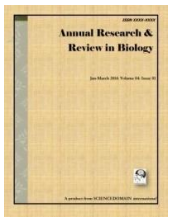

Annual Research & Review in Biology
6(6): 364-378, 2015, Article no.ARRB.2015.096
ISSN: 2347-565X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Therapeutic Effects of Neural and Other Diverse Pluripotent Stem Cells in Treating Various Neurological Disorders

Aminah Suhail Qureshi[1] and Sikander Ali[1*]

[1]Institute of Industrial Biotechnology, Government College University Lahore, Lahore, Pakistan.

Authors' contributions

This writing work was planned by the collaboration of both the authors. Author SA designed and interpreted the various sections while author ASQ gathered the information after consulting literature and presented it into a draft. The authors read and approved the final article.

Article Information

DOI: 10.9734/ARRB/2015/10480
Editor(s):
(1) George Perry, University of Texas at San Antonio, USA.
Reviewers:
(1) Anonymous, Spain.
(2) Anonymous, Italy.
(3) Anonymous, USA.
(4) Xing Li, Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo College of medicine/Mayo Clinic, USA.
(5) Anonymous, USA.
(6) Anonymous, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=865&id=32&aid=8399

Review Article

Received 29th March 2014
Accepted 5th February 2015
Published 12th March 2015

ABSTRACT

Stem cells are unipotent (producing only one cell type, i.e. their own), pluripotent (giving rise to all cell types making up a body), or multipotent (having the ability to develop into more than one type of cell but less than that possessed by pluripotent) cells possessing an unlimited ability to proliferate and differentiate under proper culturing conditions. They can be conveniently obtained from the inner cell mass of mammalian embryos in the blastocyst stage (ESCs, which are pluripotent) and adult tissues (ASCs, which are multipotent so far). Human embryonic stem cells (hESCs) proved to be fatal for the embryos owing to which induced pluripotent cells (iPSCs) have been developed by forcibly inducing the expression of specific regenerative genes. All types of pluripotent stem cells, which include ectodermal stem cells, mesenchymal stem cells and rare types of endodermal stemcells, are used in treating several early onset neurodevelopment diseases and late-onset neurodegenerative disorders. Neural stem cells (NSCs) can be found in

**Corresponding author: E-mail: alisbiotech@yahoo.com;*

the developing and adult central nervous system (CNS) and form a population comprising astrocytes, oligodendrocytes and neurones. Several researches conducted in animal models have shown structural and functional recovery as they have been researched on to treat diseases like stroke, brain tumours, metastatic tumours, human primary tumours, spinal cord injury and multiple sclerosis and, for this purpose, neural stem cells enjoy a pivotal status. Their efficacy and safety has been proven not only by the data collected from literature, but also from researches in animal models induced with various neurological disorders.

Keywords: Stem cells; embryonic stem cells; induced pluripotent stem cells; neurological disorders; neural stem cells.

1. INTRODUCTION

Stem cells are undifferentiated cells which have unlimited capacity for proliferation of which many can differentiate, under correct inducing conditions, into one of several kinds of cells; different kinds of stem cells differ markedly in terms of the kinds of cells they will differentiate into [1,2]. Their self-renewal is achieved by stimulating proliferation preceded by suppressing differentiation [3]. Stem cells can be derived from the inner cell mass of mammalian embryos in the blastocyst stage, and adult tissues [4,5]. Stem cells obtained from the cord blood of new-born infants or embryos are known as embryonic stem cells (ESCs) [6]. Stem cells can also be isolated successfully from adult tissues which are then referred to as adult-derived stem cells (ASCs). ESCs have the ability to self-renew to produce more stem cells, and can differentiate into several types of mature and specialised cells. They serve up as an inexhaustible pool for several primogenitor and stem cell populations [7].

In addition, it is only after long *in vitro* culture conditions that embryonic stem cells show signs of aging owing to the high levels of telomerase. They can be coaxed to generate any tissue of interest depending upon the type of conditions provided. Regenerative medicine uses this idea to collect stem cells from the patients suffering from genetic disorders, manipulate these cells genetically by gene therapy, and reinsert and incorporate these genetically modified cells into the referred patient. However, collecting human embryonic stem cells (hESCs) results in the destruction of the embryo [8]. To avoid this, ASCs are being promoted as safer alternatives, but they have not been isolated from many all adult tissues (except blood, muscle, teeth, mammary glands, fat, bone marrow, hair, skin, pancreas, intestine and brain) [9]. Without doubt, human embryonic stem cells still continue to serve as the pluripotent stem cells which are hoped to function as the donor cells in transplantation in regenerative medicine (the example of which has been given later in this review) [10].

Induced pluripotent stem cells (iPSCs), another type of pluripotent stem cells, have been developed to avoid the damage to potential embryos and the risk generated by using the adult-derived stem cells [11]. They are artificially educed from non-pluripotent cells (typically adult somatic cells) by forcibly inducing an expression of specific regenerative genes. The reprogramming is usually done by all 4 factors (or sometimes by 3 factors excluding c-Myc): c-Myc, SOX2, KLF4 and OCT4. This is a cusp development as ethical problems exist in using human embryonic stem cells, while induced pluripotent stem cells are produced from patients themselves and, therefore, have minuscule ethical problems. It is for this reason that iPSCs are also referred to as “personalised pluripotent cells” [12]. These somatic cells, having been induced with the ability to differentiate into a myriad species of cells, are being used as donor cells for various neurological diseases [13].

A neurological disease is any disorder of the nervous system. These may be hereditary or intermittent and unpredictable, and may result in the chronic and progressive deprivation of neural structures and functions. These are often classified depending on their onset, i.e. they are either early-onset neurodevelopment disorders or late-onset neurodegenerative diseases. Aging is considered to be the triggering factor for the onset of many neurodegenerative diseases therefore it is of paramount importance to develop novel treatments [14]. All the above-mentioned stem cell types have an ability to self-renew and undergo multilineage differentiation under adequate *in vitro* culturing conditions [2] (Fig. 1). Therefore, this review will be regarding the treatment of several neurological diseases by bringing in use more or less all types of well-researched stem cells.

2. TREATMENT OF NEUROLOGICAL DISORDERS WITH DIFFERENT STEM CELL APPLICATIONS

Human embryonic stem cells self-renew through fibroblast growth factor (FGF)-2 and the nodal-signalling pathway [15]. Phosphoinositide-3-kinase signalling also plays pivotal and critical roles in self-renewal of stem cells [16]. Adult-derived stem cells, on the other hand, can be obtained from various tissues, such as bone marrow, brain, skin, hair, pancreas, mammary glands, adipose, pancreas, intestine, teeth and blood, and have been well-characterised [9]. Also known as somatic stem cells, these are undifferentiated cells which are found all over the body after its development. They multiply by cell division to replace dead cells and replenish damaged tissues. The most proficiently studied ASCs are haematopoietic stem cells (HSCs), which undergo self-renewal cell division, differentiate and develop into mature blood elements from single progenitor cell level, and utilitarianly regenerate the severely and completely depleted haematopoietic system of humans and other animals. Other types of ASCs have only been recently examined and described among which neural stem cells (NSCs) are of major significance [4].

