target site selection is crucial in neurological applications to ensure efficient gene regulation without unintended consequences, which requires careful consideration of factors like genomic location, strand specificity, proximity to transcription start sites, and the epigenetic context to minimize the confounding effect [11, 17].

Delivery challenges should also be highlighted in neurological applications due to the blood-brain barrier (BBB), which complicates the efficient transportation of CRISPR components to target cells. Although dual-adeno-associated virus-mediated delivery shows promise due to its efficiency, limitations such as load capacity, pre-existing antibodies, and immunogenicity represent significant hurdles [11, 15, 27, 28]. The size of the dCas9-effector complex further complicates delivery, particularly given the restricted capacity of many viral vectors. This issue underscores the need for alternative approaches, such as smaller Cas9 variants or split-dCas9 systems [28].

Immunogenicity represents another crucial challenge, with the potential for an immune response against Cas proteins, which are derived from bacteria. One of the studies investigated the interaction between T-cell response and the Cas9-attached cell. A notably high frequency of anti-SpCas9 (78%) and anti-SaCas9 (67%) antibodies were detected, validating the significantly high ratio for the presentation of anti-SpCas9 (78%) and anti-SaCas9 (58%) cells within the donor samples. This hostility induced the apoptosis of Cas9-presenting cells [43]. This concern is magnified in neurological applications, where immune responses within the CNS must be carefully managed to avoid exacerbating disease conditions or triggering neuroinflammation [27, 28].

6. Prospect

Notwithstanding the challenges, the potential of CRISPRi system is nonnegligible. Many ongoing efforts are being made to address the limitations. These efforts encompass a diverse array of strategies aimed at refining the system’s precision and efficacy. Strategies include the downsizing or aggregating of the enzymatic domains, such as DNMT3ACD and DNMT3A-3L, and the augmentation by another amplifier, such as SunTag and Gly4Ser, to enhance methylation efficiency and safety. The exploration of new Cas9 orthologs with distinct PAM sequences seeks to boost specificity further. The integration of deep learning algorithms for the advanced design of sgRNAs promises higher accuracy in on-target editing [15]. Further, the CRISPRi system’s versatility and capacity for multiplexing enable the simultaneous targeting of multiple genetic factors involved in neurological diseases, offering a nuanced approach [17]. Efforts to minimize off-target effects are crucial, utilizing technologies like whole-genome bisulfite sequencing for precise target validation and developing synergistic systems to boost methylation efficacy with minimal side effects [11]. Additionally, addressing the challenge of delivering these therapies directly to brain tissues is guiding the development of novel delivery methods, including non-viral vectors such as nanoparticles, to enhance targeting specificity and genetic payload [11].

7. Conclusion

In conclusion, with the rising concern of neurological conditions such as AD and glioma among the adolescent and geriatric population, CRISPRi technology demonstrates promising therapeutic potential through targeted epigenetic modifications. The successful manipulation of the APP gene to mitigate AD pathologies and the precision targeting of MGMT and TERT genes in glioma highlight CRISPRi's capability to effectively alter disease pathways at the molecular level. These interventions, which range from reducing Aβ42 peptide levels in AD to enhancing chemosensitivity in glioma, underscore the method's specificity, effectiveness, and potential for reversibility, distinguishing it from traditional clinical treatment and canonical CRISPR-Cas9 gene editing techniques. However, the journey from preclinical success to clinical application is fraught with challenges. Issues such as off-target effects, delivery barriers, especially the BBB, and immunogenic responses, remain significant hurdles that need to be addressed. Looking forward, the journey of CRISPRi from the bench to bedside, while fraught with challenges, represents an exciting era in genomic medicine, with the promise of reshaping our approach to neurological disease treatment and individualized management.

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