

Review

Stem Cell-Based Neuroprotective and Neurorestorative Strategies

Chia-Wei Hung^{1,4,†}, Ying-Jay Liou^{2,4,†}, Shao-Wei Lu^{5,†}, Ling-Ming Tseng^{3,4,†},
Chung-Lan Kao^{3,4}, Shih-Jen Chen^{3,4}, Shih-Hwa Chiou^{3,4,*} and Charn-Jung Chang^{5,*}

¹ Department of Neurology, Zhongxiao Branch, Taipei City Hospital/No.87, Tongde Rd., Nangang Dist., Taipei City 115, Taiwan

² Department of Psychiatry, Taipei Veterans General Hospital/No.201, Sec. 2, Shih-Pai Road, Taipei 11217, Taiwan

³ Department of Medical Research and Education, Taipei Veterans General Hospital/No.201, Sec. 2, Shih-Pai Road, Taipei 11217, Taiwan

⁴ Institute of Clinical Medicine, National Yang-Ming University/No.155, Sec. 2, Linong St., Taipei 112, Taiwan

⁵ Department of Pharmacy, Tri-Service General Hospital & National Defense Medical Center/No.325, Sec.2, Chenggong Rd., Neihu District, Taipei 114, Taiwan

† These authors contributed equally to this work.

* Authors to whom correspondence should be addressed: E-Mails: shchiou@vghtpe.gov.tw (S.-H.C.), michael0938@yahoo.com.tw (C.-J.C.); Tel.: +886-2-287-652-32 (S.-H.C.), +886-2-237-111-12 (C.-J.C.); Fax: +886-2-287-574-34 (S.-H.C.), +886-2-237-135-94 (C.-J.C.).

Received: 23 March 2010; in revised form: 8 April 2010 / Accepted: 18 April 2010 /

Published: 5 May 2010

Abstract: Stem cells, a special subset of cells derived from embryo or adult tissues, are known to present the characteristics of self-renewal, multiple lineages of differentiation, high plastic capability, and long-term maintenance. Recent reports have further suggested that neural stem cells (NSCs) derived from the adult hippocampal and subventricular regions possess the utilizing potential to develop the transplantation strategies and to screen the candidate agents for neurogenesis, neuroprotection, and neuroplasticity in neurodegenerative diseases. In this article, we review the roles of NSCs and other stem cells in neuroprotective and neurorestorative therapies for neurological and psychiatric diseases. We show the evidences that NSCs play the key roles involved in the pathogenesis

of several neurodegenerative disorders, including depression, stroke and Parkinson's disease. Moreover, the potential and possible utilities of induced pluripotent stem cells (iPS), reprogramming from adult fibroblasts with ectopic expression of four embryonic genes, are also reviewed and further discussed. An understanding of the biophysiology of stem cells could help us elucidate the pathogenicity and develop new treatments for neurodegenerative disorders. In contrast to cell transplantation therapies, the application of stem cells can further provide a platform for drug discovery and small molecular testing, including Chinese herbal medicines. In addition, the high-throughput stem cell-based systems can be used to elucidate the mechanisms of neuroprotective candidates in translation medical research for neurodegenerative diseases.

Keywords: stem cells; neural stem cells; neuroprotection; neurodegenerative diseases; stem cell-based strategy

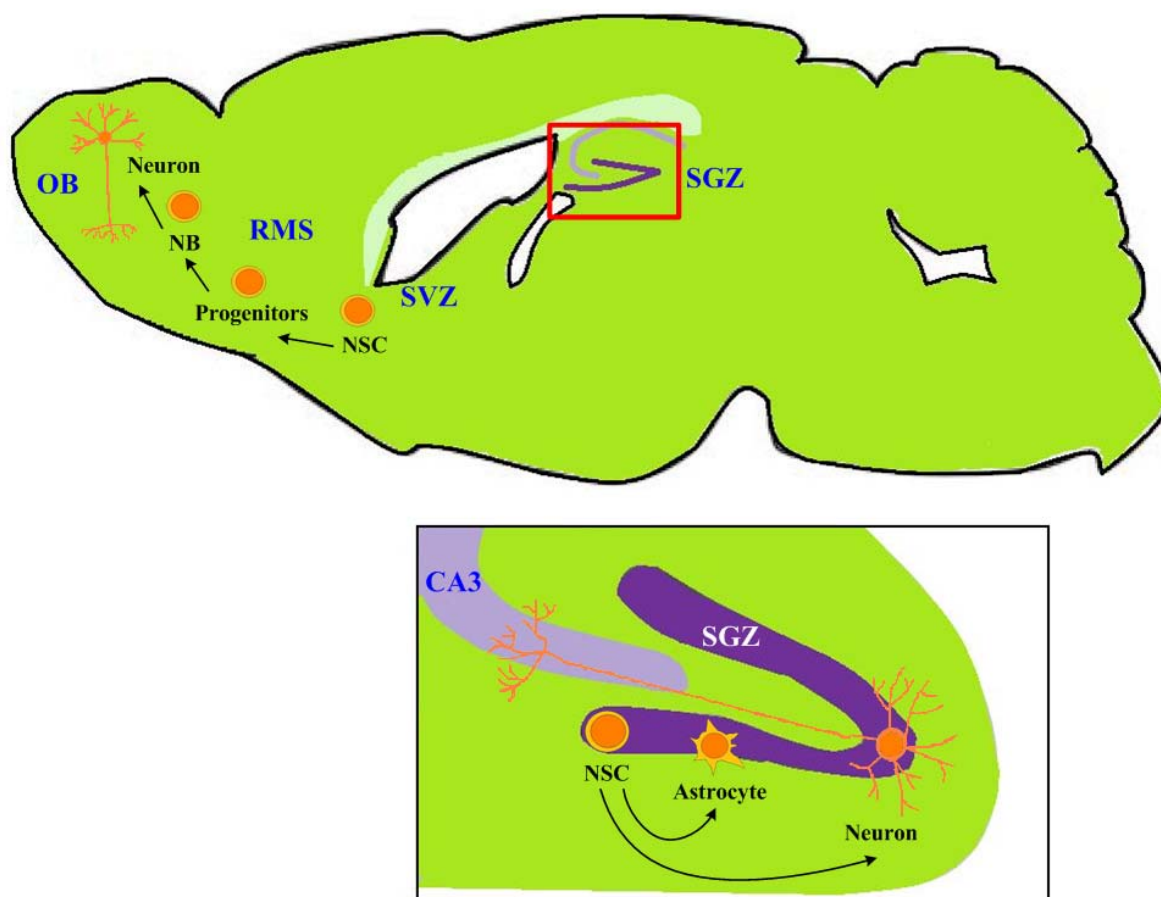
1. Introduction

Stem cells are classified into three types according to their abilities to differentiate. The first type is totipotent stem cells, which can be implanted in the uterus of a living animal and give rise to a full organism. The second type is pluripotent stem cells such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. They can give rise to every cell of an organism except extraembryonic tissues, such as placenta. This limitation restricts pluripotent stem cells from developing into a full organism. The third type is multipotent stem cells. They are adult stem cells which only generate specific lineages of cells [1]. Neural stem cells (NSCs) are multipotent stem cells which are derived from neural tissues, either from the central nervous system or peripheral nervous systems [1]. These cells are self-renewing and can give rise to all cell types (neurons, astrocytes and oligodendrocytes) of the nervous system through asymmetric cell division [1].

