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Original article

The Advances in Gene Therapy Research for Huntington's Disease

Joey Sun [1,2], Frank Fan [2]

[1]Dougherty Valley High School, San Ramon, CA 94582.
[2]Association of Applied Life Sciences, San Jose, CA 95131.

*Corresponding to Frank Fan at frank@biolifevalley.com

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Abstracts

Huntington's Disease (HD) is a debilitating neurodegenerative disorder characterized by progressive motor dysfunction, cognitive decline, and psychiatric symptoms, resulting from the expansion of CAG repeats in the HTT gene. The aggregation of mutant huntingtin (mHTT) protein is central to HD pathogenesis, leading to widespread neuronal dysfunction and eventual cell death. Advances in in vitro modeling and drug screening methodologies have significantly contributed to understanding and potentially mitigating HD's effects. In vitro models, particularly those involving induced pluripotent stem cells (iPSCs), enable detailed exploration of HD's molecular mechanisms, facilitating the development of targeted therapeutic strategies. The integration of these approaches, alongside comprehensive timelines detailing the disease's progression and intervention windows, is summarized in this review in the light of providing systematic info for advancing the development of effective treatments.

Keywords: ***Huntington's Disease, mutant huntingtin (mHTT), in vitro models, induced pluripotent stem cells (iPSCs), high-throughput drug screening, neurodegeneration, autophagy.***

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive motor dysfunction, cognitive decline, and psychiatric disturbances. The genetic basis of HD is a CAG trinucleotide repeat expansion in the huntingtin (HTT) gene, which encodes the Huntingtin protein. When the CAG repeat exceeds 35 units, it results in an abnormal protein with an expanded polyglutamine tract, leading to widespread cellular degeneration, particularly of the medium spiny neurons in the caudate nucleus and putamen of the brain. This neuronal loss underlies the hallmark symptoms of HD, including chorea, dystonia, and other motor deficits, as well as significant cognitive and behavioral changes. The pathophysiology of HD involves multiple mechanisms, including excitotoxicity, mitochondrial dysfunction, transcriptional dysregulation, disrupted proteostasis, and neuroinflammation. These complex interactions culminate in the progressive deterioration of the brain's basal ganglia and cortical structures, manifesting clinically with involuntary movements, impaired voluntary motor control, psychiatric symptoms such as depression and irritability, and cognitive impairments that often progress to dementia. Despite significant research efforts, there is still no cure available [1].

Gene therapy is a revolutionary medical technique aimed at treating or preventing diseases by modifying the genetic material within a patient's cells. This involves introducing functional genes to replace defective ones, silencing harmful genes through methods like RNA interference (RNAi) or antisense oligonucleotides (ASOs), or using precise gene-editing technologies such as CRISPR/Cas9. Delivery of therapeutic genes is typically achieved through viral vectors like adenoviruses, adeno-associated viruses (AAVs), lentiviruses, or non-viral methods including liposomes and nanoparticles. Gene therapy shows immense promise for treating monogenic disorders such as cystic fibrosis and hemophilia, various cancers, infectious diseases like HIV, and neurological disorders such as Huntington's disease. Despite challenges in delivery efficiency, long-term expression, safety, and ethical concerns particularly regarding germline editing advancements in gene-editing technologies and delivery methods continue to drive the field forward (**Figure 1**). As research progresses, gene therapy offers the potential to cure previously untreatable conditions, transforming the future of medicine [1-3].

