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Review Article

Gene Therapy, a Potential Panacea for Neurodegenerative Diseases

Asuku Abraham O[1]., Adeyemo Michael B[2]., Edward Monday O[3]., Daodu Praise J[4]., Asade Taofikat A[4]., Odeyemi Esther O[4].

[1]Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja
[2]Joint Universities Preliminary Examinations Board, Kogi State University, Ayingba
[3]Department of Bioethanol, National Biotechnology Development Agency, Abuja
[4]Department of Medical Laboratory Science, Kogi State College of Health Sciences and Technology, Idah
[5]Department of Physiology, University of Ilorin, Ilorin

[*]***Author for Correspondence:***
Asuku A. O.
Email: asufem2017@gmail.com
Telephone numbers: 08077427135

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ABSTRACT
Gene therapy has the potential to help patients with a variety of neurodegenerative diseases by protecting and restoring neurons, correcting pathogenic mechanisms directly, and controlling symptoms. Recent research suggests that experimental procedures for genetic diseases could be translated into effective patient therapies. Genetically complex disorders such as Alzheimer's disease, Parkinson's disease and Huntington's diseases are potential targets for neurodegenerative disease therapies. Targeting the central nervous system with gene therapy presents unique technical and biological challenges. Novel gene vector delivery strategies may be used to overcome these problems. We discussed various gene therapy strategies involved in the treatment of neurodegenerative diseases using adeno-associated virus (rAAVs), gene editing, and ribonucleic acid interference (RNAi) tools.
The challenges of these methods were discussed as against their potentials to be accepted as effective treatment options for neurodegenerative diseases.

INTRODUCTION

Diseases of the Neurons

The term "neurodegenerative diseases" refers to a group of disorders that affect the central nervous system and eventually result in neurodegeneration.[1] Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease are the most common neurodegenerative diseases (HD). As a result of genetic mutations and/or cell dysregulation, the morbidity rate for these diseases is increasing among the elderly. In some cases, the same mutations and pathological mechanisms are linked to different neurodegenerative diseases. All neurodegenerative diseases have different causes, but they all result in nerve cell death, axonal regeneration failure, demyelination, and/or structural and functional neural injury.[2] These characteristics can appear in various combinations that are either genetic or idiopathic. [2] These conditions could be caused by amyloid protein and Tau aggregation in Alzheimer's disease and traumatic brain injuries, misfolded proteins, RNA toxicity, or translational products within genes [2-3] Tau, α-synuclein, amyloid-ß, and huntingtin are examples of misfolded protein aggregates that can spread and invade tissues through cellular interactions. They eventually result in sensory, motor, and cognitive functions being damaged, if not completely lost. Cognitive impairment, memory loss, apathy, anxiety, muscle weakness, speech problems, and breathing problems are all common symptoms of neurodegenerative disorders.

AD is complex and heterogenous and appears to follow an age-related dichotomy: rare and highly penetrant early-onset familial AD (EOFAD) represents only a small fraction of all AD cases (≤5%) and typically presents with onset ages younger than 65 years, showing autosomal dominant transmission within affected families.[4] To date, more than 160 mutations in 3 genes have been reported to cause EOFAD. These include the Aβ precursor protein (APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin 2 (PSEN2) on chromosome 1.[4]

The most frequently mutated gene, PSEN1, accounts for the majority of AD cases with onset prior to age 50 [4] While these AD-causing mutations occur in 3 different genes located on 3 different chromosomes, they all share a common biochemical pathway, i.e., the altered production of Aβ leading to a relative over - abundance of the Aβ42 species, which eventually results in neuronal cell death and dementia. late-onset AD (LOAD), on the other hand, is classically defined as AD with onset at age 65 years or older and represents the vast majority of all AD cases [4] Mutations in at least 5 genes have now been shown to cause familial early-onset parkinsonism (α-synuclein [PARK1]; parkin [PARK2]; DJ-1 [PARK7]; PTEN-induced putative kinase I [PARK6]; and leucine-rich repeat kinase 2 or dardarin [PARK8]), with several other linkage regions pending characterization and/or replication.[4] As was the case in the study of AD, the first

