How is Parkinson's Disease Best Treated? A Comparative Analysis of CRISPR-Cas9 and iPSC-Based Gene Therapies

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Abstract. Parkinson's disease (PD) is a devastating neurological condition with little long-term treatment options. Gene therapy has emerged as a potential way to address the underlying pathology of the disease entirely. This paper has examined two main gene therapy approaches: direct in vivo gene editing with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9, and cell replacement therapy with induced pluripotent stem cells (iPSCs). The paper considers the mechanisms of action, therapeutic potential, and overall challenges and barriers to implementation for each approach. The analysis found that both CRISPR-Cas9 and iPSCs have potential as gene therapeutic approaches or modalities, with distinctions. CRISPR-Cas9 have the opportunity to correct the genetic cause of familial PD. However, there were large unresolved challenges about delivery and off-target safety. iPSCs therapy replaces the lost dopaminergic neurons and is a more generic approach, with challenges related to manufacturing the cells and clinical delivery, but these appear more tractable challenges and with more advanced methods available now. Overall, iPSCs based cell replacement therapy is a more preferable and promising short-to-medium term treatment option for a wider population of PD patients.

Keywords: Parkinson's Disease, Gene Therapy, CRISPR-Cas9, Induced Pluripotent Stem Cells (iPSCs), Neurodegeneration, Cell Replacement Therapy.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that typically results in the loss of dopaminergic neurons associated with significant motor function deficits (e.g., tremor, rigidity, bradykesia) [1]. PD, which is the fastest growing neurological disorder in the world, has more than doubled in prevalence over the last 25 years [2]. Current mainstream treatments, which are primarily small molecule drugs such as levodopa, supplement dopamine; however, they are not durable and provide diminishing efficacy, along with significant long-term side effects (motor fluctuations, dyskinesia) [3]. There appears to be a therapeutic gap that warrants more durable and fundamental treatment alternatives to PD.

In recent years, gene therapy is providing a unique opportunity to repair the underlying genetic and cellular deficiency of several diseases including PD. Gene therapy modifies the gene expression in the patients' cells to achieve beneficial effects [4]. Gene therapy generally contains two approaches: the first is direct alteration of the genome through gene editing, and the second approach is replacing damaged tissues with stem cells. Although many tools exist, there are two main areas of advanced technology: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 for genome editing and induced pluripotent stem cells (iPSCs) for cell therapy, representing two of the most powerful researched platforms for neurodegenerative disease.

The objective of this paper is to critically assess the status quo of these two leading gene therapy approaches for PD. By systematically assessing the mechanism of action, therapeutic potential, and key implementation considerations for both CRISPR-Cas9 and iPSCs-based approaches, this paper aims to assess the comparative feasibility of each and ultimately answer the overarching question: which of these approaches holds greater promise for a long-term treatment strategy for PD at the present time?

2. Literature Review

2.1. Conceptual Foundations of Gene Therapy

Gene therapies can be classified into two fundamental types: germline gene therapy (GGT) and somatic gene therapy (SGT). GGT involves altering the DNA in reproductive cells, making the changes heritable. Due to profound ethical concerns and the risk of irreversible, unforeseen consequences, GGT is widely considered illegal and professionally untenable, as exemplified by the controversial "CRISPR baby" case of 2019 [5]. In contrast, SGT modifies the DNA in a patient's non-reproductive cells, meaning the changes are confined to the individual and are not passed to offspring. Given its superior safety profile and ethical consensus, SGT is the exclusive focus of clinical and research efforts, and thus, of this paper.

Advanced SGT encompasses several techniques, including gene silencing, gene editing, and stem cell therapy. Gene editing, which alters the genome's sequence, commonly employs tools like CRISPR-Cas-associated nucleases to add, remove, or replace DNA at precise locations. Stem cell therapy, a form of regenerative medicine, uses the unique ability of stem cells to differentiate into specialized cell types to repair or replace damaged tissues. iPSCs technology, which allows for the reprogramming of a patient's own somatic cells into a pluripotent state, has become a particularly powerful tool in this domain.