In the adult brain, NSCs are primarily located in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Figure 1). In general, the quiescent or dormant NSCs might be present and can be derived from multiple areas of the adult brain [2-4]. The SVZ and SGZ niches have common cellular niche components which include astroglia, ependymal cells, vascular cells, NSC progeny and mature neurons, and common extracellular niche signals which include Wnt, Sonic Hedgehog, bone morphogenic protein antagonists, membrane-associated Notch signaling, leukemia inhibitory factor, transforming growth factor- α , fibroblast growth factors, neurotrophins and extracellular matrix. [3]. These cellular and extracellular components regulate the behaviors of NSCs in a region-specific manner [3]. For example, SVZ NSCs give rise to $Dlx2^+$ $Mash1^+$ intermediate progenitor cells which subsequently give rise to PSA - $NCAM^+$ $doublecortin^+$ (DCX^+) neuroblasts and migrate towards the olfactory bulb (OB). In contrast, SGZ NSCs do not differentiate into interneuron-lineage cells like those in the OB, but give rise to local glutamatergic excitatory dentate granule cells [3]. The region-specific development of these NSCs is

not only due to intrinsic characteristics of the NSCs themselves, but also due to the dictation of local microenvironment (*i.e.*, the niche). A detail summary of the neurogenic niche can be found in a recent review by Ma *et al.* [3].

Figure 1. The two niches of neural stem cells (NSCs). The subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus have common cellular niche components and extracellular niche signals. The development of NSCs of the two niches is different in a region-specific manner. SVZ NSCs give rise to $Dlx2^+$ $Mash1^+$ intermediate progenitor cells which subsequently give rise to $PSA-NCAM^+$ $doublecortin^+$ (DCX^+) neuroblasts (NB) and migrate towards the olfactory bulb (OB). SGZ NSCs give rise to local glutamatergic excitatory dentate granule cells. RMS: rostro-migratory stream; GL: granular layer. Adapted from Ma *et al.* [3] and Taupin and Gage [5].



Neurogenesis derived from adult NSCs is critical for a plethora of central nervous functions, such as spatial learning and memory, mood regulation and motor controls. Growing evidence also suggests the significant contribution of adult NSCs to pathological conditions like seizures, brain tumors, mood disorders or neurodegenerative diseases [3]. If the biopathological role of adult NSCs can be better understood, therefore, the therapeutic strategies that assist neuroprotection and neurorestoration can be framed and tested through collaborative efforts of both basic and translational research. In the

following sessions, we will introduce the roles of NSCs in the pathogenesis in some psychiatric and neurological diseases, and the application of stem cell-based therapies.

2. Depression and Neurogenesis: Evidence from Neural Stem Cells

Depression is one of the most common psychiatric disorders, with 10-20% lifetime prevalence [6,7]. However, the etiology and pathophysiology of depression still remain unclear. Preclinical and clinical studies suggest the involvement of hippocampus in the pathogenesis of depression. Hippocampus plays an important role in learning, memory and emotionality [8,9]. It is also one of the primary niches of NSCs. Reduction of hippocampal volume was found in patients with posttraumatic stress disorders [10]. Magnetic resonance imaging studies also showed a consistent reduction in hippocampal volume in patients with depression [11]. Two meta-analyses have demonstrated a reduction in hippocampal volume in patients with recurrent depression in comparison to age- and sex-matched controls [12,13]. In addition, most antidepressants and environmental interventions that confer antidepressant-like behavioral effects stimulate adult hippocampal neurogenesis [11].

Based on these findings, impaired hippocampal neurogenesis was considered to be one of the etiologies of depression. However, recent studies have shown some controversial evidences against the previous findings. First, preclinical and pathohistological studies showed that the reduction of hippocampal volume might be a result of decreased dendritic complexity and changes in neurophil and glial number rather than impaired hippocampal neurogenesis [14-16]. Besides, the ablation of neurogenesis did not induce or affect depression-like or anxiety-like behaviors in animals [14,17-19]. To date, hippocampal neurogenesis is not thought to be involved in the pathogenesis of depression [11,20], although the regulation of neurogenesis in adult brain may be required for antidepressant treatment [11].

Most antidepressant drugs increase the levels of monoamines serotonin (5-hydroxytryptamine; 5-HT) and/or noradrenaline (NA); this suggests that biochemical imbalances within the 5-HT/NA systems may cause mood disorders. In addition to the regulation of neurotransmitters, antidepressants also have both neuroprotective and neurorestorative effects on hippocampal cells. For example, monoamine oxidase-A inhibitor moclobemide (MB) can upregulate proliferation of hippocampal progenitor cells in chronically stressed mice [21]. MB can also provide neuroprotection by reducing intracellular pH and neuronal activity of CA3 hippocampal neurons [22]. A selective serotonin reuptake inhibitor, fluoxetine, was used to treat rats with maternal separation. Compared to the rats that did not receive fluoxetine, cell proliferation was increased and apoptosis was decreased in the dentate gyrus of the rats that receive fluoxetine [23]. To elucidate the molecular mechanism of the neuroprotective and neurorestorative effects of antidepressants, NSCs derived from the hippocampal tissues of adult rats can be used as a model for the *in vitro* drug-effect test [24].

Clinical findings have shown evidence that hippocampal volume in patients with depression is reduced in comparison to the volume in healthy people [10]. Furthermore, the clinical studies and magnetic resonance imaging (MRI) survey demonstrated that the hippocampal volume decreases in patients with depression and post-traumatic stress disorder [6,10]. Increased neurogenesis in the hippocampus by the administration of antidepressant drugs can result in altered behavior in stress-induced models and patients [14,23]. Moreover, Chen *et al.* showed the evidence that desipramine can promote neurogenesis in hippocampus and reverse the learned behavior in learned helplessness rats [25]. Taken together, these observations implicated that adult hippocampal neurogenesis is decreased by stress and this process of neuron loss may be involved in both the pathogenesis and treatment of mood disorders.

Neural stem cells (NSCs), derived from hippocampus and other germinal centers of the brain, have been isolated and defined as cells with the capacity of self-renewal and multilineage differentiation [1]. NSCs also possess the utilizing potential to develop the transplantation strategies and to screen the candidate agents for neurogenesis in neurodegenerative diseases [26]. By using *in vitro* culture of NSCs from hippocampus of adult rats, antidepressants of different classes are proved to have neuroprotective effects and can assist neurogenesis [27-31].