Somatic stem cells can be classified into three groups, namely ectodermal stem cells, mesenchymal (or mesodermal) stem cells and endodermal stem cells [17,18]. As their names imply, ectodermal stem cells are obtained from body parts and organs which originated from the outermost layer of the germ cell (ectoderm – it differentiates to form the epidermis, tooth enamel and the nervous system), mesenchymal stem cells are acquired from those organs which developed from the middle of the three primary germ layers in the early-stage embryo (mesoderm – it forms mesenchyme, mesothelium, coelomocytes, non-epithelial blood cells and parts of gonads, and lines coeloms and mesenteries), while endodermal stem cells are derived from organs originally developed from the innermost layer of the germ cell (endoderm – it forms gastrointestinal tract, respiratory tract, endocrine glands and organs, auditory system and urinary system). Ectodermal stem cells, therefore, include hair follicle stem cells, retinal stem cells [19], dental pulp stem cells and neural stem cells [20], whereas mesenchymal stem cells consist of skin-derived precursors [21], adipose-derived mesenchymal stem cells [22], bone marrow mesenchymal stem cells [23], umbilical cord blood mesenchymal stem cells [24], placenta-derived mesenchymal stem cells, multilineage-differentiating stress enduring (MUSE) stem cells [25] and peripheral blood monocytes (Fig. 2). However, endodermal stem cells seldom differentiate into pluripotent stem cells without dedifferentiation or stimulation to form induced pluripotent stem cells [26].

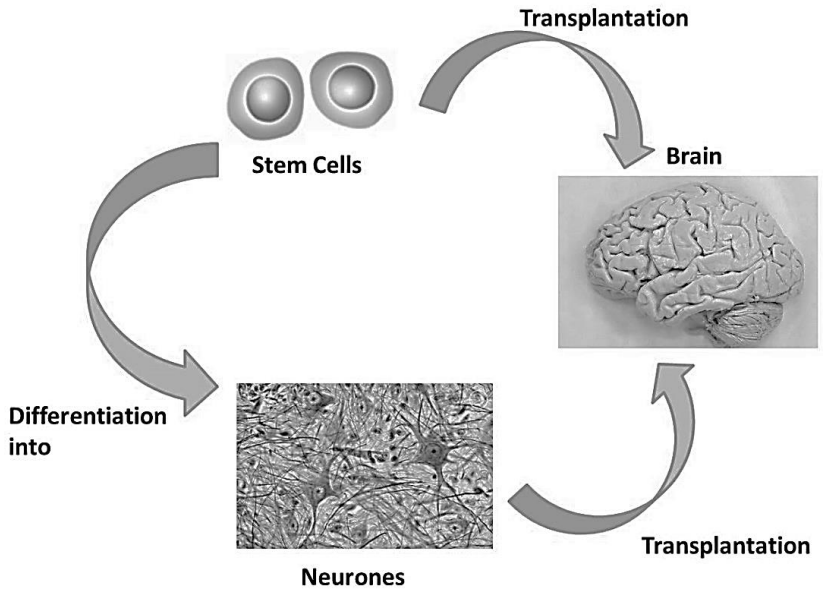

Fig. 1. Transplantation of stem cells into the central nervous system (CNS)

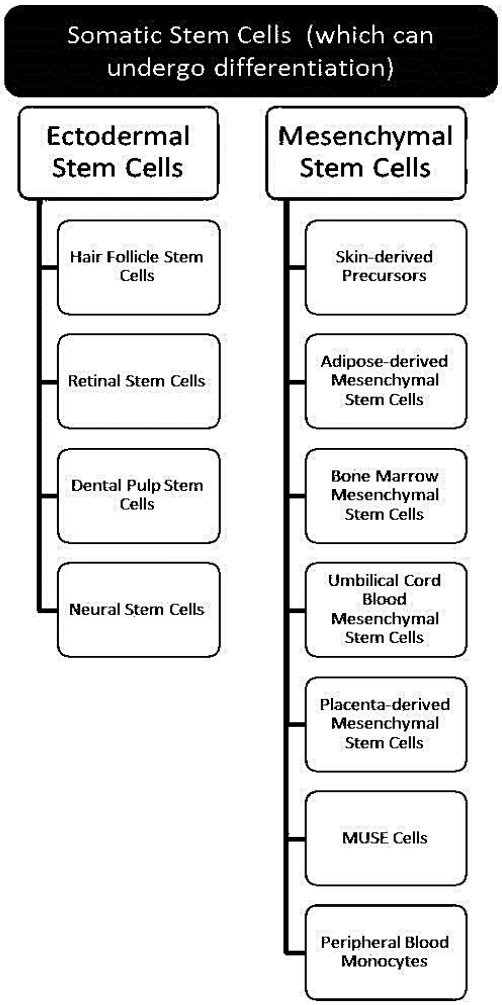

Fig. 2. Somatic stem cells which possess the ability to undergo neuronal differentiation

Among the aforementioned types of stem cells, neural stem cells (NSCs) are described as undifferentiated precursor progenitor cells which possess high ability of self-renewal and being multi-potent owing to their complex and intricate patterns of gene expression [27]. Moreover, they can be obtained from several sources and can differentiate into a variety of neurones [28]. However, in this specific spread of the review, we shall consider the therapeutic promises of other somatic stem cells for treating neurological diseases.

2.1 Hair Follicle Stem Cells

Hair follicle stem cells, which express nestin protein and reside at the bulge region and the outer root sheath of hair follicle underneath the sebaceous glands, are described to differentiate into epithelial lineage cells, such as neural cells [29]. Such hair follicle stem cells are also referred to as neural crest stem cells [30]. These cells have been reported to conduce in neural regenerative treatment and therapy, by which the central nervous system (the brain and the spinal cord) is repaired along with the peripheral nerves. Hair follicle stem cells have been reported to differentiate to dopaminergic neurones in the cerebrum thus causing improvement in the symptoms [31].

2.2 Retinal Stem Cells

Recent studies have shown that retinal stem cells (a type of ectodermal stem cells) are progenitor cells which cannot only be obtained from adult ciliary epithelium of humans and other primates but also from embryonic and new-born retina [32]. They are normally found in the non-pigmented or pigmented cilial epithelium of the

peripheral edge of the retina. Photoreceptor cells possess the highest ability to differentiate therefore these retinal stem cells can be used to treat retinal-neuronal degenerative diseases [33].

