Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/62/20241511

Advances in the induction and differentiation of neural stem cells

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Abstract. Neural stem cells (NSCs) have the capacity for self-renewal and differentiation into various neuronal cell types. In-depth research on NSCs has demonstrated their immense potential in treating neurological diseases such as neurodegenerative disorders and neural injuries. Rapid advancements in NSC research are currently focused on NSC acquisition, induced differentiation, and clinical translation. It is generally acknowledged that NSCs can be obtained from primary tissues or through the differentiation of induced pluripotent stem cells (iPSCs) and somatic cell reprogramming. Various approaches for inducing iPSC differentiation have been developed, including transcription factors, small molecule induction, and neural stem sphere differentiation methods. Each approach has unique advantages and is the subject of ongoing research. NSC-induced differentiation represents a promising avenue for treating neurological diseases, with studies underway using reprogramming factors and small molecule compounds. However, the NSC research field is extensive and rapidly evolving, with enormous potential for medical applications. Currently, there is a lack of comprehensive analytical reviews in the literature. This article consolidates and analyzes the major advancements in NSC induction, encompassing NSC isolation and culture, in vitro induction, and clinical applications. By presenting the latest developments in all aspects of NSCs, this article aims to provide guidance and insights for future regenerative medicine research in neurological diseases.

Keywords: Applied computing, Life and medical sciences, Genomics.

1. Introduction

The presence of neural stem cells (NSCs) within mammalian bodies enables these organisms to generate differentiated neuronal cells. NSCs are progenitor cells with the ability to divide, differentiate, and self-renew. In different locations within nervous tissue, the types and distribution of their progeny vary slightly. Current research indicates that NSCs are primarily found in the central nervous system (CNS). During mammalian embryonic development, NSCs originate from various brain regions, including the cerebral cortex, ventricular zone, hippocampus, striatum, telencephalon, and telencephalic ventricular zone. After embryonic development, NSCs can be identified in the subgranular zone of the hippocampal dentate gyrus, the subventricular zone (SVZ) of the brain, and the subependymal zone of the spinal cord.

In adulthood, NSC development primarily continues in two regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ)[1]. The SGZ, located near the dentate gyrus, is a neurogenic region limited to fine granular areas and intimately linked to cognitive functions

DOI: 10.54254/2753-8818/62/20241511

such as learning and memory. The presence of undifferentiated NSCs in this region provides the potential for cellular renewal and repair within the hippocampus. The SVZ, a thin layer beneath the ependymal cell layer, harbors significant neuron regeneration potential, contributing to brain cell repair and regeneration. Studies have found that CD133-positive ependymal cells can differentiate into dopaminergic neurons, safeguarding the physiological activities of mammals.

Currently, treatment options for neurodegenerative diseases and neural injuries (e.g., cerebral ischemia, stroke, spinal cord injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and brain tumors) are limited and often ineffective. Neural stem cell (NSC) transplantation emerges as a potential therapeutic strategy, with the acquisition of NSCs being a crucial step in regenerative medicine. Stem cell transplantation is particularly promising as a therapeutic option for neurodegenerative diseases. As the "seed" cells of the central nervous system, NSCs possess the ability to self-renew and generate neurons and glial cells during mammalian CNS development, making them highly relevant for treating neurodegenerative diseases. Advances in stem cell research have enabled the direct differentiation of NSCs from pluripotent stem cells, such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), by utilizing cellular signals and morphogens involved in CNS development.

In this review, we analyze the key technologies for NSC induction from the perspective of NSC research, comprehensively examining recent progress to provide theoretical support for NSC applications.

2. Neural Stem Cells (NSCS)

2.1. Isolation and Culture of Neural Stem Cells

The process of isolating NSCs from different sources varies, but generally includes microanatomical dissection of tissue sections, followed by separation and purification. To obtain high yields of NSCs, microanatomical dissection of the subventricular zone (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG) in adult mammals can be performed [2]. Zhang Xiao-feng found that commonly used separation methods, including mechanical pipetting, trypsin digestion, and PDD (papain and dispase) digestion, all yield a large number of NSCs. However, PDD digestion causes less damage to NSCs and results in faster neurosphere proliferation. For purification methods, such as differential centrifugation and filtration, Zhang Xiao-feng's experiments demonstrated that filtration results in higher purity of NSCs [3].