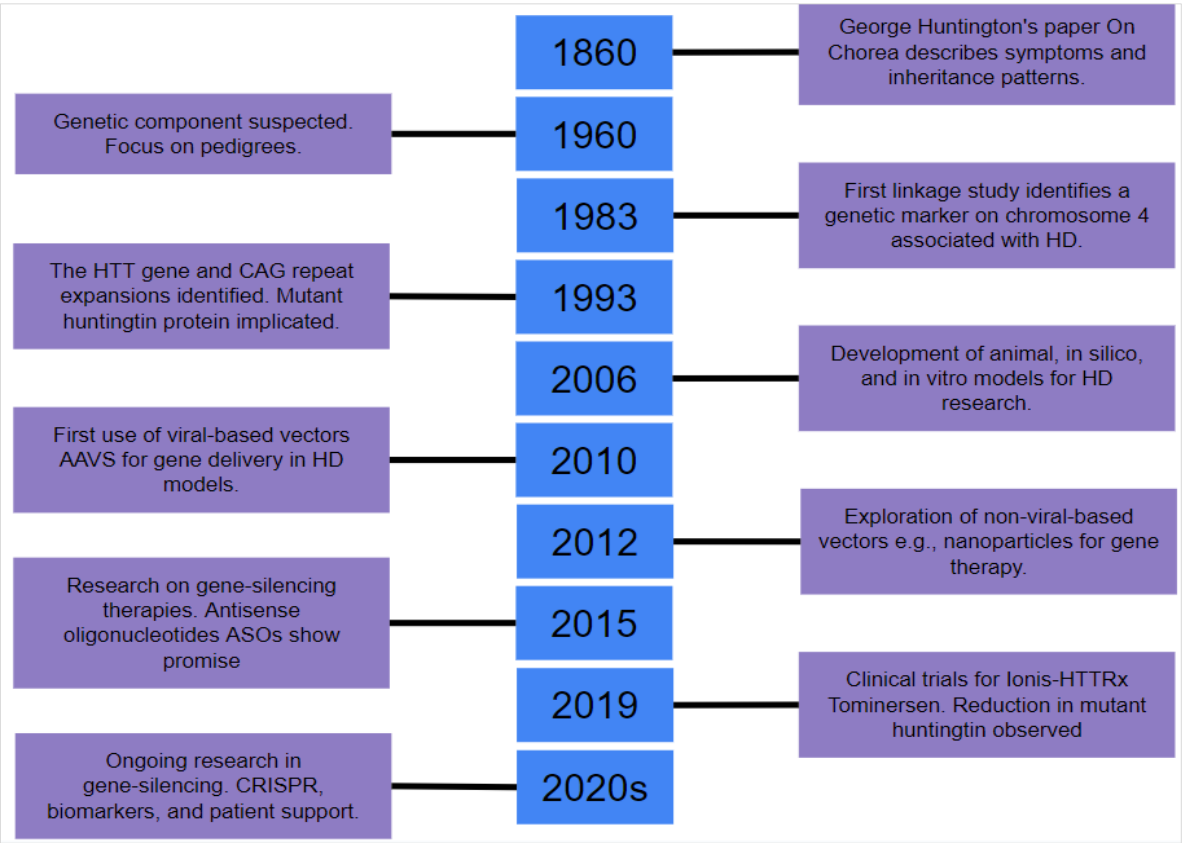

Figure 1. Milestones in the HD pathogenesis and treatment development.

Over the past years, different gene therapy methods have been tried for HD treatment, achieving significant progress. Gene therapy in HD focuses on addressing the root genetic cause by modifying or silencing the mutant Huntington (mHTT) gene responsible for the disorder. Various strategies are being explored, including the use of CRISPR/Cas9 to edit the HTT gene directly, ASOs to reduce the expression of mutant Huntingtin, and RNAi techniques to silence the harmful gene. Additionally, gene therapy approaches involve converting striatal astrocytes into GABAergic neurons to compensate for neuronal loss [1,3]. In this review, these different methods, viral vectors and non-viral vectors for gene delivery, and CRISPR-based editing, were discussed. In addition, the challenges, such as delivery efficiency, long-term expression, safety concerns, and ethical considerations, along with the promising advancements that could make gene therapy a viable treatment for HD in the future, were also discussed.

1. Mechanisms of Pathogenesis for HD

1.1. The genetics root of HD

The expanded CAG repeats in the HTT gene lead to an elongated polyglutamine tract in the Huntingtin protein, which is the hallmark of HD. This abnormal protein structure promotes its misfolding and aggregation within cells, forming insoluble intracellular inclusions that disrupt normal cellular functions. These aggregates interfere with transcriptional regulation, protein degradation pathways such as the ubiquitin-proteasome system (UPS) and autophagy, and mitochondrial function. The resulting cellular dysfunction and eventual neuronal death are central to the pathology of HD [4,5].

1.2. Oxidative Stress role in HD development

Oxidative stress plays a crucial role in the progression of Huntington's disease, exacerbated by mitochondrial dysfunction caused by mHTT. Mitochondria in HD neurons produce higher levels of reactive oxygen species (ROS) due to impaired electron transport chain function and calcium dysregulation. These ROS cause oxidative damage to cellular components, including lipids, proteins, and DNA, leading to neuronal dysfunction and eventual cell death [6,7].

1.3. Excitotoxicity roles in HD progress

Excitotoxicity, resulting from the overactivation of glutamate receptors, is another critical mechanism contributing to neuronal degeneration in HD. Excessive glutamate release leads to sustained calcium influx into neurons, triggering mitochondrial dysfunction, energy depletion, and activation of apoptotic pathways. This cascade of events culminates in neuronal death, particularly affecting medium spiny neurons in the striatum, a characteristic feature of HD pathology [8].

1.4. Mitochondrial Dysfunction in HD

Mitochondrial dysfunction is a hallmark of Huntington's disease, characterized by impaired mitochondrial biogenesis, dynamics, and energy metabolism. Mutant Huntingtin disrupts mitochondrial fusion and fission processes, leading to fragmented mitochondria and compromised ATP production. This energy deficit exacerbates neuronal vulnerability to stress and contributes to the progressive neurodegeneration observed in HD [6,9].

