Molecular Neurobiology (2023) 60:1486–1498
https://doi.org/10.1007/s12035-022-03150-5

CRISPR/Cas9 Mediated Therapeutic Approach in Huntington's Disease

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Received: 1 November 2022 / Accepted: 26 November 2022 / Published online: 9 December 2022
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Abstract

The pathogenic mechanisms of these diseases must be well understood for the treatment of neurological disorders such as Huntington's disease. Huntington's Disease (HD), a dominant and neurodegenerative disease, is characterized by the CAG re-expansion that occurs in the gene encoding the polyglutamine-expanded mutant Huntingtin (mHTT) protein. Genome editing approaches include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats/Caspase 9 (CRISPR/Cas9) systems. CRISPR/Cas9 technology allows effective gene editing in different cell types and organisms. Through these systems are created isogenic control of human origin induced pluripotent stem cells (iPSCs). In human and mouse models, HD-iPSC lines can be continuously corrected using these systems. HD-iPSCs can be corrected through the CRISPR/Cas9 system and the cut-and-paste mechanism using isogenic control iPSCs. This mechanism is a piggyBac transposon-based selection system that can effectively switch between vectors and chromosomes. In studies conducted, it has been determined that in neural cells derived from HD-iPSC, there are isogenic controls as corrected lines recovered from phenotypic abnormalities and gene expression changes. It has been determined that trinucleotide repeat disorders occurring in HD can be cured by single-guide RNA (sgRNA) and normal exogenous DNA restoration, known as the single guideline RNA specific to Cas9. The purpose of this review in addition to give general information about HD, a neurodegenerative disorder is to explained the role of CRISPR/Cas9 system with iPSCs in HD treatment.

Keywords Huntington's Disease · Neurodegenerative Disorders · CRISPR/Cas9 · Genome Editing Systems · iPSC · Isogenic Cell Lines

Introduction

Neurological disorders have a negative impact on public health and have been associated with impaired central or peripheral nervous systems [1]. Specific neurodegenerative diseases are characterized by prominent clinical profiles depending on age. Loss of neuronal function, movement disorders and cognitive disorders occur in neurodegenerative diseases, characterized by progressive atrophy of neurons and tissue. Molecular properties such as mitochondrial dysfunction, axonal damage and abnormal protein aggregation are observed before neuronal death and dysfunction in neurodegenerative diseases. Complex and distinctive pathophysiological profiles appear in various neurodegenerative proteinopathies. The reason for this may be processing and aggregation of misfolded proteins. The most important of these diseases are Alzheimer's disease, Parkinson's disease and Huntington's disease. Alzheimer's disease is characterized amyloid beta plaques and phosphorylated Tau tangles.

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Parkinson's disease is characterized by alpha synuclein-related Lewy bodies. It is also known that HD characterized by inclusion bodies containing mHTT. These aggregate proteins cause neuronal axon damage and dysfunction. There are many pathogenic mutations associated with protein processing and aggregation [2].

HD is a serious neurodegenerative disorder and stems from an autosomal dominant mutation that occurs in the first exon of the Huntingtin gene encoding the Huntingtin protein [3]. In HD pathology, expansion of cytosine-guanine-adenine (CAG) repeats occurs on chromosome 4 (4p16.3) encoding the HTT gene [4]. Although there are 16–20 CAG repeats in the HTT gene in healthy individuals; HD patients usually have more than 40 CAG repeats. mHTT contains an expanded polyglutamine stretch, which leads to misfolding of the protein aggregate formations. It is known that these aggregates affect a wide variety of molecular and cellular processes. Striatal and cortical neurons death occurs as a result of the end of these processes. In most cases diagnosed with HD, basic HD symptoms, such as movement disorders, cognitive disorders, and psychiatric disorders, occur in middle ages. A significant relationship is reported between the age of onset of HD and CAG repeats. The higher number of CAG repeats is associated with the earlier onset of the disease and the development of more serious forms [3].

Although autosomal dominant mutation, which is effective in HD pathogenesis, has been described much earlier, disease-related pathways have not been fully elucidated. Symptomatic treatment is generally used in the treatment of this disease [3]. Symptomatic treatments such as caspases targeting glutamate and dopamine pathways, aggregation inhibition, mitochondrial dysfunction, and transcriptional dysregulation are used for improving of HD [4].

HD is a slowly progressing disease. Various analyes such as transcriptom analysis, bioinformatics analysis are applied ın the investigation of disease progression mechanisms. However, in these methods, interactions between genes, proteins and cellular organelles during disease progression cannot be fully explained. Therefore, modern gene editing approaches are applied in scientific researches. DNA sequences and the expression of target genes may be changed in consequence of these approaches [3]. In addition to the symptomatic treatments applied in HD improvement, new therapeutic approaches that target the mHTT protein and the HTT gene have been used recently. Thanks to these gene editing techniques, CAG repeats can be reduced and gene editing techniques are thought to be useful, especially when applied in hereditary, neurodegenerative disorders [4].

These modern gene editing approaches include approaches such as ZFNs, TALENs, CRISPR/Cas9 [3]. These approaches enable the recognization of enlarged CAG domains in normal cells or correction of mutations in cells derived from the patient. Thus, it is aimed to create isogenic cell lines with the same genetic background. Isogenic cell lines play an important role in the study of disease mechanisms and cell-based highly productive compound scanning [5, 6]. In addition, these approaches are highly effective in multiplex analysis and functional scanning of genes associated with HD pathogenesis in neurodegenerative processes. Cells that have corrected HTT are very important in autologous cell therapy and it is thought that mutant allele-specific gene regulation may play an effective role in in vivo HD gene therapy [3].

Based on this, our aim in this study is to contribute to the literature by explaining the mechanism of HD and summarizing the studies related to the CRISPR/Cas9 approach applied with iPSC, which is one of the genome editing systems used in HD treatment.

Huntington's Disease

HD is characterized by psychiatric symptoms, motor disturbances, cognitive deficits, sleep disturbances, and weight loss. HD is a genetic neurodegenerative disease and is characterized by neuron loss, motor dysfunction [7]. HD, which is a progressive genetic disease, has symptoms such as choroic movements, behavioral and cognitive disorders, dementia. HD symptoms vary in the early stages of HD pathology, the genetic basis of which was discovered in 1993. The same pathologies occur in all patients together with the progression of the disease. The earliest observed obvious damage, occurs in the neostriatum, consisting of the caudate nucleus and putamen. It is thought that the neurons most vulnerable against damage in HD are medium spiny neurons (MSNs) in the striatum [8].