2.2. CRISPR-Cas9 as a Gene Editing Strategy for PD

2.2.1. Mechanism and Therapeutic Targets

CRISPR is a family of gene sequences that evolved in prokaryotes, such as bacteria and archaea, as a form of adaptive immunity against bacteriophages to prevent future reinfections by the same virus [6]. The CRISPR-Cas system consists of CRISPR sequences and a Cas protein, for example, the bacterial protein Cas9, or CRISPR-associated protein 9. A “single-guided RNA (sgRNA)” offers target specificity via complementary base pairings, allowing Cas9 to endonucleolytically cut the target. Thus, a custom-made sgRNA supplied with Cas9 protein can theoretically target and edit an endogenous DNA sequence by introducing double-stranded DNA breaks, potentiating therapeutic reprogrammable DNA to restore normal function [7].

The myriad of key mutations in PD offers potential CRISPR-Cas9 targets. Familial PD is closely interlinked with mutations of PD-linked genes, such as SNCA, Parkin, DJ-1, PINK1 and LRRK2. These contribute to the accumulation of the protein alpha-synuclein as well as cell death in the brain’s basal ganglia, leading to the demise of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) [8]. These complex interactions are illustrated in Figure 1.

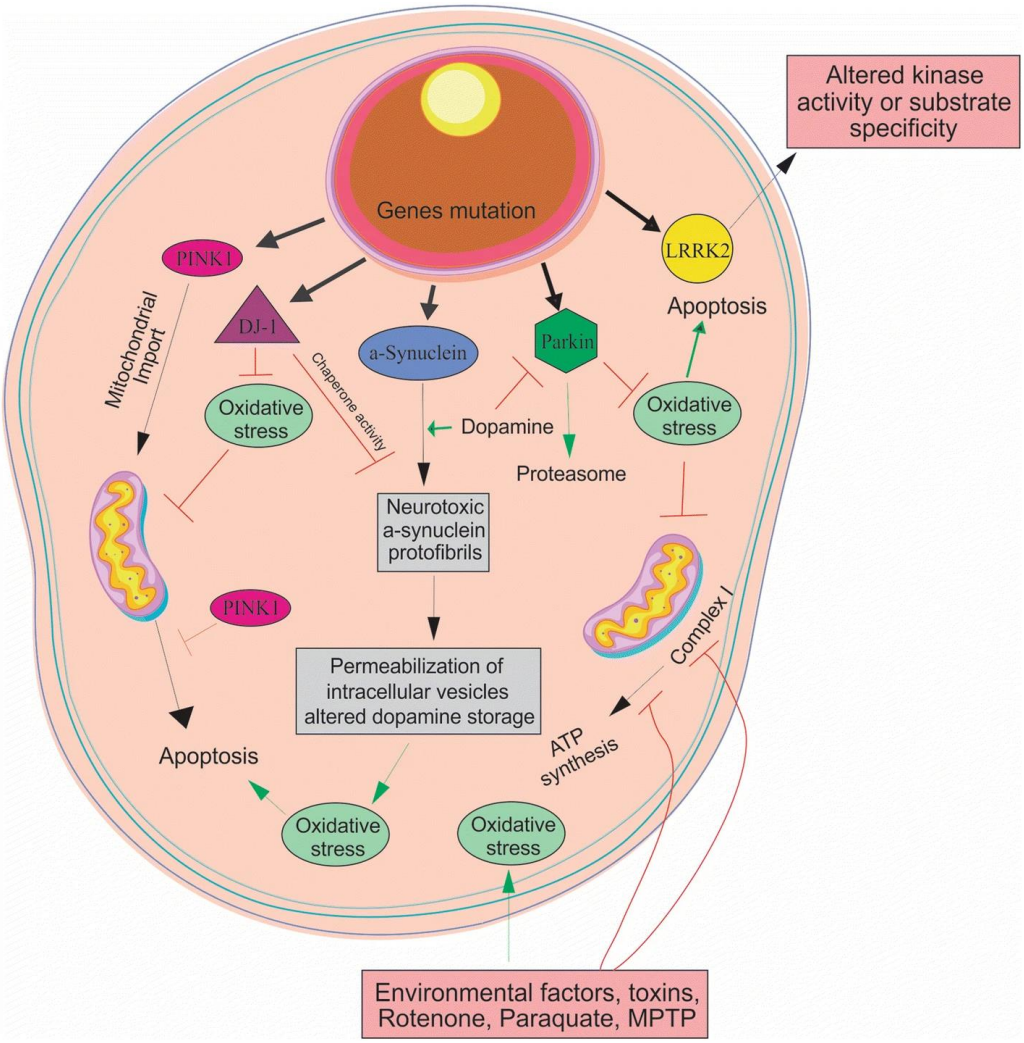

Figure 1. Illustration of the main molecular pathways involved in PD pathogenesis [9].

Key examples of targeting these genes include:

Targeting SNCA Mutation: The alpha-synuclein protein is enriched in the axon terminal of presynaptic neurons and is responsible for adequate neuron communication via neurotransmitters. The mutation of SNCA, a regulator of alpha-synuclein synthesis, is involved in the early onset of familial PD and is among the top risk factors of PD-linked mutations [10]. Thus, transcriptional and posttranslational modulation of SNCA provides a potential PD therapeutic strategy. A study by Soldner et al. [11] used Genome-wide association studies (GWAS) to identify a variant in an enhancer region that controls SNCA expression, confirming that editing this region to a wildtype state could potentially treat PD by reversing abnormal alpha-synuclein synthesis.

Targeting PINK1 Mutation: Abnormal mitochondria function contributes to selective neuron vulnerability, and studies have shown that autosomal recessive PD is associated with PARKIN and PINK1 [12]. PINK1, a protein kinase, normally passes through the mitochondrion membrane, but accumulates when mitochondria are deficient or depolarized, recruiting PARKIN, a ubiquitin ligase, to proceed with mitochondrial degradation. Mutations in their associated genes disrupt this from functioning normally. While CRISPR-Cas9 has been used to create and restore PINK1 mutants to explore this pathway, its direct therapeutic use faces significant delivery challenges [9].