1.5. Neuroglial Dysfunction

Neuroinflammation mediated by activated astrocytes and microglia is increasingly recognized as a significant contributor to HD pathogenesis. In response to mutant Huntingtin aggregates and neuronal injury, glial cells release pro-inflammatory cytokines, chemokines, and reactive oxygen species, creating a chronic inflammatory milieu that further exacerbates neuronal damage and accelerates disease progression [10].

1.6. Protein Aggregation and Proteostasis

Impairment of proteostasis mechanisms, including the UPS and autophagy, is central to the accumulation of misfolded proteins in Huntington's disease. Mutant Huntingtin disrupts protein clearance pathways, leading to the aggregation of toxic protein species that interfere with cellular function and contribute to neuronal dysfunction and death [11,12].

2. Gene Therapy Strategies for HD

2.1. Allele-Specific Silencing for HD Therapy

Allele-specific silencing approaches, such as ASOs and RNA interference (RNAi), selectively target the mutant HTT mRNA while sparing the normal allele. By binding to specific sequences in the mutant mRNA, ASOs promote its degradation, thereby reducing the synthesis of mutant Huntingtin protein. This targeted approach has shown promise in preclinical studies by effectively lowering mutant HTT levels without affecting the normal Huntingtin protein, potentially slowing disease progression [13,14].

2.2. CRISPR/Cas9 application in HD therapy

The CRISPR/Cas9 gene editing system offers a versatile platform for precise modification of the HTT gene. By introducing double-strand breaks at specific locations within the HTT gene, CRISPR/Cas9 can induce gene knockout or correction of the CAG repeat expansion. Recent advancements in CRISPR technology, such as base editing and prime editing, provide even more precise tools for therapeutic genome editing in Huntington's disease [3,15].

2.3. Astrocyte Conversion for HD treatment

Gene therapy strategies aimed at converting striatal astrocytes into functional GABAergic neurons represent a promising approach to replace lost neurons and restore neurotransmitter balance in HD. This innovative method involves reprogramming astrocytes using specific transcription factors or viral vectors to induce neuronal differentiation, thereby improving motor function and neuronal survival in preclinical models [16,17].

2.4. Stem Cell Therapy for HD

Stem cell-based therapies hold the potential for treating Huntington's disease by replacing damaged neurons and providing neurotrophic support. Engineered stem cells, such as mesenchymal stem cells (MSCs) or induced pluripotent stem cells (iPSCs), can deliver therapeutic genes or factors like brain-derived neurotrophic factor (BDNF) to promote neuronal survival and function. These approaches are currently being investigated in animal models and early-phase clinical trials for their efficacy and safety in HD treatment [18,19].

3. In Silico Modeling and Computational Biology

3.1. Molecular Dynamics Simulations for Therapeutic Targets Prediction

Molecular dynamics simulations are pivotal in exploring the structural dynamics of mutant Huntington (mHTT) and its interactions with cellular proteins. These simulations provide insights into the folding and aggregation behavior of the polyglutamine tract, aiding in the identification of potential therapeutic targets. Additionally, simulations have been used to understand how mHTT interacts with other proteins involved in cellular processes, offering clues for therapeutic intervention [20,21].

3.2. Bioinformatics application in HD models for biomarkers and therapeutic targets identification

Bioinformatics analysis integrates large datasets from genomic, transcriptomic, and proteomic studies to uncover disrupted pathways in Huntington's disease (HD). The role of bioinformatics tools in constructing comprehensive models of HD pathogenesis, which includes identifying potential biomarkers and therapeutic targets. Furthermore, such analyses can reveal correlations between genetic variations and clinical phenotypes, aiding in the development of personalized treatment strategies for HD [21,22].

3.3. In Silico Screening for HD Drug Development

In silico screening methods facilitate the rapid identification of small molecules and natural products that can modulate the activity of mHTT or its downstream effects. Virtual screening approaches have been instrumental in identifying potential drug candidates for HD therapy, which are subsequently validated through in vitro and in vivo experiments. Moreover, computational modeling allows for the prediction of compound binding affinities and interactions, guiding the selection of lead compounds for further development [23,24].

3.4. Computational tools assisting Gene Therapy Vectors design

Computational tools are crucial for designing and optimizing gene therapy vectors such as CRISPR/Cas9 guide RNAs and antisense oligonucleotides (ASOs). The importance of predictive modeling in enhancing the specificity and efficacy of gene editing tools, minimizing off-target effects, and improving therapeutic outcomes [25]. Additionally, computational algorithms aid in the design of viral and non-viral vectors for efficient delivery of therapeutic payloads to target cells in the brain [26].

3.5. Role of Computational Models in Therapy Development

Integrating in silico approaches with experimental and clinical research accelerates the development of effective therapies for Huntington's disease. Computational models help researchers understand complex disease mechanisms and predict the efficacy of novel therapeutic interventions. By simulating biological processes and disease progression, computational models contribute to the identification of critical intervention points and the optimization of treatment protocols [23,27].

