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Stem-cell-based treatment of PD

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Abstract: *The degeneration and death of dopaminergic neurons in the mesencephalic substantia nigra leads to insufficient dopamine secretion in the cerebral striatum, which leads to the corresponding clinical manifestations in patients, which is the main pathogenesis of Parkinson's disease. At present, there are two main clinical treatment methods: drug treatment and surgical treatment. However, both treatments have side effects, so it is inevitable to explore new treatment methods for Parkinson's disease. In this review, we discuss the application of stem cells in the treatment of Parkinson's disease, and specifically expand from the acquisition of stem cells, brain transplantation, and in vivo functions. We focus on the great potential of cell therapy in Parkinson's treatment and highlight the potential therapeutic applications of stem cells in clinical medicine.*

Keywords: *Parkinson's disease; embryonic stem cells; induced pluripotent stem cells; cell therapy; neurodegenerative diseases*

1. Introduction

Parkinson's disease (PD) is another common neurodegenerative disease after Alzheimer's disease. With the gradual aging of China's population, the number of PD patients is also continuing to grow, especially in the elderly aged 65 and over, the prevalence of PD is as high as 1.06%. Its main pathological changes are reflected in the continuous reduction of dopaminergic neurons and Lewy body formation in the nigra region of the midbrain. Due to the complex pathogenesis of the disease and the inability of missing dopaminergic neurons, there is a lack of effective treatment, and mainly supportive and symptomatic therapy. Patients with PD usually experience motor dysfunction such as resting tremor, and motor delay, as well as non-motor-related symptoms such as cognitive decline and dysregulation of the autonomic nervous system.

Currently, the main treatments for PD include medication centered on levodopa (L-DOPA), and surgical procedures such as deep brain stimulation. Although surgery can improve the exercise capacity of patients, the postoperative recurrence rate is high and many complications. Despite these treatments, the development of PD has still not been fundamentally curbed. Therefore, it is necessary to find new therapeutic targets in order to find better treatment approaches, improve the living standard of patients and ease the economic burden of family and society.

Recently, cell therapy has gained widespread attention as a promising treatment approach. Transplanted cells can replace lost dopaminergic neurons, potentially allowing to restore neural transmission function in the nigral-striatal region. Although early transplantation of fetal ventral midbrain tissue (fVM) has shown some efficacy in some cases, the source and quality control of primary cells are the main constraints, and there are some ethical controversies. Therefore, researchers are actively looking for other sources of cells that may be used in clinical practice, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs) and induced neurons (iNs), each cell type has its unique advantages and limitations. The purpose of this article is to make a comprehensive evaluation of the application of transplanted cells from different sources in PD treatment and the research progress.

2. Dopamine-secretable nerve cells were extracted and isolated

2.1. Stem cells used for induced differentiation

Since Parkinson's disease is caused by the specific degeneration and loss of dopamine neurons in the mesencephalic nigra, treating this disease first requires the acquisition of dopamine-secretory nerve cells. Currently, available cell sources include NSCs derived from fetal brain tissue, hESCs isolated from blastocysts, and iPSCs reprogrammed from somatic cells such as fibroblasts and blood cells[1] . When MSCs are cultured in specialized NDM (neural differentiation matrix medium), MSCs from different sources can differentiate into new cells with neurosynaptic morphology, including neural cells that can secrete dopamine. If iPSCs is cultured as neural progenitor cells in vitro and the neural progenitor cell is directly transplanted into the brains of PD patients, the cells will theoretically have the opportunity to autonomously differentiate into dopamine secretory nerve cells. Earlier studies showed that the use of somatic cell nuclear transfer (SCNT) can reprogram differentiated somatic cells to an undifferentiated state iPSCs. Exposure to specific factors in vitro guides cell differentiation into specific lineages (such as NSCs or DA neurons)[2] . When cell therapy with the ventral midbrain (VM) from aborted embryos, tissue was prepared as a solid graft or dissociated cell suspension and transplanted into the patient's putamen via an injection needle[3].