Targeting LRRK2 Mutation. Another critical target is the Leucine-rich repeat kinase 2 (LRRK2) gene. Mutations in LRRK2 are among the most common causes of both familial and sporadic PD. The G2019S mutation, for example, leads to a hyperactive kinase that is toxic to neurons. Therapeutic strategies using CRISPR-Cas9 aim to either correct this specific mutation or, more commonly, to introduce a premature stop codon to knock out the expression of the mutated allele entirely, thereby reducing the levels of the toxic protein [13].

2.2.2. Challenges and Potential Solutions

a) Off-target effects. Off-target effect refers to editing unwanted, or unspecified genes caused by the endonuclease Cas9 binding to regions other than the targeted site [9], disrupting otherwise normal and sometimes essential genes, with oftentimes fatal results. Minimizing off-target effects is a crucial challenge to overcome for the clinical use of CRISPR technology. One possible solution is to enhance the sgRNA specificity and therefore reduce the risk of it binding to similar sequences [14]. A more advanced strategy is prime editing, which uses a modified Cas9 enzyme that only nicks a single DNA strand, greatly reducing the chance of catastrophic errors even if the complex goes off-target [15].

b) Delivery across the Blood-Brain Barrier. A major obstacle is how to deliver the CRISPR system successfully and efficiently into the patient's brain. The blood-brain barrier is made up of glial cells or astrocytes in the blood capillaries, creating tight junctions that prevent toxic or harmful substances from entering the brain [16]. Viral vectors, particularly certain Adeno-Associated Virus (AAV) vectors like AAV-PHP.eB, are considered viable options due to their ability to penetrate the blood-brain barrier with high efficiency and their weak immunogenicity [17].

c) Other Technical Hurdles. Beyond delivery, challenges remain in achieving complete transduction of the target structure while avoiding leakage into neighboring regions [18]. Furthermore, after genes are edited, it is difficult to control for unintended consequences, as a single gene can be responsible for a variety of metabolic functions. Finally, the overall efficiency of editing a sufficient number of cells in the brain to achieve a therapeutic effect remains low with current technology.

