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Review

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Development and application of neural stem cells for treating various human neurological diseases in animal models

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Stem cells derived from adult tissues or the inner cell mass (ICM) of embryos in the mammalian blastocyst (BL) stage are capable of self-renewal and have remarkable potential for undergoing lineage-specific differentiation under *in vitro* culturing conditions. In particular, neural stem cells (NSCs) that self-renew and differentiate into major cell types of the brain exist in the developing and adult central nervous system (CNS). The exact function and distribution of NSCs has been assessed, and they represent an interesting population that includes astrocytes, oligodendrocytes, and neurons. Many researchers have demonstrated functional recovery in animal models of various neurological diseases such as stroke, Parkinson's disease (PD), brain tumors, and metastatic tumors. The safety and efficacy of stem cell-based therapies (SCTs) are also being evaluated in humans. The therapeutic efficacy of NSCs has been shown in the brain disorder-induced animal models, and animal models may be well established to perform the test before clinical stage. Taken together, data from the literature have indicated that therapeutic NSCs may be useful for selectively treating diverse types of human brain diseases without incurring adverse effects.

Key words: Neural stem cells, Parkinson's disease, stroke, brain tumor, metastatic tumor

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The most important characteristics of stem cells are their ability to self-renew, multilineage differentiation, and capability of promoting *in vivo* functional reconstitution of a given tissue [1]. The self-renewal of stem cells is achieved by suppressing differentiation and stimulating proliferation [2]. This enables extensive *ex vivo* and *in vivo* expansion of progenitor cell populations in a targeted tissue, a key feature for generating a sufficient number of cells to meet the potential demand for tissue replacement [3]. The multi-lineage differentiation potential of stem cells presents both an opportunity and challenge since differentiation at the wrong time, place, or into an undesired cell type may lead to the development of a pathophysiological state or non-functional tissue [4]. These unique properties allow stem cells to have the potential for revolutionizing medicine by offering therapeutic options for a wide range of diseases and disorders for which no treatments currently exist [5]. Stem cell-based therapy (SCT) has garnered significant interest over the last decade as a strategy for treating a wide range of diseases [6]. Many stem cell-based techniques have shown great promise in preclinical studies. For example, promising breakthroughs for SCTs against bone disease, heart disease, puerperal vascular disease, spinal cord injury, cancer, and neurological disease have been reported [7].

Stem cells derived from adult tissues or the inner cell mass (ICM) of mammalian embryos in the blastocyst

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(BL) stage can self-renew and have remarkable potential for undergoing lineage-specific differentiation under *in vitro* culturing conditions [4,8,9]. Embryonic stem (ES) cells derived from the ICM/epiblast of pre-implantation embryos first obtained from mice can be cultured *in vitro* in an undifferentiated state for several passages and induced to differentiate into the three primary germ layers (the ectoderm, mesoderm, and endoderm) *in vitro* and *in vivo* [10,11]. Mouse ES cells can be maintained in an undifferentiated state in medium containing bone morphogenetic proteins (BMPs), leukemia inhibitory factor (LIF), and neural supplements, N2/B27 [12]. In contrast, human ES cells undergo self-renewal via fibroblast growth factor (FGF)-2 and the activin/nodal signaling pathway [13]. LIF/STAT3, BMP/inhibitor of differentiation (ID), phosphoinositide-3-kinase (PI3K)/Akt, and Src signaling cascades have been shown to play critical roles in stem cell self-renewal [14,15].

Adult stem cells that are intrinsic to various tissues, such as bone marrow, skin, amnion, and brain, have been described and characterized [16]. The best studied adult stem cells, hematopoietic stem cells (HSCs), undergo self-renewing cell division, differentiate at the single cell level into mature blood elements, and functionally repopulate the hematopoietic system of myeloablated animals or humans [17]. Other types of adult stem cells were more recently defined and have therefore been less frequently studied. Nevertheless, neural stem cells (NSCs), mesenchymal stem cells (MSCs), and epidermal stem cells all fulfill the basic stem cells criteria.