2.3 Dental Pulp Stem Cells

Dental pulp stem cells (DPSCs) are purported to have been derived from neural crest cells [34] and, therefore, differentiate into neurones. Hence, they serve to be a pool of donor neural cells for neuronal regenerative therapies, for instance in transplantation to treat spinal cord injury. It is reported that when provided with adequate growing and developing conditions, dental pulp stem cells can differentiate into functional neurones and stimulate endogenous axon steering [35].

2.4 Skin-derived Precursors

Skin-derived precursors (SKPs), also known as dermal papilla stem cells (DPSCs) [36], are capable to differentiate into a variety of cells including neural cells [37]. Though they putatively develop from mesenchymal tissues in dermis, they differentiate to epithelial lineage cells (glia, neurones and keratinocytes), along with adipose cells, smooth muscle cells and mesenchymal-derived cells. It is worth mentioning that glia is sustentacular tissue which surrounds and nurtures the neurones in the central nervous system and composes the tissue of the central nervous system along with the neural cells [38].

2.5 Adipose Tissue-derived Mesenchymal Stem Cells

Adipose-derived stem cells are similar to bone marrow stromal cells in that they can differentiate into a myriad of cells, originally derived not only from mesenchymal organs but also from epithelial and endogenous organs. Lately, human adipose-derived stem cells have been provided with retinoic acid which resulted in their directed differentiation into motor neurone-like cells [39]. This breakthrough has provided potentials for treating diseases like Huntington's [40] and intra-cerebral haemorrhage [41]. VEGF and HGF, the trophic factors of adipose-derived mesenchymal stem cells, contribute in repairing ischemic brain tissues and patients suffering from Parry-Romberg syndrome (progressive hemi-facial atrophy) [42].

2.6 Bone Marrow Mesenchymal Stem Cells

Bone marrow mesenchymal stem cells, also known as bone marrow stromal cells (BMSCs), can differentiate into a variety of cells such as those of neural tissues, liver, adipose tissues, bone and cartilage [43]. Their transplantation can be used to treat patients suffering from Alzheimer's disease [44], Parkinson's disease [45], multiple sclerosis [46], amyotrophic lateral sclerosis [47], cerebral infarction [44] and spinal cord injury [48], but success is fractional. When transplanted through intravenous transfusion, they can penetrate the blood-brain barrier (BBB) but seldom survive to differentiate into neural cells [4]. Despite this problem, they may positively affect the neural tissue repair by secreting neurotrophic factors (a family of proteins which promotes the growth and sustenance of developing and mature neurones) [49]. Many clinical trials on humans suffering from spinal cord injuries involved the transplantation of bone marrow stromal cells, which gave positive results of improved motor neurone functioning. This shows great potential of relying on bone marrow stromal cells in treating neurological disorders [50].

2.7 Umbilical Cord Blood Mesenchymal Stem Cells

Human umbilical cord blood contains, in plentiful amount, haematopoietic stem cells (HSCs) and mesenchymal stem cells. Among them, the mesenchymal stem cells, also specifically known as umbilical cord blood-derived mesenchymal stem cells (UCBSCs), are capable to differentiate into neural cells which can be essentially used in regenerative cell therapies for treating neuronal disorders. Protein kinase A is supposed to facilitate differentiation into neural cells [51]. Moreover, oestrogen is known to stimulate this differentiation of human UCBSCs [52].

2.8 Placenta-derived Mesenchymal Stem Cells

This type of mesenchymal adult-derived stem cells can differentiate into cells similar to dopaminergic neural cells only if provided with appropriate developing conditions [53]. Moreover, their inter-cerebral transplantation is a promising therapy to treat several neural diseases [54].

2.9 MUSE Cells

Multilineage-differentiating stress enduring (MUSE) stem cells are obtained from mesenchymal stromal cells and skin fibroblasts [25]. In fact, they can be harboured from all organ tissues. They resemble human embryonic stem cells and induced pluripotent stem cells in their pluripotency [55]. In a study when fibroblasts were kept under stress conditions (in other words, on treatment with heparin), MUSE cells were released. These are capable to differentiate into tri-dermal cells. They are expected to function as donor cells for neural regenerative therapies as they can be differentiated to neural lineage cells. The generative rate of multilineage-differentiating stress enduring (MUSE) cells is small yet stable because of which they can be obtained from autologous fibroblasts and mesenchymal stem cells. However, the obligation of cell sorting by using anti-SSEA-3 antibody is a limiting factor in its genesis [25].

2.10 Peripheral Blood Monocytes

Peripheral blood monocytes comprise multi-potential mesenchymal stem cells which have the ability to differentiate into neural lineage cells. These can be easily obtained by involving minimal invasive procedures. These cells express neuronal markers (microtubule-associated protein 1-B, neurone specific enolase and neurofilament) when treated with nerve growth factor (NGF) [56]. These then specialise to microglia, the supportive nurturing tissues of neural tissues, and therefore prove to be essential donor cells of autologous transplantation [26].

2.11 Endoderm-derived Somatic Stem Cells

Very few endoderm-derived somatic stem cells have the ability of neuronal differentiation [26]. But thyroid cells obtained from endoderm are capable to differentiate to neurones which can be proven by the fact that on treating thyrocytes with non-serum small airway growth medium (NSAGM), they express beta-III-tubulin, a neuronal marker. Hepatocytes derived from endoderm can be differentiated to neurones by reprogramming them as induced pluripotent stem cells (iPSCs) [57]. Generally, it is challenging to differentiate endoderm-derived stem cells to neurones or any other type of cells.

3. MAJOR ROLE OF NEURAL STEM CELLS IN THE TREATMENT OF VARIOUS NEUROLOGICAL DISORDERS

Neural stem cells (NSCs) are undifferentiated stem cells that can undergo self-renewal and differentiate into major types of cells of brain that exist in the developing and fully-developed adult central nervous system (CNS). They can be obtained from embryonic, foetal, neonatal and adult central nervous tissues and form neurospheres (free floating multicellular spheres) which extemporaneously differentiate into a fascinating population of neural cells, including neurones, astrocytes and oligodendrocytes [28]. It is grimly difficult to obtain human neural stem cells from adults but can be easily obtained from human foetal brains [26]. Previously, obtaining neural stem cells from foetal brains often required an abortion foetus which posed a grave ethical problem [58]. Adult neural stem cells are seldom used also owing to histocompatibility [14].