2.3. iPSCs as a Cell Replacement Strategy for PD

2.3.1. Mechanism and Therapeutic Rationale

iPSCs technology, pioneered by Shinya Yamanaka in 2006, demonstrated the possibility that somatic cells can be reprogrammed to have functions similar to embryonic stem cells [19]. The therapeutic approach for PD involves taking a patient's own cells (e.g., skin cells), reprogramming them into iPSCs in vitro, inducing them to differentiate into dopaminergic neurons, and then surgically implanting these new neurons back into the patient's brain [20]. This differentiation is a complex, multi-step process that mimics embryonic development, often involving the use of small molecules and growth factors to activate key transcription factors like LMX1A and NURR1, which are crucial for specifying the midbrain dopaminergic neuron fate [21]. The quality and purity of the final cell product are critical for therapeutic success. The characteristics of PD make it a highly suitable candidate for cell-based therapy, and previous cases of fetal cell transplantation provide a proof of concept for it [22]. The iPSCs technique aims to compensate for the loss of function for PD, which is dopamine production, by replacing the dead or dying neurons.

2.3.2. Challenges and Potential Solutions

a) Surgical and Clinical Feasibility. The most troublesome challenge is the surgical aspect of iPSCs therapy, because there are no large-scale, well-established clinical applications of it yet. However, a patient with age-related macular degeneration was part of a successful case of iPSCs-derived cell transplantation in 2017, and no adverse reactions have occurred since the disease was cured, which further proves the feasibility of iPSCs [23]. Although fewer risks and uncertainties are identified in iPSCs than in CRISPR, they are still present and vary from person to person [22].

b) Tumorigenicity Risk. According to Shinya Yamanaka [24], cells that are reprogrammed back to an embryonic-like state have the ability to divide infinitely. If any undifferentiated iPSCs remain in the final cell product and are transplanted, their potency is not controlled strictly, and it can lead to tumorigenesis. This can result from incorrect patterning during differentiation, residual activity of reprogramming factors, or potential gene mutations that might arise during in vitro culture.

c) Immunogenicity. Immune rejection is a very significant point to pay attention to in surgery, because the recipient's immune system can automatically detect and attack transplanted cells that it thinks may be foreign. Autologous iPSCs, which is the use of the recipient's own cells for transplantation, theoretically, should not cause an immune response. However, in practice, this

problem cannot be completely avoided because reprogrammed stem cells have a higher immunogenicity than differentiated cells. For instance, Zhao et al. [25] performed an experiment which demonstrated that mouse immune systems were able to reject teratomas derived from even autologous iPSCs. One approach to this problem could be to use selected and screened autologous iPSCs and derived grafts [24]. Further, in recognition of the immunogenicity and cost of personalized autologous therapy, the idea of "iPSCs banks" (i.e. banks that store collections of iPSCs lines from healthy donors with common HLA (human leukocyte antigen) genotypes) has become more popular. Potentially, these banks could allow the patient to be matched to a banked cell line and produce a cell product that is "off-the-shelf" with a low risk of immune rejection. This would improve scale-up and accessibility of the therapy [26].

3. Discussion

After reviewing the processes and obstacles of both CRISPR-Cas9 and iPSCs-based therapies, it is important to come back to each therapy and have an overall discussion about the decision as to which therapy is the "best" treatment option for PD. This discussion focuses on three important considerations: the therapeutic rationale, the safety profile, and the potential to implement.

First, the therapies fundamentally differ in therapeutic rationale. CRISPR-Cas9 will attempt to "cure" familial PD through correction of a genetic defect. The elegance is that CRISPR-Cas9 would halt the disease process as a cure. Unfortunately, CRISPR-Cas9 can usually only apply to the minority of cases of PD that are known to have a single-gene, known mutation. On the other hand, iPSCs therapy did not intend to change the underlying genetic predisposition of the disease, and instead is a replacement therapy that targets restoring the lost dopaminergic neurons. This approach opens up the possibility for iPSCs to offer a treatment option more widely, potentially all PD patients regardless of cause, since iPSCs therapy corrects the ultimate source of the PD motor symptoms: the loss of dopamine-producing cells.