NSCs are undifferentiated precursor cells defined by their capacity for self-renewal and multipotency, and show complex patterns of gene expression that vary in space and over time [18]. These cells are obtained from embryonic, fetal, neonatal, or adult CNS tissues, and form multicellular free floating spheres (neurospheres) that spontaneously differentiate into neurons, astrocytes, neurons, or oligodendrocytes [19]. In the postnatal mammalian brain, NSCs are retained in a unique compartment after embryonic development and generate new cells throughout the life of the animal. Under normal conditions, postnatal neurogenesis occurs only in two major neurogenic regions: the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the dentate gyrus of the hippocampus [20]. This specialized microenvironment is called the NSC niche and provides appropriate cues that regulate NSC behaviors such as maintenance, self-renewal, and proliferation [21]. The NSC niche is composed of cellular components and extracellular substrates that collectively provide a residing milieu for the NSCs and regulate NSC behaviors. This region has been defined as a highly specialized CNS germinal niche that contains slowly proliferating putative CNS stem cells positive for glial fibrillary acidic protein (GFAP), nestin, and the radial glial marker RC2 [22]. NSCs are slowly dividing cells possessing a self-renewal capacity, astrocyte-like features, and include actively dividing transit amplifying progenitors (TAPs) [23]. Endogenous NSCs residing in germinal niches might be beneficial for nervous system repair owing to their ability to promote neurogenesis and gliogenesis during adulthood.

NSC-based therapies for treating nervous system disorders, stroke, Parkinson's diseases (PD), Huntington's disease (HD), multiple sclerosis, spinal cord injury (SCI), brain tumors, and brain trauma have been successfully developed [24]. Most of these have been reported in experimental models. Thus, there are still important issues that need to be resolved before any potential human applications can be developed. Transplantation approaches at the experimental level must also be further refined before clinical application can be considered.

NSC-based therapies for treating stroke in animal models

Stroke is one of the most common causes of neurological diseases related death in the worldwide [25]. Extensive ischemic injury is a neurological disorder caused by multiple factors including hypoxia and severe damage to the cerebral parenchyma that result in the formation of a cystic cavity and consequential loss of neural cells and their connections. This leads to the death of oligodendrocytes, astrocytes, and endothelial cells [26]. In the past, fetal brain tissue transplants have been shown to promote limited recovery in animal models of stroke, but ethical considerations and a scant supply of human fetal tissue have limited this approach [27]. Successful isolation and transplantation of adult NSCs has demonstrated the feasibility of using autologous NSCs transplantation as a regenerative strategy after stroke. Most animal models of ischemic stroke involve occlusion of the arterial blood supply to the brain using surgical techniques [28]. Jeong *et al*. reported that human NSCs delivered intravenously are beneficial in an animal model of stroke [29]. Intravenously transplanted NSCs can enter the brain of rats with intracerebral hemorrhage (ICH), survive, migrate,

and improve functional recovery. Transplanted NSCs selectively migrate to the perihematomal areas and differentiated into neurons (approximately 10%) and astrocytes (approximately 75%). Kelly *et al*. also studied the effects of human neurospheres derived from CNS stem cells transplanted into the ischemic cortex of rats 7 day after distal middle cerebral artery occlusion [30]. The neurospheres continued to survive in both naïve and ischemic brains 4 weeks after transplantation. Additionally, the microenvironment was found to influence neurosphere migration and fate.

NSCs are a relatively quiescent cell population, and stem cell proliferation in the SVZ is tightly controlled under physiological conditions. Stroke upregulates the expression of mitogens, including epidermal growth factor (EGF) and bFGF, which may contribute to increased NSC numbers [31]. Zhang *et al*. found that after stroke, neuroblasts derived from NSCs proliferate and migrate into the ischemic striatum [32]. This might have important implications for targeting endogenous NSCs and progenitor cells in the areas of damaged brain tissues undergoing repair. Moreover, increased neurogenesis and migration of NSCs to the site of injury indicates that stromal derived factor-1 (SDF-1) and angiopoietin-1 contribute to the targeting of newly formed neural progenitors to the injury sites [33].