locus to be characterized – PARK1, on chromosome 4q21 – involves the protein that is the major constituent of one of the classic neuropathological hallmarks of the disease, i.e., α-synuclein, which can be found at the core of Lewy bodies. Approximately 90% of HD cases are hereditary and transmitted in an autosomal dominant fashion. [4] As a matter of fact, the HD gene was the first autosomal disease locus to be mapped by genetic linkage analysis (to chromosome 4q16). It took some years after to identify the underlying gene defect, which proved to be a poly-CAG (encoding glutamine [Q]) repeat in exon 1 of a 350-kDa protein (huntingtin; gene: HD). The precise function of huntingtin remains elusive, but cloning experiments show that it is highly conserved throughout evolution, which suggests an essential functional role of this protein in neuronal development and homoeostasis. HD is virtually always attributable to a defect in a single gene, i.e., poly-Q expansion in huntingtin, although such defects only account for 50% of the inter individual onset age variation.[4]

Gene therapy

Gene therapy is a technique for delivering functional genetic material to cells in order to repair a malfunctioning gene. The product encoded by a therapeutic gene [its messenger RNA (mRNA) and/or proteins] will be continuously synthesized within the cell using the cell's own transcriptional and translational machinery if a copy of that gene is delivered to affected cells.[5] The main advantage of this technology is that it has the potential to provide a long-term therapeutic effect without the need for repeated administration. RNA interference (RNAi) for the silence of the mutant allele, introducing a disease-modifying gene, or using gene-editing technology are all examples of gene therapy technique.[6-8] Viral or non-viral gene therapy vectors are available. Without the use of viral vectors, therapeutic genes can be delivered to cells using a variety of physical and chemical systems. Non-viral vectors do not limit the size of the therapeutic gene, have a low risk of immunogenicity, and are relatively inexpensive.[9] However, most gene therapies now rely on viral vectors because non-viral technologies require high therapeutic doses and the resulting gene expression is usually brief. Adenovirus, lentivirus, -retrovirus, herpes simplex virus (HSV), and adeno associated virus (AAV) are among the viral vector types that have been tested in clinic.[10] AAV is currently the most common vector for *in vivo* gene delivery.

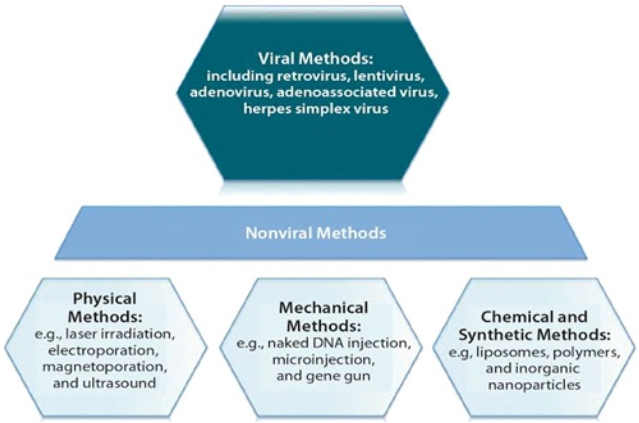

Fig 1: An overview of gene therapy vectors [11]

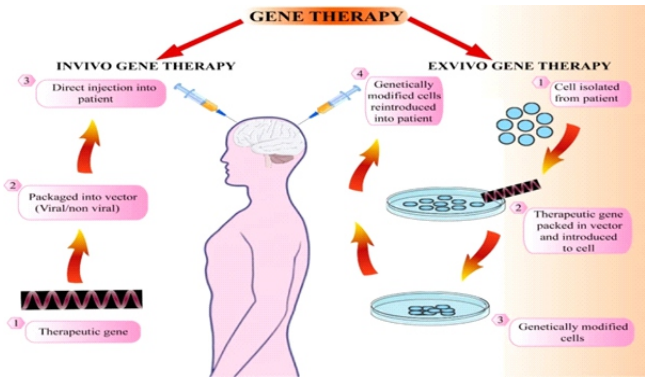

Fig. 2: Illustration of gene therapy approaches [12]