2.2. Engineering editing of cell

Lmx1a and Msx1 act as lineage determinants of DA neural cells in the ventral midbrain, triggering the production of DA neurons with a midbrain identity. Suggesting that Lmx1a and Msx1 types play important roles in the differentiation and maturation of DA neurons[4]. An additional overexpression of the transcription factor ASCL1 is able to restore neurogenesis from human neural progenitor cells and to produce neurons with more axons[5]. Moreover, studies in PD patients suggest a regulatory role of NURR1 in the development of DA neurons, so forced overexpression of NURR1 enhances the ability of mouse neural stem cells to differentiate into DA neurons and survive in a rat model of PD[6]. In order to improve the survival rate of stem cells in vivo and reduce immune rejection, engineering editing of stem cells is common in the academic community. In the application research of genetically engineered stem cells, Zhang Jianxin et al. have deeply explored the action mechanism of cell therapy in the treatment of cardiovascular diseases[7]. Although this study is not directly directed at PD, it provides important insights into understanding the role of stem cells in the treatment of other diseases.

3. In vivo assessment of neural cells

3.1. Cell survival studies

Effective survival of cells is central to stem cell therapy in PD, and the results directly affect the therapeutic effect. In the early stages after transplantation, monitoring of cell survival often relies on imaging techniques such as positron emission tomography (PET) and magnetic resonance imaging (MRI), which provide an intuitive picture of live cell distribution and survival[8]. In addition, using bioluminescence imaging (BLI) technology, the researchers were able to monitor the survival of labeled stem cells in real time [9]. At the cellular level, methods such as flow cytometry and immunohistochemical staining are widely used to assess the survival rate of transplanted cells. Flow cytometry quantifies the numbers of specific cell populations by detecting specific markers, whereas immunohistochemical staining identifies and quantifies the of transplanted nerve cells by labeling specific cellular proteins, such as neuron-specific enolase (NSE) or tyrosine hydroxylase (TH)[10]. These methods provide precise quantification data for assessing cell survival. To further improve cell survival ability, multiple strategies have been explored. For example, pretreatment of stem cells to enhance their anti-apoptotic capacity and antioxidant capacity can improve cell survival after transplantation [11]. In addition, the use of the extracellular matrix (ECM) provides a more suitable living environment for the transplanted cells, contributing to the maintenance of cell survival and function. In the long-term observation after transplantation, cell survival is influenced by many factors, including the source of the transplanted cells, the method of transplantation, the immune status of the host, and the pathological stage of PD. Studies have shown that transplanted sources of cells, such as hESCs and iPSCs, have a significant impact on cell survival and differentiation capacity [8]. Transplantation methods, such as direct injection, intraventricular injection, or via vascular routes, can also affect cell distribution and survival [9]. The immune status of the host and the pathological stage of PD determine the cell microenvironment after

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transplantation, which in turn affects the cell survival and function [12]. Despite the challenges, researchers have made significant progress through continuous experimental and clinical research. For example, using gene editing techniques, such as CRISPR/Cas 9, stem cells can be specifically genetically modified to improve their survival in vivo and therapeutic efficacy [13]. Moreover, the combination of multiple therapeutic strategies, such as the combination of cell transplantation with drug therapy or neuroprotective factors, has also been shown to improve the success rate of cell therapy [14].

3.2. Maturation ability of the cells

In the stem cell therapy study of PD, examining the maturity of transplanted cells is an important step in assessing their therapeutic efficacy. Mature nerve cells should have complete morphological structure, stable electrophysiological properties, and capable neurotransmitter synthesis and release. These properties underlie the ability of cells to exert their expected function in the host. Morphological analysis is a common method to test cell maturity, observing the morphological changes of cells by light and electron microscopy, such as the development of cell dendrites and axons. These morphological features reflect the maturation state and structural integrity of the cell. For example, mature dopaminergic neurons often have large cell bodies and developed dendritic structures, a marker of their functional maturation [15]. The detection of electrophysiological properties provides functional evidence for assessing cell maturity. With the patch-clamp technique, researchers can record the membrane potential, action potential, and synaptic transmission function, which are important indicators of nerve cell maturity. Mature neurons should be able to generate regular action potentials and release the neurotransmitter upon appropriate stimulation. The detection of the synthesis and release capacity of neurotransmitters is also critical to assessing cell maturity. The maturity of dopaminergic neurons can be assessed by detecting their TH activity and the synthesis and release of dopamine. TH is the key enzyme in dopamine synthesis, and its high and low activity directly reflects the function [16] of cells. In experimental studies, cell maturity can also be assessed by gene expression analysis. For example, gene expression levels such as neural maturation, such as neuron-specific enolase (NSE) and brain-derived neurotrophic factor (BDNF). Moreover, the ability of cells to integrate in vivo is also an important aspect in assessing their maturity. The cell maturity and functionality can be assessed by observing the migration, differentiation within the host brain, and connection to the host neural network. The integration ability of the cell reflects not only its maturity but also a key factor in its long-term efficacy [17]. In preclinical studies, assessing the maturity and functional recovery of transplanted cells in animal models can provide an important reference for clinical application. For example, in a rat model of PD, the maturity and treatment efficacy [18].

