Brain-Targeted Nano delivery System for CRISPRa-Mediated Activation of the Arc Gene in Alzheimer's Disease

Yibing Lu *

School of Pharmacy, China Pharmaceutical University, Nanjing, 211198, China

* Corresponding Author Email: 2020240341@stu.cpu.edu.cn

Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline and synaptic dysfunction, for which effective treatments remain lacking. Recent advances in CRISPR activation (CRISPRa) systems and nanodelivery platforms have opened new therapeutic avenues, particularly through modulation of Arc gene expression to improve synaptic plasticity. However, efficient brain-targeted delivery remains a critical challenge. This study systematically analyzes the molecular mechanisms of CRISPRa-mediated Arc gene regulation and its therapeutic potential in AD, with a focus on optimizing delivery strategies such as lipid nanoparticles (LNPs) with ligand modifications and improved immunocompatibility to enhance brain-targeting efficiency. Current evidence demonstrates considerable promise in vitro and in animal models, though the precise activation of Arc by CRISPRa requires further validation. The findings provide valuable insights for AD gene therapy and nanodelivery technologies, while emphasizing the need for further optimization of delivery system safety and scalability. Future work should focus on multi-ligand synergistic targeting, immune modulation, and standardized manufacturing processes to facilitate clinical translation.

Keywords: Alzheimer's disease, CRISPR activation (CRISPRa), Arc gene, Nanodelivery, Brain-targeted gene therapy.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and synaptic dysfunction. Current pharmacological interventions can only alleviate symptoms but fail to halt disease progression, underscoring the urgent need to explore novel therapeutic strategies at the mechanistic level.

The activity-regulated cytoskeleton-associated protein (Arc) gene plays a pivotal role in synaptic plasticity, long-term potentiation (LTP), and memory consolidation. Its expression is markedly downregulated in both AD patients and animal models. Evidence suggests that Arc may participate in β-amyloid pathology and synaptic dysfunction by regulating AMPA receptor endocytosis, influencing LTP maintenance, and modulating amyloid precursor protein (APP) processing, thereby contributing to cognitive impairment development [1, 2]. Consequently, restoring Arc expression has been considered a promising therapeutic approach.

The CRISPR activation system (CRISPRa) employs a catalytically inactive Cas9 (dCas9) fused to transcriptional activators to upregulate endogenous gene expression without inducing DNA cleavage. This approach offers high specificity, reduced risk, and regulation closer to physiological levels—features particularly suitable for Arc activation. However, the application of CRISPR-based systems to the nervous system is hindered by the blood–brain barrier (BBB).

Nanodelivery systems (NDS) utilize structurally tunable nanocarriers with surface functionalization to overcome the limitations of conventional drugs, including poor solubility, low stability, and suboptimal biodistribution. Compared with free drugs, nanosystems not only enhance targeting and controlled release [2,3] but also improve safety by employing biodegradable or natural carrier materials [4, 5]. These features significantly expand their potential for central nervous system (CNS) therapeutics. Strategies such as ligand modification and PEGylation have been shown to effectively enable brain delivery [5].

In recent years, NDS have demonstrated substantial promise in treating CNS disorders, particularly AD and other cognitive impairment-related conditions. Studies have reported 2–6-fold increases in

brain drug accumulation and significant improvements in learning and memory performance in animal models [6-8]. Nevertheless, their clinical translation still faces challenges such as safety concerns, difficulty in large-scale production, uncertainty in in vivo delivery efficiency, and insufficient regulatory standardization. Thus, enhancing brain-targeting efficiency while ensuring stable, controllable fabrication remains a critical direction for future development.

This article systematically reviews and evaluates the research progress of CRISPR-based gene editing systems and nanodelivery platforms in AD therapy, with a focus on their mechanisms of action, application strategies, technical bottlenecks, and future perspectives, aiming to provide a reference for both basic research and clinical applications in related fields.