Gene therapy using the adeno-associated virus (AAV)

Adeno-associated virus belongs to the Parvoviridae family and is preferred for its gene immunogenicity profile. It does not cause disease.[13] Between two inverted terminal repeats (ITRs) is the genome of wild-type AAV.[14] A replication (Rep) and a capsid (Cap) gene are both found in the ITRs. Genome replication, integration, and packaging into the capsid all require ITRs. A growing number of naturally occurring and engineered AAV serotypes with various viral capsids have altered tissue tropism, transduction rate, and ability to cross the blood–brain barrier.[15-16] Replacing the Rep and Cap genes with an expression cassette containing a therapeutic gene of interest allows for the production of recombinant AAV (rAAV). The ITRs are the smallest regions in rAAV that must be kept in order for the genome to be packaged. More than 200 human clinical trials have used rAAVs, and they've been shown to be safe.[17] Because of its neuronal tropism and good safety profile shown in clinical studies, AAV-based gene therapies are very reliable for the treatment of neurodegenerative diseases. Furthermore, when in vivo AAV administration requires invasive procedures, a single administration results in long-term, potentially life-long gene expression. Intrathecal administration can be done via lumbar puncture, direct injection into the cisterna magna, or intraventricular injection into the cerebral ventricle.[18] As a result, the vector enters the CNS without passing through the blood–brain barrier. When compared to systemic administration, this is less invasive than intracerebral administration and typically results in less leakage to peripheral organs. Its main drawback is that, when compared to intracerebral administration, the dilution of the vector and the resulting transduction is usually less. AAVs include serotypes depending on capsid profiles. Multiple AAV serotypes have been identified. They vary in tropism, making each of them suitable for the transduction of specific cells or tissue types with the AAV receptor's aid. These include AAV1, 2, 3b, 4, 5, 6, 7, 8, and 9 with varying ability to transduce specific cell types. AAV capsid interaction with glycans and proteins in the cell surface as well as the serotypes of varying protein composition is the major factor that determines the efficiency of transduction. AAV9 is an excellent vector that can be directly introduced into the brain and can produce a global expression in the spinal cord and the brain following a peripheral systemic administration route in animal models. Thus, affecting the entire CNS without being injected into the CNS seems promising for gene therapy. AAV2 is considered a gold standard in neurosurgical gene

therapy because of its phenotype specificity for neurons and the safety profile. It is being studied in several clinical trials [19].

Ribonucleic acid Interference (RNAi-based gene therapy)

Many neurodegenerative diseases are caused by genetic mutations, and gene silencing technologies can be used to reduce the expression of the disease-causing genes. RNA interference (RNAi) is a gene-silencing technology. By providing protection against foreign nucleic acids from pathogens, RNAi plays a vital physiological role in gene regulation and the innate immune response of cells.[20] The RNAi-induced silencing complex (RISC) is a nuclease that can cleave mRNA and reduce its expression. It was one of the first mediators of RNAi to be discovered. Endogenous and exogenous double-stranded RNAs can both activate RNAi.[21]

The first step of RNAi mechanism of action involves processing and cleavage of longer double-stranded RNA into siRNAs, generally bearing a 2 nucleotide overhang on the 3′ end of each strand. The enzyme responsible for this processing is an RNase III-like enzyme termed Dicer When formed, siRNAs are bound by a multiprotein component complex referred to as RISC (RNA - induced silencing complex). Within the RISC complex, siRNA strands are separated and the strand with the more stable 5′-end is typically integrated to the active RISC complex. The antisense single-stranded siRNA component then guides and aligns the RISC complex on the target mRNA and through the action of catalytic RISC protein, a member of the argonaute family (Ago2), mRNA is cleaved.[22]