4. In Vitro Models for HD Therapy Development

4.1. Cells used for in vitro models

4.1.1. Primary Neurons

Cultured primary neurons from HD animal models or genetically modified human cells provide valuable insights into the cellular effects of mutant Huntingtin are crucial for studying neuronal morphology, synaptic function, and cell viability in HD [28]. Moreover, primary neuron cultures allow for detailed investigations into cellular responses to mHTT expression, screening of potential therapeutic compounds, validation of therapeutic targets in a physiologically relevant environment, and elucidation of their mechanisms of action in a controlled laboratory setting [28,29].

4.1.2. Immortalized Human Cell Lines

Immortalized human cell lines such as SH-SY5Y neuroblastoma cells or HEK293 cells engineered to express mutant Huntingtin are essential tools for high-throughput screening potential therapeutic compounds and mechanistic studies in HD research [30,31]. These cell lines are used to evaluate the effects of mHTT on cellular pathways and screen for potential therapeutic agents. Furthermore, cell-based assays using these models provide insights into the molecular mechanisms underlying HD pathogenesis and the efficacy of candidate drugs [32,33].

4.1.3. Induced Pluripotent Stem Cells (iPSCs)

IPSCs derived from HD patients are increasingly used to model disease pathogenesis and test personalized therapeutic approaches. iPSCs derived from HD patients can differentiate into neurons that exhibit hallmark features of HD, including mHTT aggregation and neuronal dysfunction, providing a human-specific model for studying disease mechanisms and testing therapeutic interventions. Moreover, iPSCs-based models enable researchers to study the impact of genetic variability on disease progression and treatment response [32,34].

4.2. Gene therapy application of the in vitro models

4.2.1. In Vitro CRISPR/Cas9 Gene Editing

In vitro CRISPR/Cas9 gene editing allows researchers to perform precise modifications on the HTT gene in cell lines and primary neurons. Wang et al. demonstrate successful targeting and disruption

of the mutant allele using CRISPR/Cas9 technology, highlighting its potential to reduce mHTT levels and alleviate cellular toxicity. In addition, CRISPR/Cas9 gene editing technology was employed in vitro to precisely modify the HTT gene, either by correcting the CAG repeat expansion or disrupting the mutant allele responsible for Huntington's disease. These experiments provide proof-of-concept for gene editing strategies aimed at reducing mHTT expression and improving disease outcomes. Moreover, these studies contribute to refining gene editing techniques for potential clinical applications, emphasizing safety and efficacy ("Advancements in CRISPR/Cas9 technology for therapeutic genome editing" by Smith and colleagues), and hold potential for future therapeutic applications [3,35].

4.2.2. Antisense Oligonucleotides (ASOs)

Antisense oligonucleotides (ASOs) are designed to bind specifically to mHTT mRNA, promoting its degradation and reducing protein expression. Preclinical studies demonstrating the effectiveness of ASOs in reducing mHTT levels and improving cellular viability in HD models. Furthermore, ASO therapies are being evaluated in clinical trials to assess their safety and therapeutic potential in HD patients [36].

4.2.3. RNA Interference (RNAi)

RNA interference (RNAi) approaches utilize small interfering RNAs (siRNAs) to target and degrade mHTT mRNA, thereby reducing its expression levels. RNAi-based therapies have shown promise in preclinical models by mitigating mHTT toxicity and improving neuronal survival [37]. In vitro models play a crucial role in optimizing RNA interference (RNAi)-based therapies aimed at reducing mHTT expression in Huntington's disease. These studies involve testing the efficacy and specificity of various small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) across different cell types and disease conditions, facilitating the development of targeted therapies. RNAi-mediated silencing of mHTT holds promise as a potential disease-modifying treatment by reducing toxic protein levels and alleviating neuronal dysfunction in HD models, in addition with the advances in delivery methods and siRNA design have enhanced the specificity and efficacy of RNAi-based treatments, paving the way for their translation into clinical settings [38].

4.3. Other applications of the in vitro models.

4.3.1. High-Throughput Screening

In vitro models are instrumental in the high-throughput screening of small molecules and natural products that can modulate mHTT toxicity and cellular pathways affected in HD. These screens facilitate the identification of lead compounds with potential therapeutic benefits, which can then be further validated in animal models and clinical trials. Furthermore, advancements in automated screening technologies have accelerated the drug discovery process, enabling the rapid evaluation of large compound libraries.

4.3.2. Molecular Pathway Investigations

In vitro systems allow for detailed investigations into the molecular pathways disrupted by mHTT in HD. Using cell culture models to elucidate the role of altered Ca2+ signaling, mitochondrial dysfunction, and synaptic protein dysregulation in disease pathogenesis. These studies not only enhance our understanding of HD mechanisms but also provide critical insights for developing targeted therapies aimed at restoring cellular homeostasis [39].

