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History of Neural Stem Cell Research and Its Clinical Application

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Abstract

"Once development was ended…in the adult centers, the nerve paths are something fixed and immutable. Everything may die, nothing may be regenerated," wrote Santiago Ramón y Cajal, a Spanish neuroanatomist and Nobel Prize winner and the father of modern neuroscience. This statement was the central dogma in neuroscience for a long time. However, in the 1960s, neural stem cells (NSCs) were discovered. Since then, our knowledge about NSCs has continued to grow. This review focuses on our current knowledge about NSCs and their surrounding microenvironment. In addition, the clinical application of NSCs for the treatment of various central nervous system diseases is also summarized.

Key words: neural stem cell, neurogenesis, clinical application

Introduction

"Once development was ended…in the adult centers, the nerve paths are something fixed and immutable. Everything may die, nothing may be regenerated," wrote Santiago Ramón y Cajal, a Spanish neuroanatomist and Nobel Prize winner and the father of modern neuroscience. This has been the central dogma in neuroscience for a long time. However, in the 1960s, neural stem cells (NSCs) were discovered. Since then, a great amount of research has focused on increasing our understanding of NSCs.

The method of neural induction has also been clarified, and in 2006, Takahashi and Yamanaka first reported on induced pluripotent stem (iPS) cells.[1)] Numerous studies have provided new hope for the development of cell therapy to treat central nervous system (CNS) diseases. Recently, clinical trials using stem cells to treat CNS diseases have been undertaken. In this review, the history and characteristics of NSCs are described, and the clinical applications of stem cells, mainly focusing on NSCs, are introduced.

Adult NSC and Neurogenesis

I. The discovery of adult NSCs and neurogenesis

The discovery of adult NSCs and neurogenesis occurred in the 1960s. Gage and Temple have summarized the events in a recent review.[2)] The observations of cell division and differentiation in the adult brain emerged from studies of brain development and were greatly advanced by the application of tritiated thymidine, which incorporates into the DNA of dividing cells and can be detected by autoradiography. Using this labeling technique, Smart and Leblond observed and concluded that glial cells were likely dividing throughout the parenchyma.[3)] Following these pioneering studies, Altman, using the same techniques, observed dividing cells in the subventricular zone (SVZ) and speculated that neurogenesis occurred in the adult rat and cat dentate gyrus (DG).[4,5)] Then, in 1965, Altman and Das provided the first strong evidence for neurogenesis in the adult brain,[6)] reporting on the migration of cells born postnatally in the SVZ, which matured into neurons in the olfactory bulb. In 1969, Altman was the first to describe the rostral migratory stream, which is located between the SVZ and olfactory bulb and serves as the migratory path for the NSCs born in the SVZ.[7)] Surprisingly, there was little follow-up to these discoveries for about 10 years.

The next phase in the early history of adult neurogenesis focused on the avian brain, where Goldman and Nottebohm first detected what they reported as neurogenesis in adult birds.[8)] Then, in the 1990s, there was the first evidence that proliferation levels of the early progenitor cells and subsequent numbers of newborn neurons are regulated; Gould et al. demonstrated that stress levels negatively affect the number of proliferating cells in the DG.[9)] This

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finding was followed by a series of observations demonstrating that neurogenesis could be substantially increased by running,[10] that housing animals even for short periods in complex enriched environments robustly increased the number of surviving newborn neurons,[11] that learning itself could influence adult neurogenesis,[12,13] and that antidepressant drugs, as well as alcohol,[14] could influence components of the adult neurogenesis process.[15] Around this same time, neurogenesis was shown to decrease with age, but persist throughout life.[16]

The next development was the advancement in immunohistological techniques combined with the application of confocal microscopy, which allowed researchers to visualize adult neurogenesis and to follow individually labeled cells. Importantly, the application of stereological techniques for quantifying dividing cells [in particular cells labeled with bromodeoxyuridine (BrdU)] and neuron-specific antibodies (initially NeuN), has allowed researchers to determine the absolute numbers of dividing and surviving cells in various brain regions. These techniques convincingly demonstrated that dividing cells from the DG indeed differentiated into neurons.[17,18] Subsequently, through the use of BrdU labeling in cancer patients, these techniques were also applied to determine that adult neurogenesis also takes place in humans.[19]

Another important development was the establishment of methods to isolate, propagate, and differentiate neuronal progenitors from the adult CNS in special culture conditions. This breakthrough was first achieved by dissecting the lateral wall of the lateral ventricle to obtain cells from the SVZ; these cells were then expanded in culture in proliferating populations that are now referred to as "neurospheres."[20,21] The ability to isolate, maintain, expand, and differentiate these precursor cells *in vitro* allowed for researchers to explore in greater detail the cellular and molecular nature of these cells, as well as the mechanisms that regulate their behavior.