MicroRNA (miRNAs), small interfering RNA (siRNA), and piwi interacting RNAs are three forms of short non-coding RNAs that use the RNAi pathway. Negative regulators of gene expression, miRNAs and siRNAs, whereas piRNAs protect organisms from transposable elements, serve as negative regulators of gene expression. [23] Therapeutics like miRNA mimics, anti-miRs, and artificial miRNAs are based on the capacity to manufacture artificial miRNAs that target quiet disease-related genes. MiRNA mimics are double-stranded miRNAs synthesized artificially to match a matching miRNA, with the goal of compensating for miRNAs that are down regulated in illnesses. Anti-miRs are single-stranded miRNAs that are synthesized artificially and utilized to bind and inhibit the action of target miRNAs. Artificial miRNAs are created by substituting the guide strand sequence of an endogenous miRNA precursor with the sequence of a target mRNA, allowing upregulated genes to be silenced.

Gene Editing

Gene editing is a unique method of removing, adding, or changing DNA sequences in a sequence-specific manner. The revelation that targeted DNA double strand breaks (DSBs) can be generated by endogenous cellular repair machinery via homology-directed repair (HDR) or non-homologous end-joining was essential in the development of gene editing. Engineered nucleases with a sequence-specific DNA-binding domain linked to a DNA cleavage module can be utilized to introduce DNA DSBs at specific locations. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the RNA-guide clustered regulatory interspaced short palindromic repeats (CRISPR) and CRISPR associated system 9 (Cas9) (CRISPR/Cas9) are the most common nucleases. All gene editing techniques, including zinc-fingers, can cause unintended DNA mutations or deletions. ZFNs and TALENs are both based on DNA–protein interactions, but TALE repeats are a different DNA binding domain. A single TALE motif can recognize and bind to a single nucleotide, while a cluster of TALEs can recognize and bind to a longer DNA sequence. TALENs are easier to design than ZFNs, and the TALE repeat array can easily be extended to the desired length. [24] Off-target effects are a major drawback for TALENs, as are their larger sizes compared to ZFNs, making viral vector delivery more difficult. The most recent of the nuclease systems mentioned is CRISPR/Cas9. CRISPR/Cas9, unlike ZFN and TALEN endonucleases, uses an RNA-guided system to edit genes rather than a protein. As a result, it has a simple and inexpensive design. A large number of preclinical studies show that this technology, in combination with gene therapy, has great potential for treating neurodegenerative diseases. In Alzheimer's disease, for example, the defective (amyloid precursor protein) APP gene was successfully edited in human fibroblasts using a CRISPR/cas9 construct, resulting in lower levels of amyloid beta in AD patients' brains. [25] The G4C2 repeat in the non-coding region of the C9orf72 gene was successfully removed in transfected patient-derived iPS cells, which prevented RNA foci formation and promoter hypermethylation, both of which are common in ALS.[26] In the striatum of HD140Q-knock-in mice, CRISPR/Cas9 permanently suppressed mutant Huntingtin and its aggregates. [27]