4.4. In vitro model applications in discovering the underlying mechanisms for HD

4.4.1. Protein Aggregation Investigation

In vitro models have been pivotal in elucidating the mechanisms underlying the formation and toxicity of mutant Huntingtin (mHTT) aggregates in Huntington's disease (HD). These studies highlight the role of polyglutamine (polyQ) expansions within mHTT in promoting misfolding and aggregation, which contributes to cellular dysfunction and neuronal toxicity. Understanding the dynamics of protein aggregation in HD pathology informs therapeutic strategies aimed at preventing or clearing these toxic aggregates to alleviate disease symptoms and slow progression.

4.4.2. Autophagy Dysfunction Investigation

Research using in vitro models has demonstrated that mHTT disrupts autophagy, a cellular process crucial for clearing protein aggregates and damaged organelles. Impaired autophagy leads to the accumulation of toxic mHTT aggregates and dysfunctional mitochondria, exacerbating neuronal toxicity in HD. Therapeutic approaches targeting autophagy enhancement hold promise for mitigating mHTT-induced cellular stress and preserving neuronal function, underscoring autophagy modulation as a potential disease-modifying strategy in HD.

4.4.3. Synaptic Dysfunction Investigation

In vitro studies have elucidated the detrimental effects of mHTT on synaptic function, including impaired synaptic vesicle recycling, neurotransmitter release deficits, and altered receptor trafficking. These synaptic abnormalities contribute to the cognitive deficits observed in HD patients and are critical targets for therapeutic intervention. Strategies to restore synaptic integrity and function may alleviate cognitive impairments and improve overall neurological outcomes in HD, highlighting synaptic dysfunction as a pivotal aspect of disease pathophysiology [40].

4.4.4. Cellular Stress Responses Investigation

mHTT triggers cellular stress responses including oxidative stress, endoplasmic reticulum (ER) stress, and DNA damage responses, all of which contribute to neuronal dysfunction and cell death in Huntington's disease. These stress pathways exacerbate neurodegeneration and underscore the need for therapeutic interventions that mitigate cellular stress and promote neuronal survival. Targeting stress response pathways represents a promising approach to alleviating disease progression and enhancing the quality of life for HD patients [41].

5. Animal Models for Investigation HD

5.1. Rodent models for HD

5.1.1. Transgenic Mouse Models

Transgenic mouse models, such as the R6/2 and YAC128 mice, express mutant Huntingtin (mHTT) and mimic key aspects of Huntington's Disease (HD) pathology. The utility of these models in studying disease progression, testing therapeutic interventions, and understanding the underlying mechanisms [42]. These models have been pivotal in evaluating gene therapy approaches, pharmacological treatments, and behavioral changes associated with HD progression [43].

5.1.2. Knock-in Mouse Models

Knock-in mouse models, which incorporate CAG repeat expansions into the endogenous Htt gene, provide a more accurate representation of the genetic mutation seen in HD. Slow and colleagues discuss how these models recapitulate progressive motor and cognitive impairments, offering insights into disease mechanisms and therapeutic development. Researchers can better understand genotype-phenotype correlations in HD by studying the interaction between CAG repeat length and disease severity.

5.2. Large Animal Models

Large animal models, including transgenic pigs and sheep, aim to better replicate human HD pathology due to their physiological and anatomical similarities. Yan and colleagues discuss how these models offer advantages in studying disease progression, evaluating therapeutic interventions, and assessing safety profiles. These models are crucial for bridging the gap between preclinical research

and clinical trials, facilitating the translation of experimental therapies to human patients [44].

5.3. Non-human Primate Models

Non-human primate models, such as transgenic macaques, provide valuable insights into HD pathology and therapeutic development due to their genetic, anatomical, and behavioral similarities to humans. Yang and colleagues emphasize how these models recapitulate motor and cognitive deficits observed in human HD patients, facilitating the evaluation of novel therapies. These models play a crucial role in assessing the safety and efficacy of gene therapy approaches, neuroprotective strategies, and cognitive enhancement therapies [45].

5.4. Clinical trial

Clinical trials and observational studies provide critical insights into the symptoms and prognosis of Huntington's disease (HD), helping to refine diagnostic criteria and disease management strategies. The progressive nature of HD, characterized by motor dysfunction, cognitive decline, and psychiatric symptoms. These studies underscore the importance of multidisciplinary care and personalized treatment plans tailored to address the diverse needs of HD patients.

5.5. Gene Editing Application and Strategies for in vivo HD treatment development

5.5.1. CRISPR/Cas9 Gene Editing

CRISPR/Cas9 gene editing techniques are advancing towards in vivo applications, targeting the mutant HTT allele directly within the brain. Recent advancements in CRISPR technology, including base editing and prime editing, hold promise for correcting genetic mutations associated with HD, potentially offering long-term therapeutic benefits by addressing the underlying cause of the disease. Studies are in highlights for viral vectors, lipid nanoparticles, and other delivery systems designed to transport CRISPR/Cas9 components across the blood-brain barrier. These developments aim to optimize gene editing efficiency while minimizing off-target effects, paving the way for clinical trials in HD patients [46,47].