3.3. Safety test of the cells

The safety of cell therapy is a key factor in determining its successful application in the clinic. In stem cell therapy studies of PD, examining the safety of transplanted cells involves multiple levels, including immune rejection, risk of tumor formation, and cell stability and long-term effects. Immune rejection is one of the important priorities in cell therapy. Since the transplanted cells may be viewed as foreign substances, the host immune system may attack these cells. To reduce the risk of immune rejection, multiple strategies were used, such as using immunosuppressive agents, or by transplanting with the patient-derived cells[19]. In addition, the modification of stem cells by genetic engineering to express immunoregulatory molecules is also an effective method to reduce immune rejection [20]. The risk of tumor formation is another important aspect of assessing the safety of cell therapy. Because of the ability of stem cells to self-renew and proliferate, cells with incomplete differentiation may form tumors. To this end, the researchers took strict quality control measures during cell preparation to ensure the genetic stability and complete differentiation of cells. Meanwhile, abnormally proliferating cellular [21] can be eliminated when necessary by introducing specific safety genes, such as suicide genes. Long-term monitoring of the survival status and functional performance of the transplanted cells can reveal the behavior and potential risks of the cells in the host. By using non-invasive imaging techniques, such as PET and MRI, the researchers are able to monitor the survival and distribution of cells in real time and detect any abnormal changes in time [22]. Animal models are widely used to assess the safety of cell therapy in preclinical studies. By making long-term observations in animal models of PD, researchers can evaluate the effects of transplanted cells on host behavior, physiological function, as well as pathological changes. These studies provide important preliminary data for clinical applications and help to predict and circumvent potential safety concerns [23].

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3.4. In vivo efficacy assessment of cells

3.4.1. Using primate PD model

Primate models have become an important tool to study the efficacy of stem cell therapeutic PD because of their physiological and genetic similarities with humans. These models can simulate the pathological process of human PD, providing a platform close to human conditions for assessing the safety and efficacy of stem cell therapy. In primate PD models, behavioral assessment after stem cell transplantation is a key part of observing efficacy. By monitoring the animal's motor function, such as motor coordination, gait stability, and bradykinesia, the researchers were able to evaluate the effect of the transplanted cells on improving PD symptoms. In addition to behavioral assessment, neuropathological analysis is also important for examining the efficacy of stem cells. By staining and microscopic pathological sections of animal brain tissue, researchers can assess the survival, differentiation of transplanted cells, and their effects on neural tissue. For example, the distribution and maturation of transplanted dopaminergic neurons in the host brain by TH[24]. Neuroimaging techniques play an important role in assessing the clinical effects of stem cell therapy. Using techniques such as PET and MRI, researchers can monitor the survival and functional status of the transplanted cells under in vivo conditions. These imaging techniques can not only provide spatial information on cellular distribution, but also assess the active of the dopaminergic system with specific tracers. Electrophysiological approaches are also important tools for assessing the efficacy of stem cells. The long-term effects and safety of stem cell therapy have also received attention in primate PD models. With long-term follow-up studies, researchers can monitor the long-term survival, functional stability of the transplanted cells, and the tendency for abnormal proliferation or tumor formation. These long-term data are critical for assessing the sustainability and safety of stem cell therapy [25].