Culturing NSCs is challenging and requires numerous attempts during the exploration process, which is essential for research. In early experiments, B.A. Reynolds and S. Weiss in 1992 explored the culture conditions for NSCs, initially using a medium containing epidermal growth factor (EGF). This resulted in the death of most cells, but a small subset proliferated to form floating aggregates of cells with immunocytochemical features of neuroepithelial cells, known as the initial neurospheres.

In 2001, Fiona Doetsch, from Harvard Medical School's Department of Molecular and Cellular Biology, further developed these studies. She stated that transit-amplifying cells retain stem cell competence under the influence of growth factors, although only a fraction of these cells are NSCs [4].

Currently, the primary method for culturing NSCs involves a medium composed of DMEM/F12 (98%), B27 (2%), bFGF (20 μg/L), and EGF (20 μg/L). Main culture methods include suspension and adherent cultures, with medium exchanges usually performed using a half-volume exchange method. During passaging, NSCs can be passaged after 6-8 days of culture using methods such as trypsin digestion, Accutase digestion, and mechanical pipetting. Mechanical pipetting is suitable for primary and first-generation neurospheres, while Accutase digestion yields better cell status for passages beyond the second generation. Prompt removal of metabolites during culturing is also essential. However, NSCs may exhibit neurosphere adherence after multiple passages (beyond the 9th generation), with their suspension properties weakening as passage numbers increase [5].

Recently, 3D NSC culture methods have been developed that better mimic the in vivo environment and offer new approaches to neural cell culture [6].

DOI: 10.54254/2753-8818/62/20241511

2.2. Methods for Verifying Neural Stem Cells

NSCs possess the potential for self-renewal and differentiation into multiple types of neural cells. To verify cells as NSCs, their self-renewal ability can first be confirmed using the limited dilution method for single-cell cloning. Subsequently, cells are mechanically separated into a single-cell suspension. All cells from the clone are then cultured. If these cells produce a large number of clones similar to the primary culture after continuous passaging and still retain their cloning ability, their self-renewal capability can be confirmed.

To validate their differentiation potential, cells can be labeled with BrdU, subjected to clonal induction of differentiation using a medium containing 5% fetal bovine serum and DMEM/F12, and then analyzed through immunofluorescence experiments. The presence of various morphologies of dividing and proliferating newborn neural cells indicates differentiation potential [7].

Markers discovered for NSCs include Nestin, Musashi, neuron-specific enolase, vimentin, and cell adhesion molecules. Detection of these markers can identify NSCs, with Nestin commonly used due to its temporal expression pattern, which is high in NSCs and gradually decreases during differentiation into other neural cells [5].

3. Methods of Inducing Neural Stem Cells

3.1. Differentiation of Induced Pluripotent Stem Cells (iPSCs) into Neural Stem Cells (NSCs)

Differentiating iPSCs into NSCs is currently a significant method for obtaining NSCs. Kazutoshi Takahashi and Shinya Yamanaka of Kyoto University first induced iPSCs in 2006. Under conditions typical for ES cell culture, they introduced four factors—Oct3/4, Sox2, c-Myc, and Klf4—to reprogram mouse embryonic or adult fibroblasts into cells, which they termed induced pluripotent stem cells (iPSCs) [8]. These iPSCs exhibit morphological and growth characteristics similar to those of embryonic stem cells (ES cells) and express the marker genes typical of ES cells. Takahashi and Yamanaka's experiments demonstrated that fibroblasts can be reprogrammed into pluripotent stem cells by introducing a few defined factors.

To guide the differentiation of iPSCs into NSCs, the iPSCs are first suspended and cultured to form embryonic bodies (EBs). These EBs further differentiate into rosette structures, which are then isolated and cultured in NSC medium. Here, the rosettes form a monolayer of bipolar-shaped NSCs. These NSCs are then mechanically dissociated into neurospheres, which are self-renewing and multipotent, consisting predominantly of NSCs.

3.1.1. Small Molecule Compound Induction

Currently, commonly used small molecule compounds for promoting neural differentiation include retinoic acid (RA) and sonic hedgehog (SHH). RA regulates neural differentiation processes by modulating the expression of certain transcription factors, thereby exhibiting favorable effects on neuronal development, regeneration, and maintenance. SHH is a secreted protein that controls neuron formation during vertebrate development, with production occurring in the ventral and dorsal neural tubes to regulate neural differentiation and promote axon formation.