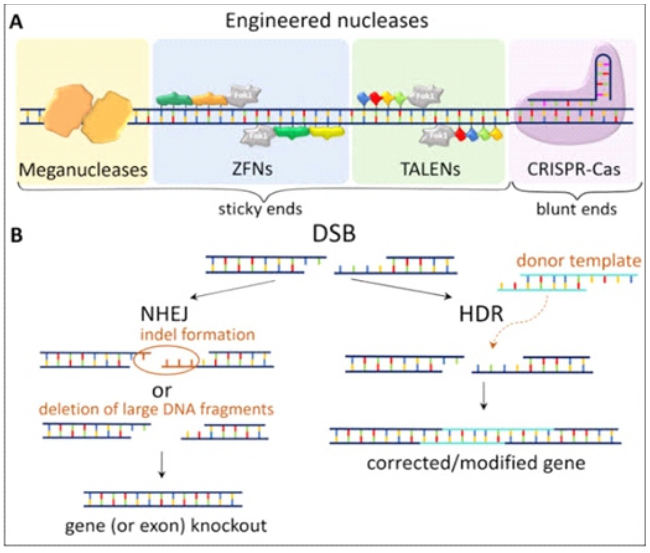

Fig. 3: gene editing [28]

Gene therapy for Alzheimer's disease

Alzheimer's disease is the most common and fatal neurodegenerative disease that affects the elderly. Progressive cognitive decline and morphological changes in the central nervous system (CNS), such as amyloid peptide (b-amyloid-Ab) accumulation, neurofibrillary degeneration, synapse loss, and cell death in most cortical structures,

characterize the disease. The loss of cholinergic neurons in the basal forebrain causes memory impairment in Alzheimer's disease. These neurons have widely diffuse projections to the cerebral cortex and use acetylcholine as a neuronal transmitter. The most difficult task for gene therapy is to find a cure for the neuropathology of Alzheimer's disease. The use of trophic factors to improve cholinergic neurons and an autoantibody approach to remove amyloid-beta accumulation are the two main treatments currently available. Nerve growth factor is a trophic factor that increases acetylcholine synthesis and improves memory in cholinergic neurons of the basal forebrain. Another gene therapy approach for Alzheimer's disease is an anti-amyloidogenic strategy. The reduction of amyloid-beta peptide synthesis and the removal of amyloid-beta peptide accumulation were used. Since intramuscular or oral administration of rAAV expressing amyloid-beta cDNA resulted in a safer immunization modality with reduced amyloid deposits in an Alzheimer's disease rat model without inflammation, gene therapy methods targeting the amyloid-beta have been promising.[29]

