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

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...
(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 proteinaceous 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.
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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References

1. Paulklin S, Pedersen RA, Vallier L. Mouse pluripotent stem cells at a glance. J Cell Sci 2011; 124( Pt 22): 3727-3732.
2. Ogawa K, Saito A, Matsui H, Suzuki H, Ohtsuka S, Shimosato D, Morishita Y, Watabe T, Niwa H, Miyazono K. Activin-Nodal signaling is involved in propagation of mouse embryonic stem cells. J Cell Sci 2007; 120( Pt 1): 55-65.
3. Dado D, Sagl M, Levenberg S, Zemel A. Mechanical control of stem cell differentiation. Regen Med 2012; 7(1): 101-116.
4. Maul TM, Chew DW, Nieponice A, Vorp DA. Mechanical stimuli differentially control stem cell behavior: morphology, proliferation, and differentiation. Biomech Model Mechanobiol 2011; 10(6): 939-953.
5. Ying QL, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nat Biotechnol 2003; 21(2): 183-186.
6. Soncin F, Mohamet L, Eckardt D, Risdon S, Eastham AM, Bobola N, Russell A, Davies S, Klemmer R, Mercy CL, Ward CM. Abrogation of E-cadherin-mediated cell-cell contact in mouse embryonic stem cells results in reversible LIF-independent self-renewal. Stem Cells 2009; 27(9): 2069-2080.
7. Harting MT, Jimenez F, Xue H, Fischer UM, Baumgartner J, Dash PK, Cox CS. Intraocular mesenchymal stem cell therapy for traumatic brain injury. J Neurosurg 2009; 110(6): 1189-1197.
8. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981; 292(5819): 154-156.
9. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines from human blastocysts: somatic differentiation in collaboration with STAT3. Cell 2003; 115(3): 281-292.
10. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 2000; 18(4): 399-404.
11. Smith AG. Embryo-derived stem cells: of mice and men. Annu Rev Cell Dev Biol 2001; 17: 435-462.
12. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 2003; 115(3): 281-292.
13. Vallier L, Alexander M, Pedersen RA. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J Cell Sci 2005; 118( Pt 19): 4495-4509.
14. C. Anneren, C. A. Cowan and D. A. Melton. The Scl family of tyrosine kinases is important for embryonic stem cell self-renewal. J Biol Chem 2004; 279(Number): 31590-31598.
15. Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. J Biol Chem 2004; 279(46): 48063-48070.
16. Körbling M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? N Engl J Med 2003; 349(6): 570-582.
17. Verfaillie CM. Adult stem cells: assessing the case for pluripotency. Trends Cell Biol 2002; 12(11): 502-508.
18. Lee C, Hu J, Ballas S, Kitaumura T, Loh YP, Yang Y, Mukoyama YS, Ahn S. The molecular profiles of neural stem cell niche in the adult subventricular zone. PLoS One 2012; 7(11): e50501.
19. Galli R, Gritti A, Bonfanti L, Vescovi AL. Neural stem cells: an overview. Circ Res 2003; 92(6): 598-608.
20. Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 2005; 28: 223-250.
21. Alvarez-Buylla A, Lim DA. For the long run: maintaining germinial niches in the adult brain. Neuron 2004; 41(5): 683-686.
22. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci 2006; 7(5): 395-406.
23. Mu Y, Lee SW, Gage FH. Signaling in adult neurogenesis. Curr Opin Neurobiol 2010; 20(4): 416-423.
24. Pluchino S, Zanotti L, Deleidi M, Martino G. Neural stem cells and their use as therapeutic tool in neurological disorders. Brain Res Brain Res Rev 2005; 48(2): 211-219.
25. Davenport R, Dennis M. Neurological emergencies: acute stroke. J Neurol Neurosurg Psychiatry 2000; 68(3): 277-288.
26. Lindvall O, Kokaia Z. Stem cells in human neurodegenerative disorders--time for clinical translation? J Clin Invest 2010; 120(1): 29-40.
27. Riolobos AS, Heredia M, de la Fuente JA, Criaud JM, Yajeya J, Campos J, Santacana M. Functional recovery of spared forelimb use in rats obliged to use the impaired limb after grafting of the frontal cortex lesion with homotopic fetal cortex. Neurobiol Learn Mem 2001; 75(3): 274-292.
28. Ding DC, Shyu WC, Chiang MF, Lin SZ, Chang YC, Wang HJ, Su CY, Li H. Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. Neurobiol Dis 2007; 27(3): 339-353.
29. Jeong SW, Cha K, Jung KH, Kim SU, Kim M, Roh JK. Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. Stroke 2003; 34(9): 2258-2263.
30. Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci U S A 2004; 101(32): 11839-11844.
31. Tanaka N, Sasahara M, Ohno M, Higashiyama S, Hayase Y, Shimada M. Heparin-binding epithelial growth factor-like growth factor mRNA expression in neonatal rat brain with hypoxic/ischemic injury. Brain Res 1999; 827(1-2): 130-138.
32. Zhang R, Zhang Z, Wang L, Yang G, Gousev A, Zhang L, Ho KL, Morshed C, Chopp M. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct border in adult rats. J Cereb Blood Flow Metab 2004; 24(4): 441-448.
33. Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci 2006; 26(50): 13007-13016.
34. Ding DC, Lin CH, Shyu WC, Lin SZ. Neural Stem Cells and Stroke. Cell Transplant 2013; 22(4): 619-630.
35. Franke TF, Hornik CP, Segev L, Shostak GA, Sugimoto C. PI3K/Akt and apoptosis: size matters. Oncogene 2003; 22(56): 8983-8998.
36. Chong ZZ, Kang QJ, Maiese K. Erythropoietin fosters both intrinsic and extrinsic neuronal protection through modulation of microglia, Akt1, Bad, and caspase-mediated pathways. Br J Pharmacol 2003; 138(6): 1107-1118.
37. Lee HH, Kim MK, Kim HJ, Kim SU. Human neural stem cells genetically modified to overexpress Akt1 provide neuroprotection and functional improvement in mouse stroke model. PLoS One
Stem cell-based therapy for diverse human neurological diseases