II. NSCs in the adult mammalian brain

A neurogenic niche is a region where neurogenesis takes place.[22] There are two major neurogenic niches that exist in the adult mammalian brain where endogenous NSCs reside—the SVZ lining the lateral ventricles and the subgranular zone (SGZ) within the DG of the hippocampus (Fig. 1). The SVZ resides next to the ependymal cell layer, which is directly exposed to the cerebral spinal fluid and separates the ventricular space from the SVZ (Fig. 2A). Adult NSCs from the SVZ (also named "type B cells") extend a basal process that terminates on blood vessels, as well as an apical process with a primary cilium that pokes through the ependymal cell layer to contact the cerebrospinal fluid (CSF) in the ventricle.[23] Type B NSCs give rise to transient amplifying progenitors (C cells),[24] which undergo multiple divisions before becoming neuroblasts (A cells). Neuroblasts then form a chain and migrate into the olfactory bulb where they radially migrate and differentiate into different subtypes of interneurons. Radial glia-like NSCs (named RGLs or type 1 cells) in the SGZ, which reside at the border between the inner granule cell (GC) layer and hilus, give rise to intermediate progenitor cells (IPCs),[25] which exhibit

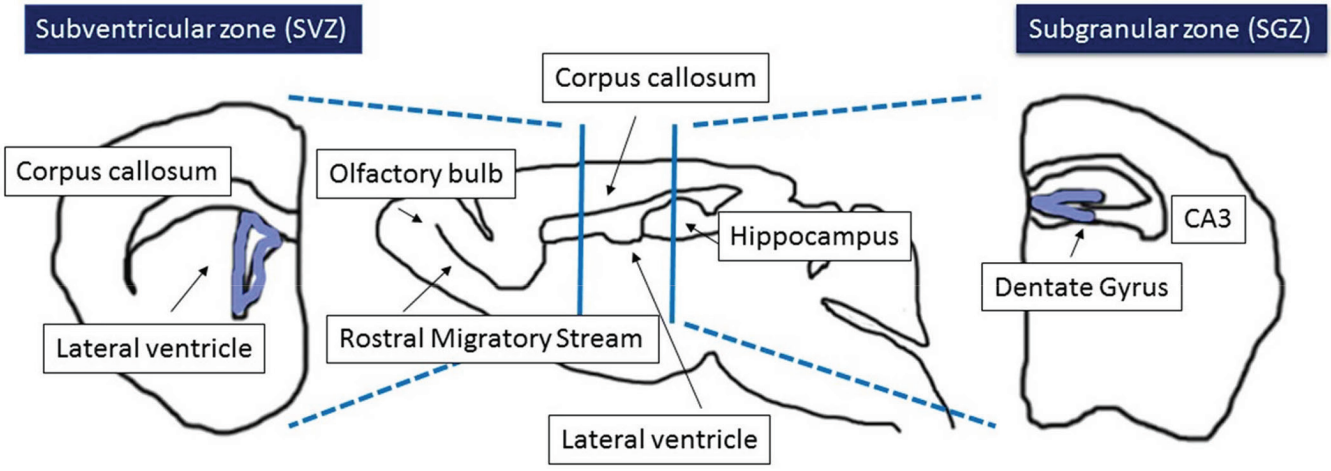

Fig. 1 Behavior of neural stem cells (NSCs) in the adult rodent brain. A sagittal view of the adult rodent brain, focusing on two major niches where adult NSCs reside: the subventricular zone (SVZ) and the subgranular zone (SGZ). The SVZ is located along the lateral ventricle in the forebrain and the SGZ is located in the hippocampus along the dentate granule cell layer, where it abuts the hilus.[22]