When examined neuropathologically, HD is characterized by neurodegeneration that progresses in the striatum and to a lesser extent in the cortex. In HD, HTT misfolded in neurons and HTT protein aggregates containing molecules that can interact with HTT occur [9]. HD, which is an incurable neurodegenerative disorder, also includes loss of GABAergic MSN, progressive motor, psychiatric, and cognitive symptoms in the striatum. GABAergic MSNs with dopaminergic and glutamatergic inputs are projection neurons. While striatum takes dopaminergic inputs from the pars compacta of substantia nigra, takes glutamatergic inputs from the cortex and thalamus. Some MSNs besides dopaminergic and glutamatergic receptors, also express cholinergic and adenosinergic receptors [10].

Various animal models have been developed in the investigation of HD pathogenesis. It is aimed that mHTT protein levels can be reduced and change the affected neurons with the experimental approaches used in HD treatment [7]. In studies with HD animal models, it has been shown that

disease phenotypes may be improved and neuropathology may be reversed as a result of reducing mHTT [8].

In addition to experimental animal models in therapy for HD, embryonic stem cells, mesenchymal stem cells, neuronal stem cells may be used [7]. Nowadays, proximally targeted treatments are being developed in HD pathogenesis using HTT, DNA, RNA and protein. As a result of these approaches, it is aimed to reduce mHTT levels and to improve pathogenic effects [8].

Pathological Mechanisms of Huntington's Disease

The CAG codon encodes the alpha amino acid. It is known that glutamine is synthesized from glutamate and ammonia by glutamine synthetase enzyme. Glutamate, the precursor of the neurotransmitter glutamine, is mainly produced in the muscles, lungs and brain. CAG with non-toxic, has glutamine amino acids in the HTT gene. As a result of polyglutamine expansion, aggregate formation emerges and becomes toxic. Aggregates in the brain are an important factor in the emergence of HD. Inflammatory responses, mitochondrial dysfunction, apoptosis, excitotoxicity, and transcriptional regulation develop as a result of aggregate formations. The first exon of the HTT gene has a CAG nucleotide repeat and polyQ stretch occur at the N-terminus of the HTT protein in translation [4].

CAG repeats in HD onseting in adults, reaches 40 or more expansions. However, in patients (with Juvenile HD) less than 20 years old, a mutation with more than 55 CAG repeats is usually described. The presence of HTT exon 1 mRNA was determined in fibroblasts of juvenile HD patients and was detected especially in the post-mortem brains of early HD individuals [11].

Wild-type HTT has a complex structure with multiple interaction domains. So, it is thought to be wild-type HTT, a scaffolding protein that helps coordinate other proteins and cellular functions [12]. Wild-type HTT plays an important role in transcriptional regulation, in the production of brain-derived neurotrophic factor (BDNF). BDNF is an important factor for the survival of striatal and cortical neurons. Also wild-type HTT plays an important role in axonal transport, exchange of endosomes and organelles and vesicular recycling [13]. mHTT causes disease through a dominant toxic function gain mechanism [14, 15]. These pathological mechanisms include mechanisms such as early transcription dysregulation, synaptic dysfunction, proteasome dysfunction, aggregate pathology, oxidative damage, mitochondrial dysfunction and extrasynaptic excitotoxicity [16, 17].

It is thought that as one of these pathological mechanisms, proteasome dysfunction to be effective in the pathogenesis of HD. There are uncertainties regarding the regulation of proteasome activity in stem cells and somatic cells affected by HD. It has been determined that HD-iPSCs show high proteasome activity. It has been detected that neural progenitor cells (NPCs) derived from HD-iPSC exhibit lower levels of proteasome activity. It has also been shown that HD-NPCs form HTT aggregates under oxidative stress [9].

HTT Gene as Therapeutic Target

It is known that the HTT gene responsible for HD was identified many years ago. A polymorphic region containing CAG repeats encoding a glutamine domain called polyQ is found in first exon of the HTT gene. Various approaches have been developed to block mHTT expression and prevent toxic neurodegeneration. Gene silencing strategies are applied involving RNA interference (RNAi) and Antisense Oligonucleotide (ASO) compounds to induce partial degradation of the target mRNA. Also, in these approaches continuous expression is required for therapeutic molecules. HTT gene regulation is a treatment approach that causes permanent inactivation of the HTT gene. Genome editing systems are used in neurodegenerative diseases such as HD. Genome regulation is implemented as disease-modifying therapy, and this regulation has certain stages [18].

Current Treatment Approaches of Huntington's Disease

The HTT protein is required for neuronal development, and mutations in this protein have also been associated with HD development. In the mutated HTT protein, increased CAG repeats within the HTT gene result in expansion of the polyglutamine pathway. The molecular basis of HD is known, but there is no known treatment for the disease other than the symptomatic treatment approach. In addition to treatment approaches developed for specific symptoms in HD, disease-modifying treatments should also be explored. In HD, striatal medium spiny neurons (MSN) degenerate and mutHTT toxicity affects this degeneration more. The causes of striatal MSN loss are not fully known, but a significant loss of striatal MSN has been identified in HD patients compared to healthy individuals. Various therapeutic strategies are being investigated in preclinical models and clinical trials. Therapeutic approaches to decrease mutHTT content at transcription and translation levels, therapeutic approaches that induce mutHTT proteosomal degradation are very important. In addition, post-translational modification of mutHTT as a pharmacological approach is also being investigated. Besides these approaches, stem cell therapies targeting patient-derived induced pluripotent stem cells to replace lost striatal neurons are also important therapeutic

strategies. Therapeutic approaches for HD may be classified as therapies associated with mutHTT modification and degradation, therapies associated with signaling pathways, therapies associated with decreased mutHTT content, stem cell therapies, and pharmacological therapies [19] (Fig. 1).