Antidepressants can increase the viability and promote the differentiation of NSCs. They also decrease the level of proinflammatory cytokines. [27-31]. Antidepressants are able to prevent Fas ligand (FasL)- or lipopolysaccharide (LPS)-induced apoptosis of NSCs through the upregulation of Bcl-2 and Bcl-XL expression [27-31]. Higher expression level of phosphorylated ERK 1/2 in addition to Bcl-2 was detected in NSCs treated with MB, and the expression was inhibited by a MAPK/ERK kinase inhibitor PD98059 [29]. The MAPK inhibitor U0126 also enhances the apoptotic activities and decreases cell viability in LPS- and imipramine-treated NSCs [30]. These results suggest antidepressants upregulate Bcl-2 expression through the MAPK/ERK pathway.

In addition to MAPK/ERK signaling modulation, cellular FLICE-inhibitory protein (c-FLIP) may also be involved in the prevention of apoptosis of NSCs by antidepressant [28]. C-FLIP is a cytoplasmic protein that has sequence homology to FLICE (FADD-like IL-1 β -converting enzyme) [32]. c-FLIP is capable of binding to FADD, but is unable to be cleaved to an active caspase because of a substitution of tyrosine from an active site cysteine. The substitution of tyrosine prevents the initiation of the death pathway [32,33]. Chiou *et al.* demonstrated that fluoxetine upregulated the expression of c-FLIP [28]. This upregulation involved PI3k/AKT pathway, since administration of PI3-K inhibitor LY294002 dose-dependently reduced fluoxetine-mediated activation of c-FLIP promoter and protein expression of c-FLIP [28].

It has been well-documented that antidepressants present the potential to upregulate the expression of brain-derived neurotrophic factor (BDNF) in animal models as well as the patients with depression [30,34-37]. BDNF is the most abundant neurotrophin in the brain. It regulates neuronal cell survival, differentiation, synaptic strength and morphology [38]. Blocking endogenous BDNF activity leads to aggravated death of a subpopulation of hippocampal neurons after global forebrain ischemia [39]. The neuroprotective role of endogenous BDNF is further supported by the observed correlation between BDNF protein levels and resistance to ischemic damage in hippocampal subregions [40]. Peng *et al.*

demonstrated that imipramine, a tricyclic antidepressant, increased Bcl-2 expression and differentiation of rat hippocampal NSCs [30].

Imipramine also decreased apoptotic activities and proinflammatory cytokines, and improved cell viability of LPS-treated NSCs. These effects were all achieved through the upregulation of BDNF [30]. Taken together, hippocampal neurogenesis is required for antidepressant therapies. Using cultured rat hippocampal NSCs, the molecular mechanisms of antidepressant effects are explored, which include the MAPK/ERK pathway, the PI3k/AKT pathway, and the upregulation of BDNF, Bcl-2 and c-FLIP.

3. Diseases of Central Nervous System and Neural Stem Cells – Stem Cell Therapy and the Development of New Target Drug

Diseases of the central nervous system (CNS) such as stroke, traumatic brain injury, dementia, Parkinson's disease or multiple sclerosis, usually cause morbidity and mortality as well as increase social and economic burdens of patients and caregivers. However, most treatments for these diseases are symptomatic or preventive, and are not effective. Many attempts have been made to develop a neuroprotective treatment to reduce the volume of brain injury, but the translation of neuroprotection from experimental therapies to clinical use has not been very successful [41]. Along with the development of stem cell studies and the discovery of neural stem cells in the adult brain, transplantation of stem cells or their derivatives, and mobilization of endogenous stem cells within the adult brain have been proposed as future therapies for the CNS diseases [42]. We herein introduce the role of stem cell-based therapies in the possible treatment for Parkinson's disease and ischemic stroke. The two diseases have different etiologies and pathophysiologies, and therefore, different strategies of treatment are required.

3.1. Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disease. Its main pathology is cellular loss of the substantia nigra pars compacta dopamine neurons that project to the striatum [43]. Clinical signs of PD, which include rest tremor, rigidity and bradykinesia, are evident when about 80% of striatal dopamine and 50% of nigral neurons are lost [44]. Because PD results from the loss of dopaminergic neurons, the prospect of utilizing cell replacement therapies has attracted substantial interests. The first attempt was to use fetal mesencephalic tissue for transplantation, and the results were successful in the earliest reports [42,45,46]. However, not all trials showed beneficial outcomes. The sham surgery-controlled study also demonstrated some clinical benefits in younger but not in older patients [47]. Another study showed no significant treatment effects [48]. Moreover, fetal mesencephalic transplantation is associated with several problems. First, off-medication dyskinesia increased 6-12 months after the transplantation in 15-56% of patients [47-49]. Second, graft-induced inflammatory responses might influence the longevity of transplanted cells [50]. Third, tissue availability limits the clinical use [42]. As a result, fetal mesencephalic transplantation is not recommended as a conventional therapy for PD.

Graft-induced dyskinesia is thought to be caused by unfavorable composition of the fetal mesencephalic grafts. The fetal mesencephalic tissue includes not only dopaminergic but also

non-dopaminergic neurons [42]. The exclusion of serotonin and GABA neurons, and enrichment of substantia nigra dopamine neurons may decrease the occurrence of dyskinesia [50]. To achieve this goal, probable solutions include refinement of dissection methods for fetal tissue transplantation, isolation of desired cell types and/or removal of unwanted cellular populations using fluorescence- and/or magnetic-activated cell sorting (FACS/MACS), and using stem cells (ES cells and iPS cells) as an alternative cell source [50]. Recent evidence has shown that dopamine neurons derived from ES cells and bone marrow-derived neural progenitors are functional when grafted into parkinsonian rats [42,51-52]. Several methods are able to improve the effectiveness of midbrain dopamine neuron generation and/or retrieval from stem cells. These include manipulating transcription factor like Nurr1, Pitx3 or Lmx1a, co-culture with astrocytes and using fluorescence-activated cell sorting [50]. The replacement of fetal tissue by stem cells also solve the problem of availability and ethical issue [50]. The ability of deriving large quantities of correctly differentiated dopamine neurons makes stem cells promising cell sources for transplantation in PD.

3.2. Ischemic Stroke

Ischemic stroke is a major cause of morbidity and mortality worldwide. The only effective treatment for acute ischemic stroke is thrombolytic agents such as rt-PA [53]. For patients receiving thrombolytic therapy shortly after the stroke (3–4.5 h), only 31-50% of them obtained favorable outcomes, and 6.4% of patients developed brain hemorrhage [54]. New therapeutic strategies with neuroprotection or neurorestoration are crucial for improving the prognosis of patients with stroke.