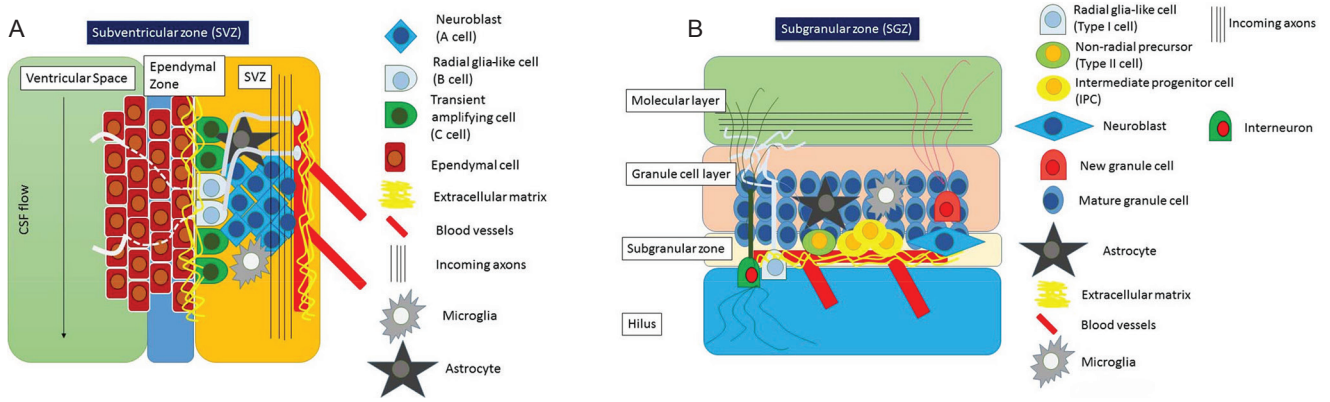

Fig. 2 Adult neural stem cell (NSC) niches. A: A schematic diagram depicting cellular and molecular components of the SVZ niche. Ependymal cells line the lateral ventricle and border the SVZ. Radial glia-like neural stem cells (B cells) reside along the ependymal zone in the SVZ and extend a radial process to contact blood vessels. They also extend a single cilium through the ependymal rosettes to contact the cerebrospinal fluid in the ventricular space. Radial glia-like NSCs generate transit-amplifying cells (C cells), which generate neuroblasts (C cells). Neuroblasts migrate along the rostral migratory stream to the olfactory bulb, where they differentiate into olfactory bulb interneurons. In addition to the aforementioned cell types, astrocytes and microglia contribute to the cellular architecture of the niche.[22] B: A schematic diagram depicting cellular and molecular components of the SGZ niche. Radial glia-like NSCs (type 1 cells) reside in the SGZ and extend a radial process through the granule cell layer of the dentate gyrus into the molecular layer. Radial glia-like NSCs generate intermediate progenitor cells (IPCs), which generate neuroblasts, and these progenitor cells are closely associated with the vasculature. Neuroblasts differentiate into dentate granule cells, which migrate into the granule cell layer of the dentate gyrus. In addition to the aforementioned cell types, astrocytes, microglia, and interneurons contribute to the cellular architecture of the niche. CSF: cerebrospinal fluid.[22]

limited rounds of proliferation before generating neuroblasts (Fig. 2B).[26] Neuroblasts tangentially migrate along the SGZ and develop into immature neurons, which radially migrate into the GC layer to differentiate into dentate granule neurons.[27]

Stem cells are characterized by two fundamental properties: the ability to self-renew and the ability to rise to differentiated progeny (Fig. 3A). It had long been postulated that adult neurogenesis originates from tri-potent NSCs with the capacity to generate neurons, astrocytes, and oligodendrocytes. The existence of self-renewing, multipotent adult NSCs was originally suggested by the long-term expansion and differentiation of neurospheres (nonadherent, spherical cultures of clonally derived precursors), or monolayer cultures that differentiated into three neural lineages.[21,28] However, recent genetic fate-mapping and clonal lineage-tracing of NSCs in the adult hippocampus *in vivo* have determined that these cells generate neurons and astrocytes, but not oligodendrocytes as originally thought.[29] In the adult SVZ, results from population fate-mapping studies had suggested the generation of both neurons and oligodendrocytes, but recent *in vivo* clonal analysis has determined only neuronal lineages from individual NSCs.[30] Nevertheless, *in vitro* time-lapse analysis revealed the generation of either neurons or oligodendrocytes from acutely isolated individual precursor cells, but never both (Fig. 3B).[31]

Studies using an anti-mitotic drug to eliminate dividing precursors showed that adult NSCs are largely quiescent *in vivo*.[32] The quiescent state has long been viewed as dormant and passive, and quiescence is thought to allow adult stem cells to withstand metabolic stress and to preserve genome integrity over a lifetime. Emerging evidence suggests just the opposite. Single-cell transcriptome analysis of quiescent adult SGZ NSCs revealed active expression of various receptors for niche signals and downstream signaling components, the majority of which are downregulated once NSCs become activated.[33] In most adult somatic stem cell systems, quiescent and active stem cell populations co-exist.[34] The most obvious example of heterogeneity among adult NSCs is the difference in their progeny between two niches: SVZ NSCs generate olfactory bulb interneurons and corpus callosum oligodendrocytes, whereas SGZ NSCs generate dentate granule neurons and astrocytes. Notably, NSCs derived from both niches generate all three neural lineages once propagated in culture with high concentrations of growth factors,[21,28] suggesting that the *in vivo* niche may limit adult NSC potential.