Drugs that Prevent Excitotoxicity

Riluzole, which is among the drugs that inhibit excitotoxicity, is a glutamate inhibitor and provides reduction of abnormal movement especially in Amyotrophic Lateral Sclerosis (ALS) disease. In a previous study, it was determined that Riluzole is not neuroprotective and is not effective in the reduction of HD symptoms. Memantine, an antagonist of extrasynaptic N-methyl-D-aspartate (NMDA) receptors, plays an important role in Alzheimer's disease and mild-low dementia. It is known that memantine prevents the progression of HD by decreasing striatal cell death. Thus, HD-related cognitive functions may develop [4]. In another study, it was determined to prevent Memantine and Risperidone combination, motor symptoms, cognitive decline, and predicted progression of psychosis [20]. In a study conducted on rodents, it was determined to reduce pathology by applying Memantine at a low dose. Also, in the same study, it was reported that high doses of Memantine support cell death.

Tetrabenazine (TBZ) inhibits vascular monoamine transporter type 2. Thus, the dopamine pathway is also inhibited. The existing dopamine in the synapse reduce and its interaction with the postsynaptic dopamine receptors is limited. Deutetrabenazine containing deuterium atom is also known as vascular monoamine transporter type 2 inhibitor. In some treatment studies, it has been found that Deutetrabenzine more is tolerated than TBZ. In studies conducted by creating mouse models, it was determined that TBZ improve motor symptoms and reduce striatal neuronal cell loss [4].

Targeting of Caspase Activities

Minocycline, known as a tetracycline analog, plays a role in inhibiting caspase-3 and caspase-1 expression by crossing the blood–brain barrier. In patients treated with minocycline, it was determined that neuroprotective effect occurred and improvement was observed in disease phenotype. In a trial study conducted with human, motor and cognitive improvements were observed in HD patients who received 100 mg of Minocycline for 6 months. In another pilot study, motor and cognitive improvements were observed in HD patients applying Minocycline treatment for 6 months [4].

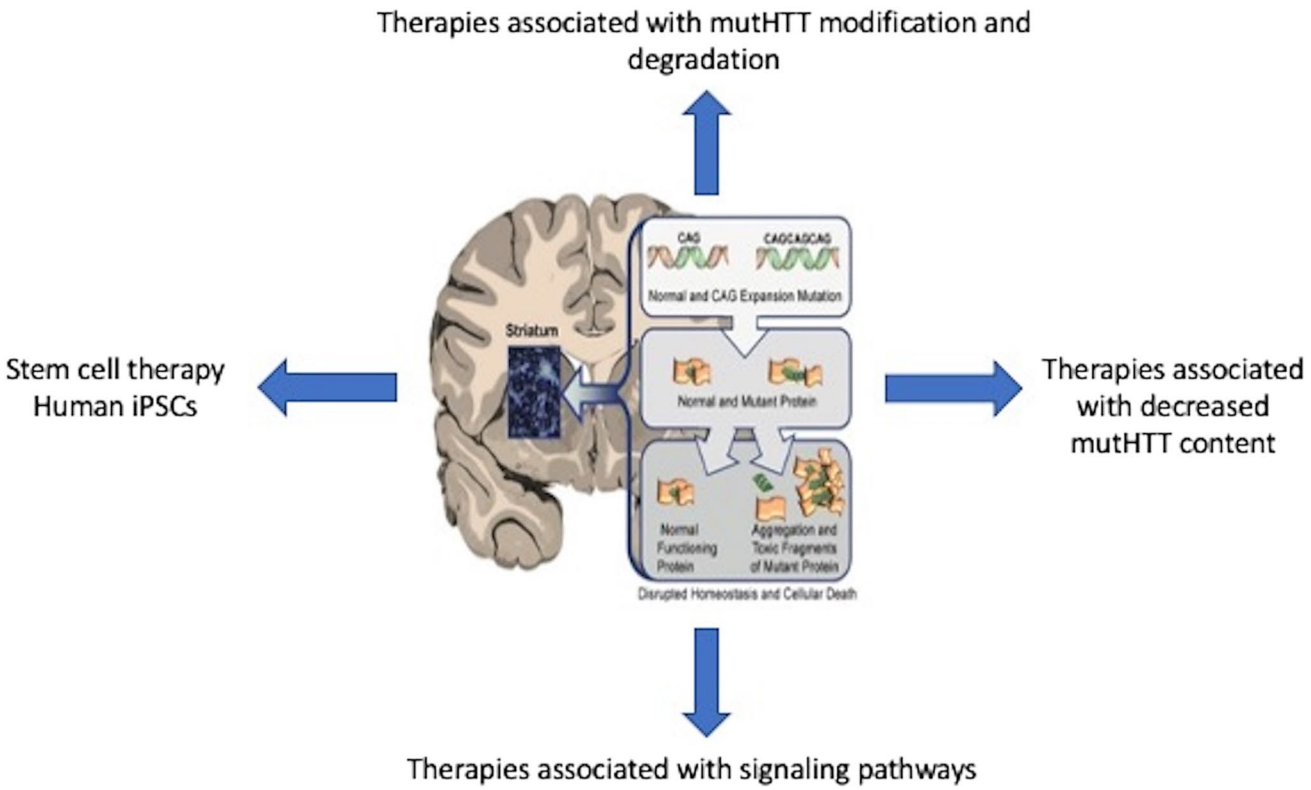


Fig. 1 Current treatment approaches for HD [19]

Targeting of HTT Aggregation and Clearance

It is known that dye called as Congo Red bind to beta layers containing amyloid fibrils. When this dye is injected into mice with HD, it has been determined that the dye is effective in the recovery of motor functions by preserving normal protein synthesis and degradation. The dye also supports the clearance of enlarged polyQ repeats, thereby prevents polyglutamine oligomer formation. Besides these, there are also studies showing that this dye prevents ATP exhaustion and caspase activation. Trehalase disaccharide is effective in inhibiting the formation of nuclear residues. In studies carried out with mice, it has been reported that modified motor functions improve. The C2-8 compound, which inhibits polyglutamine aggregates in brain slices and cell cultures, may reduce the amount of neuronal atrophy by improving motor functions [4]. The mammalian target of rapamycin (mTOR), which phosphorylates many proteins, is an important protein kinase and plays a role in various cellular functions such as autophagy and transcription. It is known that mTOR interacts with mHTT. As a result of this interaction, mTOR activity, autophagy and mHTT clearance decrease. mTOR phosphorylates S6K1, an important regulator of cell volume. mTOR disorder, which is known to be associated with mHTT, is an important factor in explaining brain atrophy in HD. In some studies in HD mouse models, it has been determined that Rapamycin develops motor performance and decreases striatal neuropathology [21–23].