2. Editing Principles of the CRISPR System

The CRISPR-Cas system uses a single-guide RNA (sgRNA) to direct a Cas protein to a specific genomic sequence. In gene therapy, CRISPRa—formed by fusing a dCas9 with transcriptional activators such as VP64, p65, and Rta—can upregulate endogenous gene expression without the risk of DNA cleavage [9]. Compared with traditional overexpression techniques, CRISPRa more closely mimics physiological expression patterns and reduces the risk of abnormal protein aggregation.

This strategy works by recruiting RNA polymerase and auxiliary regulatory proteins to the target promoter region, thereby enhancing transcription without inducing genomic disruption [10]. It offers good controllability, safety, and reversibility—qualities particularly valuable in therapeutic research for nervous system diseases where safety is paramount [11]. Furthermore, unlike plasmid- or virus-mediated cDNA overexpression, CRISPRa activation maintains expression patterns closer to natural regulation, minimizing risks of abnormal post-translational modifications or protein misfolding.

A typical CRISPRa system consists of two core components: (1) a dCas9–transcriptional activator fusion protein expressed under a neuron-specific or ubiquitous promoter, and (2) an sgRNA sequence targeting the promoter region of the gene of interest. The choice of sgRNA binding site is critical, with optimal locations typically lying ~−400 to −50 bp upstream of the transcription start site, a region rich in transcription factor binding sites and open chromatin [12]. In the nervous system, neuron-specific promoters such as SYN can markedly improve activation efficiency while reducing off-target expression in non-neuronal cells [12].

Beyond single-gene activation, CRISPRa can regulate multiple genes simultaneously by introducing multiple sgRNAs, enabling the study of gene interaction networks and complex pathological processes [11]. In the context of AD, this capability could allow concurrent modulation of multiple endogenous genes involved in synaptic plasticity, energy metabolism, and neuroprotection, thereby intervening at multiple pathological stages.

Although CRISPRa design principles have been validated in vitro, in vivo applications face significant hurdles. The BBB limits the delivery of large macromolecular gene-editing tools to neuronal targets. Existing viral vectors such as lentiviruses and adeno-associated viruses (AAVs) can achieve partial brain delivery but suffer from limitations—AAVs have small packaging capacities, while lentiviruses carry integration risks [2]. Therefore, achieving efficient, safe, and precise CRISPRa delivery to target neurons is a key technical challenge, and represents a promising opportunity for integration with nanodelivery systems.

3. Association Between the Arc Gene and AD

Arc (activity-regulated cytoskeleton-associated protein) is an immediate early gene whose expression is tightly regulated by neuronal activity. Its functions encompass multiple aspects, including synaptic structural remodeling, maintenance of long-term potentiation (LTP) and long-term depression (LTD), and regulation of neural circuit stability [13, 14]. Pastuzyn et al. discovered that Arc protein can form capsid-like structures resembling those of retroviral Gag proteins, encapsulating its own mRNA and transferring it between neurons via exosomes [15]. This process mediates non-

cell-autonomous LTD, thereby modulating neural circuit function and establishing a unique mechanism of inter-synaptic communication [15]. Such a mechanism provides a molecular basis for large-scale neural circuit plasticity and suggests a network-level coordinating role for Arc. However, in pathological conditions such as AD, this property may be attenuated or disrupted, thereby impairing network stability and adaptability.

In AD, Arc expression is markedly downregulated in both patients and animal models [16]. This reduction correlates closely with morphological changes such as decreased postsynaptic density (PSD) protein levels and reduced dendritic spine density, as well as impaired LTP induction and deficits in learning and memory. At the molecular level, Arc regulates AMPA receptor endocytosis and surface recycling, thereby modulating the strength of excitatory synaptic transmission [14]. In the AD pathological context, Arc downregulation disrupts AMPA receptor homeostasis, reduces synaptic transmission efficiency, and exacerbates synaptic dysfunction. Moreover, Arc is thought to be involved in the regulation of amyloid precursor protein (APP) processing and β-amyloid (Aβ) deposition, potentially via modulation of endocytic pathways and intracellular trafficking [16]. Together, these mechanisms constitute a multidimensional role for Arc in AD pathogenesis, providing a novel therapeutic target. However, conventional overexpression approaches have difficulty achieving precise regulation of endogenous Arc. Thus, selective activation of endogenous Arc expression has been proposed as a promising intervention strategy [16].