Ischemia affects the behavior and proliferation status of NSCs. For example, focal ischemia of brain enhances endogenous neurogenesis, angiogenesis, axonal sprouting and synaptogenesis [41,55]. However, the proportion of damaged or dead neurons replaced by the new neurons is small [55]. Besides, neurogenesis does not occur in some ischemic regions. This is probably either due to an unfavorable microenvironment of the ischemic sites, or because these sites are distant from the SVZ and SGZ, which are most abundant in NSCs [55]. Pharmacological treatments aimed at enhancing neurogenesis, angiogenesis and axonal outgrowth were successful in animal studies. These included erythropoietin, statins, phosphodiesterase 5 inhibitors, granulocyte-colony stimulating factor, nicotinic acid and minocycline [41]. Limited clinical data have indicated beneficial therapeutic potential of these drugs in human [56], but further clinical survey is required. The difficulties of the cell-replacement therapy are due to variable cell types involved in ischemic stroke, which include neurons, astrocytes, oligodendrocytes and endothelial cells of blood vessels [41]. Although transplantation of bone marrow-derived mesenchymal stem cells promoted functional recovery, the effect was caused by activation of endogenous restoration of injured brain rather than cell replacement [41,42,57]. ES cells have been demonstrated to have greater developmental potential and more significant survival rate than adult stem cells after transplantation [58]. Transplantation of ES cells also recovered behavioral dysfunction induced by middle cerebral arterial occlusion in an animal model [59]. However, the ethical consideration, the limited availability and the possibility of immune rejection after transplantation restrict the accessibility of ES cells.

3.3. The Hope and Hype of Induced Pluripotent Stem Cells in Cell Replacement Therapy of Neurological Diseases

The recent progresses in stem cell research have demonstrated that induced pluripotent stem (iPS) cells could be generated from mouse embryonic fibroblasts as well as from adult human fibroblasts via the retrovirus-mediated transfection of four transcription factors, that is, Oct3/4, Sox2, c-Myc, and Klf-4 [60-62]. The development of iPS cells provides an additional option for replacement therapy. They are indistinguishable from ES cells in morphology, proliferative abilities, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity [62,63]. They are also capable of self-renewal and differentiation into three germ layers, offering potential for clinical cell therapies [64,65]. Because iPS cells can be derived from the somatic cells, potential immune rejection and ethical consideration can be avoided by autologous transplantation. Recently, Wernig *et al.* demonstrated that neuronal and glial cell types could be derived from iPS cells *in vitro* and that transplantation of iPS cell-derived neuronal cells into the brain was able to improve behavior in a rat model of PD [66]. We also demonstrated an efficient method to differentiate iPS cells into astrocyte-like and neuron-like cells which displayed functional electrophysiological properties [67]. Our *in vivo* study showed that direct injection of iPS cells into damaged areas of rat cortex significantly decreased the infarct size, improved the motor function, attenuated inflammatory cytokines, and mediated neuroprotection after middle cerebral artery occlusion (MCAO) [67]. Subdural injection of iPS cells with fibrin glue was as effective and as the direct-injection method, and provided a safer choice for cell replacement therapy [67].

The ability to form teratomas *in vivo* has been a landmark and routine assay for evaluating the pluripotency of ES as well as iPS cells [64,68]. However, teratoma or tumor formation is an unacceptable adverse effect for cell transplantation therapy. Preventing teratoma formation or tumorigenesis has become an emergent issue [69-73]. One of the methods is elimination of nonneural progenitors, which can be achieved by the elaboration of differentiation protocols that allow maximal homogeneity of the transplant [74] or by cell sorting before transplantation [75-78]. Exclusion of poorly-differentiated ES or iPS cells can also reduce the rate of teratoma or tumor formation [79]. Some antioxidants may prevent tumorigenesis after cell transplantation. Resveratrol, a natural polyphenol antioxidant, is demonstrated that it can inhibit teratoma formation *in vivo* [65]. Our recent study result also found that docosahexaenoic acid can inhibit teratoma formation in addition to promoting dopaminergic differentiation in iPS cells in PD-like rats [80]. It has been only two years since the development of iPS cells. Enhancement of effectiveness and eliminating adverse effects of this cell-transplantation therapy required more extensive studies.

4. Diet and Neurogenesis

Recent reports suggested that the environmental factors, especially the detrimental factors induced by neuronal injury, have a critical impact on adult neurogenesis. Several environmental factors are also involved in adult neurogenesis, diet being one of them. Interested readers can refer to a recent comprehensive review by [81]. Briefly, The influence of diet on adult neurogenesis comes from four domains: meal content, meal texture, meal frequency and calorie intake [81]. With regards to meal content, zinc, thiamine and vitamine-A deficiencies decrease cell proliferation in adult hippocampus [81]. Similarly, excess in retinoic acid and increased homocysteine levels also decrease or inhibit cell proliferation in adult hippocampus. In contrast, low-dose curcumin and flavonoids have beneficial effects on adult hippocampal cell proliferation in rodents [81]. It is worthy noting that most flavonoids are extensively metabolized *in vivo* and the bioavailability of flavonoids after the consumption of flavonoid-rich food can only reach very low concentrations in human plasma [82]. In order for adult hippocampal neurogenesis to take place, the purity of flavonoid intake needs to be high. An example is the extract from a traditional Chinese herbal decoction Xiaobuxin-Tang [83]. It is also interesting that calorie restriction and extending the time between meals increase adult hippocampal neurogenesis while diets with high-fat content are noxious and weaken neurogenesis in male rates [81].

5. Neural Stem Cell, Chinese Herbs, and New Drug Screening

Natural plant products and phytochemicals have been used as medicinal agents for hundreds of years in oriental medicine [84]. Based on clinical experiences and recent studies, Chinese herbs and their constituents can be the sources for the development of new drugs for many important human disorders, such as cancers [85,86]. Accumulating evidences have pointed to the fact that some herb-derived substances have neuroprotective effects. For example, Lee *et al.* reported that wogonin, a flavonoid derived from the root of *Scutellaria baicalensis* Georgi, is neuroprotective *in vitro* and *in vivo* [87]. It has an anti-inflammation effect by inhibiting the activation of TNF- α , interleukin-1 β , and nitric oxide (NO) production induced by LPS in cultured brain microglia, and protects co-cultured PC12 cells against microglial cytotoxicity [87]. In two experimental brain injury models, transient global ischemia by 4-vessel occlusion and excitotoxic injury by systemic kainite injection, wogonin reduced induction of inflammatory mediators (ex. iNOS and TNF- α) in hippocampus, inhibits micorgial activation, and attenuates ischemic death of hippocampal neurons [87]. Tetramethylpyrazine (TMP) is another example. It is an alkaloid extracted from the Chinese herbal plant *Ligusticum wallichii* Franchat (*chuanxiong*). Previous experimental studies have demonstrated its beneficial effects on cardiac and cerebral blood flow and reperfusion, as well as its role on calcium antagonism, on vascular tissues, on ROS scavenger and on inhibition of inflammation [88]. In addition, systemic administration of TMP protects neuronal cells from ischemic or traumatic brain or spinal cord injury, promotes functional recovery and attenuates learning and memory impairment induced by D-galactose in animals [89-92]. Furthermore, systemic administration of TMP following the onset of seizure induced by kainite significantly reduced the number of TUNEL-positive cells in hippocampus and piriform cortex, indicating TMP attenuates neuronal degeneration and has neuroprotective efficacy against neuro-excitotoxic attack [88]. Another popular plant which is used in oriental food and

medicine, ginger, is able to inhibit β -amyloid peptide-induced cytokine and chemokine expression in cultured monocytes [93]. This *in vitro* study suggests the potential role of ginger in delaying the onset and progression of neurodegenerative disorder involving chronically activated microglial cells in CNS [93].