Second, safety profiles are comparable in having both genomic and cellular risks. The CRISPR-Cas9 risk is genomic in the sense that those off-target mutations may have very, very serious and unforeseen implications for genomic integrity. Technologies such as prime editing will lessen that risk but the residual risk of making permanent changes to a patient's DNA will remain significant. In contrast, the main risk in iPSCs therapy is cellular; tumorigenicity attributed to residual pluripotent cells and possible immune rejection. Overall, these concerns, while serious, seem to be more manageable than genetic alterations. With excellent cell sorting and purification techniques, it is possible to minimize the tumor risk; and autologous cells, while problematic, generally will not elicit catastrophic immune responses. Biological safety risks associated with iPSCs will likely be largely procedural or quality-control related, while safety risks in CRISPR are intrinsic to the mechanism of action.

Finally, in terms of implementation feasibility, both face formidable but distinct obstacles. For CRISPR-Cas9, the central challenge is the in vivo delivery of the editing machinery across the blood-brain barrier to a sufficient number of target cells. While AAV vectors show promise, achieving widespread, efficient, and safe delivery to the brain remains a major unresolved issue. iPSCs therapy bypasses the delivery problem by performing all genetic and cellular manipulation ex vivo. Its main hurdle is the invasive neurosurgical procedure required for implantation and the subsequent survival and integration of the transplanted neurons into existing neural circuits. While neurosurgery is inherently risky, it is a well-established medical field. The successful proof-of-concept in macular degeneration and historical data from fetal cell transplants suggest that the surgical and cell integration challenges, though significant, are part of a more clinically advanced and tangible research pathway.

It is also important to consider the possibilities of synergy within these technologies. Rather than using these technologies in an exclusionary manner, they can be combined in a myriad of ways. For patients with a known genetic mutation, CRISPR-Cas9 could be used ex vivo to correct the defect in

their somatic cells before they are reprogrammed to iPSCs. These gene-corrected, patient-specific iPSCs could then be differentiated into healthy dopaminergic neurons and used for autologous transplantation. This combined strategy would surely be the best of both worlds: a permanent genetic fix and a functional cellular replacement, tackling both the causal agent of disease and damage already accumulated by the patient, and would further reduce the likelihood of immune exclusion.

In addition to the scientific and technical challenges, the path to clinical translation for both treatments will be long and will also face both regulatory and socioeconomic challenges. The manufacture of clinical grade viral vectors, as well as iPSCs-derived cells, is exorbitant and will be produced with severe good manufacturing process (GMP) requirements. Ample preclinical and/or clinical trial data showing safety and efficacy is needed to obtain approval from regulators such as the U.S. Food and Drug Administration (FDA). Also, due to the high costs of the final treatment, it could pose serious problems with respect to access and health equity, which must be considered as the technologies develop.

4. Conclusion

The objective of this study was to assess, compare, and analyze different gene therapies for potential PD therapy and then select the best therapy based on its efficacy and feasibility. Both CRISPR-Cas9 and iPSCs therapies are groundbreaking treatments with expansive possibilities for treating PD. CRISPR-Cas9 provides the incredible promise of a true genetic cure for certain forms of the disease, but has many fundamental obstacles on the way to clinical utility that can be hard to fully navigate with respect to delivery and safety issues. While iPSCs therapy is not a cure for the genetics of the underlying disease, it has a more universal plan for restoring function, with its biggest obstacles related to cell manufacturing, surgical implantation, and immune management that appear more tractable with existing and imminent technologies. While acknowledging the promise of gene editing in the future, this analysis therefore concludes that iPSCs therapy is a more feasible and broadly viable treatment option for PD. But from a surgical perspective, there have been no cases to demonstrate clinical application, so application stands as its next challenge.

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