Notably, Arc expression levels correlate positively with cognitive performance. Animal studies have shown that either exogenous overexpression or enhanced endogenous Arc expression can improve spatial memory and synaptic plasticity parameters in AD models [16]. These findings provide direct evidence for targeting Arc as a therapeutic strategy. However, conventional overexpression typically relies on exogenous cDNA, which may lead to protein overproduction and non-physiological post-translational modifications, potentially resulting in toxicity. In contrast, CRISPRa-mediated activation of the endogenous Arc gene can enhance expression while preserving the integrity of its regulatory network, achieving a pattern closer to physiological regulation [9].

Savell et al. developed a neuron-optimized dual-lentiviral CRISPR/dCas9 transcriptional activation system, which employs neuron-specific promoters to enhance CRISPRa expression efficiency in the nervous system both in vitro and in vivo [12]. This system enables tunable, multi-gene targeting and demonstrates transcript-specific regulation within complex multi-promoter genes such as brain-derived neurotrophic factor (BDNF). Although Savell's study did not examine Arc, the successful activation of synaptic function-related genes such as BDNF suggests that CRISPRa is equally applicable to Arc gene regulation.

4. Current Applications of LNPs and CRISPR in AD Therapy

LNPs, as non-viral gene delivery platforms, have gained significant attention in recent years in the fields of mRNA vaccines and gene therapy. Notably, surface modification with brain-targeting ligands such as rabies virus glycoprotein (RVG)—which specifically binds nicotinic acetylcholine receptors (nAChRs) and p75 neurotrophin receptors on neuronal membranes—enables receptor-mediated transport and retrograde axonal transport across the BBB. This strategy markedly enhances brain-targeting efficiency and drug accumulation in neural tissue.

For CRISPRa delivery, LNPs offer the advantage of co-encapsulating mRNA encoding the dCas9–activator fusion protein together with sgRNA, thereby avoiding the risk of genomic integration associated with viral vectors [9]. More importantly, mRNA delivery can produce short-term, high-peak protein expression, reducing immune responses and potential toxicity associated with prolonged exogenous protein accumulation. Zhu et al. developed guanidinium-rich lipopeptide LNPs that achieved over 70% delivery efficiency of Cas9/sgRNA ribonucleoproteins to muscle tissue while maintaining good biocompatibility [17]. The high charge density design of these nanoparticles not only promotes nucleic acid condensation and cellular uptake but also offers valuable structural insights for delivering large gene-editing tools to the brain.

Similarly, Tang et al. constructed peptide-functionalized LNPs (pLNPs) by conjugating brain-targeting peptides (e.g., RVG39, T7, Angiopep-2, mApoE) onto the surface of LNPs composed of ionizable lipids, phospholipids, cholesterol, and PEG–lipids. These modifications enable binding to receptors highly expressed on brain capillary endothelial cells, followed by receptor-mediated endocytosis and transcytosis across the BBB [8]. In an in vitro Transwell-BBB model, this approach increased neuronal transfection efficiency by approximately 100-fold, and intravenous administration in vivo significantly enhanced brain delivery while reducing peripheral organ distribution. Integrating this approach with an Arc-targeted CRISPRa system could improve both the precision and efficiency of brain-specific gene editing.

Preclinical studies have provided preliminary validation of LNP-mediated CNS delivery of gene-editing tools. For example, RVG peptide-modified LNPs increased brain delivery efficiency by 3–5 times compared to unmodified LNPs in mouse models [18]. In AD models, this strategy not only improved drug distribution in the hippocampus and cortex but also significantly enhanced learning and memory performance as well as synaptic morphology [13]. These findings suggest that LNPs hold promise for achieving similar spatial targeting advantages when delivering large CRISPRa components.