Genetically modified NSCs have also been shown to improve function in a mouse model of stroke. This might be accomplished by overexpressing brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), or Akt which is known as a general mediator of cell survival [34]. Akt, a serine/threonine kinase, exerts anti-apoptotic effects against a variety of pro-apoptotic factors including withdrawal of extracellular signaling molecules, oxidative and osmotic stress, and ischemic shock [35]. This protein prevents cerebellar granule cells from undergoing apoptotic cell death, and promotes the survival of hippocampal neurons under hypoxic conditions [36]. In mouse ICH models, human NSCs genetically modified to express Akt1 improve motor performance as determined by rotarod and limb placement tests, and increase the survival of grafted NSCs or differentiation into neurons and astrocytes [37]. Taken together, the results of several previous studies suggest that NSC transplantation is a potential regenerative therapy for treating stroke. Currently, the development of stem cell therapy for stroke patients is in its infancy.

NSC-based therapies for treating PD in animal models

PD is common neurodegenerative disease characterized by an extensive loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and DA neuronal terminals in the striatum [38]. Another major pathological feature is the presence of Lewy bodies (LBs) which are intraneronal proteinacious cytoplasmic inclusions in the surviving neurons [39]. Clinically, patients with PD exhibit rigidity, bradykinesia, resting tremors, and postural instability [40]. One of the earliest biochemical changes seen in these individuals is decreased levels of reduced glutathione (GSH), a major component of cellular antioxidant defenses [40]. Reduced GSH levels are also associated with incidental LB diseases thought to be asymptomatic precursors to PD [41].

Animals models of PD have been generated with toxins including neurotoxins used to induce dopaminergic neurodegeneration, 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP), paraquat, and rotenone [42]. 6-OHDA is relatively selective for monoaminergic neurons, resulting from preferential uptake by DA and noradrenergic transporters. Since this compound cannot permeate the blood-brain barrier (BBB), it must be administered by local stereotaxic injection into the SNpc, median forebrain bundle (MFB) which forms ascending dopaminergic and serotonergic projections into the forebrain, or striatum to target the nigrostriatal dopaminergic pathway [43]. Yasuhara *et al*. transplanted green fluorescent protein-labeled NSCs into lesions in the striatum of a 6-OHDA-treated rat model of PD. The grafted NSCs survived in the brain lesions which are positive for the neuronal marker, mitogen-activated protein 2 (MAP2), and synaptophysin-positive terminals. Furthermore, endogenous neurogenesis was observed in the rat SVZ. In another rat PD model generated with 6-OHDA, Kim *et al*. transplanted NSCs expressing genes encoding tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GTPCH1) in order to create dopamine-producing NSCs [44]. Marked improvement was observed in the PD rats that received NSCs expressed TH and GTPCH.

In humans and monkeys, treatment with MPTP produces an irreversible and severe Parkinsonian syndrome characterized by features of PD including tremor, rigidity, slowness of movement, postural instability, and freezing [42]. The susceptibility to MPTP increases with age in both monkeys and mice [45]. In a previous study,

a small number of human NSC progeny were found to differentiate into TH and/or dopamine transporter (DAT)-positive cells, suggesting that the microenvironment within and around the SNpc lesions in adult monkeys permits the development of a DA phenotype in responsive progenitor cells [46]. These results indicated that naïve or genetically modified NSCs have a great potential for use in cell replacement therapy for patients suffering from PD.

NSC-based therapies for treating brain tumor in animal models

Brain tumors are the leading cause of cancer mortality in children, and remain difficult to cure despite advances in surgical techniques and adjuvant therapy [47]. Malignant brain tumors, including glioblastoma multiforme, remain virtually untreatable and lethal. Currently available treatments for brain tumors include radical surgical resection followed by radiation or chemotherapy, and have substantially improved the survival rate in patients suffering from these lesions. However, brain tumors remain incurable in large proportion of patients. Therefore, there is an urgent need for effective and minimally toxic therapies for treating these tumors.