Targeting of Mitochondrial Dysfunction

In previous studies, creatinine which has antioxidant properties decreases serum 3-hydroxy-2-deoxyguanosine levels. In addition, it was determined that creatine administration delays the functional decrease in the early emergence of HD. In another study, it has been reported that creatine treatment administered at a certain dose, improved muscle function capacity in neuromuscular diseases. However, there is also a study showing it was not improved in the I–III stages in HD, in the cognitive conditions and neuromuscular functions. It has been determined that Coenzyme Q cofactor, which plays a role in ATP production in the electron transport chain of mitochondria, improves mitochondrial functions in HD patients [24]. In another study performed with mice, it has been detected that coenzyme Q provides neuroprotective effect and delays motor deficiency, atrophy, inclusion [4]. However, coenzyme Q was not effective in phase II randomized clinical trials [25]. Ethyl-Eicosapentaenoic Acid (EPA) is known as a derivative of EPA that binds to the peroxisome proliferator-activated mitochondria receptor [26]. It is thought that Ethyl-EPA be able to reduce mitochondrial damage by inhibiting caspase, thereby improving neuronal function [4]. However, in a study conducted with stage III HD patients, significant difference was not determined between Ethyl-EPA and placebo on the total motor score 4 scale [27]. It has been detected that a significant improvement in this scale in patients with fewer CAG repeats. In a phase III randomized control trial, it was determined that Ethyl-EPA did not improve cognitive functions [28]. It has been determined that cystamine and 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) blockers increase the survival effect in HD cells by preventing oxidative damage [29]. It has also been shown that Meclizine, an antihistamine drug showing neuroprotective effect in the Drosophila model, inhibits oxidative metabolism and apoptosis [4].

Targeting of Transcriptional Dysregulation

In a study performed with N171-82Q symptomatic mice, when sodium phenylbutyrate was administered to these mice, less brain atrophy has been observed. It has been determined that sodium phenylbutyrate is effective in regulating caspases that play a role in apoptosis. In studies conducted with HD mouse models, DACi4b known as Histone deacetylase (HDAC) inhibitor, has been also determined to reduce neurodegeneration by improving motor impairment. There are also studies showing that HDACi4b regulates mRNA expression. In studies conducted with transgenic mice, it was determined that histone acetylation in the brain increased with Suberoylanilide Hydroxamic Acid (SAHA) and thus motor disorders were improved. Mitrahramycin and Chromomycin treatments known as anthracycline derivatives form the basis for clinical studies for HD. It has been reported that these derivatives support epigenetic histone modifications in transgenic cell lines [4].

Targeting of mHTT

Modified ASO, known as Peptide Nucleic Acid (PNA), selectively recognizes the mutant allele. In addition, it has been determined that ASO selectively inhibit mHTT expression in human fibroblasts [30]. RNAi, which plays a role in the development of motor behavior, may play an effective role in HD treatment by reducing neuropathy. In various studies conducted with transgenic mice with HD using intrabodies and artificial peptides, it has been determined that they play a role in the development of motor and cognitive functions [4].

Therapeutic Approaches Targeting DNA

DNA targeting approaches include ZFNs, TALENs, and CRISPR/Cas9 approaches. Each of these approaches has different mechanisms in terms of DNA binding and modes of action. Zinc finger Proteins (ZFPs), one of the highest protein groups play an important role in the regulation

of DNA, RNA and protein functions. They may be connected to specific DNA sequences using therapeutic compounds. ZFPs that do not affect wild-type HTT expression reduce mHTT expression. CRISPR/Cas9, which is associated with viral defense mechanisms of bacteria that recognize and destroy foreign DNA, plays a role in excising CAG repeats to silence mHTT expression. In studies performed with mice, it has been shown that the CRISPR/Cas9 system may be applied in improving motor functions by reducing mHTT [4].

Therapeutic approaches targeting HTT DNA modulate gene transcription and they are effective in genome regulation by modifying the HTT gene directly. In approaches towards DNA targeting, a specific form of DNA binding element is used, combined with effector elements such as nucleases, epigenetic modulators or transcription factors. While DNA binding elements ensure productive and precise DNA targeting, effector elements alter gene sequence or expression. Nuclease effectors are considered as genomic scissors. These effectors separate the targeted DNA to produce double strand break that stimulates the endogenous DNA repair mechanisms of the cell. They then repair DNA damage using repair mechanisms [31].

Genome Editing Systems

In gene editing prosess, a double-strand break (DSB) is produced in the targeted DNA sequence. Four important nuclease systems are used for the induction of DSBs. ZFNs, TALENs, meganucleases and CRISPR/Cas9 nuclease systems are found among these nuclease regulation systems (Fig. 2) [32–34]. In the administration of the CRISPR/Cas9 approach, a sgRNA sequence complementary to any desired target region is designed and Cas9 nuclease is directed to this region. Unlike this system ZFNs, TALENs and meganuclease systems are based on the production of a new protein specific to the individual target DNA sequence. In CRISPR/Cas9 system that only at the same time multiple sgRNA can be used multiplex gene changes are possible [33].

Genome editing is a technology that involves the formation of genome modifications at specific regions in the genomes of living organisms. These genome editing technologies are based on engineered endonucleases and these nucleases consist of sequence-specific DNA binding domains [35]. Genome editing systems are developed from prokaryotic systems so that relevant genes can be specially modified [18]. ZFN, TALEN and CRISPR/Cas are