Nevertheless, current LNP applications in AD gene therapy face several limitations. First, while ligand modification can enhance BBB permeability, cross-species applicability and long-term safety require further validation [13]. Lessons from tumor immunotherapy—such as non-viral chimeric antigen receptor-engineered natural killer (CAR-NK) cell delivery systems, which have achieved long-term safe delivery in preclinical studies via optimized nanocarrier formulations—indicate that mature, cross-disciplinary strategies for material selection and immune modulation are available. Second, large-scale manufacturing of LNPs demands rigorous batch-to-batch consistency and stability control to meet regulatory requirements for clinical translation [18]. Finally, in multi-target editing or chronic treatment scenarios, avoiding immune rejection against repeated administrations of carrier components remains a pressing technical challenge.

5. Discussion

As a core regulator of synaptic plasticity, the Arc gene plays a pivotal role in maintaining synaptic function, and its downregulation is closely associated with synaptic dysfunction and abnormal Aβ metabolism in AD [16]. The CRISPRa system enables precise activation of endogenous Arc gene expression while avoiding the DNA double-strand break risks associated with conventional gene-editing approaches, offering a novel strategy for AD therapy [9]. However, its clinical application faces a major hurdle in delivery systems. Among existing delivery strategies, non-viral vectors such as LNPs have demonstrated unique advantages due to their tunability and safety profile [18]. Recent studies have shown that functional modifications can significantly improve the delivery efficiency of LNPs: guanidinium-rich lipopeptide nanoparticles enhance cellular uptake by optimizing charge density [17], while peptide-modified LNPs can effectively traverse the BBB [8]. These advances lay a solid foundation for brain-targeted delivery of Arc-CRISPRa systems.

Future development may focus on several key directions. First, surface modification strategies for LNPs should be further optimized, incorporating multi-ligand synergistic designs to enable precise targeting of distinct neuronal subpopulations. Second, improving carrier immunocompatibility will be essential to reduce the risks associated with repeated administration. Finally, establishing standardized manufacturing processes is critical to ensure clinical translation feasibility.

6. Conclusion

This study systematically explored a CRISPRa-based therapeutic approach for activating the Arc gene in AD and analyzed the critical role of brain-targeted nanodelivery systems in enabling this strategy. The Arc gene is central to the maintenance of synaptic plasticity and the regulation of Aβ

metabolism, and its downregulation is strongly correlated with cognitive deficits. CRISPRa technology can precisely activate endogenous Arc expression while avoiding DNA cleavage risks, providing a safe and feasible pathway for restoring synaptic function.

However, the BBB remains a significant obstacle to CNS applications. Functionalized LNPs and other non-viral vectors can markedly improve brain delivery efficiency, thereby providing the technical basis for implementing this therapeutic strategy. By integrating gene editing with nanodelivery, this study presents a comprehensive framework for molecular intervention in AD, with broader implications for precision regulation strategies in other neurodegenerative diseases.

Limitations of the current work include the absence of specific carrier designs and animal model validation, with some conclusions relying on indirect evidence. Future research should advance multi-target activation, optimize vector immunocompatibility, and develop standardized manufacturing processes to achieve safe, efficient, and clinically translatable brain-targeted gene-editing therapies.

References

[1] Shepherd J D, Bear M F. New views of Arc, a master regulator of synaptic plasticity. Nature Neuroscience, 2011, 14 (3): 279 - 284.

[2] Zhang X, Hou X, Te L, Zhongsheng Z, Jiang J, Wu X. Mesenchymal stem cells and exosomes improve cognitive function in the aging brain by promoting neurogenesis. Frontiers in Aging Neuroscience, 2022, 14: 1010562.