In 2011, Mark E. Hester et al. proposed that RA and SHH alone were insufficient for complete differentiation. They developed an adenoviral gene delivery system encoding MN-inducing transcription factors—neurogenin 2 (Ngn2), islet-1 (Isl-1), and LIM/homeobox protein 3 (Lhx3)—which increased the neural induction efficiency of human iPSCs (hiPSCs) to over 60%–70%, significantly shortening the induction time (specifically for motor neurons, MN) [9].

In addition to RA and SHH, Matthew B. Jensen, MD, and colleagues supplemented their approach with growth factors (brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, and insulin-like growth factor 1) and cyclic adenosine monophosphate to promote iPSC neural differentiation in 2011, successfully converting human iPSCs into early NSCs. Following focal cerebral ischemia, they intracerebrally injected these cells or vectors into rats, observing their survival and further differentiation in the rat brain without tumor formation [10].

DOI: 10.54254/2753-8818/62/20241511

In the same year, Mitne-Neto et al. also demonstrated that growth-derived neural factors promote neural differentiation.

3.1.2. Neural Stem Sphere Method

Nakayama pioneered the neural stem sphere method, which facilitates the conversion of ESCs into neurons through three distinct stages. In 2011, Hayashi et al. applied this technique to transform mouse iPSCs into neural stem spheres (NSS), subsequently culturing them on a matrix gel-coated plate. These NSSs formed spherical colonies of NSCs capable of differentiating into various types of neural cells.

The neural stem sphere differentiation method is faster than the small molecule compound induction method. This method requires only 4 days to transform suspended ES cells into neural stem cells. By continuing the culture for at least another 4 days, neural stem spheres can be obtained through the expansion of neural stem cells. Neural stem cells induced by this method can expand approximately 1000 times and possess differentiation capabilities [11].

3.2. Direct Reprogramming of Somatic Cells into Neural Stem Cells

3.2.1. Reprogramming Factors—Genetic Induction Methods

In 2011, Janghwan Kim et al. from Harvard Medical School explored the direct reprogramming of fibroblasts into neural stem cells (NSCs) or neural progenitor cells (NPCs) without the intermediate step of induced pluripotent stem cells (iPSCs), which is time-consuming [12]. Their experiments demonstrated that the transient induction of four reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) efficiently converted fibroblasts into functional NSCs/NPCs that responded to appropriate signaling inputs. These cells could differentiate into various neural cell types except oligodendrocytes, indicating partial NSC differentiation potential.

In 2012, Marc Thier et al. from the University of Bonn, Germany, utilized retroviruses expressing iPSC reprogramming factors Sox2, Klf4, and c-Myc, leveraging their endogenous expression in NSCs. Within 18 days, NSC spheres emerged and could be maintained in adherent culture, confirming the successful conversion of fibroblasts into induced NSCs (iNSCs) using this method. The resultant iNSCs displayed characteristic differentiation into neurons, astrocytes, and oligodendrocytes [13].

In the same year, Dong Wook Han et al. utilized transcription factors from both pluripotent stem cells and NSCs, including Brn4/Pou3f4, Sox2, Klf4, c-Myc, and E47/Tcf3, to convert mouse fibroblasts into induced NSCs (iNSCs). Similar to Thier et al., these iNSCs exhibited characteristics akin to brain-derived NSCs. Remarkably, they could be passaged for over 130 generations without tumor formation, with no presence of pluripotent stem cells in the cultures [14].

In 2012, Karen L. Ring et al. achieved the reprogramming of mouse or human fibroblasts into iNSCs solely by overexpressing the pluripotent stem cell transcription factor Sox2. These iNSCs demonstrated self-renewal and differentiation potential suitable for human iNSC production. They expressed NSC markers such as Sox2, Nestin, and Sox1 while abstaining from pluripotent stem cell gene expression like Oct4 and Nanog. This approach mitigates risks associated with oncogenic gene overexpression, such as c-Myc, thereby reducing the potential for tumorigenesis. However, reliance on retroviral infection introduces challenges related to exogenous gene integration into the cellular genome, and the reactivation of Sox2 remains a concern, potentially posing risks of tumor formation in neural epithelia [15].

3.2.2. Small Molecule-Induced Reprogramming

Compared to transcription factor-mediated reprogramming, small molecule-induced direct reprogramming of somatic cells into NSCs in vitro mitigates the risk of tumorigenesis associated with viral transduction vectors. Moreover, it offers advantages in terms of applicability, optimization, manufacturability, and potential development into pharmaceuticals.