3.4.2. Using mouse PD model

Using mouse PD models, researchers were able to obtain important information about the effects of stem cell therapy, especially in terms of the efficacy of intervention and potential therapeutic mechanisms in the early stages of the disease. In the mouse PD model, behavioral assessment is fundamental for evaluating the efficacy of stem cell therapy. By designing a range of behavioral tests, such as the rotational behavior test, gait analysis, and the suspended tail test, researchers can quantitatively assess motor function and behavioral changes in mice. These tests were able to reflect the neurological recovery of mice after stem cell treatment, providing intuitive evidence of therapeutic effect [26]. A series of advances have been made in studying stem cell therapy in mouse PD models. For example, by using specific genetically engineered stem cells, researchers have found that improved cell survival and therapeutic efficacy. Furthermore, the combination of multiple therapeutic strategies such as cell transplantation with pharmacological therapies or neuroprotective factors has also been shown to improve therapeutic efficacy [27].

3.4.3. Combination therapy

As an emerging therapeutic strategy, combination therapy has shown great potential in the treatment of PD research. This strategy, by combining stem cell transplantation with other treatments, aims to improve the treatment efficacy, reduce side effects, and enhance the overall quality of life. In combination therapy, physiotherapy, gene therapy, and neuroprotective strategies. In terms of pharmacotherapy, the purpose of combination therapy is to exploit the neuroprotective effects or symptom relief effects of drugs to enhance the long-term efficacy of stem cell transplantation. For example, studies have shown that when combined with traditional drugs such as L-DOPA, it can improve the survival and functional integration of stem cells in PD models, thus improving the therapeutic efficacy [28]. In addition, drug pretreatment has also been used to improve the transplantation effect of stem cells, such as through the use of antioxidants and anti-inflammatory drugs to reduce the inflammatory response and oxidative stress[29] after cell transplantation. The application of physical therapy in combination therapy aims to improve the effectiveness of stem cell therapy by promoting neuroplasticity and enhancing neurological recovery. Physical therapy includes exercise therapy, electrical stimulation therapy, which can be combined with stem cell transplantation to promote the recovery of motor function in PD patients [30]. Gene therapy, as a potential therapeutic approach, improves neural function by delivering specific genes to cells to repair or replace defective genes. In stem cell therapy in PD, gene therapy can be used to enhance the dopamine production capacity of cells or to improve the survival rate of cells. For example, the differentiation of into dopaminergic neurons could be promoted by introducing specific transcription factors into the stem cells[31]. The application of neuroprotective strategies in combination therapy aims to improve the efficacy of stem cell transplantation by protecting nerve cells from injury. This includes the use of antioxidants, anti-inflammatory drugs, as well as specific neurotrophic factors, to reduce neuroinflammation and cell death in the PD model[32]. Progress has been made in preclinical research with combination therapy. In an animal model of PD, by combining stem cell transplantation and the treatments described above, the researchers observed better therapeutic effects, including improved motor function, enhanced neuroprotection, and fewer side effects[33]. Moreover, optimization of combination treatment strategies is ongoing to find the optimal treatment combination and dosing regimen. Although combination therapy has shown positive promise in PD treatment, there are still some challenges in clinical application. How to determine the optimal combination regimen, how to evaluate long-term efficacy and safety, and how to overcome potential drug interactions all need to further explore in future studies[34]. With the deepening of research, combination therapy is expected to provide more effective and safe treatment options for PD patients.

4. Conclusions

In summary, Parkinson's disease is a common neurodegenerative disease, and the current treatment methods are mainly medical treatment and surgical treatment, but these treatments have certain limitations. Stem cell therapy, as a promising treatment, brings new hope for the treatment of Parkinson's disease. By extracting and isolating dopamine-secreting nerve cells, and engineering and editing them, it is possible to improve the viability and therapeutic effect of stem cells in the body. In vivo evaluation of nerve cells, a comprehensive assessment of the cell's viability, maturation, safety, and in vivo efficacy is required. Although stem cell therapy has made some progress in the treatment of Parkinson's disease, further research and exploration are still needed to determine the best treatment regimen and dosage to improve the treatment effect and safety. At the same time, the combination of other treatments, such as drug therapy, physical therapy, gene therapy, and neuroprotective strategies, is expected to provide more effective treatment options for patients with Parkinson's disease.

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