[3] Amjady N. Short-term hourly load forecasting using time series modeling with peak load estimation capability. IEEE Transactions on Power Systems, 2001, 16 (4): 798 - 805.

[4] Sanadgol N, Abedi M, Hashemzaei M, Kamran Z, Khalseh R, Beyer C, Voelz C. Exosomes as nanocarriers for brain-targeted delivery of therapeutic nucleic acids: advances and challenges. Journal of Nanobiotechnology, 2025, 23 (1): 453.

[5] Asha Spandana K M, Bhaskaran M, Reddy Karri V V S N, Natarajan J. A comprehensive review of nano drug delivery system in the treatment of CNS disorders. Journal of Drug Delivery Science and Technology, 2020, 57: 101628.

[6] Da Silva-Candal A, Brown T, Krishnan V, Lopez-Loureiro I, Ávila-Gómez P, Pusuluri A, Pérez-Díaz A, et al. Shape effect in active targeting of nanoparticles to inflamed cerebral endothelium under static and flow conditions. Journal of Controlled Release, 2019, 309: 94 - 108.

[7] Zhang X, Hu H, Li Q, Zhao Y, Zhang Z, Zhao S, et al. Cascade-targeting multifunctional nanochaperone remodels brain microenvironment for synergistic therapy of Alzheimer's disease. Nano Today, 2024, 55: 102168.

[8] Tang S, Han E L, Mitchell M J. Peptide-functionalized nanoparticles for brain-targeted therapeutics. Drug Delivery and Translational Research, 2025. doi: 10.1007/s13346 - 025 - 01705 - 1.

[9] Becirovic E. Maybe you can turn me on: CRISPRa-based strategies for therapeutic applications. Cellular and Molecular Life Sciences, 2022, 79 (2): 130.

[10] Makarova K S, Wolf Y I, Iranzo J, Shmakov S A, Alkhnbashi O S, Brouns S J, et al. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. Nature Reviews Microbiology, 2020, 18 (2): 67 - 83.

[11] Bendixen L, Jensen T I, Bak R O. CRISPR-Cas-mediated transcriptional modulation: The therapeutic promises of CRISPRa and CRISPRi. Molecular Therapy, 2023, 31 (7): 1920 - 1937.

[12] Savell K E, Bach S V, Zipperly M E, Revanna J S, Goska N A, Tuscher J J, et al. A neuron-optimized CRISPR/dCas9 activation system for robust and specific gene regulation. eNeuro, 2019, 6 (1): ENEURO.0495 - 18.2019.

[13] Van Haasteren J, Li J, Scheideler O J, et al. The delivery challenge: fulfilling the promise of therapeutic genome editing. Nature Biotechnology, 2020, 38: 845 - 855.

[14] Martin K C, Barad M, Kandel E R. Local protein synthesis and its role in synapse-specific plasticity. Current Opinion in Neurobiology, 2000, 10 (5): 587 - 592.

[15] Pastuzyn E D, Day C E, Kearns R B, Kyrke-Smith M, Taibi A V, McCormick J, et al. The neuronal gene Arc encodes a repurposed retrotransposon Gag protein that mediates intercellular RNA transfer. Cell, 2018, 172 (1 - 2): 275 - 288.e18.

[16] Leung H W, Foo G, VanDongen A. Arc regulates transcription of genes for plasticity, excitability and Alzheimer's disease. Biomedicines, 2022, 10 (8): 1946.

[17] Zhu M, Wang X, Xie R, Wang Y, Xu X, Burger J, Gong S. Guanidinium-rich lipopeptide-based nanoparticle enables efficient gene editing in skeletal muscles. ACS Applied Materials & Interfaces, 2023, 15 (8): 10464 - 10476.

[18] Khodaverdi K, Bakhshi A, Mozafari M R, Naghib S M. A review of chitosan-based nanocarriers as drug delivery systems for brain diseases: Critical challenges, outlooks and promises. International Journal of Biological Macromolecules, 2024, 278 (3): 134962.