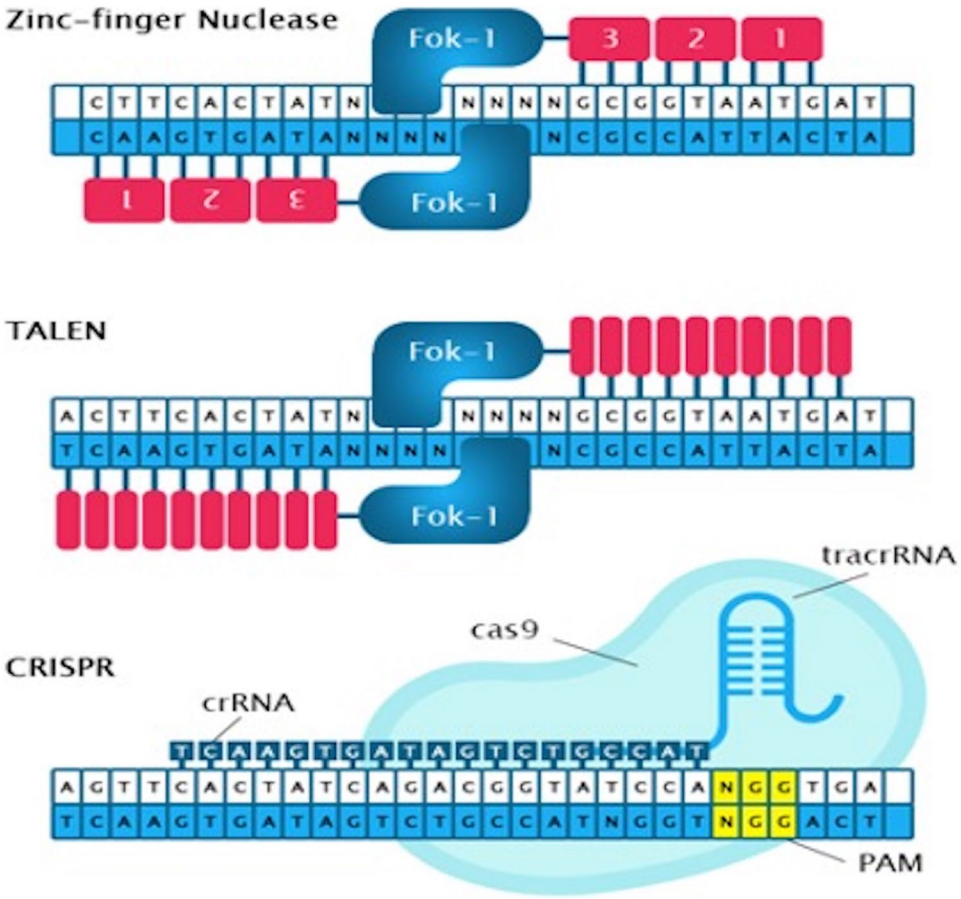


Fig. 2 ZFN, TALEN and CRISPR Genome editing approaches [34]

commonly used genome editing tools. These tools cover processes such as cell and nucleus entry, transcription and post-process alteration, gene expression alteration [36]. ZFN and TALEN are the first systems that are determined and applied in gene therapy. In these systems, a target sequence is recognized and separated via designed site-specific nucleases. These nucleases have protein recognition domains, and these domains are designed to potentially recognize any sequence of the genome. CRISPR/Cas9, another genome-editing system, is characterized by regular intermittent clustered short palindromic repeats derived from the bacterial immune system, and this system is RNA-guided nuclease (RGEN), which can provide precise modification of genes by recognizing DNA DSBs. ZFN and TALEN may utilize protein-DNA interactions to recognize the target genomic DNA. However, RGENs use synthetic guide Protospacer adjecent motif (PAM). Since CRISPR/Cas9 can target more than one gene at the same time, it has an easier design than other systems [18]. The major advantage of the CRISPR system is the ability to target more sites using a Cas 9 protein and multiple sgRNAs. Moreover, the efficiency of the method is due to the fact that it diverts uniform expression of each sgRNA using a single co-promoter [36].

CRISPR system is also a system that may be widely used in genome editing, gene expression, epigenetic regulation and cell imaging. In gene editing administrations, a PAM must follow the target sequence (for spCas9 protein) and, PAM consists of NGG/NAG nucleotides. sgRNA consists of approximately 20 nucleotides targeting the gene and a scaffold RNA domain that can interact with the nuclease. sgRNA directs Cas9 nuclease to the target sequence [18].

It is known that genome editing systems are used in the treatment of diseases in recent years. The use of these systems enables the study of the functions of genes and regulatory sequences that will facilitate the knock-out of target locus. In addition, hereditary disease models are constituted using these systems and may develop new methods for the treatment of these diseases. These systems have various structures that mediate some important functional parameters [3].

ZFNs

ZFN is a chimeric nuclease and consists of two parts. The first part consists of 3–4 zinc finger domains. Nuclease domain of endonuclease Fok-1 is known as the second part. Nuclease domains may form double chain breakage after dimerization of two ZFN subunits close together, and these nuclease domains are the same [37, 38]. The transcriptional repressor protein or specific nuclease is connected to the DNA-binding element consisting of a series of multiple zinc-finger peptides. Each zinc-finger can interconnect 3 or 5 different nucleotide sequences of the DNA strand [39]. Zinc-finger proteins do not have nuclease activities and ZFPs only can bind to DNA and reduce gene expression levels. Thus, gene transcription is also prevented. ZFPs are designed to be selectively linked to extended CAG repeats. Thus, they can specifically connect to the mHTT gene. Zinc-finger transcriptional repressor approaches can reduce mHTT levels by targeting without changing the DNA. In contrast, direct genome editing approaches, such as CRISPR/Cas9, may permanently correct CAG expansion causing disease in HD by disrupting or correcting the mutant gene. ZFNs use the nuclease effector domain linked to a DNA recognition domain, and TALENs are similar to ZFNs [3].

TALENs

TALENs consist of an artificial DNA binding domain and the DNA cleavage domain of endonuclease Fok-1, such as ZFN. DNA binding domains consist of monomers containing consecutive repeats of 34 amino acids. These monomers that localized at 12 and 13 positions are those with variable sequence repetition and they are responsible for recognizing the nucleotide. There are Repeat Variable Diresidues (RVD) in the target DNA region that bind the A, T, G, C nucleotides, respectively. It is known that the DNA binding domains of the two subunits are known to be associated with DNA chains, and the C-terminal Fok-1 domains dimerize and divide the target DNA region [40]. It is known that TALEN has higher effectivity and specificity than ZFN. However, TALEN requires T in the 5' end of the target region which limits the options of the target sequences. TALEN uses a number of specific amino acid repeats that bind to a specific nucleotide. Different combinations of amino acid repeats are produced to recognize the specific DNA sequences. The TALEN-based approach has higher efficiency and specificity than the ZFN-based approach. However, TALENs require a specific nucleotide and this nucleotide may limit targets at the end of the DNA sequence [3]. In studies conducted in HD fibroblasts using the TALEN-based approach, a reduction was determined in mHTT expression and aggregation. It has been shown that it is possible for allele-specific HTT gene modification using the TALEN-based approach [8].