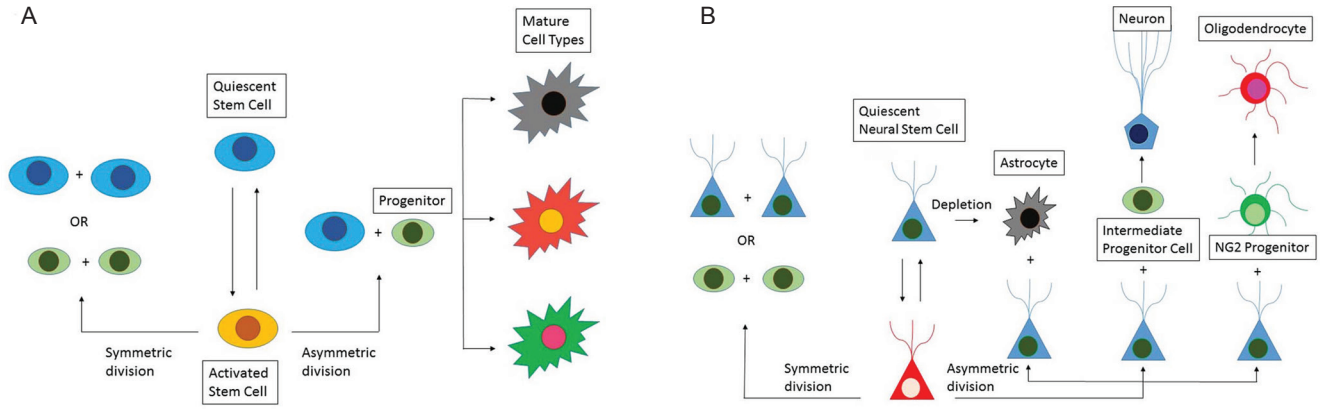

Fig. 3 Behavior of neural stem cells (NSCs) within adult niches. A schematic diagram illustrating the potential behavior of an adult stem cell (A) and, more specifically, of an adult NSC (B) over its life cycle. Adult NSCs can transition between quiescent and active states by exiting and entering the cell cycle, respectively. Once activated, NSCs choose between different modes of division. Asymmetric division is self-renewing and yields an NSC and a progenitor, whereas symmetric division yields either two NSCs (self-renewing) or two progenitors (not self-renewing). Progenitors may be fate-restricted, meaning that they can only differentiate into a particular cell type, or they may be multipotent and must make a fate choice before differentiation. It is also possible that NSCs may directly differentiate into mature glial cell types.[22]

III. Functions of adult neurogenesis

Once the evidence for the existence of adult neurogenesis was generally accepted, the question of its functional relevance emerged. A series of correlational studies clearly revealed that upregulated neurogenesis in the DG also increased behavioral performance in a variety of hippocampus-related tasks and, conversely, downregulated neurogenesis resulted in behavioral impairments. Experiments designed to decrease neurogenesis by irradiation, virus manipulation, antimitotic agents, or the engineering of transgenic animals that allowed for genetic or pharmacological regulation of adult neurogenesis, confirmed a functional role for adult neurogenesis in the DG.[35]

To more completely understand the functional importance of adult neurogenesis, it is important to consider adult neurogenesis in the context of the hippocampus and its theoretical function as a whole. Individual GCs in the DG receive inputs from thousands of entorhinal cortex neurons, suggesting that they are capable of simultaneously representing a highly complex combination of spatial and object features. Several studies[36–38] have suggested that the DG's encoding role can be thought of as a computational perspective that has attracted considerable attention in recent years, where the DG is critical for "pattern separation." Pattern separation, as related to the DG, can be described as recoding cortical input information into a sparse, essentially orthogonal representation.[39,40] By manipulating the rate of adult neurogenesis, several studies have used ablation techniques or the overexpression of adult neurogenesis in tests that reflect the computational characteristics of pattern separation to show that newborn neurons are critical for making fine discriminations between neighboring spatial locations or highly similar environments.[41–45] Together, these studies support the concept that a DG network dominated by young GCs is biased toward interpreting similar, but not identical inputs, as distinct, whereas older GCs are biased toward interpreting similar inputs as equivalent.

Although adult neurogenesis in the DG is now generally accepted to occur in all adult mammals, further studies are needed to determine the mechanisms involved in the functional contribution of hippocampus-mediated behaviors. Additionally, a greater understanding of how hippocampal circuits mediate behaviors is needed. Future studies focused on adult neurogenesis and its contribution to hippocampal function will greatly advance this field of study.

Clinical Application of NSCs for the Treatment of CNS Diseases

I. Parkinson disease

Parkinson disease (PD), which is characterized by an extensive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta and their striatal terminals, affects more than 500,000 people in the United States, with 50,000 new cases reported annually.[46,47] While the etiology of idiopathic PD is not

known, several predisposing factors for dopamine depletion associated with the disease have been suggested, including programmed cell death, viral infection, and environmental toxins. As an effective treatment for PD, patients have been administered L-dihydroxyphenyl alanine (L-DOPA), a precursor of dopamine. However, long-term L-DOPA administration results in grave side effects.[48,49] More recently, surgical deep-brain stimulation has been adopted as a more successful treatment for PD patients.[50]

Since the late 1980s, the transplantation of human fetal ventral mesencephalic tissues into the striatum of PD patients has been used as a successful therapy for patients with advanced disease.[51–54] However, fetal tissue transplantation has been associated with ethical and religious questions, as well as problematic logistics of acquiring fetal tissues. Additionally, recent reports have indicated that the survival of transplanted fetal mesencephalic cells in the patient brain was very low, and it was difficult to obtain sufficient fetal tissues needed for transplantation.[55] To overcome these difficulties, neurons with a DA phenotype have been generated from ESCs, iPSCs, MSCs, or NSCs and could serve as a practical and an effective alternative for the transplantation of fetal brain tissues. DA neurons have been generated from mouse ESCs following treatment with fibroblast growth factor 8 (FGF8) and sonic hedgehog,[56,57] overexpression of Nurr1[58,59] or Bcl-XL,[60] or co-culture with a mouse bone marrow stromal cell line.[61] Neurons with a DA phenotype have also been generated from monkey ESCs by co-culturing with mouse bone marrow stromal cells; behavioral improvement was observed in MPTP-lesioned monkeys following intra-striatal transplantation of these cells.[62]