Gene therapy for Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by the progressive loss of dopaminergic neurons in the substantia nigra, resulting in dopamine deficiency in the striatum. Tremor, bradykinesia, and rigidity are primary motor deficits, but there are also non-motor issues like cognitive and autonomic dysfunction. The delivery of a dopamine synthesizing enzyme to restore dopamine production or neuroprotective molecules such as glial cell-derived neurotrophic factor (GDNF) to reduce dopaminergic neuron degeneration are two gene therapy methods for the treatment of Parkinson's disease.[30] In the early stages of Parkinson's disease, pharmacological dopamine replacement (L-Dopa therapy) is effective and reduces symptom severity. It is, however, linked to motor and psychiatric side effects (dyskenesia or hallucination) and does not stop the disease from progressing. Tyrosine hydroxylase converts tyrosine to L-3 4-hydroxyphenylalanine (L-Dopa), and aromatic L-amino acid decarboxylase (AADC) decarboxylates L-Dopa to produce dopamine in the biosynthetic pathway for dopamine. Because dopamine is unable to cross the blood–brain barrier, L-Dopa, which can, has been developed as a pharmacological treatment for Parkinson's disease. Because it is converted to dopamine, L-Dopa can have a therapeutic effect once in the brain. However, as PD progresses, nigral neurons (the striatum's primary source of AADC) are consistently lost, and AADC levels fall. The conversion of L-Dopa to dopamine is severely hampered by a lack of precursors. To achieve a clinical response, more L-Dopa must be administered more frequently and at higher doses, resulting in overstimulation and side effects. A potential gene therapy strategy would restore a sufficient level of AADC in the striatum, allowing for the exogenous administration of L-Dopa to restore dopamine levels to effective levels. Controlling dopamine overproduction caused by excess transgene product synthesis is necessary for successful gene therapy of PD with minimal side effects. Intranigral, intrastriatal, or intraventricular injection of GDNF has both protective and restorative effects in rodent and primate models of Parkinson's disease, according to the initial research. If given before or after lesions in dopaminergic neurons caused by the toxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA), GDNF protects dopaminergic neurons and restores behavioral deficits. [31] Because of inefficient GDNF distribution in the striatal parenchyma, the first attempt to use the technique in humans failed: intraventricular administration of GDNF protein resulted in no therapeutic benefits.[32] Nonetheless, a phase I trial in which GDNF was delivered directly into the putamen of human patients resulted in significant symptomatic relief.[33] Direct and continuous GDNF administration in specific sites appears to be therapeutic, but it comes with a slew of practical and safety issues. The most promising future strategy for achieving long-term and safer GDNF delivery is direct in-vivo gene transfer. With recombinant adenoviral, rAAV, and lentiviral vectors, effective long-term expression of GDNF has been achieved. In preclinical and clinical studies, two GDNF delivery strategies have been studied, including AAV-dependent gene transfer and direct infusion of proteins. [34] The initially documented preclinical studies regarding GDNF gene therapy targeted substantia nigra as well as striatum of MPTP -lesioned and normal aged monkeys utilizing a lentiviral vector. [35] AAV2-GDNF putamenal administration produced better clinical scores, raised dopamine turnover, and a raised uptake of PET-FMT in monkeys showing symptoms of PD for 6 months. The possibility of using gene therapy for the treatment of PD has been studied extensively and found safe and effective in phase I clinical trials, despite most of them have failed to show improvement in phase II trials, except for direct injection in the subthalamic nucleus by AAV2-GAD. [36] Another phase II clinical study of postmortem brain tissue found that neurturin (NTN), a neuroprotective substance delivery to the putamen led to a rise in NTN I of the striatum/putamen, but not in the substantia nigra, that can be attributed to the failure of the retrograde transport of dopaminergic neurons. [37] Another phase II trial was undertaken to resolve this transduction pattern, and NTN was injected into the putamen and substantia nigra. In such an approach, scientists hypothesized that gene therapy might be a success only when the growth factor is delivered before the neurodegeneration progresses extensively.

Huntington's disease gene therapy

An excessive number of trinucleotide CAG repeats on exon 1 of chromosome 4 causes Huntington's disease (HD), which results in a polyglutaminated huntingtin (mHtt) protein. The pathological phenotype seen in HD is caused by increased levels of mHtt in striatal medium spiny neurons and pyramidal cortical neurons. Motor, cognitive, and psychiatric symptoms are frequently present in patients. As the disease progresses, motor symptoms change. Patients first exhibit choreiform movements, which progress to bradykinesia and rigidity as their gait deteriorates.[38] Psychiatric manifestations include impulsivity, depression, and apathy, while cognitive problems include attention deficits and mental flexibility.[39] Designing drugs that reduce mHtt levels in an attempt to stop disease progression has become a popular experimental therapeutic approach to address the underlying pathology. Because gene therapy allows for direct targeting of dysfunctional nuclei in the hopes of lowering

mHtt levels, it is a promising treatment option for HD. The RNA-induced silencing complex destroys mRNA molecules using small RNA molecules such as short interfering RNA, short hairpin RNA, and microRNA.[40] These methods allow for more precise targeting of the mHtt mRNA while reducing the risk of off-target effects. In addition, nonhuman primate studies suggest that normal Htt reductions of about 45 percent may be well tolerated.[41-42] AAV5 with a transgene encoding an engineered miRNA against HTT mRNA (AAV5-miHTT) has been used to deliver an engineered microRNA targeting human huntingtin.[43]