CRISPR/Cas9 Approach

The CRISPR/Cas9 system can target specifically any region in the genome. This system, which can lead to undesirabe changes in non-target areas, enables genome editing with high efficiency. Genomic imbalance may develop as a result of the emergence of non-target effects. Genomic imbalance can impair the functionality of normal genes. Therefore, a new modified Cas9 nuclease is created. Cas9 specificity has been developed with translocations in the

Cas9 sequence. These displacements cause some hydrogen bonds to block between Cas9 and the target DNA chain. As a result of these modifications, the energy of the Cas9-sgRNA complex change, thereby off-target binding and off-target effects reduce [3]. CRISPR is a commonly used method as genome editing technology in basic biomedical research. This technology which is used in the research and treatment of human diseases, is applied to correct DNA mutations that can cause disease in cell and animal models. CRISPR/Cas9 gene regulation, besides the treatment of genetic diseases, it is also used in immunology-focused therapies to support AIDS treatment or anti-tumor immunotherapy. The CRISPR/Cas9 system acts as an adaptive immune defense based on the destruction of viral pathogens by cutting of the target DNA with Cas nucleases at the end of the CRISPR sequences. Cas nucleases have been made specific to the pathogen by the specific feature of the enzyme required for an RNA guide that activates the enzyme and selectively targets the nuclease complementary DNA sequences. Cas nucleases are applied as high quality nuclease in this respect and DNA breaks or notches can be produced in any region of genomic DNA in vivo by these nucleases. The specific CRISPR/Cas9 system has two basic components as Cas9 nuclease and the required sgRNA. The function of sgRNA is to determine the specificity of a target DNA sequence by linking to complementary DNA sequences through the base pair. Cas9 co-localizes in the same specific region by connecting of sgRNA, it causes in cuts and DSB formation in the DNA backbone. sgRNA and Cas9 are introduced to the cells through vectors that depends on administration using recombinant DNA technology [33]. CRISPR/Cas9 system is an approach that recognizes and destroys foreign DNA and forms the basis of the bacterial immune system [41].

In the CRISPR/Cas9 approach, the Cas9 protein is known as an RNA-guided nuclease, which separates double strand breaks in certain DNA regions. In this approach, Cas9 nuclease does not use a protein-based DNA recognition domain. To target specific regions of DNA, Cas9 protein is directed by specific guide RNAs. In gene editing administrations, it should be followed by a specific recognition region known as the PAM sequence. PAM usually contains 2 or 5 nucleotides and it consists of PAM, NGG or NAG nucleotides for the first protein used in gene editing [42]. Cas9 nuclease forms are combined with synthetic guide RNAs so ribonucleoprotein structures (RNP) can be produced that can be targeted to selected DNA regions [31]. The CRISPR/Cas9 approach, alter the HTT gene encoding without permanent genome modification and it is an effective therapeutic approach for HD (Fig. 3) [3, 43, 44]. The CRISPR/Cas9 system is also used to inactivate mHTT genes that target genetic variations associated with the CAG-expanded allele in fibroblasts derived from patients. Thus, a total decrease occurs in RNA and mHTT protein [45]. It has been showed that using this method, mHTT expression in the brain inactivated in mouse models targeting genetic variations associated with CAG expansion and in differentiated iPSCs in humans [46, 47].

Applications of CRISPR/Cas 9 System

CRISPR/Cas 9 system applications are classified as research applications, therapeutic applications, and diagnostic applications. The CRISPR/Cas 9 system has been applied in genetic studies, the production of cellular models, and the production of animal models. Therapeutic gene editing or gene therapy is used for various diseases such as

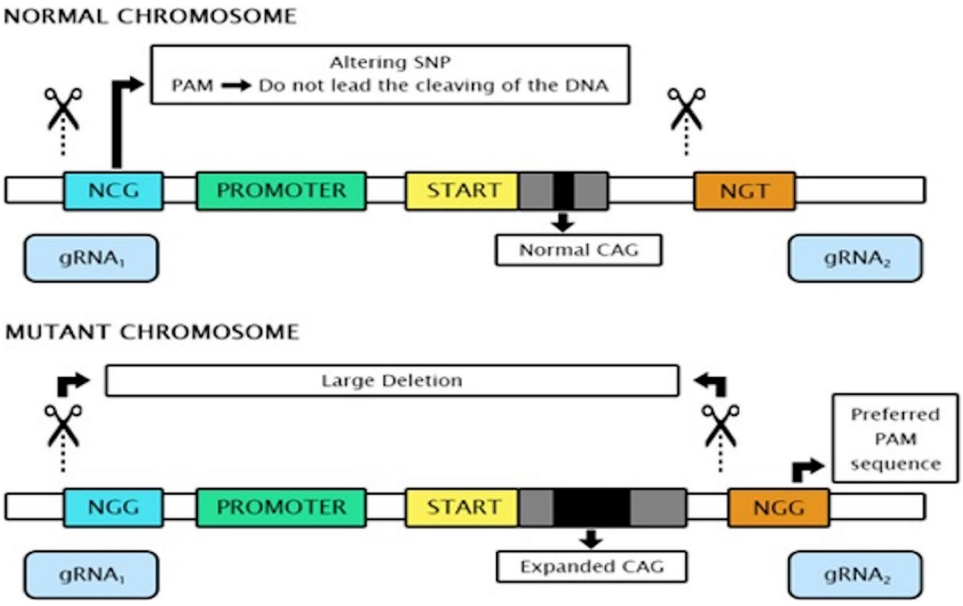


Fig. 3 CRISPR/Cas9 approach to reduction and permanent inactivation of HD [43]

cardiovascular disorders, hematological diseases, different types of cancer and neurodegenerative disorders. These approaches are accomplished by ex vivo manipulation of cells or in vivo delivery of genome editing tools. In addition, the CRISPR/Cas 9 system is used as an important diagnostic tool for microbial diseases and other diseases. CRISPR-based nucleic acid detection methods are applied in the diagnosis of COVID-19. This system is also applied in the detection of viral, fungal and bacterial pathogens, in the diagnosis of infectious diseases and various types of cancer [48].