Neural stem cells are kept in a compartment in the postnatal mammalian brain after embryonic development, which generate new neural cells throughout the life of the mammal. This neurogenesis (the development of nerve tissues) occurs in only two neurogenic regions under normal conditions: the subgranular zone (SGZ) of dentate gyrus of the hippocampus, and the subventricular zone (SVZ) of the lateral ventricle [59]. This NSC niche is a specialised micro-level environment which provides adequate prompts to regulate the behaviours of neural stem cells such as maintenance, self-renewal and production [60]. This niche comprises extracellular substrates and cellular components that provide neural stem cells with the resting environmental conditions to monitor the above-mentioned behaviours of neural stem cells. Neural stem cells divide slowly, possess astrocyte-like traits and also include efficiently-dividing transit amplifying progenitors (TAPs) owing to this highly specialised central nervous system germinal niche. It also contains slowly multiplying cells expressing nestin, glialfibrillary acidic protein (GFAP) and RC2 (the radial glial marker) which will later constitute the central nervous system [61]. In actuality, nestin was primarily described as a neural stem cell marker and was characterised as class VI intermediate filament protein because of its appearance during the development of the central nervous system [62].

This promotes neurogenesis and gliogenesis after maturity.

Therapies based on neural stem cells are being used in treating various nervous system disorders (Fig. 3). Most of the work is done on experimental models and transplantation applications must be further worked on to be considered for their vital clinical applications [4].

3.1 Stroke

Most deaths related to neurological disorders are caused by cerebrovascular accident (CVA), also widely known as stroke. This is caused by the death of endothelial cells, astrocytes and oligodendrocytes resulting from the formation of a cystic cavity and loss of neural cells along with their connections due to devastating damage to the cerebral parenchyma and hypoxia [63]. Patients have to suffer from lifelong functional disabilities due to extremely large loss of neural circuitry in the brain (cerebral arteries). Their recovery is most of the times incomplete even after receiving physical therapies. Hence stem cell therapies were welcomed with exuberance, which can be used to replace the dead cells and tissues after brain injury [14].

Stroke normally occurs due to thrombosis or embolism in a principal cerebral artery, most frequently the middle cerebral artery (MCA) [14]. Antecedently, foetal brain tissue transplants were being done, but resulted in limited recovery. Moreover, ethical issues and limited supply of foetal brain tissues have majorly limited its application. To study other prospects, experimental focal and global cerebral ischaemia models were developed. Global ischaemia is the restriction of blood flow which affects the entire brain area. On the contrary, focal ischaemia is the reduction in cerebral blood flow in a specified region of the brain. Thrombotic blockage was introduced by injecting blood clots or thrombin into the middle cerebral artery [64].

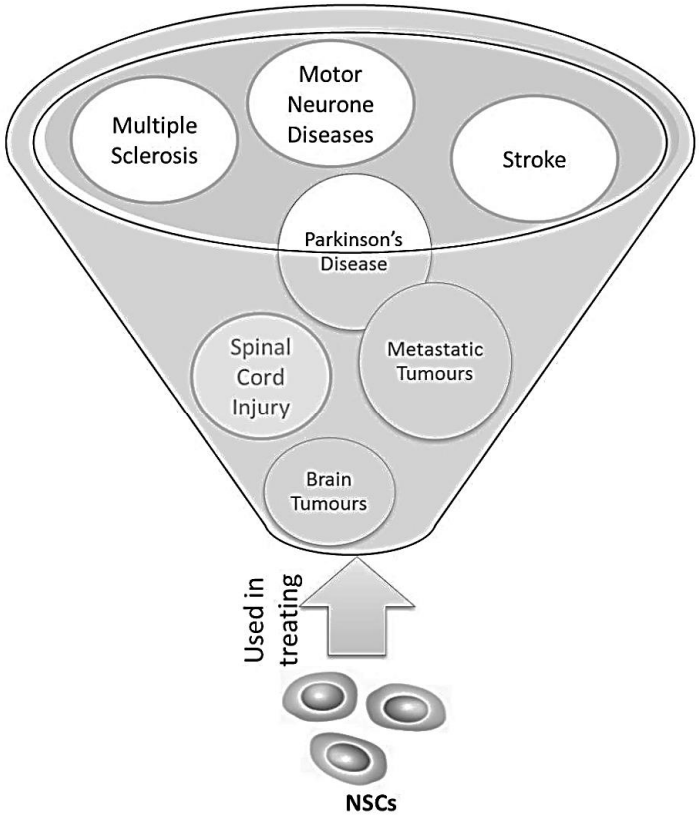

Fig. 3. Neural stem cells are used in the treatment of various neurological disorders including stroke, Parkinson's disease, brain tumour, metastatic tumours, spinal cord injury, motor neurone diseases and multiple sclerosis

The subsequent findings of these studies suggested that intravenously transplanted neural stem cells can effectively enter the brain of mammals suffering with intra-cerebral haemorrhage, sustain and improve the functional recovery. These transplanted neural stem cells then selectively move to the perihaematomal areas to differentiate to neurones and astrocytes. Consequently, the transplantation of these human pluripotent stem cells substantially decreased the infarct volume [65]. Yet the success stories in transgenic animal models are very few and the development of stem cell therapy to treat stroke is at its infant stages.

3.2 Parkinson's Disease

Parkinson's disease is a common neurodegenerative disorder recognised by its motor symptoms and caused due to an extensive and progressive degeneration and subsequent loss of mesencephalic dopamine (DA) neurones in the substantianigra pars compacta (SNpc) and dopamine neuronal terminals in the striatum [66]. Loss of dopamine-producing cells causes speech problems, tremors, weakness, inability to swallow, poor balance, muscle rigidity, loss of dexterity, bradykinesia, postural instability and reduced sense of smell. The earliest biochemical change which can be observed in the patients suffering from PD is the decrease in the levels of glutathione (GSH) [67]. The pharmacological drug L-DOPA (L-dihydroxyphenylalanine) can meliorate the symptoms but loses its effectiveness after a long-term use [68,69]. Systematic injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the gold standard of generating Parkinson's Disease animal models in laboratories. When induced, it subsequently converts into MPP+ in the brain and is later aspired into the dopamine neurones by the dopamine transporter (DAT). These block the electron transport chain reaction occurring in mitochondria of dopamine neurones and produce reactive oxygen species (ROS) which kill these neurones [70].

In another approach, a hole is drilled in the skull and neurones are injected surgically into the most-affected areas of the brain. The foetal tissues are obtained by legally-induced aborted embryos or accident victims [8]. After 1-2 weeks of study, the MPTP-treated animal models show therapeutic effects of transplanted cells at different stages. No secondary effect was observed after transplantation. However, foetal tissue grafting has not provided full recovery, with only 40% success which showed reduced tumour growth [71]. Nevertheless, neural stem cells have a great hidden potential to treat patients suffering from Parkinson's disease by using cell replacement therapy [14].