2009; 4(5): c5586.

38. Storch A, Schwarz J. Neural stem cells and Parkinson's disease. J Neurol 2002; 249 Suppl 3: III/30-32.

39. Przedborski S. Pathogenesis of nigral cell death in Parkinson's disease. Parkinsonism Relat Disord 2005; 11 Suppl 1: S3-7.

40. Martin HL, Temsamann P, Glutathione—a review on its role and significance in Parkinson's disease. FASEB J 2009; 23(10): 3263-3272.

41. Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. Biochem Pharmacol 2002; 64(5-6): 1019-1026.

42. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39(6): 889-909.

43. Yasahara T, Matsukawa N, Hara K, Yu G, Xu L, Maki M, Kim SU, Borlongan CV. Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. J Neurosci 2006; 26(48): 12497-12511.

44. Kim SU, Park IH, Kim TH, Kim KS, Choi KB, Hong SH, Bang JH, Lee CA, Jo JS, Lee CS, Kim YS. Brain transplantation of human neural stem cells transduced with tyrosine hydroxylase and GTP cyclohydrolase I provides functional improvement in animal models of Parkinson disease. Neuropharmacology 2006; 26(2): 129-140.

45. Ovadia A, Zhang Z, Gash DM. Increased susceptibility to MPTP toxicity in middle-aged rhesus monkeys. Neurobiol Aging 1995; 16(3): 931-937.

46. Redmond DE Jr, Bjugstad KB, Teng YD, Ourednik V, Ourednik J, Wakeman DR, Parsons XH, Gonzalez R, Blanchard BC, Kim SU, Gu Z, Lipton SA, Markakis EA, Roth RH, Elsworth JD, Sladek JR Jr, Sidman RL, Snyder EY. Behavioral improvement in a primate Parkinson’s model is associated with multiple homeostatic effects of human neural stem cells. Proc Natl Acad Sci U S A 2007; 104(29): 12175-12180.

47. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire RL, Snyder EY. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 2000; 97(23): 12846-12851.

48. Przedborski S. Pathogenesis of nigral cell death in Parkinson's disease. Neuropathology 2006; 26(2): 129-140.

49. Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Kim SU. Neural stem cell-based gene therapy for brain tumors. Cancer Gene Ther 2003; 10(4): e5586.

50. Yi BR, Kwak KA, Kang NH, Kim SU, Jeung EB, Kim HC, Choi KC. Antitumor therapeutic effects of cytosine deaminase and interferon-β against endometrial cancer cells using genetically engineered stem cells in vitro. Anticancer Res 2011; 31(9): 2853-2861.

51. Yi BR, Park MA, Lee HR, Kang NH, Choi KJ, Kim SU, Choi KC. Suppression of the growth of human colorectal cancer cells by therapeutic stem cells expressing cytosine deaminase and interferon-β via their tumor-tropic effect in cellular and xenograft mouse models. Mol Oncol 2013; 7(3): 543-554.

52. Yi BR, Kang NH, Hwang KA, Kim SU, Jeung EB, Choi KC. Therapeutic potential of stem cells targeting human breast cancer cells: evidence that they exert tumoricidal effects via tumor tropism (review). Int J Oncol 2012; 41(3): 798-804.

53. Biswas G, Bhagwat R, Khurana R, Menon H, Prasad N, Parikh PM. Brain metastasis—evidence-based management. J Cancer Res Ther 2006; 2(1): 5-13.

54. Ma S, Xu Y, Deng Q, Yu X. Treatment of brain metastasis from non-small cell lung cancer with whole brain radiotherapy and Gefitinib in a Chinese population. Lung Cancer 2009; 65(2): 198-203.

55. Barnholtz-Sloan JS, Sloan AE, Davis FG, Vigneau FD, Lai P, Savaya RE. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. J Clin Oncol 2004; 22(14): 2865-2872.

56. Yi BR, Choi KJ, Kim SU, Choi KC. Antitumor effects of genetically engineered stem cells expressing suicide genes that selectively target human breast cancer cells: evidence that they exert tumoricidal effects via tumor tropism. Int J Oncol 2012; 41(3): 798-804.