It is also interesting to review the evidence of phytochemicals as sources of antidepressants. Lim *et al.* showed that ginger oil possessed antidepressant-like action by reducing immobility in the forced swim test (FST) in mice after the inhalation of ginger oil [94]. Xu *et al.* also showed that the mixture of honokiol and magnolol had an antidepressant effect because the mixture significantly attenuated the reduction of 5-HT levels in frontal cortex, hippocampus, striatum, hypothalamus and nucleus accumbens, and raised serum corticosterone concentration induced by chronic mild stress (CMS) in rats [95]. The mixture of honokiol and magnolol also decreased immobility time in the mouse FST and tail suspension test (TST) significantly, and reversed CMS-induced anhedonia in rats [95]. In our experiments, we also found that mice treated with *Scutellaria baicalensis*, *Phellodendri Cortex* and *Ligusticum wallichii* had increased number of Brd-U positive cells in dentate gyrus and reduced serum levels of corticosterone after the exposure to CMS. Compared with those which were exposed to CMS alone without the three traditional Chinese medicinal herbs, these animals had increased body weight and reduced immobility time in FST [96]. The cellular, biochemical and behavioral effects of the three herbs were similar to the effects of fluoxetine and duloxetine [96]. Furthermore, we also found that the three traditional Chinese medicinal herbs increased the cell viability of NSCs, with superior effect on the index than fluoxetine treatment [96]. These recent progresses not only support the future niche of Chinese medicinal herbs as the useful antidepressants, but also indicate the potential of the use of NSC-based screening system for new drug discovery and characterization from Chinese herbs and medicines.

6. Conclusions

The development of stem cell studies has provided a promising future for the treatment of neurological and psychiatric diseases in several ways. First, understanding the biology and pathology of NSCs will help us elucidate the pathophysiology of several neurological and psychiatric diseases, such as depression, Parkinson's disease or ischemic stroke. The growing knowledge also helps us develop neuroprotective and neurorestorative therapies. Second, NSCs can provide a platform to clarify the mechanism and to test the efficacy of drugs, including Chinese herbal medicines. Third, the development of ES cells and iPS cells make cell transplantation therapies promising in the treatment of ischemic stroke or neurodegenerative diseases. There are still lots of unsolved problems like the formation of teratomas from implanted stem cells, or the viability and the ability of differentiation of implanted cells. In addition, the collaborative efforts of both basic and translational research are needed in the future. Finally, stem cell-based neuroprotective and neurorestorative strategies preserve the utilizing potential to develop the transplantation strategies and to screen candidate agents for neurogenesis, neuroprotection, and neuroplasticity in neurodegenerative diseases.

Acknowledgements

This study was supported by research grants from NSC-(97-3111-B-075-001-MY3), Taipei Veterans General Hospital (98-C1-099/E1-003/ ER3-001), the Joint Projects of VGHUST (98-G6-6; 98-P1-01), Yen-Tjing-Ling Medical Foundation, National Yang-Ming University (Ministry of Education, Aim for the Top University Plan), and Technology Development Program for Academia, Department of Industrial Technology (98-EC-17-A-19-S2-0107), Ministry of Economic Affairs, Taiwan.

References

1. Gage, F.H. Mammalian neural stem cells. *Science* **2000**, *287*, 1433-1438.
2. Alvarez-Buylla, A.; Lim, D.A. For the long run: Maintaining germinal niches in the adult brain. *Neuron* **2004**, *41*, 683-686.
3. Ma, D.K.; Bonaguidi, M.A.; Ming, G.L.; Song, H. Adult neural stem cells in the mammalian central nervous system. *Cell Res.* **2009**, *19*, 672-682.
4. Ma, D.K.; Ming, G.L.; Song, H. Glial influences on neural stem cell development: Cellular niches for adult neurogenesis. *Curr. Opin. Neurobiol.* **2005**, *15*, 514-520.
5. Taupin, P.; Gage, F.H. Adult neurogenesis and neural stem cells of the central nervous system in mammals. *J. Neurosci. Res.* **2002**, *69*, 745-749.
6. Gurvits, T.V.; Shenton, M.E.; Hokama, H.; Ohta, H.; Lasko, N.B.; Gilbertson, M. W.; Orr, S.P.; Kikinis, R.; Jolesz, F.A.; McCarley, R.W.; *et al.* Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biol. Psychiatry* **1996**, *40*, 1091-1099.
7. Wong, M.L.; Licinio, J. Research and treatment approaches to depression. *Nat. Rev. Neurosci.* **2001**, *2*, 343-351.
8. Moser, M.B.; Moser, E.I. Functional differentiation in the hippocampus. *Hippocampus* **1998**, *8*, 608-619.
9. Kempermann, G.; Krebs, J.; Fabel, K. The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. *Curr. Opin. Psychiatry* **2008**, *21*, 290-295.
10. Sapolsky, R.M. Why stress is bad for your brain. *Science* **1996**, *273*, 749-750.
11. Sahay, A.; Hen, R. Adult hippocampal neurogenesis in depression. *Nat. Neurosci.* **2007**, *10*, 1110-1115.
12. Videbech, P.; Ravnkilde, B. Hippocampal volume and depression: A meta-analysis of MRI studies. *Am. J. Psychiatry* **2004**, *161*, 1957-1966.
13. Campbell, S.; Marriott, M.; Nahmias, C.; MacQueen, G.M. Lower hippocampal volume in patients suffering from depression: A meta-analysis. *Am. J. Psychiatry* **2004**, *161*, 598-607.
14. Santarelli, L.; Saxe, M.; Gross, C.; Surget, A.; Battaglia, F.; Dulawa, S.; Weisstaub, N.; Lee, J.; Duman, R.; Arancio, O.; *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **2003**, *301*, 805-809.
15. Czeh, B.; Lucassen, P.J. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur. Arch Psychiatry Clin Neurosci.* **2007**, *257*, 250-260.
16. McEwen, B.S. Glucocorticoids, depression, and mood disorders: Structural remodeling in the brain. *Metabolism* **2005**, *54*, 20-23.