3.3 Brain Tumours

Brain tumours remain to be a challenge for medical science as they are still difficult to cure in spite of all the advances in surgical and therapeutic techniques [72]. It is one of the major causes of the development of cancers and subsequent deaths of children. Glioblastoma multiforme and other malignant brain tumours are still practically not curable and, therefore, are fatal. The survival rate in patients has been substantially increased by prevailing treatments which include radiation or chemotherapy preceding radical surgical resection [4]. Nevertheless, brain tumours are untreatable in majority of the patients. Effective therapies possessing little toxicity to the patients are, hence, urgently being studied and researched on.

The latest research being conducted on human brain tumours focuses largely on the cellular and molecular analysis of tumour masses in bulk. Non-toxic pro-drugs are converted into toxic anti-cancerous drugs by expressing the gene for exogenous enzymes. This molecular approach, called suicide gene-based therapy, is being used most widely. Furthermore, genes expressing immune-stimulating cytokines can also be transferred by using a technique known as genetic immunotherapy [73]. Most prominently, genetically modified neural stem cells can be used in treating many human brain tumours. For instance, *in vivo* tumour growth can be effectively reduced by 50% via using pro-drug 5-fluorocytosine (5-FC) and neural stem cells simultaneously, which in effect produce a bioactive factor, cysteine deaminase (CD). Transplanted neural stem cells mediate the conversion of 5-flurocytosine into 5-fluorouracil which in turn stimulates tumour regression. These neural stem cells can also produce an agent which kills tumour cells and themselves undergo apoptosis. Research shows that 5-fluorocytosine is ineffective in the absence of medium offered by neural stem cells expressing cysteine deaminase [74].

In order to examine the ability of neural stem cells to secrete TRAIL (the pro-apoptotic protein), athymic nude mice were used. TRAIL was secreted in high levels within the tumour masses followed by subsequent decrease in tumour

volume which proved that neural stem cells expressing TRAIL had migrated into the tumour outgrowths. In another medulloblastoma model, human neural stem cells expressing the cysteine deaminase gene (mesendephlic neurones) were introduced into the brain's contralateral hemisphere. These models were then systematically injected with 5-fluorocytosine. Histological findings reveal a 76% decrease in the tumour volume owing to the migration of neural stem cells to the tumour bed and lesion boundary [75]. The aforementioned findings provide an enlightened potential for treating gliomas, medulloblastomas and many other human brain tumours.

3.4 Metastatic Tumours

Metastatic tumours are malignant and, therefore, spread to other parts of the body. Quite a few researches have vindicated the therapeutic effects of neural stem cells in animal models of metastatic tumours. Under normal physiological conditions, the brain receives 15-20% of the blood flow circulated in the body. Most of the patients suffering from metastatic brain tumours sustain two or more metastases [76]. Each of these cannot be treated individually owing to the impermeability of the blood-brain barrier (BBB) to most chemotherapeutic drugs [4].

Majority of the brain metastatic tumours originate from primary cancers of skin, breast and lung (primary cancer of lungs possesses the highest probability of developing brain metastases with more or less 40% of lung cancer patients developing metastatic tumours, while breast cancer is the second most commonly associated primary cancer) [77]. Animal models can be prepared by two methods, namely blood-born brain metastasis and direct implantation of tumour cells into the brain. In models of metastatic breast cancer, tumours were treated by employing neural stem cells expressing cysteine deaminase (CD) or carboxyl esterase (CE) apoptotic genes. Carboxyl esterase (CE) converts CPT-11 pro-drug to the toxic compound SN-38. Furthermore, neural stem cells expressing yeast cysteine deaminase (yCD) have proven to be far more operative than the bacterial cysteine deaminase, equally *in vitro* and *in vivo* conditions; the former neural stem cells in the presence of 5-flurocytosine regressed the comportment of lung cancer cells. This drug, furthermore, easily penetrates the blood-brain barrier (BBB) and, therefore, can be introduced into the brain parenchyma. Consequently, this pro-drug is relied upon to possess a concealed potential in treating various metastatic brain tumours [78].

3.5 Spinal Cord Injury

The therapeutic effects of transplanted stem cells can also be studied in animal models induced with an important neurological disorder known as spinal cord injury (SCI) [14]. It reduces the rate of regeneration and proliferation in the central nervous system (CNS) owing to which patients suffer from permanent paralysis. After spinal cord injury, robust cell death occurs in injured regions because of which cysts and cavities form and, consequently, block neurotransmission, both ascending and descending. Neuronal fibre damage, glial scar formation and mass ischaemic neural cell necrosis along with the subsequent apoptosis occur instantly after spinal cord injury with consequent injuries of secondary tissues. This injury is also pigeonholed (characterised) by commotion of axonal functions, loss of axonal proliferation and failure of sensory and motor function [79].

The biggest hurdle in treating spinal cord injury with stem cell therapy is the transplantation into the injured central nervous system. Transplanted cells are selected on their capability to supplant the injured site, promote and steer axonal growth, form myelin protein to promote neurotransmission, promote malleability in the spinal cord, and secrete neuroprotective trophic factors. In majority of the transplantations, neural stem cells, in few microliters of amount, are injected directly into the injured area through fine glass needles or capillaries [79]. In animal models, transplantation is done 1-2 weeks concomitant to the injury and is known as sub-acute therapy; this wait is required as transplantations done immediately after the injury seldom render expected recovery due to the robust cell death. When done at compromised optimal time, the transplantation yields positive recovery in the form of re-myelination, promotion of axon and neuronal proliferation, and inhibition of necrosis and neuronal apoptosis [80].

3.6 Motor Neurone Diseases

Motor neurones (MNs) derived from human pluripotent stem cells are being used in cell-based modelling in understanding the adjuvant development of motor neurone diseases. Motor neurones are specifically obtained from the ventral horn of the spinal cord. Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are the most prominent motor

neurone diseases [14]. Amyotrophic lateral sclerosis (ALS) is also widely known as Lou Gehrig's disease and is a late-onset neurodegenerative disorder, described by progressive and devastating loss of cortical and spinal motor neurones. It results in gradual myasthenia and amyotrophy with sequent paralysis and death. Transgenic mice overexpressing human superoxide dismutase 1 (SOD1) gene can be used as animal models for studying human amyotrophic lateral sclerosis. Spinal muscular atrophy (SMA), on the other hand, is described by hypotonia (muscles lacking normal tone or tension), symmetrical proximal muscle weakness, lack of motor ontogeny, and severe muscle weakness [81]. These effects result in reduction in survival of motor neurones (SMN). The pathogenesis of this disorder can be studied by deleting the SMN gene to produce a transgenic model [14].

Therapies involving stem cells can be used to treat SMA and ALS. Motor neurones obtained from an SMA patient showed castrated neurite extensions and reduced survival in culture as compared to the healthy motor neurones. *In vivo* and *in vitro* transplantation of motor neurones derived from human embryonic stem cells exhibited remarkable positive therapeutic effects as they survived at least six week in the model's spinal cord [82]. This study enlightened the future possibilities to employ this technique in its translational applications.