In addition to delivery systems, optimizing CRISPR/Cas9 guide RNA sequences and protein engineering is critical for enhancing gene editing precision and efficacy. Researchers have been exploring strategies to design highly specific guide RNAs that can accurately target the mutant HTT allele while avoiding unintended genetic modifications. These advancements support the development of safer and more effective CRISPR-based therapies for treating genetic disorders like HD [48].

5.5.2. Allele-Specific Silencing strategies in HD treatment

Allele-specific silencing strategies target the mutant HTT allele using approaches such as antisense oligonucleotides (ASOs) and RNA interference (RNAi), while sparing the wild-type allele to maintain normal Huntingtin protein function. These therapeutic approaches aim to reduce mutant Huntingtin protein levels selectively, offering potential disease-modifying benefits by alleviating toxicity without disrupting essential cellular functions.

ASOs have shown efficacy in reducing mutant Huntingtin protein levels and improving disease symptoms in preclinical models. It is a promising approach for targeting mutant Huntington (mHTT) mRNA and reducing its expression levels in HD patients. Recent clinical trials have demonstrated the safety and potential efficacy of ASOs in lowering mHTT levels and improving clinical outcomes. These trials highlight ongoing efforts to develop disease-modifying therapies that can slow disease progression and improve patient outcomes [14].

5.5.3. Astrocyte Conversion

Gene therapy approaches can reprogram striatal astrocytes into functional neurons, aiming to replenish lost neurons and restore neurotransmitter balance in HD. This innovative strategy has demonstrated efficacy in preclinical models, suggesting its potential for neurodegenerative therapies to improve motor function and cognitive outcomes in HD patients.

5.5.4. Stem Cell Therapy

Stem cell-based therapies involve transplanting engineered stem cells to express healthy Huntingtin protein or neurotrophic factors, aiming to replace lost neurons and provide trophic support in HD models [18,49]. Viral vectors, such as adeno-associated virus (AAV) and lentivirus, can be used to deliver therapeutic genes or RNA molecules to the brains of HD models. These approaches have shown promise in reducing mHTT expression and ameliorating disease symptoms in vivo [25]. These approaches are under investigation in animal models and early-phase clinical trials, exploring their potential for neuronal replacement and disease-modifying effects in HD patients.

5.6. Outcome evaluation

5.6.1. Behavioral and Cognitive Assessments

In vivo models are indispensable for evaluating the behavioral and cognitive impacts of Huntington's disease (HD) and potential therapies. These assessments encompass a range of tests including motor coordination tasks (e.g., rotarod), cognitive function assessments (e.g., Morris water maze), and evaluations of psychiatric symptoms such as anxiety and depression-like behaviors. Such models allow researchers to monitor disease progression, assess therapeutic interventions, and correlate behavioral changes with underlying neuropathological findings.

5.6.2. Neuroimaging Studies

Advanced neuroimaging techniques like magnetic resonance imaging (MRI) and positron emission tomography (PET) are pivotal in elucidating the structural and functional changes in the brains of HD models and patients. These studies provide insights into cortical and subcortical atrophy, altered connectivity patterns, and metabolic abnormalities [50]. By tracking these changes over time and assessing responses to therapeutic interventions, neuroimaging aids in refining treatment strategies aimed at preserving brain function and improving the quality of life for HD patients.

5.6.3. Neuropathological Analysis

Post-mortem analysis of brain tissues from HD models and patients reveals critical pathological features such as mutant Huntingtin aggregation, neuronal loss, and gliosis. These analyses provide essential insights into disease mechanisms, validating preclinical findings and guiding the development of targeted therapies. By correlating pathological changes with clinical symptoms and experimental outcomes, researchers can tailor treatments to mitigate specific aspects of HD pathology and improve patient outcomes.

5.7. Mechanisms

5.7.1. Transcriptional Dysregulation

In Huntington's disease (HD), mutant Huntingtin protein (mHTT) disrupts transcriptional regulation by interacting with transcription factors and co-activators, altering gene expression profiles crucial for neuronal function and survival. Dysregulated gene expression contributes to the progressive neurodegeneration observed in HD, highlighting the therapeutic potential of restoring normal transcriptional activity to alleviate disease symptoms and slow disease progression.

5.7.2. Impaired Protein Degradation

mHTT impairs protein degradation pathways such as the ubiquitin-proteasome system (UPS) and autophagy, leading to the accumulation of misfolded proteins and cellular stress [51]. This impairment exacerbates neuronal toxicity and contributes to the selective vulnerability of neurons in HD. Therapeutic strategies aimed at enhancing protein clearance mechanisms hold promise for

reducing protein aggregation and ameliorating cellular dysfunction in affected brain regions.