DA neurons have also been generated from neural progenitor cells derived from fetal brain and were shown to induce functional recovery following transplantation into the brains of parkinsonian monkeys.[63] Transplantation of NSCs in the brain attenuates anatomical or functional deficits associated with injury or disease in the CNS via cell replacement, the release of specific neurotransmitters, and/or the production of neurotrophic factors that provide neuroprotection and promote neuronal growth.

Previous studies have reported that mouse or human ESC-derived DA neurons are efficacious in PD animal models; however, considerable safety concerns exist, such as risk of tumor formation and neural overgrowth. More recent studies have indicated that functional human DA neurons could be efficiently generated from human ES cells, and upon transplantation in rat PD models, the ES cell-derived DA neurons induced behavioral recovery.[64–66]

Human DA neurons derived from iPS cells may provide an ideal cellular source for PD transplantation therapy because they can be generated from the patient's own fibroblasts and do not cause immune rejection. However, the development of an effective cellular therapy for PD using iPS cells relies on the optimization of *in vitro* production of iPS cell-derived DA neurons, as well as the prevention of teratoma formation *in vivo*. A recent study reported on the generation of DA neurons from iPS cells derived from fibroblasts and improved behavior following transplantation of these DA neurons in PD model rats.[67] When multiple human iPSC lines derived by virus- and protein-based reprogramming were compared, DA neurons derived from protein-based iPSCs were best suited for transplantation, because they induce gene expression and exhibit physiological and electrophysiological properties similar to human midbrain DA neurons.[68] DA neurons have also been generated from iPS cells from PD patients; these DA neurons were transplanted without signs of neurodegeneration into a PD animal model. Additionally, the neurons survived at high numbers and mediated functional effects in PD animals.[69] These PD iPS cell-derived DA neurons could be used for screening novel drugs in the development of PD therapeutic strategies.

More recently, human fibroblasts were directly converted into DA neuron-like cells through the use of a combination of five transcriptional factors: *Mash1*, *Ngn2*, *Sox2*, *Nurr1*, and *Pitx3*. Results showed that the reprogrammed cells expressed various markers for DA neurons. Although further research is still required, cell therapy strategies based on iPS-derived DA neurons[70] or DA neurons directly converted from fibroblasts may become a promising treatment strategy for PD patients in the coming years.

II. Huntington disease

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder characterized by involuntary choreic movements, cognitive impairment, and emotional disturbances.[71,72] Despite the identification of the HD gene and associated protein, the mechanisms involved in HD pathogenesis remain largely unknown, thereby hampering effective therapeutic interventions. The transplantation of fetal human brain tissue may serve as a useful strategy in reducing neuronal damage in the HD brain. A recent study documented improvements in motor and cognition performance in HD patients following fetal cell transplantation.[73] This trial follows previous reports in HD experimental animals describing the positive effects of fetal striatal cell transplantation in ameliorating neuronal dysfunction[74] and that striatal

graft tissue could integrate and survive within the progressively degenerated striatum in a transgenic HD mouse model.[75] This latter study is consistent with results obtained from HD patients indicating survival and differentiation of implanted human fetal tissue in the affected regions.[76] Cell replacement therapy using human fetal striatal grafts has shown clinical success in HD patients.

It is, however, important to note that a recent study reported neural overgrowth of grafted tissue in a HD patient who survived 5 years post-transplantation.[77] Overgrown grafts were composed of neurons and glia embedded in a disorganized neuropil. This report highlights the safety concerns related to fetal cell grafts and the potential risk of neural overgrowth following transplantation in the brain of HD patients. The use of NSC transplantation to replace degenerated neurons or genetically modified NSCs producing neurotrophic factors has been employed to protect striatal neurons against excitotoxic insults.[78] At present, little is known regarding whether NSC implantation prior to neuropathological damage could alter the progressive degeneration of striatal neurons and motor deficits that occur in HD. This question is important, because genetic studies of HD gene mutations[79] and neuroimaging have provided details about the factors involved in HD progression,[80,81] suggesting that early intervention using brain transplantation could be effective in "pre-clinical" HD patients that express the mutant HD gene.

III. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a relentlessly progressive, adult-onset, neurodegenerative disorder characterized by the degeneration and loss of motor neurons in the cerebral cortex, brain stem, and spinal cord, leading to muscle wasting and weakness, and eventually to death within 5 years after onset of clinical symptoms.[82,83] The proposed pathogenetic mechanisms of ALS, which have not been fully determined, include oxidative stress, protein aggregation, mitochondrial dysfunction, impaired axonal transport, glutamate-mediated excitotoxicity, and insufficient production of neurotrophic factors.[84] To date, there is no effective treatment for patients suffering from ALS. Recent studies have indicated that it is possible to generate motor neurons in culture from stem cells, such as ESCs and NSCs.[85–88] A phase I trial of intraspinal injections of fetal-derived NSCs in ALS patients was conducted in the United States. Ten total injections were made into the lumbar spinal cord at a dose of 100,000 cells per injection in 12 ALS patients. Clinical assessments, ranging from 6 months to 18 months after transplantation, demonstrated no evidence of acceleration of disease progression due to the intervention.[89] A previous study reported that iPSCs isolated from an ALS patient were differentiated into motor neurons,[90] suggesting that these patient-derived neurons could serve as an ideal cellular source for screening new drug candidates. Neurons and glia induced from patient-derived iPSCs are autologous and easily accessible, and also lack the problems associated with immune rejection and ethical concerns.

It is unrealistic to expect transplantation of stem cells or stem cell-derived motor neurons to replace lost neurons in ALS patients or to integrate into existing neural circuitry and restore motor function. Rather, preventing cell death in host motor neurons via provision of neurotrophic factors by transplanted stem cells or stem cell-derived motor neurons is more realistic and an achievable approach.[91]

IV. Alzheimer disease

Alzheimer disease (AD) is characterized by the degeneration and loss of neurons and synapses throughout the brain, particularly in the basal forebrain, amygdala, hippocampus, and cortical area. Symptoms include progressive decline in memory and cognitive function, as well as dementia and premature death.[92–94] To date, there is no effective treatment. Acetylcholinesterase inhibitors have been shown to augment cholinergic function, but this is not curative and is only a temporary measure.

With regard to AD pathogenesis, the amyloid cascade hypothesis postulates that memory deficits are caused by increased levels of both soluble and insoluble amyloid beta (Aβ) peptides, which are derived from the larger amyloid precursor protein (APP) sequential proteolytic processing.[92–94]

Previously, a phase 1 clinical trial utilizing *ex vivo* NGF gene delivery was performed in eight mild AD patients; autologous fibroblasts genetically modified to express human NGF were implanted into the forebrain. After a mean follow-up of 22 months in six subjects, long-term adverse effects were not observed. Evaluation by Mini-Mental State Examination and Alzheimer's Disease Assessment Scale-Cognitive Subcomponent (ADAS-Cog) suggested improvement in the rate of cognitive decline, and serial positron emission tomography (PET) scans showed significant increases in cortical fluorodeoxyglucose after treatment.[95] In AD patients, a dysfunctional presynaptic cholinergic system is one of the causes for the cognitive disorders involved in patients where decreased activity of choline acetyltransferase (ChAT), which is responsible for acetylcholine (ACh) synthesis, is observed.[96] To date, AD therapy has been largely

based on small molecules designed to increase ACh concentration by inhibiting acetylcholinesterase.[97] Because therapies with these drugs is only palliative and does not provide protection against progressive tissue destruction, there is a continued need for effective therapies for AD patients. Stem cell-based therapeutic approaches targeting AD provide hope for fulfilling this requirement.

A recent review article indicated that stem cell transplant therapy is an extension of NSC use in other neurological treatments, such as PD and stroke, and could serve as a highly effective therapeutic approach for AD.[98]

V. Cell-based therapies against stroke

In a recent review by George and Steinberg, the use of stem cell therapy to treat stroke patients was discussed.[99] Stem cell therapy is an exciting area of research that has entered the clinical arena with multiple ongoing trials. Stem cells are pluripotent or multipotent cells that have the ability to transform into multiple cell types and are self-perpetuating. Endogenous therapeutic strategies have focused on increasing mobilization, longevity, and production of NSCs in the SVZ and dentate gyrus. Exogenous stem cell treatments refer to transplanted cells from another source into a patient. Exogenous stem cells have been delivered to the brain via the blood stream, or direct transplantation and have shown great promise in stroke animal models to enhance recovery.

VI. Endogenous stem cells

NPCs from the SVZ, which normally migrate along the rostral migratory system to the olfactory lobe, have also been shown to traverse to injured brain areas following neurological insult.[100] Brain ischemia results in the upregulation of endogenous NPCs and occasional differentiation into the predominant cell type of the injured region.[101,102] Therapeutic approaches have focused on augmenting the brain's normal endogenous reaction to injury. Multiple pathways have been used to induce neurogenesis, including those triggered by numerous neurotrophic and growth factors such as GDNF, BDNF, granulocyte colony-stimulating factor (G-CSF), and insulin growth factor (IGF-1).[103,104] Alternative mechanisms of increasing endogenous NPC proliferation include anti-inflammatory drugs like indomethacin, non-coding RNA, and hormones such as erythropoietin.[105–107] The delivery of G-CSF and IGF-1 to alter key survival pathways, such as the phosphoinositide 3-kinase-Akt pathway, has been shown to reduce NPC death.[108] Current clinical trials are investigating the ability of G-CSF to mobilize endogenous bone marrow cells, as well as utilizing the neuroprotective effects of G-CSF for stroke recovery.[109,110] Increasing the number of migrating endogenous stem cells can be achieved using various chemokine receptors, such as stromal-derived factor 1 and integrin beta-1.[111,112] However, this research is currently limited to the preclinical arena.