17. Airan, R.D.; Meltzer, L.A.; Roy, M.; Gong, Y.; Chen, H.; Deisseroth, K. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science* **2007**, *317*, 819-823.
18. Meshi, D.; Drew, M.R.; Saxe, M.; Ansorge, M.S.; David, D.; Santarelli, L.; Malapani, C.; Moore, H.; Hen, R. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nat. Neurosci.* **2006**, *9*, 729-731.
19. Saxe, M.D.; Battaglia, F.; Wang, J.W.; Malleret, G.; David, D.J.; Monckton, J.E.; Garcia, A.D.; Sofroniew, M.V.; Kandel, E.R.; Santarelli, L.; *et al.* Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17501-17506.
20. Thomas, R.M.; Peterson, D.A. Even neural stem cells get the blues: Evidence for a molecular link between modulation of adult neurogenesis and depression. *Gene Expr.* **2008**, *14*, 183-193.
21. Li, Y.F.; Zhang, Y.Z.; Liu, Y.Q.; Wang, H.L.; Yuan, L.; Luo, Z.P. Moclobemide up-regulates proliferation of hippocampal progenitor cells in chronically stressed mice. *Acta. Pharmacol. Sin* **2004**, *25*, 1408-1412.
22. Bonnet, U.; Leniger, T.; Wiemann, M. Moclobemide reduces intracellular pH and neuronal activity of CA3 neurones in guinea-pig hippocampal slices-implication for its neuroprotective properties. *Neuropharmacology* **2000**, *39*, 2067-2074.
23. Lee, H.J.; Kim, J.W.; Yim, S.V.; Kim, M.J.; Kim, S.A.; Kim, Y.J.; Kim, C.J.; Chung, J.H. Fluoxetine enhances cell proliferation and prevents apoptosis in dentate gyrus of maternally separated rats. *Mol. Psychiatry* **2001**, *6*, 725-728.
24. Chiou, S.H.; Sheu, B.C.; Chang, W.C.; Huang, S.C.; Hong-Nereng, H. Current concepts of tumor-infiltrating lymphocytes in human malignancies. *J. Reprod. Immunol.* **2005**, *67*, 35-50.
25. Chen, H.; Pandey, G.N.; Dwivedi, Y. Hippocampal cell proliferation regulation by repeated stress and antidepressants. *Neuroreport* **2006**, *17*, 863-867.
26. Goldman, S. Stem and progenitor cell-based therapy of the human central nervous system. *Nat. Biotechnol.* **2005**, *23*, 862-871.
27. Chen, S.J.; Kao, C.L.; Chang, Y.L.; Yen, C.J.; Shui, J.W.; Chien, C.S.; Chen, I.L.; Tsai, T.H.; Ku, H.H.; Chiou, S.H. Antidepressant administration modulates neural stem cell survival and serotonergic differentiation through bcl-2. *Curr. Neurovasc. Res.* **2007**, *4*, 19-29.
28. Chiou, S.H.; Chen, S.J.; Peng, C.H.; Chang, Y.L.; Ku, H.H.; Hsu, W.M.; Ho, L.L.; Lee, C.H. Fluoxetine up-regulates expression of cellular FLICE-inhibitory protein and inhibits LPS-induced apoptosis in hippocampus-derived neural stem cell. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 391-400.
29. Chiou, S.H.; Ku, H.H.; Tsai, T.H.; Lin, H.L.; Chen, L.H.; Chien, C.S.; Ho, L.L.; Lee, C.H.; Chang, Y.L. Moclobemide upregulated Bcl-2 expression and induced neural stem cell differentiation into serotonergic neuron via extracellular-regulated kinase pathway. *Br. J. Pharmacol.* **2006**, *148*, 587-598.
30. Peng, C.H.; Chiou, S.H.; Chen, S.J.; Chou, Y.C.; Ku, H.H.; Cheng, C.K.; Yen, C.J.; Tsai, T.H.; Chang, Y.L.; Kao, C.L. Neuroprotection by Imipramine against lipopolysaccharide-induced apoptosis in hippocampus-derived neural stem cells mediated by activation of BDNF and the MAPK pathway. *Eur. Neuropsychopharmacol.* **2008**, *18*, 128-140.

31. Huang, C.J.; Cheng, H.H.; Chou, C.T.; Kuo, C.C.; Lu, Y.C.; Tseng, L.L.; Chu, S.T.; Hsu, S.S.; Wang, J.L.; Lin, K.L.; *et al.* Desipramine-induced Ca²⁺ movement and cytotoxicity in PC3 human prostate cancer cells. *Toxicol. In Vitro* **2007**, *21*, 449-456.
32. Irmiler, M.; Thome, M.; Hahne, M.; Schneider, P.; Hofmann, K.; Steiner, V.; Bodmer, J.L.; Schroter, M.; Burns, K.; Mattmann, C.; *et al.* Inhibition of death receptor signals by cellular FLIP. *Nature* **1997**, *388*, 190-195.
33. Schulze-Bergkamen, H.; Brenner, D.; Krueger, A.; Suess, D.; Fas, S.C.; Frey, C.R.; Dax, A.; Zink, D.; Buchler, P.; Muller, M.; *et al.* Hepatocyte growth factor induces Mcl-1 in primary human hepatocytes and inhibits CD95-mediated apoptosis via Akt. *Hepatology* **2004**, *39*, 645-654.
34. Xu, H.; Chen, Z.; He, J.; Haimanot, S.; Li, X.; Dyck, L.; Li, X.M. Synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in cell proliferation and BDNF expression in rat hippocampus. *Hippocampus* **2006**, *16*, 551-559.
35. Hayley, S.; Poulter, M.O.; Merali, Z.; Anisman, H. The pathogenesis of clinical depression: Stressor- and cytokine-induced alterations of neuroplasticity. *Neuroscience* **2005**, *135*, 659-678.
36. Manji, H.K.; Chen, G. PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. *Mol. Psychiatry* **2002**, *7*, S46-S56.
37. Shirayama, Y.; Chen, A. ; Nakagawa, S.; Russell, D.S.; Duman, R.S. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci.* **2002**, *22*, 3251-3261.
38. Einat, H.; Manji, H.K. Cellular plasticity cascades: Genes-to-behavior pathways in animal models of bipolar disorder. *Biol. Psychiatry* **2006**, *59*, 1160-1171.
39. Larsson, E.; Nanobashvili, A.; Kokaia, Z.; Lindvall, O. Evidence for neuroprotective effects of endogenous brain-derived neurotrophic factor after global forebrain ischemia in rats. *J. Cereb. Blood Flow Metab.* **1999**, *19*, 1220-1228
40. Kokaia, Z.; Nawa, H.; Uchino, H.; Elmer, E.; Kokaia, M.; Carnahan, J.; Smith, M.L.; Siesjo, B. K.; Lindvall, O. Regional brain-derived neurotrophic factor mRNA and protein levels following transient forebrain ischemia in the rat. *Brain. Res. Mol. Brain. Res.* **1996**, *38*, 139-144.
41. Zhang, Z.G.; Chopp, M. Neurorestorative therapies for stroke: Underlying mechanisms and translation to the clinic. *Lancet Neurol.* **2009**, *8*, 491-500.
42. Lindvall, O.; Kokaia, Z.; Martinez-Serrano, A. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat. Med.* **2004**, *10*, S42-S50.
43. Samii, A.; Nutt, J.G.; Ransom, B.R. Parkinson's disease. *Lancet* **2004**, *363*, 1783-1793.
44. Fearnley, J.M.; Lees, A.J. Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain* **1991**, *114* (Pt 5), 2283-2301.
45. Lindvall, O.; Hagell, P. Clinical observations after neural transplantation in Parkinson's disease. *Prog. Brain Res.* **2000**, *127*, 299-320.
46. Kordower, J.H.; Freeman, T.B.; Snow, B.J.; Vingerhoets, F.J.; Mufson, E.J.; Sanberg, P.R.; Hauser, R.A.; Smith, D.A.; Nauert, G.M.; Perl, D.P.; *et al.* Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N. Engl. J. Med.* **1995**, *332*, 1118-1124.