Challenges

There are several limitations currently faced in gene therapy which affects its safety and efficacy. There are barriers such as biological barriers as well as immune responses, rate limiting steps in the expression of transgene based on cell type, and possible undesirable effects associated with vectors or treatment strategies. [12] Overcoming these barriers and limitations greatly enhances the benefits of gene therapy. Selection of vectors is important and AAV vectors are less immunogenic compared to other viral vectors. However, the nucleic acid sequence and the capsid proteins delivered are capable of triggering our immune system by secreting neutralizing or non-neutralizing antibodies which in turn either eliminates transduced cells or opsonize the viral particles thereby diminishing the clinical efficacy of AAV mediated therapeutic agents. [12] There comes a challenge of how to deliver therapeutic dose of AAV in patients with an immunological memory against AAV. This can be overcome by the selection of specific AAV variants that are not yet circulated in human population so that there can be lesser chances of developing memory responses against T cells and neutralizing antibodies. Another option to minimize immunological responses can be the selection of specific administration routes for different therapeutic strategies [44]. The specificity of RNAi is not as robust as it was initially thought to be. It is now well established that siRNA off-targets exist for many siRNA and that most siRNA molecules are probably not as specific as once thought. The introduction of siRNA can result in off-target effect, i.e. the suppression of genes other than the desired gene target, leading to dangerous mutations of gene expression and unexpected consequences.[45] The majority of the off-target gene silencing of siRNA is due to the partial sequence homology, especially within the 3'untranslated region (3'UTR), exists with mRNAs other than the intended target mRNA. [46] This mechanism is similar to the microRNA (miRNA) gene silencing effect. The off-target effect can also be a result of the immune response. RNA is recognized by immunoreceptors such as TLRs (Toll-like receptors) [47], leading to the release of cytokines and changes in gene expression.

For gene editing, off target effects, Cas9 specificity, and potential mutagenesis are major challenges for the CRISPR/Cas9 system when it comes to targeting disease-causing genes. Cas9 protein expression at high levels has been linked to toxicity, as expected. [48] The likelihood of off-target binding in the host genome is estimated to be relatively high (50 percent) using this system. [49] Another challenge is the delivery method, as CRISPR/Cas9 components are much longer than AAV vector packaging capacity. As a result, the CRISPR/Cas9 system has a lot of potential for therapeutic development, but there are a lot of issues that need to be addressed before this technology can be used to treat neurodegenerative diseases.

RECOMMENDATION/CONCLUSION

For delivering therapeutic genes to treat neurodegenerative disorders, gene therapy holds a lot of promise. AAV vectors are one of the safest ways to treat neurodegenerative diseases. New AAV vectors with a better transduction profile, better distribution, and higher transduction in the target organs via less invasive administration routes are highly sought after. These drawbacks could be overcome with genetically engineered AAV vectors. A key factor in the success of gene therapy is the selection of appropriate and suitable delivery routes. For efficient and effective transduction of the CNS, direct administration of AAV vectors into the parenchyma is currently used. Although this method of administration is more invasive than injections into the venous system or other fluid-filled compartments, the benefits are clear. Intra-parenchymal administration of the transgene results in a high concentration of the transgene in the target cells, high local transduction, less distribution to other organs, and a lower risk of immune responses or toxicities from AAV particles or ectopic transgene expression. AAV delivery via systemic or intrathecal routes necessitates higher doses, posing a greater risk of toxicity. At the transgene level, there is also much room for improvement. After in vivo delivery of viral vectors, many parts of the CNS are difficult to reach, resulting in a smaller number of cells expressing the therapeutic transgene, which may be insufficient to achieve overall therapeutic levels. [50] In addition, finding biomarkers for early disease diagnosis will be critical for identifying new patients and predicting clinical outcomes. More importantly, improving efficacy will require delivering treatments before the onset of neurodegeneration. The development of better pre-clinical models and success will be aided by a better understanding of neurodegenerative diseases pathogenesis. Future trials should combine these gene therapy approaches, such as nerve growth factor, which has been shown to reduce neuronal degeneration and cell death, with anti-amyloidogenic approaches to reduce amyloid-beta accumulation in Alzheimer's patients.

Conflicts of interest

There is no conflict of interest.

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