DNA Repair Pathways in Genom Editing Systems

Cellular mechanisms repair the defect after Cas9-mediated DNA cleavage. These cellular mechanisms are classified as nonhomologous end joining (NHEJ) and homologous-directed repair (HDR). NHEJ, which is more prone to error, is usually characterized by insertion or deletion (INDEL) mutations (Fig. 4) [18, 49, 50]. When sgRNA targets 5' end of a coding sequence, it causes frame shift and early stop codon. Thus, the functional impairment of the gene occurs. HDR system is a weakly active system in postmitotic cells. In this system, endogenous or exogenous DNA templates are used around the target sequence to repair DSB [18]. The HDR mechanism uses another DNA sequence to correct the fragmentation. In genome editing applications, exogenous DNA sequence with a desired sequence is formed by using nuclease. Then the new DNA sequence is introduced directly to the target area and repair is provided [31].

Stem Cells in Regenerative Medicine

Regenerative medicine is a therapeutic approach using stem cells. The aim of regenerative medicine is to restore the functions of injured and affected cells, tissues, and organs. This therapeutic approach is integrated with CRISPR/Cas technology. Various diseases may be treated with cell-based therapeutic strategies. In these cell-based therapies, single cell suspensions of stem cells are cultured and these cells are delivered to the target specific organ by direct injection. Stem cells are unique cells that can transform into specific cell types and regenerate damaged tissues. Induced pluripotent stem cells (iPSCs), one of the stem cells that form the basis of regenerative medicine, are used in various disorders such as organ failures, spinal cord injuries, skin disorders, neurological damages. CRISPR/Cas systems result in applications based on iPSC stem cells. iPSC stem cell sources through the CRISPR/Cas system are used in the production of various regenerative drugs. iPSCs are known as patient-specific pluripotent stem cells and play an important role in therapeutic applications of neurodegenerative disorders. CRISPR/Cas 9 genome editing technology allows editing the genome of stem cells using modified stem cells as regenerative drugs in therapeutic applications [51]. The CRISPR/Cas 9 system is also used for live cell imaging of genomic loci and for monitoring RNA in living cells [52].

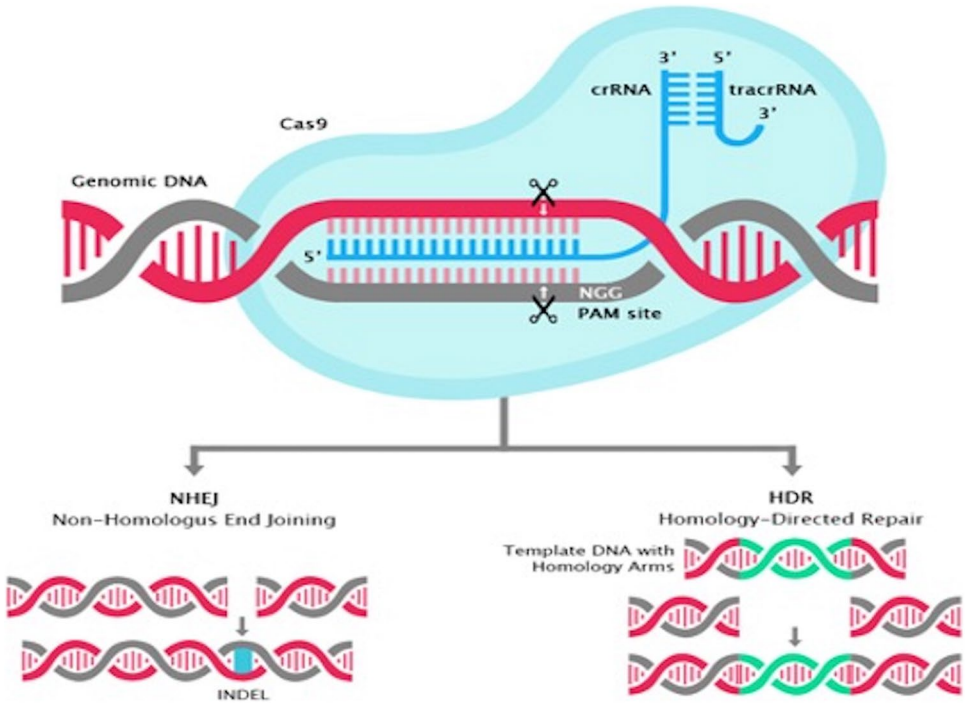


Fig. 4 DNA repair mechanisms in gene editing systems [49]

Huntington's Disease Treatment Approach in Cell and Animal Models

CRISPR/Cas9 approach is applied to individuals with specific genetic diseases in the treatment of the disease through the production and characterization of patient-derived iPSCs derived from these individuals. The production of human iPSCs derived from the disease occur through advanced translational research. It was known that iPSCs are used in in vitro modeling in neurodegenerative disorders such as Alzheimer's disease, Parkinson's Disease, and Huntington's disease. The CRISPR/Cas9 system is also used in the modeling of human diseases in vivo and in the production of genome-regulated animals carrying genetic mutations responsible for human diseases. These models are very important in defining the pathology of the diseases and thus in developing the treatments for the diseases. In CRISPR/Cas9-mediated gene editing therapy, it is aimed to correct the mutations causing diseases in vivo [33].

Features of iPSC

iPSCs are similar to embryonic stem cells in terms of self-renewal and pluripotent properties. They are obtained from virus-mediated through induction from human iPSC fibroblasts. It has been reported that HD-iPSC line with different CAG repeat lengths and control iPSC lines has been created in HTT [10]. iPSCs derived from patients can be differentiated into disease-related neurons, thereby therapeutic strategies are developed by in vitro modeling. iPSCs derived from patients can be used for modeling of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [2]. It was determined that cells derived from HD patients show cellular changes, protein expression changes and mitochondrial dysfunction compared to control cell lines [10]. Patient somatic cells can be reprogrammed to iPSCs so that disease-related cells can be produced in in vitro disease modeling. Human iPSCs can differentiate into any cell type in the human body. Therefore, these cells may play a role in genetic variations associated with pathogenesis. iPSCs can be used in human disease models such as developmental and adulthood diseases. The use of these cells can be in the form of two-dimensional (2D) cell cultures or three-dimensional (3D) organoids. Cells derived from patient iPSCs are used to determine the phenotypes of various human neurodegenerative diseases. In addition, phenotype-based drug screening can be performed on disease target cells produced from iPSCs. Gene editing technologies are combined with derived iPSCs from patient. Thus, a series of genetically identifiable human iPSC lines can be created for disease modeling. iPSCs have organizational ability themselves. These capabilities are extremely important for progenitor region organization, neurogenesis, and gene expression [2].