47. Freed, C.R.; Greene, P.E.; Breeze, R.E.; Tsai, W.Y.; DuMouchel, W.; Kao, R.; Dillon, S.; Winfield, H.; Culver, S.; Trojanowski, J.Q.; *et al.* Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N. Engl. J. Med.* **2001**, *344*, 710-719.
48. Olanow, C.W.; Goetz, C.G.; Kordower, J.H.; Stoessl, A.J.; Sossi, V.; Brin, M.F.; Shannon, K.M.; Nauert, G.M.; Perl, D.P.; Godbold, J.; *et al.* A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann. Neurol.* **2003**, *54*, 403-414.
49. Hagell, P.; Piccini, P.; Bjorklund, A.; Brundin, P.; Rehncrona, S.; Widner, H.; Crabb, L.; Pavese, N.; Oertel, W.H.; Quinn, N.; *et al.* Dyskinesias following neural transplantation in Parkinson's disease. *Nat. Neurosci.* **2002**, *5*, 627-628.
50. Hedlund, E.; Perlmann, T. Neuronal cell replacement in Parkinson's disease. *J. Intern. Med.* **2009**, *266*, 358-371.
51. Yang, D.; Zhang, Z.J.; Oldenburg, M.; Ayala, M.; Zhang, S.C. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. *Stem Cells* **2008**, *26*, 55-63.
52. Glavaski-Joksimovic, A.; Virag, T.; Chang, Q.A.; West, N.C.; Mangatu, T.A.; McGrogan, M.P.; Dugich-Djordjevic, M.; Bohn, M.C. Reversal of dopaminergic degeneration in a parkinsonian rat following micrografting of human bone marrow-derived neural progenitors. *Cell Transplant.* **2009**, *18*, 801-814.
53. Adams, H.P., Jr.; del Zoppo, G.; Alberts, M.J.; Bhatt, D.L.; Brass, L.; Furlan, A.; Grubb, R.L.; Higashida, R.T.; Jauch, E.C.; Kidwell, C.; *et al.* Guidelines for the early management of adults with ischemic stroke: A guideline from the American Heart Association/American Stroke Association Stroke Council, Clinical Cardiology Council, Cardiovascular Radiology and Intervention Council, and the Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups: The American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. *Stroke* **2007**, *38*, 1655-1711.
54. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N. Engl. J. Med.* **1995**, *333*, 1581-1587.
55. Arvidsson, A.; Collin, T.; Kirik, D.; Kokaia, Z.; Lindvall, O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat. Med.* **2002**, *8*, 963-970.
56. Farooq, M.U.; Naravetla, B.; Moore, P.W.; Majid, A.; Gupta, R.; Kassab, M.Y. Role of sildenafil in neurological disorders. *Clin. Neuropharmacol.* **2008**, *31*, 353-362.
57. Chopp, M.; Li, Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurol.* **2002**, *1*, 92-100.
58. Takahashi, K.; Yasuhara, T.; Shingo, T.; Muraoka, K.; Kameda, M.; Takeuchi, A.; Yano, A.; Kurozumi, K.; Agari, T.; Miyoshi, Y.; *et al.* Embryonic neural stem cells transplanted in middle cerebral artery occlusion model of rats demonstrated potent therapeutic effects, compared to adult neural stem cells. *Brain Res.* **2008**, *1234*, 172-182.
59. Yanagisawa, D.; Qi, M.; Kim, D.H.; Kitamura, Y.; Inden, M.; Tsuchiya, D.; Takata, K.; Taniguchi, T.; Yoshimoto, K.; Shimohama, S.; *et al.* Improvement of focal ischemia-induced rat dopaminergic dysfunction by striatal transplantation of mouse embryonic stem cells. *Neurosci. Lett.* **2006**, *407*, 74-79.

60. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. *Nature* **2007**, *448*, 313-317.
61. Park, I.H.; Zhao, R.; West, J.A.; Yabuuchi, A.; Huo, H.; Ince, T.A.; Lerou, P.H.; Lensch, M.W.; Daley, G.Q. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **2008**, *451*, 141-146.
62. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **2007**, *131*, 861-872.
63. Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Stewart, R.; *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* **2007**, *318*, 1917-1920.
64. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663-676.
65. Kao, C.L.; Tai, L.K.; Chiou, S.H.; Chen, Y.J.; Lee, K.H.; Chou, S.J.; Chang, Y.L.; Chang, C.M.; Chen, S.J.; Ku, H.H.; *et al.* Resveratrol promotes osteogenic differentiation and protects against dexamethasone damage in murine induced pluripotent stem cells. *Stem Cells Dev.* **2010**, *19*, 247-258.
66. Wernig, M.; Zhao, J.P.; Pruszak, J.; Hedlund, E.; Fu, D.; Soldner, F.; Broccoli, V.; Constantine-Paton, M.; Isacson, O.; Jaenisch, R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5856-5861.
67. Chen, S.J.; Chang, C.M.; Tsai, S.K.; Chang, Y.L.; Chou, S.J.; Huang, S.S.; Tai, L.K.; Chen, Y.C.; Ku, H.H.; Li, H.Y.; *et al.* Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells Dev.* **2010**, Mar 1. [Epub ahead of print].
68. Yamanaka, S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. *Cell Prolif.* **2008**, *41*, S51-S56.
69. Bjorklund, L.M.; Sanchez-Pernaute, R.; Chung, S.; Andersson, T.; Chen, I.Y.; McNaught, K.S.; Brownell, A.L.; Jenkins, B.G.; Wahlestedt, C.; Kim, K.S.; *et al.* Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2344-2349.
70. Erdo, F.; Buhrle, C.; Blunk, J.; Hoehn, M.; Xia, Y.; Fleischmann, B.; Focking, M.; Kustermann, E.; Kolossov, E.; Hescheler, J.; *et al.* Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. *J. Cereb. Blood Flow Metab.* **2003**, *23*, 780-785.
71. Hedlund, E.; Pruszak, J.; Ferree, A.; Vinuela, A.; Hong, S.; Isacson, O.; Kim, K.S. Selection of embryonic stem cell-derived enhanced green fluorescent protein-positive dopamine neurons using the tyrosine hydroxylase promoter is confounded by reporter gene expression in immature cell populations. *Stem Cells* **2007**, *25*, 1126-1135.
72. Pruszak, J.; Sonntag, K.C.; Aung, M.H.; Sanchez-Pernaute, R.; Isacson, O. Markers and methods for cell sorting of human embryonic stem cell-derived neural cell populations. *Stem Cells* **2007**, *25*, 2257-2268.