In 2016, Jie Zheng et al. introduced seven small molecules—VPA, BIX0294, RG108, PD0325901, CHIR99021, vitamin C, and A83-01—into the culture medium, resulting in the formation of

DOI: 10.54254/2753-8818/62/20241511

neurospheres expressing typical NSC markers. However, the process proved cumbersome, and induction efficiency remained low [16].

That same year, Mingliang Zhang et al. employed nine different small molecules—CHIR99021, LDN193189, A83-01, RG108, Parnate, SMER28, retinoic acid, HhAg1.5, and bFGF—to directly reprogram MEFs into NSCs under hypoxic conditions [17].

Due to the stringent hypoxic conditions required in previous induction methods, Jinjin Fu et al., in 2022, employed the same small molecule M9 medium used by the Zhang research group to successfully reprogram fibroblasts into NSCs without the need for hypoxic conditions. Further analysis of differentially expressed genes identified significant upregulation of neuro-related genes such as Grm7, C1ql2, Gabrg3, Lrrn4, Nxph1, and Nlgn1. These genes were primarily enriched in signaling pathways associated with neurogenesis, neuronal differentiation, nervous system development, neurogenesis regulation, brain development, and axonogenesis [18].

In 2018, Yan-Chuang Han et al. successfully induced mouse embryonic fibroblasts (MEFs) into NSCs using small molecules PD0325901, Bix01294, RG108, CHIR99021, A83-01, VPA, and vitamin C. These cells exhibited stable and uniform proliferation for two years without significant reduction in self-renewal ability and morphologically resembled classical NSCs. Termed SMINS-MEF-7, these cells demonstrated robust differentiation potential into astrocytes, neurons, or oligodendrocytes, underscoring them in vitro multipotency [19].

3.2.3. microRNA Induction

microRNAs (miRNAs) constitute a class of non-coding, single-stranded small RNAs encoded by endogenous genes, discovered in animals, plants, and some viruses. Mature miRNAs regulate transcription or expression by binding to the mRNA of target genes, specifically targeting the 6-8 nucleotide-long “seed sequence” located at the 3’ UTR end of mRNA. They play pivotal roles in cell proliferation, apoptosis, aging, and differentiation. Approximately 70% of miRNAs are expressed in the nervous system, where they significantly influence the proliferation, differentiation, and cell cycle of neural stem cells (NSCs). Each miRNA can target multiple genes [20].

In 2015, Weizhong Xiao et al. discovered that miR-146 inhibits NSC proliferation and promotes the spontaneous differentiation of NSCs into glial cells. Notch1 serves as a critical target gene of miR-146, and its modulation by miR-146 regulates NSC differentiation by suppressing Notch1 expression [21].

In the same year, Jyung-Rok Yu investigated the potential of miRNA activity to enhance the reprogramming of human dermal fibroblasts (hDFs) into human induced neural stem cells (hiNSCs). They transfected Sox2-transduced cells with miR-124, miR-9-5p, miR-9-3p, anti-let-7b, let-7b, and miR-CTL. Remarkably, overexpression of let-7b significantly reduced the efficiency of reprogramming into NSCs. Further experiments elucidated that let-7b plays a crucial role in promoting the reprogramming efficiency of Sox2-bound hiNSCs and in regulating their proliferation, migration, and self-renewal [22]. These findings highlight the positive impact of miRNAs on enhancing reprogramming efficiency into NSCs, underscoring their potential as a pivotal research area for advancing stem cell sources in clinical therapies.

3.3. Directed Induction of Other Types of Stem Cells into Neural Stem Cells

As early as 2008, Wang Jing et al. explored the feasibility of in vitro induction of human embryonic stem cells (hESCs) into highly purified neural stem cells (NSCs). Researchers simulated various stages and developmental environments of neuronal cell differentiation in vivo, employing a three-stage approach to induce hESCs toward NSC differentiation. Surface markers were assessed using immunofluorescence cytochemistry, flow cytometry, and RT-PCR, while the differentiation potential of the induced NSCs was comprehensively evaluated. This study demonstrated that the described method effectively induces hESCs to generate high-purity NSCs capable of redifferentiation [23].