VII. Exogenous stem cells

Exogenous stem cells are typically divided into three categories: (1) immortalized cell lines, (2) NPCs or NSCs, and (3) bone marrow-derived hematopoietic/endothelial progenitors and stromal cells.[113] Immortalized cell lines have been developed from tumor cells or from oncogene manipulation (such as myc in the human fetal neural cell line ReN001 of ReNeuron). NT2N cells, which are derived from teratocarcinomas, differentiate into post-mitotic neuron-like cells with the addition of retinoic acid and mitotic inhibitors,[114,115] and have been shown to improve outcome in several ischemic models.[116] ReNeuron's cells have shown to exhibit dose-dependent recovery in stroke rodent models,[117] and have been engineered to be immortal only in the presence of tamoxifen to reduce the risk of tumor formation.[118]

Human NPCs are derived from embryonic and fetal tissue and have the ability to produce astrocytes, neurons, and oligodendrocytes.[119] In stroke models, NPCs are able to migrate to the injured regions and improve recovery.[120–122] NPCs sometimes integrate into the host tissue, differentiate, and exhibit neuronal characteristics, including expression of synaptic proteins, synapse formation, and electrophysiological properties.[123–125] Progenitor cells derived from bone marrow, umbilical cord blood, and adipose tissue have all been shown to improve recovery in stroke models.[126] Many of these sources are already used for the clinical treatment of other disorders, such as malignancy, and can be obtained from autologous harvesting.

Although many cell types are included in each of these sources and it appears that the mononuclear or marrow stromal cell component mediates recovery, it is not clear which subtype is responsible for improving functional outcomes. Multiple trials using exogenous stem cells have been performed or are ongoing.

The discovery of iPS cells created a paradigm shift in cell therapy. The ability to transform host somatic cells, such as fibroblasts, into pluripotent stem cells bypassed many of the concerns of traditional stem cell therapy, such as ethical discussions, supply limitations, and the possible requirement of immunosuppression.[1,127,128] Further development has led to vector- and transgene-free techniques to

derive iPS cells that improve functional outcome after brain ischemia.[129] A recent study was able to generate neural cells directly from mouse or human fibroblasts using transcription factors, without passing through a pluripotent phase, which may ultimately have clinical relevance.[130]

Apart from efficacy, safety is an important consideration to further develop cell transplantation therapy. Careful biological classification and understanding will be critical for reducing any possibility of tumor formation or adverse effects.[131] Immortalized cell lines (NT2N) were the first human cells used in a clinical phase I stroke trial and were implanted into the infarcted region of 12 patients, 6 months to 6 years after a basal ganglia stroke.[132] There were no significant adverse events and functional improvement was observed in this small patient group. A subsequent phase II trial with NT2N cells implanted into the peri-infarct or peri-hemorrhagic cavity also showed no increase in adverse events.[133] An open-label, single-blinded, randomized trial using mesenchymal stem cells (MSCs) showed significant improvement in functional outcome based on the modified Rankin scale (a functional outcome scale with 0–3 representing the ability to walk with varying degrees of disability) in the treatment group without a difference in adverse events, and multiple other trials have indicated safety and feasibility.[134–136] In addition, trials using bone marrow mononuclear cells (BMMNCs) have shown safety and feasibility in the acute and chronic phases of recovery.[137–139] A phase 1/2A study that transplanted human-modified bone marrow-derived stromal cells were safe and feasible for direct intracerebral transplantation 6 months to 5 years post-stroke, with improvement in neurological outcomes.[140] Many questions have been raised about the translation of cell therapy to clinical applications, and numerous clinical trials are underway (Table 1) to determine whether cell-based therapy will become the next modality for restorative stroke therapeutics.[99]

Table 1 List of completed or ongoing trials using exogenous stem or progenitor cells

Study type	Sponsor	Cell type	Planned enrollment	Timing of delivery	Delivery route	Status/Results
Ph1-NR-OL	Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)	BMMNC	15	3–90 days	IA or i.v.	Complete, no reported results
Ph1-NR-OL	Neurogen Brain and Spine Institute (Mumbai, India)	BMMNC	30	chronic	IT	No reported results
Ph2-R-OL	Manipal Acunova Ltd. (Manipal, India)	BMMNC	11	7–30 days	i.v.	Safe, feasible
Ph1/2-R-DB	University of California (Irvine, USA)	BMMNC	40	1–3 days	i.v.	Not currently recruiting
Ph1-NR-OL	The University of Texas Health Science Center (Houston, USA)	BMMNC	10	1–3 days	i.v.	Safe, feasible
Ph2-R-SB	All India Institute of Medical Sciences (New Delhi, India)	BMMNC	120	7–30 days	i.v.	Safe, feasible, no efficacy benefit
Ph1/2-NR-OL	Chaitanya Hospital (Pune, India)	BMMNC	50	chronic	IT	Recruiting
Ph1-NR-OL	Neurogen Brain and Spine Institute (Mumbai, India)	BMMNC	200	subacute/ chronic	IT	Recruiting
Ph1/2-NR-OL	India	BMMNC	11	3–12 months	i.v.	Safe, feasible, improved neurologic outcomes
Ph2-R-OL	Andalusian Initiative for Advanced Therapies (Andalusia, Spain)	BMMNC	76	1–7 days	IA	Recruiting
Ph2-R-DB	Athersys, Inc. (Cleveland, USA)	multistem	126	1–2 days	i.v.	Safe, feasible, no efficacy benefit
Ph2-NR-OL	ReNeuron Limited (Guildford, UK)	CTX0E03, NSC	41	2–3 months	IC	Safe, improved neurologic outcomes