5.7.3. Mitochondrial Dysfunction

Mitochondrial dysfunction is a hallmark of HD pathology, characterized by disrupted mitochondrial dynamics, bioenergetic deficits, and increased oxidative stress. These mitochondrial abnormalities contribute to neuronal dysfunction and cell death, underscoring the importance of targeting mitochondrial health as a therapeutic strategy in HD. Mitochondria-targeted therapies aim to restore energy production, reduce oxidative damage, and improve neuronal survival in affected brain regions [52].

5.7.4. Excitotoxicity

Dysregulation of glutamate signaling and impaired calcium homeostasis contribute to excitotoxicity in HD, where excessive glutamate release leads to neuronal hyperexcitability and subsequent cell death. Modulating glutamate receptors and restoring calcium balance represent potential therapeutic avenues to mitigate excitotoxic neuronal damage and preserve striatal function in HD. Targeted therapies aimed at reducing glutamate-mediated neurotoxicity may help alleviate motor and cognitive symptoms associated with HD progression [53].

5.7.5. Neuroinflammation

Activation of microglia and astrocytes leads to the release of pro-inflammatory cytokines and other mediators, contributing to neuroinflammation and exacerbating neuronal damage in HD. Chronic neuroinflammation is implicated in disease progression and contributes to the degenerative process in HD. Therapeutic strategies targeting neuroinflammatory pathways may provide neuroprotective effects by reducing inflammatory-mediated neurotoxicity and preserving neuronal integrity in affected brain regions.

5.7.6. Synaptic Dysfunction

mHTT disrupts synaptic function and plasticity, impairing neurotransmission and synaptic connectivity in HD. These synaptic abnormalities contribute to cognitive deficits and psychiatric symptoms observed in HD patients. Therapeutic approaches aimed at preserving synaptic integrity and enhancing neurotransmission may alleviate cognitive impairments and improve overall neurological function in individuals affected by HD [40].

5.7.7. Altered Cellular Signaling

mHTT interferes with various cellular signaling pathways involved in survival, apoptosis, and stress responses, contributing to overall cellular dysfunction in HD. Dysregulated signaling cascades exacerbate neuronal vulnerability and contribute to disease progression in HD. Therapeutic interventions targeting cellular signaling pathways aim to restore normal cellular function, promote neuronal survival, and mitigate disease severity in affected individuals [54].

6. Other Therapeutic Modalities for HD treatments

6.1. Small Molecule Inhibitors

Small molecule inhibitors targeting mutant Huntingtin aggregation, histone deacetylases (HDACs), and other pathogenic pathways have demonstrated efficacy in preclinical studies [55]. These inhibitors offer potential therapeutic benefits by restoring cellular homeostasis, mitigating disease progression, and improving motor and cognitive function in HD patients.

6.2. Neurotrophic Factors

Delivery of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) has shown neuroprotective effects in HD models, supporting neuronal survival and function. Gene therapy approaches aimed at enhancing neurotrophic factor expression offer potential benefits for promoting neuronal health and mitigating disease progression in HD patients [56,57].

6.3. Immunotherapy

Immunotherapy strategies targeting mutant Huntingtin aggregates and other pathogenic proteins are under investigation for their potential to enhance clearance mechanisms and reduce neurotoxicity in HD. These approaches include monoclonal antibodies and vaccination strategies designed to stimulate immune responses against toxic protein species, offering novel therapeutic avenues for disease modification in HD [58,59].

6.4. Drug Screening

High-throughput drug screening using in vitro models allows for the systematic evaluation of small molecules that modulate mHTT toxicity, enhance autophagy, or improve neuronal function in Huntington's disease. These screens facilitate the discovery of potential therapeutic compounds by identifying candidates that target specific disease mechanisms or pathways implicated in HD pathogenesis. Optimizing drug screening methodologies in vitro accelerates the identification and development of novel treatments that address the complex molecular and cellular processes underlying Huntington's disease [60].

7. Conclusion

HD remains a challenging neurodegenerative disorder with a pressing need for effective therapies. The in vitro models advance, such as those utilizing induced pluripotent stem cells (iPSCs), elucidating the complex molecular mechanisms of HD. High-throughput drug screening has emerged as a powerful tool for identifying compounds that modulate key pathogenic processes, including mHTT aggregation, impaired autophagy, and neuronal dysfunction. Along with the power of gene therapy (**Figure 2**), integrating these approaches with detailed timelines of disease progression, researchers can better identify critical intervention windows, improving the likelihood of successful therapeutic outcomes.