Most current research on human brain tumors is focused on the molecular and cellular analysis of bulk tumor masses. A widely used molecular approach is suicide gene-based therapy that relies on the conversion of non-toxic prodrugs into toxic anticancer drugs via the expression of exogenous enzymes. Additionally, genetic immunotherapy involving the transfer of genes expressing immune-stimulating cytokines has also been developed [48]. Genetically modified NSCs may also be used to treat various human brain tumors. For example, Aboody *et al*. suggested that the prodrug 5-fluorocytosine (5-FC) along with NSCs able to express cytosine deaminase (CD), a bioactive factor, could dramatically reduce tumor burden *in vivo* [49]. Transplanted NSCs expressing CD can convert 5-FC into 5-fluorouracil (5-FU) and induce tumor regression. NSCs expressing the CD gene can also generate an agent that kills tumor cells and undergo self-elimination should the NSCs themselves become mitotic [49]. In another study, a significant decrease of glioma tumoral mass (approximately 50%) was observed in rat neural progenitor cells expressing CD gene treated with 5-FC [50]. The 5-FC itself had no effect on the tumor in the absence of cells expressing CD.

In athymic nude mice, the ability of NSCs secreting the pro-apoptotic protein TRAIL to treat human gliomas was investigated [51]. High levels of TRAIL secretion was observed within the main tumor mass as well as the tumor pockets and satellites, indicating that NSCs expressing TRAIL migrated into the tumor outgrowths. TRAIL-induced cell death led to a highly significant decrease in tumor volume compared to transplantation with NSCs expressing LacZ or the saline-inoculated control.

In an murine intracranial medulloblastoma model, human NSCs expressing the CD gene were injected into the contralateral hemisphere of the brain [52]. The mice were then treated systemically with 5-FC. Histologic analyses showed that the NSCs migrated to the tumor bed and lesion boundary, resulting in a 76% reduction of tumor volume [52]. This finding provides a rationale for further evaluating NSC-based cellular delivery systems for treating human brain tumors, including gliomas or medulloblastomas.

NSC-based therapies for treating various human primary tumor in animal models

The efficacy of modalities using NSCs based on gene direct enzyme/prodrug therapy (GEPT) has been examined in various animal models of human cancer [53,54]. Hepatocellular carcinoma (HCC) is the sixth most common cancer and third most common cause of cancer-related death in the world [55]. For treating HCC in a xenograft SCID mouse model, Yi *et al*. compared NSCs with HB1.F3.CD and HB1.F3.CD.IFN-β cells expressing the CD and/or interferon-beta (IFN-β) gene [56]. Results of this experiment showed that NSCs expressing the therapeutic genes have the potent advantage of selective migration toward HCC cells *in vivo*. After 8 weeks, mice in the negative control group (without stem cells or prodrug) bearing tumors reached the endpoint of ethical death. However, tumor growth in animals treated with the HB1.F3.CD or HB1.F3.CD.IFN-β cells was inhibited approximately 40-50% compared to the control group [56]. Additionally, fluorescence pre-stained NSCs were detected in HCC tumor mass of animal models. These data indicate that NSCs possess tumor-specific tropism in cases of HCC as well as brain lesions. Yi *et al.* also studied the activities of NSCs expressing therapeutic genes in mice bearing tumors arising from MDA-MB-231 human breast carcinoma, colorectal cancer, or Ishikawa endometrial cancer cells