In 2024, Han Xia, Zhao Ruidong, and Yang Junli from the Affiliated Hospital of Inner Mongolia Medical University investigated the feasibility of directing human umbilical cord mesenchymal stem cells (UC-MSCs), cultured in human peripheral blood serum, toward neural stem cell (NSC)

DOI: 10.54254/2753-8818/62/20241511

differentiation. Umbilical cord tissue was extracted, and UC-MSCs were induced using DMEM/F12 medium supplemented with 10% human peripheral blood serum. Subsequently, third-generation UC-MSCs were directed into NSCs using DMEM/F12 medium containing 0.5% N2, 1.5% B27, 20 ng/ml basic fibroblast growth factor, and 20 ng/ml epidermal growth factor. Surface markers were assessed again by flow cytometry. UC-MSCs exhibited high expression of surface markers CD44, CD105, CD29, and CD73. Following directed differentiation into NSCs, these cells showed high expression of CD44, CD105, CD29, CD73, Nestin, NF-L, and GALC. After 8 days of NSC induction, typical cell morphology was observed, and immunofluorescence staining for microtubule-associated protein 2 and glial fibrillary acidic protein was positive. This experiment demonstrated that human umbilical cord mesenchymal stem cells cultured in human peripheral blood serum could be effectively directed into NSCs, capable of further differentiation into other neuronal cell types with extended culture time [24].

3.4. Differences and Challenges in Various Induction Techniques

Currently, research on different induction methods for iPSCs primarily focuses on improving reprogramming efficiency. However, there is a lack of in-depth understanding of the genetic, epigenetic, and signal transduction mechanisms involved in reprogramming. The research discussed in this paper emphasizes studying the molecular mechanisms of reprogramming. (1) Core Transcription Factors: Under the introduction of core transcription factors Oct4 and Sox2, reprogramming efficiency is generally positively correlated with the number of exogenous factors introduced. The research group led by Xiao Lei used six transcription factors—Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28—to induce human fibroblasts, achieving a reprogramming efficiency 10.4 times higher than with the four factors (Oct4, Sox2, Klf4, and c-Myc). (2) Small Molecules: The chemical small molecule Repsox can compensate for the absence of exogenous Oct4 and Sox2, enabling the reprogramming of somatic cells into iPS cells. In melanoma cell reprogramming, exogenous Sox2 is non-essential, indicating that traditional core reprogramming factors are not irreplaceable but can enhance cell reprogramming efficiency. (3) RNA Involvement: Studies show that introducing the mRNA of transcription factors (Oct4, Sox2, Lin28, and Nanog) can reprogram human fibroblasts into iPS cells. Lin et al. used only the ES cell-enriched miRNA-302 to reprogram human melanoma cells and prostate cancer cells into ES cell-like pluripotent stem cells. Additionally, introducing the mouse ES cell-specific miR-290 family members (miR-291-3p, miR-294, and miR-295, which can replace c-Myc) or inhibiting miR-let7 in somatic cells can improve reprogramming efficiency.

4. Application of Neural Stem Cells in Neurological Diseases

4.1. Application in the Treatment of Central Nervous System Diseases

Stroke and traumatic brain injury primarily result from neuronal damage. Under these pathological conditions, endogenous neural stem cells in the spinal cord are often unable to produce the neurons required for neural reconstruction, necessitating the introduction of exogenous cells to address this issue. Induced neural stem cells (iNSCs) can proliferate and undergo neurogenesis. They can migrate to the damaged area and differentiate into new neurons to replace the damaged ones. Transplanted iNSCs can promote neuronal growth, induce myelin regeneration, and protect nerves, thereby having a therapeutic effect in repairing nerve damage.

Neural stem cells (NSCs) hold immense promise for treating neurological diseases, and researchers are exploring various approaches. Despite significant progress, several challenges impede their clinical application, primarily concerning cell delivery, selection, and in vivo growth regulation.

NSC transplantation typically involves intracerebral or intraspinal injection, demonstrating good surgical tolerance and substantial improvements in patients' quality of life [25]. To meet clinical requirements, NSCs must be purified into specific subsets or differentiated into desired cell types. Researchers commonly employ surface marker-based positive or negative selection for screening purposes. However, variations in cell cycle stages, cell sources (iPSCs/ESCs), and culture conditions can influence the expression levels of these surface markers. As a result, researchers use combinations

DOI: 10.54254/2753-8818/62/20241511

of differentiation cluster antigens (CD antigens) to enhance specificity, ensuring the exclusion of partially differentiated cells during screening to prevent aberrant differentiation post-transplantation.

NSCs can enhance the survival and regeneration of endogenous neurons by secreting neurotrophic factors, which mitigate neuronal cell death, promote axonal and dendritic connections, and thereby bolster the survival of transplanted NSCs. This is typically achieved through the attenuation of neuroinflammation [26].