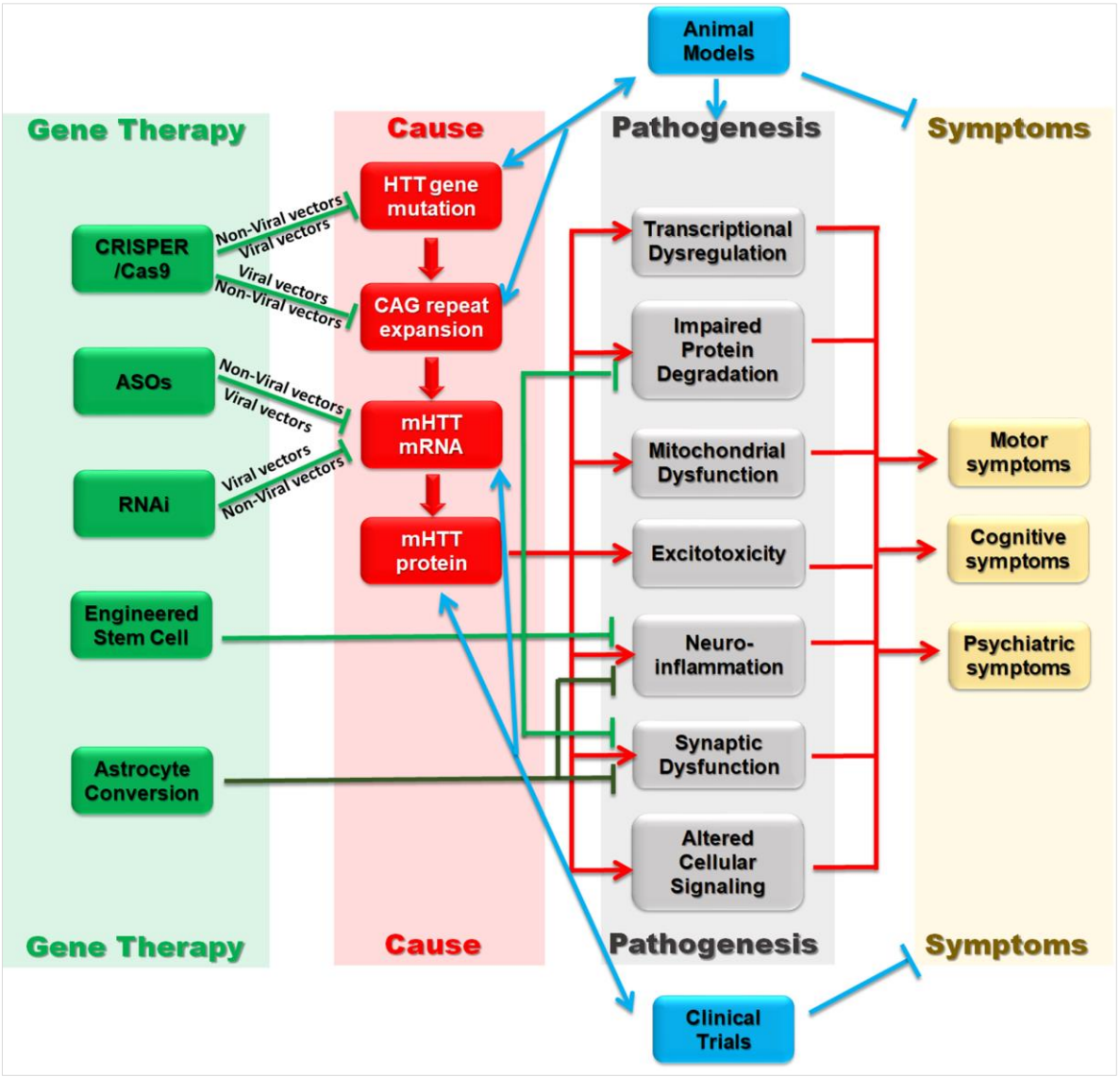

Figure 2: Gene Therapy Application in Pathogenesis Study and Treatment Discovery of Huntington's Disease (HD). The green block lists all the current gene therapy methods or gene-engineered cells for HD therapy. The red block highlights the root cause of HD. The grey block enumerates the potential pathological changes that promote HD progression. The light-yellow block shows the functional systems affected by HD. ASOs: antisense oligonucleotides; RNAi: RNA interference; mHTT: mutant huntingtin.

List of Abbreviations

AAVs: adeno-associated viruses
ASOs: antisense oligonucleotides
BDNF: brain-derived neurotrophic factor
HD: Huntington Disease
HDACs: histone deacetylases
iPSCs: induced pluripotent stem cells
mHTT: mutant huntingtin
MRI: magnetic resonance imaging
MSCs: mesenchymal stem cells
PET: Positron emission tomography
polyQ: polyglutamine
RNAi: RNA interference
ROS: reactive oxygen species
UPS: ubiquitin-proteasome system

Declarations

Ethics approval

Does not apply as this is a literature review of already published data. We have not performed any experiments and directly collect or use any data from a patient.

Consent to participate

Does not apply as this is a literature review of already published data. We have not performed any experiments and directly collect or use any data from a patient.

Data Availability

We did not have any additional data to share. All the data is from published research and cited appropriately.

Conflicts of Interest

The authors declare no any conflict interest.

Funding Statement

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Authors' contributions

J.S. and F.F. conceived the idea, J.S. did the literature search, J.S. and F.F. organized the data, J.S. write the manuscript, and J.S. and F.F. revised and final approved the manuscript.

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