iPSC-based Disease Modelling in Huntington's Disease

HD, which is an autosomal dominant, monogenic, fatal, progressive neurodegenerative disease, is characterized by exonic CAG repeats in the HTT gene. The extended CAG repeats encode a polyglutamine region. CAG repeats cause a toxic function gain and lead to preferential deaths of GABAergic projection neurons in the striatum. It is known that HD symptoms occur in middle age. Although there is a specific treatment for HD, there is generally no available treatment [53]. It is known that the main sensitive cell type in HD is MSNs. Human iPSCs are used to form the MSNs. For HD modeling, it is possible to form MSNs from iPSCs derived from HD patients. Thus, HD-related phenotypes such as neuronal degeneration and mHTT protein aggregation can be determined in nerves derived from HD-iPSCs. There are studies showing that the formation of mHTT aggregates in HD-iPSC-derived neurons is rare. Better in vitro HD modeling can be performed by creating isogenic HD-iPSC pairs. HD-iPSCs can provide to improving of CAG locus in HTT gene (Fig. 5) [3, 54]. Recovery of disease phenotypes such as mitochondrial abnormalities can be achieved by neurons derived from isogenic lines. In addition, the production of genetically corrected isogenic lines for in vitro neuronal induction may be carried out using the CRISPR/Cas9 system. In various studies, neurons derived from iPSCs are used to investigate HD pathogenesis and develop effective therapeutic strategies for HD [2].

CRISPR/Cas9 Approach with iPSC for HTT Gene Editing

In a previous study, it has been shown that HD-iPSCs can be genetically correctable. In this study, cellular HDR system was used together with antibiotic selection. There are also studies showing that some of the iPSC clones can be corrected after antibiotic selection using a pair of sgRNA and Cas9 D10A nickase. Wild-type Cas9 protein catalyzes sgRNA-targeted DSBs. In contrast, Cas9 D10A nickase create sgRNA-targeted single-strand break in DNA. Therefore, it is thought that Cas9 D10A shows improved target sequence specificity. In another study, it was found that the HTT allele carrying the CAG expansion was selectively inactivated by CRISPR/Cas9 mediated excision in fibroblasts derived from HD patients. Suppression of non-target

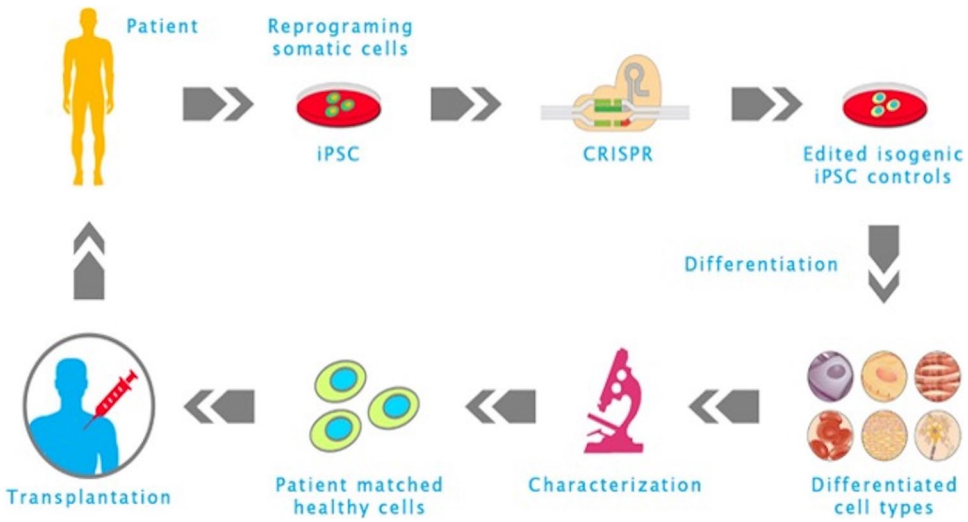


Fig. 5 CRISPR gene editing with human iPSCs [54]

effects in non-consensus areas should be ensured in order for CRISPR/Cas9-mediated gene therapy to be widely used in the clinical environment [10].

Conclusion

HD is an inherited neurodegenerative disease and degrades motor and cognitive functions by targeting striatal MSNs. As a result of the expansion of the PolyQ pathway, the mutHTT protein becomes toxic to neurons. Therapeutic strategies are being investigated in various preclinical models and clinical trials. These therapeutic strategies have been associated with a reduction in mutHTT content at the level of genome, mRNA, or protein degradation. There are also therapeutic strategies associated with post-translational modification of mutHTT. In addition to these, stem cell therapy may also be used due to the loss of striatal neurons. As a result of these clinical studies, important biomarkers may be obtained for the treatment of HD disease. Comprehensive modeling and analysis of human diseases at cellular and molecular levels are possible by developing new technologies for gene editing. The ZFN, TALEN, CRISPR/Cas9 approaches reveal different modifications. These systems regulate gene expression in different cell types, such as iPSCs and gene sequences. These systems, which are used to create isogenic cell lines carrying different numbers of CAG repeats, can also be used to correct mutations that cause HD. In recent years, many studies have been carried out to modeling molecular mechanisms and genetic diseases. ZFN and TALEN are the first artificial nuclease. These systems are simpler than CRISPR system. Human iPSCs are used to create cell types associated with diseases to investigate the mechanisms underlying human diseases. In order to explain cellular progression for neurodegeneration in vitro, iPSCs derived from the patient can spread to specific neuronal subtypes and can be differentiated effectively. Differentiated iPSCs can be used in various neurodegenerative disease modeling such as Alzheimer's disease, Parkinsons disease, and Huntington's disease. Many obstacles must be overcome in order for iPSC-based technology to be used in clinical administrations. However, the combination of iPSC technology with genome editing technology will enable the development of new treatments and drugs for human neurodegenerative diseases.

Author Contribution SSA and NA conceived the idea for the article. SSA, NA, AA and IA searched the literature. SSA and NA wrote the manuscript. SSA created the figures. All authors read and approved the final manuscript. The corresponding author attests that all listed authors meet the authorship criteria and that no other authors meeting the criteria have been omitted.

Data Availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Consent for Publication The author, SSA, NA, AA and IA have read and approved the final manuscript for submission. We confirm the figures are original for this article.

Conflict of Interest The authors declare no competing interests.

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