3.7 Multiple Sclerosis

Sclerosis is any pathological hardening or thickening of tissues [14]. Multiple sclerosis (MS) is an autoimmune-arbitrated inflammatory disease. Inflammation and de-myelination occurs at multifocal regions which results in neuronal loss. It is characterised by oodles of signs and symptoms. Majority of the patients fail responding to many approved treatments for multiple sclerosis. Demyelinating disease models are used in laboratories to conduct researches and studies on this neurodegenerative disease. Experimental autoimmune encephalomyelitis (EAE) is used in animal models for studying multiple sclerosis as it is among the most frequently described disease [83]. Induced models develop signs of gliosis, demyelination, inflammation and loss of axons. It is also suggested that models for multiple sclerosis can be developed by viral infection. Numerous human pathogenic viral particles possess the ability to infiltrate the central nervous system and cause damage to the myelin sheath, thus affecting the neurotransmission [84]. However, remyelination in persistently infected mice with MHV (mouse hepatitis virus) has been observed when oligodendrocyte progenitor cells (OPCs) derived from human ESCs were transplanted in the MS models. This ability of OPCs to promote remyelination can prove to be a vital progress in the development of therapies for the treatment of multiple sclerosis [85].

4. BIOMEDICAL RESEARCH ON HPSC-DERIVED NEURONES, CLINICAL APPLICATIONS AND SAFETY CONSIDERATIONS

Human pluripotent stem cells have been successfully differentiated into neurones and glia [14]. These stem cells have been used in modelling various neurological diseases for preclinical trials in laboratories and still have great hidden potential to pose a worthful impact on biomedical research being carried on therapeutic techniques and regenerative medicine. Human induced pluripotent stem cells (hiPSCs) have edge over transplantations by the fruitful prospect of rectifying mutations by using homologous recombination technology which happens, for example, in adult brain. Neural stem cells in neurogenic regions such as cortex, olfactory bulb, subependymal zone and hippocampus have the ability to proliferate and differentiate into glia or neurons [4].

For individuals, genetically rectified iPSCs can be generated by using genome-editing technology; this surely is a progression in iPSC technology. These iPSCs could be utilised as a pool of neurons required for therapeutic transplantation [86]. Furthermore, majority of the patients suffering from neurological disorders are given physiotherapy trainings along with stem cell transplantations which result in raised levels of satisfactory results [87].

However, production of specific neurone subtypes is one of the many possible risks in the aforementioned procedures because of the high variability in protocols. This further hinders the ability of these subtypes to faux disease-specific phenotypes [86]. Though human pluripotent stem cells have proved to be very functional and effective *in vitro*, the purity of cells to be transplanted, sites of transplantation, tumourigenesis and accumulation (integration) of transplanted cells are the some of the many obstacles in their clinical translation into patients. For this purpose, safer induced pluripotent stem

cells need to be developed to avoid integration of exogenous DNA which can consequently alter genome and cause tumour formation. In order to select the desired and required types of cells for transplantation, specific biomarker can be used to sort cells. To overcome tissue rejection and histocompatibility-associated problems, induced pluripotent cells, which are immunogenically specific to patients, should be developed [88].

Human pluripotent stem cells offer a resource of innumerable disease-specific progenitor cells which can be used in regenerative medicine. Cell types may vary in accordance to the culture conditions, protocols of differentiation and sources of materials. Before applying preclinical assessments on clinical cases, these aspects must be considered. Pluripotent cells should be efficiently and effectively introduced and nurtured in order to get the desired phenotype. Remarkable advancements have been made in research on human pluripotent stem cells and induced pluripotent stem cells. Nevertheless, clinical studies should be designed carefully and made safer for the patients to obliterate the potential hidden in stem cell-based therapies.

5. CONCLUSION

Stem cell therapy has great potential in treating various neurodevelopmental and neurode-generative disorders. Much of the work relies on the ability of self-renewal of mesodermal and ectodermal stem cells, and endodermal stem cells are rarely used to serve the purpose. Much progress has been made by employing neural stem cells, the transplantation of which have been useful in treating neurological disorders, namely stroke, Parkinson's disease, brain tumours, metastatic tumours, spinal cord injury, ALS, SMA and multiple sclerosis. However, the safety and feasibility of these procedures need to be tested and confirmed before applying them clinically. Without doubt, therapeutic effects of pluripotent stem cells are still to be unleashed in the future.

ACKNOWLEDGEMENTS

I would like to thank GC University Lahore and respected Vice Chancellor, Prof. Dr. M. Khaleeq-ur-Rahman (Izaz-i-Kamal), for providing me with this platform. This piece of writing would have never been written without the continuous backing of and counselling from my instructor, Dr.Sikander Ali (IIB, GC University Lahore). Doubtlessly, I cannot forget the invariant and ceaseless encouragement and support of my grandfather, parents and sister. I apologise to all whose works could not be cited due to space constraints.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saeed H, Iqtedar M. Stem cell function and maintenance - end that matter: Role of telomeres and telomerase. J. Biosci. 2013;38:641-649.
2. Zhang Y, Khan D, Delling J, Tobiasch E. Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. Sci. World. J. 2012;793823.
3. 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(1):55-65.
4. Yi B, Kim S, Choi K. Development and application of neural stem cells for treating various human neurological diseases in animal models. Lab Anim Res. 2013;29(3): 131-137.
5. Evans M, Kaufman M. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292(5819):154-156.
6. Reuinoff B, Pera M, Fong C, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol. 2000;18(4):399-404.
7. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res. 2008;100:133-158.
8. Denker H. Potentiality of embryonic stem cells: an ethical problem even with alternative stem cell sources. J Med Ethics. 2006;32:665-671.
9. Kørbling M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? N Engl J Med. 2003;349(6):570-582.
10. Li L, Baroja M, Majumdar A, et al. Human embryonic stem cells possess immune-privileged properties. Stem Cells. 2004;22(4):4448-4456.
11. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomodo K, Yamanaka S. Induction of pluripotent stem cells from