Continued

Table 1 Continued

Study type	Sponsor	Cell type	Planned enrollment	Timing of delivery	Delivery route	Status/Results
Ph1-NR-OL	ReNeuron Limited (Guildford, UK)	CTX0E03, NSC	12	6–60 months	IC	Not currently recruiting
Ph1/2- NR-OL	Ageless Regenerative Institute (FL, USA)	ASC	10	subacute	IA	Not currently recruiting
Ph1/2-R-DB	Stempeutics Research Pvt Ltd (Whitefield, India)	MSC	78	< 10 days	i.v.	Not currently recruiting
Ph1/2-R-OL-SB	South Korea	MSC	85	5–7 weeks	i.v.	Safe, feasible, improved neurologic outcomes
Ph2-R-OL	Stempeutics Research Pvt Ltd (Whitefield, India)	MSC	30	< 6 weeks	i.v.	Not currently recruiting
Ph1/2-NR-OL	Stemedica Cell Technologies, Inc. (San Diego, USA)	MSC	35	> 6 months	i.v.	Recruiting
Ph1/2-R-DB	Instituto de Investigación Hospital Universitario La Paz (Madrid, Spain)	MSC	40	< 14 days	i.v.	Not currently recruiting
Ph1-NR-OL	Japan	MSC	12	1–4 months	i.v.	Safe, feasible, decreased infarct
Ph3-R-OL	Samsung Medical Center (Seoul, South Korea)	MSC	60	< 90 days	i.v.	recruiting
Ph1/2-R-DB	Sean Savitz (Houston, USA)	MSC	48	3–10 days	i.v.	Not currently recruiting
Ph1/2-R-DB	Southern Medical University (GuangZhou, China)	MSC, EPC	90	5 weeks	i.v.	Recruiting
Ph1/2-NR-SB	Hospital Universitario Central de Asturias (Asturias, Spain)	CD34+	20	5–9 days	IA	Safe, feasible, increased b-NGF
Ph2-R-OL	China Medical University Hospital (Taichung City, Taiwan)	CD34+	30	6–60 months	IC	Complete, no reported results
Ph1/2-NR-OL	Imperial College London (London, UK)	CD34+	5	7 days	IA	Safe, feasible, reduced infarct volume
Ph1-R-OL	Zhejiang Hospital (Zhejiang, China)	CD34+	40	< 12 months	IA	Recruiting
Ph1-NR-OL	China Medical University Hospital (Taichung City, Taiwan)	CD34+	6	6–60 months	IC	Not currently recruiting
Ph2-R-DB	Celgene Corporation (Summit, USA)	PDC	44	acute	i.v.	Stopped by sponsor
Ph1/2-NR-OL	SanBio, Inc. (Mountain View, USA)	SB623	18	6–36 months	IC	Safe, improved neurologic outcomes
Ph2-R-OL-SB	University of Pittsburgh (Pittsburgh, USA)	NT2	18	1–5 years	IC	Safe, feasible, improved neurologic outcomes in secondary endpoints
Ph1-NR-OL	University of Pittsburgh (Pittsburgh, USA)	NT2	12	6–72 months	IC	Safe, improved neurologic outcomes

ASC: adipose-derived stromal cell, DB: double blind, EPC: endothelial progenitor cell, IA: intra-arterial, IC: intracranial, i.v.: intravenous, NR: nonrandomized, NSC: neural stem cell, NT2: tetracarcinoma cell-derived neurons, OEC: olfactory ensheathing cell, OL: open label, P1: Phase 1 trial, P2: Phase 2 trial, PDC: placenta-derived stem cell, R: randomized, SB: single blind, SB623: human mesenchymal stromal cells.

Conclusion

Recent scientific advancements have suggested the novel use of NSC for therapy, with numerous clinical trials underway. Results from these studies will provide valuable information for future strategies. However, the demonstration of efficacy in randomized, double-blinded trials is needed to prove the efficacy of cell therapy for the treatment of CNS diseases.

Conflicts of Interest Disclosure

None declared. The author is a member of the Japan Neurosurgical Society and has registered online self-reported COI disclosure statement forms.

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