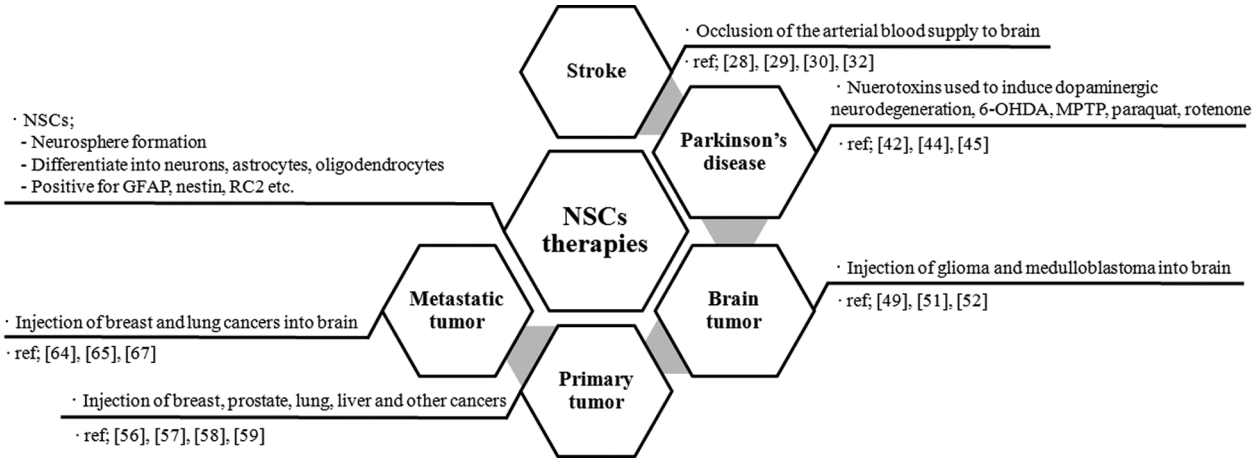

Figure 1. Application of neural stem cells (NSCs) therapies. NSCs the self-renew and differentiate into major cell types of the brain exist, such as astrocytes, oligodendrocytes, and neurons, in the developing and adult central nervous system (CNS). There have been describing the effects of NSC transplantation for achieving functional recovery from CNS damage. Therefore, NSCs may be a suitable component for treating neurological diseases such as stroke, Parkinson's diseases (PD), brain tumors, primary and metastatic tumors. GFAP; glial fibrillary acidic protein, RC2: radial glial cell marker, 6-OHDA; 6-hydroxydopamine, MPTP; 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine.

[57-59]. Results of these experiments demonstrated that tumor volume is regulated in animals treated with the NSCs and prodrug. Aggressive behavior of tumor cell masses is also substantially reduced by injection with stem cells and prodrug treatment in mice [60].

NSC-based therapies for treating metastatic tumor in animal models

Several researchers have confirmed the therapeutic effect of NSCs in animal models of metastatic tumors. The brain receives 15-20% of the body's blood flow, thereby increasing the chance of circulating tumor cells reaching the brain. The reported incidence of brain metastases ranges from 12-35% [61]. Many patients with brain metastasis harbor two or more metastases. Treatment of these individuals is hampered by the fact that intact blood-brain barrier (BBB) is largely impermeable to most chemotherapeutic drugs.

The most common origins of brain metastasis include primary cancers of the lung, breast, and skin [62]. Primary lung cancer has the highest incidence of brain metastasis with approximately 40% of all lung cancer patients developing brain metastasis. Breast cancer is the second most common cancer associated with brain metastasis [63]. Animal models of metastatic tumors have been produced using two methods: direct tumor cell implantation into the brain or blood-born brain metastasis [64]. In an animal model of metastatic breast cancer, NSCs expressing the CD or carboxyl esterase (CE) suicide genes were used to treat tumors [65]. CE can convert the prodrug CPT-11 to the toxic compound SN-38 [66]. Yi *et al*. also investigated the effect of NSCs expressing the yeast CD (yCD) gene in an animal model of metastatic lung cancer [67]. Expression of yCD appears to be far more effective for converting 5-FC into 5-FU compared to bacterial CD, both *in vitro* and *in vivo* [68]. NSCs expressing yCD in the presence of 5-FC reduced the density and aggressive behavior of lung cancer cells compared to the negative control or NSCs without 5-FC. The cytotoxic drug 5-FU is effective for treating brain metastases but cannot penetrate the BBB. However, 5-FC readily crosses the BBB or is transported into the brain parenchyma [69]. Therefore, this prodrug may have great potential for treating brain metastases.

Conclusion

The adult brain has a capacity for self-repair and replacing lost neurons in several CNS regions such as the olfactory bulb, hippocampus, subependymal zone, and cortex [70]. NSCs within these neurogenic regions can proliferate and differentiate into neurons or glia, thus providing a reservoir for the replacement of cells lost during normal cell turnover and after brain injury [71]. There have been a number of recent reports describing the effects of NSC transplantation for achieving functional recovery from CNS damage. Evidence from these investigations suggests that NSCs may be a suitable

component for treating neurological diseases such as stroke, PD, brain tumors, primary tumors, and metastatic lesions (Figure 1). In summary, naïve or genetically modified NSCs may represent an effective new modality for treating various human brain diseases without inducing injurious effects commonly associated with more conventional therapies.

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