adult human fibroblasts by defined factors. Cell. 2007;131:861-872.
12. Noisa P, Parnpai R. Technical challenges in the derivation of human pluripotent cells. Stem Cells International. 2011;7:907-961.
13. Harting M, Jimenez F, Xue H, Fischer U, Baumgartner J, Dash P, Cox C. Intravenous mesenchymal stem cell therapy for traumatic brain injury. J Neurosurg. 2009;110(6):1189-1197.
14. Jongkamonwiwat N, Parinya N. Biomedical and clinical promises of human pluripotent stem cells for neurological disorders. Biomed Res Int. 2013;656531.
15. Vallier L, Alexander M, Pedersen R. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J Cell Sci. 2005;118(Pt19):4495-4509.
16. Anneren C, Cowan CA, Melton DA. The Src family of tyrosine kinases is important for embryonic stem cell slef-renewal. J Biol Chem. 2004;279:31590-31598.
17. Tsai C, Hung S. Functional roles of pluripotency transcription factors in mesenchymal stem cells. Cell Cycle. 2012;11:3711-3712.
18. Zhao H, Sun N, Young S, Nolley R, Santos J, Wu J, Peehl D. Induced pluripotency of human prostatic epithelial cells. PLoS One. 2013;8:e64503.
19. Daadi M, Davis A, Arac A, Li Z, Maag A, Bhatnagar R, Jiang K, Sun G, Wu J, Steinberg G. Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. Stroke. 2010;41:516-523.
20. Selden N, Al-Uzri A, Huhn S, Koch T, Sikora D, Nguyen-Driver M, Guillaume D, Koh J, Gultekin S, Anderson J, et al. Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis. J Neurosurg Pediatr. 2013;11:643-652.
21. Yang L, Zheng J, Liu X, Hui G, Guo L. Culture of skin-derived precursors and their diffrentiation into neurons. Chin J Traumatol. 2004;7:91-95.
22. Dhanasekaran M, Indumathi S, Poojitha R, Kanmani A, Rajkumar J, Sudarsanam D. Plasticity and banking potential of cultured adipose tissue derived mesenchymal stem cells. Cell Tissue Bank. 2013;14:303-315.
23. Siegel G, Kluba T, Hermanutz-Klein U, Bieback K, Northoff H, Schäfer R. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. BMC Med. 2013;11:146.
24. Qiu P, Song W, Niu Z, Bai Y, Li W, Pan S, Peng S, Hua J. Platelet-derived growth factor promotes the proliferation of human umbilical cord-derived mesenchymal stem cells. Cell Biochem Funct. 2013;31:159-165.
25. Wakao S, Kitada M, Kurodo Y, Shigemoto T, Matsuse D, Akashi H, Tanimura Y, Tsuchiyama K, Kikuchi T, Goda M, et al. Multilineage-differentiating stress-enduring (Muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. Proc Natl Acad Sci USA. 2011;108:9875-9880.
26. Kanno H. Regenerative therapy for neuronal diseases with transplantation of somatic stem cells. World J Stem Cells. 2013;5(4):163-171.
27. Lee C, Hu J, Ralls S, Kitamura T, Loh Y, Yang Y, Mukouyama Y, Ahn S. The molecular profiles of neural stem cell niche in the adult subventricular zone. PLoS One. 2012;7(11):e50501.
28. Galli R, Gritti A, Bonfanti L, Vescovi A. Neural stem cells: an overview. Circ Res. 2003;92(6):598-608.
29. Liu F, Uchugonova A, Kimura H, Zhang C, Zhao M, Zhang L, Koenig K, Duong J, Aki R, Saito N, et al. The bulge area is the major hair follicle ource of nestin-expressing pluripotent stem cells which can repair the spinal cord compared to the dermal papilla. Cell Cycle. 2011;10:830-839.
30. Krejčí E, Grim M. Isolation and characterization of neural stem cells from adult human hair follicles. Folia Biol (Praha). 2010;8:134-140.
31. Higashida T, Jitsuki S, Kubo A, Mitsushima D, Kamiya Y, Kanno H. Skin-derived precursors differentiating into dopaminergic neuronal cells in the brains of Parkinson disease model rats. J Neurosurg. 2010;113:648-655.
32. Ahmad I, Dooley C, Thoreson W, Rogers J, Afiat S. *In vitro* analysis of a mammalian retinal progenitor that gives rise to neurons and glia. Brain Res. 1999;831:1-10.
33. Coles B, Angénieux B, Inoue T, Del Rio-Tsonis K, Spence J, McInnes R, Arsenijevic Y, van der Kooy D. Facile isolation and the characterization of human retinal stem cells. Proc Natl Acad Sci USA. 2004;101:15772-15777.

34. Chai Y, Jiang X, Ito Y, Bringas P, Han J, Rowitch D, Soriano P, McMahon A, Sucov H. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development. 2000;127: 1671-1679.
35. Arthur A, Shi S, Zannettino A, Fujii N, Gronthos S, Koblar S. Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells. 2009;27:2229-2237.
36. Hunt D, Morris P, Sterling J, Anderson J, Joannides A, Jahoda C, Compston A, Chandran S. A highly enriched niche of precursor cells with neuronal and glial potential within the hair follicle dermal papilla of adult skin. Stem Cells. 2008;26:163-172.
37. Biemaskie J, Sparling J, Liu J, Shannon C, Plemel J, Xie Y, Miller F, Tetzlaff W. Skin-derived precursors generate myelinating Schwann cells that promote remyelination and functional recovery after contusion spinal cord injury. J Neurosci. 2007;27:9545-9559.
38. Toma J, McKenzie I, Bagli D, Miller F. Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells. 2005;23:727-737.
39. Abdanipour A, Tiraihi T. Induction of adipose-derived stem cell into motoneuron-like cells using selegiline as preinducer. Brain Res. 2012;1440:23-33.
40. Lee S, Chu K, Jung K, Im W, Park J, Lim H, Won C, Shin S, Lee S, Kim M, et al. Slowed progression in models of Huntington disease by adipose stem cell transplantation. Ann Neurol. 2009;66:671-681.
41. Chen J, Tang Y, Liu Y, Chen K, Hu X, Liu N, Wang S, Zhang Y, Zeng W, Ni H, et al. Transplantation of adipose-derived stem cells is associated with neural differentiation and functional improvement in a rat model of intracerebral hemorrhage. CNS Neurosci Ther. 2012;18:847-854.
42. Koh K, Oh T, Kim H, Chung I, Lee K, Lee H, Park E, Jung J, Shin I, Ra J, et al. Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry-Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera. Ann Plast Surg. 2012;69:331-337.
43. Dezawa M, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, et al. Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest. 2004;113:1701-1710.
44. Lee J, Jin H, Endo S, Schuchman E, Carter J, Bae J. Intracerebral transplantation of bone marrow-derived mesenchymal stem cells reduces amyloid-beta deposition and rescues memory deficits in Alzheimer's disease mice by modulation of immune responses. Stem Cells. 2010;28:329-343.
45. Bahat-Stroomza M, Barhum Y, Levy Y, Karpov O, Bulvik S, Melamed E, Offen D. Induction of adult human bone marrow mesenchymal stromal cells into functional astrocyte-like cells: Potential for restorative treatment in Parkinson's disease. J MolNeurosci. 2009;39:199-210.
46. Liang J, Zhang H, Hua B, Wang H, Wang J, Han Z, Sun L. Allogenic mesenchymal stem cells transplantation in treatment of multiple sclerosis. Mult Scler. 2009;15:644-646.
47. Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S, Carriero A, Monaco F, Fagioli F. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: A long-term safety study. Cytotherapy. 2012;14:56-60.
48. Karamouzian S, Nematollahi-Mahani S, Nakhaee N, Eskandary H. Clinical safety and primary efficacy of bone marrow mesenchymal cell transplantation in subacuteapinal cord injured patients. Clin Neurol Neurodurg. 2012;114:935-939.
49. Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis J. A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. Brain Res. 2004;1007:1-9.
50. Sharma A, Gokulchandran N, Chopra G, Kulkarni P, Lohia M, Badhe P, Jacob V. Administration of autologous bone marrow-derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. Cell Transplant. 2012;21(Supplement 1):79-90.
51. Wang T, Tio M, Lee W, Beerheide W, Udolph G. Neural differentiation of mesenchymal-like stem cells from cord blood is mediated by PKA. Biochem Biophys Res Commun. 2007;357:1021-1027.