73. Roy, N.S.; Cleren, C.; Singh, S.K.; Yang, L.; Beal, M.F.; Goldman, S.A. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat. Med.* **2006**, *12*, 1259-1268.
74. Brederlau, A.; Correia, A.S.; Anisimov, S.V.; Elmi, M.; Paul, G.; Roybon, L.; Morizane, A.; Bergquist, F.; Riebe, I.; Nannmark, U.; *et al.* Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: Effect of *in vitro* differentiation on graft survival and teratoma formation. *Stem Cells* **2006**, *24*, 1433-1440.
75. Hedlund, E.; Pruzsak, J.; Lardaro, T.; Ludwig, W.; Vinuela, A.; Kim, K.S.; Isacson, O. Embryonic stem cell-derived Pitx3-enhanced green fluorescent protein midbrain dopamine neurons survive enrichment by fluorescence-activated cell sorting and function in an animal model of Parkinson's disease. *Stem Cells* **2008**, *26*, 1526-1536.
76. Chung, S.; Shin, B.S.; Hedlund, E.; Pruzsak, J.; Ferree, A.; Kang, U.J.; Isacson, O.; Kim, K.S. Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. *J. Neurochem.* **2006**, *97*, 1467-1480.
77. Guillaume, D.J.; Johnson, M.A.; Li, X.J.; Zhang, S.C. Human embryonic stem cell-derived neural precursors develop into neurons and integrate into the host brain. *J. Neurosci. Res.* **2006**, *84*, 1165-1176.
78. Tabar, V.; Panagiotakos, G.; Greenberg, E.D.; Chan, B.K.; Sadelain, M.; Gutin, P.H.; Studer, L. Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain. *Nat. Biotechnol.* **2005**, *23*, 601-606.
79. Seminatore, C.; Polentes, J.; Ellman, D.; Kozubenko, N.; Itier, V.; Tine, S.; Tritschler, L.; Brenot, M.; Guidou, E.; Blondeau, J.; *et al.* The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. *Stroke* **2010**, *41*, 153-159.
80. Chiou, S.H.; Huang, C.W. Docosahexaenoic acid, teratoma formation and dopaminergic differentiation in iPS cells in parkinson disease-like rats. **2009**, unpublished work.
81. Stangl, D.; Thuret, S. Impact of diet on adult hippocampal neurogenesis. *Genes Nutr.* **2009**, *4*, 271-282.
82. Lotito, S.B.; Frei, B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? *Free Radic Biol. Med.* **2006**, *41*, 1727-1746.
83. An, L.; Zhang, Y.Z.; Yu, N.J.; Liu, X.M.; Zhao, N.; Yuan, L.; Chen, H.X.; Li, Y.F. The total flavonoids extracted from Xiaobuxin-Tang up-regulate the decreased hippocampal neurogenesis and neurotrophic molecules expression in chronically stressed rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2006**, *32*, 1484-1490.
84. Gong, X.; Sucher, N.J. Stroke therapy in traditional Chinese medicine (TCM): Prospects for drug discovery and development. *Trends. Pharmacol. Sci.* **1999**, *20*, 191-196.
85. Li-Weber, M. New therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer. Treat. Rev* **2009**, *35*, 57-68.
86. Newman, D.J.; Cragg, G.M.; Snader, K.M. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Procol.* **2003**, *66*, 1022-1037.

87. Lee, H.; Kim, Y.O.; Kim, H.; Kim, S.Y.; Noh, H.S.; Kang, S.S.; Cho, G.J.; Choi, W.S.; Suk, K. Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. *FASEB. J.* **2003**, *17*, 1943-1944.
88. Tan, Z. Neural protection by naturopathic compounds-an example of tetramethylpyrazine from retina to brain. *J. Ocul. Biol. Dis. Infor.* **2009**, *2*, 57-64.
89. Fan, L.H.; Wang, K.Z.; Cheng, B.; Wang, C.S.; Dang, X.Q. Anti-apoptotic and neuroprotective effects of Tetramethylpyrazine following spinal cord ischemia in rabbits. *BMC Neurosci.* **2006**, *7*, 48.
90. Kao, T.K.; Ou, Y.C.; Kuo, J.S.; Chen, W.Y.; Liao, S.L.; Wu, C.W.; Chen, C.J.; Ling, N.N.; Zhang, Y.H.; Peng, W.H. Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. *Neurochem. Int.* **2006**, *48*, 166-176.
91. Ni, J.W.; Matsumoto, K.; Watanabe, H. Tetramethylpyrazine improves spatial cognitive impairment induced by permanent occlusion of bilateral common carotid arteries or scopolamine in rats. *Jpn. J. Pharmacol.* **1995**, *67*, 137-141.
92. Zhang, C.; Wang, S.Z.; Zuo, P.P.; Cui, X.; Cai, J. Protective effect of tetramethylpyrazine on learning and memory function in D-galactose-lesioned mice. *Chin. Med. Sci. J.* **2004**, *19*, 180-184.
93. Grzanna, R.; Phan, P.; Polotsky, A.; Lindmark, L.; Frondoza, C.G. Ginger extract inhibits beta-amyloid peptide-induced cytokine and chemokine expression in cultured THP-1 monocytes. *J. Altern. Complement. Med.* **2004**, *10*, 1009-1013.
94. Lim, W.C.; Seo, J.M.; Lee, C.I.; Pyo, H.B.; Lee, B.C. Stimulative and sedative effects of essential oils upon inhalation in mice. *Arch. Pharm. Res.* **2005**, *28*, 770-774.
95. Xu, Q.; Yi, L.T.; Pan, Y.; Wang, X.; Li, Y.C.; Li, J.M.; Wang, C.P.; Kong, L.D. Antidepressant-like effects of the mixture of honokiol and magnolol from the barks of *Magnolia officinalis* in stressed rodents. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2008**, *32*, 715-725.
96. Chiou, S.H.; Lu, S.W. Effect of chiese herb medicine on cellular, biochemical and animal models of depression. **2009**, unpublished work.