Additionally, NSCs secrete extracellular vehicles (EVs) composed of cell membranes bearing membrane proteins, surrounding an aqueous core containing soluble molecules such as proteins and nucleic acids (mRNA and miRNA). These EVs can either remain in close proximity to cells or travel over long distances, facilitating bidirectional information exchange between donor and host cells following NSC transplantation [27]. This exchange contributes to mitigating cell apoptosis, pathogenesis, and inflammation [28].

Following NSC transplantation, chemokines expressed by NSCs often aggregate at the injection site, leading to NSC clustering with restricted migratory capability. Studies indicate that blocking these signaling factors using antibodies or receptor antagonists can enhance NSC diffusion and engraftment rates. The migration and integration abilities post-transplantation are influenced by the differentiation stage of donor cells, with early and intermediate differentiated cells demonstrating greater transplantation efficacy compared to mature cells [29]. Limited migration in adult brains is partly attributed to the absence of radial glial cells, which are crucial for migration in developing brains. Furthermore, environmental cues often favor glial cell generation over neurogenesis, and the presence of perineuronal nets (PNNs) reduces plasticity. However, researchers have identified that CSPG-degrading proteases can modify PNNs under specific conditions, and treatments such as chondroitinase ABC can partially remove PNNs, thereby enhancing NSC migration and integration post-transplantation [30].

Effective integration of transplanted NSCs into the host nervous system is essential for their functional role. Given the restricted axonal growth capacity in adult central nervous systems, researchers have explored various strategies to promote axonal growth. These include supplementing pro-axonal growth factors during donor cell culture, genetic modification to overexpress pro-axonal growth genes, and manipulating the host environment.

Enhancing the therapeutic effects of NSCs can also be achieved by modulating the expression of selected growth factors. Studies have demonstrated that overexpressing IGF-1, a growth factor involved in neurogenesis and synaptogenesis, or neurotrophins such as GDNF, BDNF, NT-3, and NGF through genetic modification can enhance the longevity and survival rate of transplanted NSCs.

Genetic modification of NSCs can further promote the differentiation of transplanted cells. By engineering cells to express deficient neurotransmitters in neurodegenerative diseases, researchers aim to improve therapeutic outcomes.

4.2. Application in Regenerative Medicine

Certain organic diseases can be repaired and reconstructed using organ or cell transplantation methods. However, traditional organ or cell transplantation faces significant issues such as severe donor shortages, immune rejection, and ethical concerns, which constrain the development of regenerative medicine. The methods summarized in this article offer new therapeutic hope for regenerative medicine. In particular, disease-specific iPSCs contain the patient's own genetic material, and iNSCs obtained through reprogramming can also be generated from the patient's own somatic cells, making them autologous and thereby avoiding immune rejection and ethical issues.

Parkinson’s disease is a neurodegenerative disorder that severely impacts patients' motor function and self-care abilities, and its global incidence continues to rise annually. Current treatments primarily involve the supplementary administration of levodopa or surgical ablation of the thalamus. While these methods can alleviate symptoms, they also lead to many complications. Researchers hope to regenerate dopaminergic neurons using iPSCs to fundamentally treat Parkinson’s disease. Some researchers have induced iPSCs from skin cells of Parkinson's patients and successfully differentiated them into

dopaminergic neurons in vitro. This suggests that iPSCs hold great promise for the regenerative treatment of Parkinson's disease and for research into the disease.

5. Conclusion

This paper provides a systematic analysis of the screening, culture, and induced transformation of neural stem cells (NSCs), exploring current major research areas, their achievements, historical context, and academic background. NSCs hold considerable promise for the treatment of neurological diseases, with research efforts focused on NSC acquisition, induced differentiation, and clinical application. NSCs can be sourced from primary tissues, differentiated from iPSCs, or generated via somatic cell reprogramming, each method carrying inherent drawbacks such as genetic instability, potential side effects, and the risk of in vivo tumor formation. This article summarizes the research progress on the induction and differentiation of NSCs and offers guidance for addressing issues and improving methods related to the acquisition of NSCs in future regenerative medicine. Challenges in the cell culture system include immature techniques and low cultivation efficiency, which necessitate further exploration to optimize cell generation for transplantation. Limitations in NSC survival, localization, and post-transplantation tracking contribute to suboptimal outcomes, underscoring the need for continued research into effective transplantation methods. Moreover, transplanted NSCs may express pain-related molecules like BDNF or differentiate into astrocytes, potentially exacerbating pain. Thus, future investigations are needed to address and resolve these concerns.

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