52. Kang J, Lee C, Kim J, Yu S, Jo J, Do B, Kim H, Kang S. Estrogen stimulates the neuronal differentiation of human umbilical cord blood mesenchymal stem cells. Neuroreport. 2007;18:35-38.
53. Chen L, He D, Zhang Y. The differentiation of human placenta-derived mesenchymal stem cells into dopaminergic cells *In vitro*. Cell MolBiol Lett. 2009;14:528-536.
54. Nazaroy I, Lee J, Soupene E, Etemad S, Knapik D, Green W, Bashkirova E, Fang X, Matthay M, Kuypers F, et al. Multipotent stromal stem cells from human placenta demonstrate high therapeutic potential. Stem Cells Transl Med. 2012;1:359-372.
55. Kuroda Y, Wakao S, Kitada M, Murakami T, Nojima M, Dezawa M. Isolation, culture and evaluation of multilineage-differentiating stress-enduring (Muse) cells. Nat Protoc. 2013;8:1391-1415.
56. Zhao Y, Glesne D, Huberman E. A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. Proc Natl Acad Sci USA. 2003;100:2426-2431.
57. Suzuki K, Mitsutake N, Saenko V, Suzuki M, Matsuse M, Ohtsuru A, Kumagai A, Uga T, Yano H, Nagayama Y, et al. Differentiation of human primary thyrocytes into multilineageprogenitor cells without gene introduction. PLoS One. 2011;6: e19354.
58. Boer G. Ethical guidelines for the use of human embryonic or fetal tissue for experimental and clinical neurotransplantation and research. Network of European CNS Transplantation and Restoration (NECTAR) J Neurol. vol. 1994;242:1-13.
59. Lazarov O, Marr R. Of mice and men: neurogenesis, cognition and Alzheimer's disease. Front Aging Neurosci. 2013;7(11): e50501.
60. Alvarez-Buylla A, Lim D. For the long run: maintaining germinal niches in the adult brain. Neuron. 2004;41(5):683-686.
61. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci. 2006;7(5):395-406.
62. Lendahl U, Zimmeran L, McKay R. CNS stem cells express a new class of intermediate filament protein. Cell. 1990;60:585-595.
63. Lindvall O, Kokaia Z. Stem cells in human neurodegenerative disorders - time for clinical translation? J Clin Invest. 2010;120 (1):29-40.
64. Graham S, McCullough L, Murphy S. Animal models of ischemic stroke: balancing experimental aims and animal care. Comparative Medicine. 2004;54(5): 486-496.
65. Kelly S, Bliss T, Shah A, Sun G, Ma M, Foo W, Masel J, Yenari M, Weissman I, Uchida N, Palmer T, Steinberg G. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci USA. 2004;101(32):11839-11844.
66. Storch A, Schwarz J. Neural stem cells and Parkinson's disease. J Neurol. 2002;249(Supplement 3):III/30-III/32.
67. Martin H, Teismann P. Glutathione - a review on its role and significance in Parkinson's disease. FASEB J. 2009;23 (10):3263-3272.
68. Hoehn M, Yahr M. Parkinsoniam: Onset, progression and mortality. Neurology. 1967;17:427-442.
69. Molina J, Sainz-Artiga M, Fraile A, Jimenez-Jimenex F, Villanueva C, Orti-Pareja M, Bermejo-P F. Pathological gambling in Parkinson's disease: A behavioral manifestation of pharmacologic trearment. Movement Disorders. 2000;15(5):869-872.
70. Dauer W, Przedborski S. Parkinson's disease: Mechanisms and models. Neuron. 2003;39(6):889-909.
71. Doi D, Morizane A, Kikuchi T, et al. Prolonged maturation culture favors a reduction in the tumorigenicity and dopaminergic function of human ESC-derived neural cells in a primate model of Parkinson's disease. Stem Cells. 2012;30(5):935-945.
72. Singh S, Clarke I, Terasaki M, Bonn V, Hawkins C, Squire J, Dirks P. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003;63(18):5821-5828.
73. Kim S. Neural stem cell-based gene therapy for brain tumors. Stem Cell Rev. 2011;7(1):130-140.
74. Barresi V, Belluardo N, Sipione S, Mudò G, Cattaneo E, Condorelli D. Transplantation of prodrug-converting neural progenitor cells for brain tumor therapy. Cancer Gene Ther. 2003;10(5):396-402.
75. Kim S, Kim S, Park I, Bang J, Aboody K, Wang K, Cho B, Kim M, Menon L, Black P, Carroll R. Human neural stem cells target experimental intracranial medulloblastoma

and deliver a therapeutic gene leading to tumor regression. Clin Cancer Res. 2006;12(18):5550-5556.
76. Biswas G, Bhagwat R, Khurana R, Menon H, Prasad N, Parikh P. Brain metastasis - evidence based management. J Cancer Res Ther. 2006;2(1):5-13.
77. Barnholtz-Sloan J, Sloan A, Davis F, Vigneau F, Lai P, Sawaya R. 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.
78. Vermes A, Guchelaar H, Dankert J. Flucytosine: A review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. J Antimicrob Chemother. 2000;46(2):171-179.
79. Borner J, Blesch A, Neuhuber B, Fischer I. Promoting directional axon growth from neural progenitors grafted into the injured pinal cord. Journal of Neuroscience Research. 2010;88(6):1182-1192.
80. Garbossa D, Boido M, Fontanella M, Fronda C, Ducati A, Vercelli A. Recent therapeutic strategies for spinal cord injury treatment: Possible role of stem cells. Neurosurgical Review. 2012;35(3):293-311.
81. Zanoteli E, Maximino J, Conti Reed U, Chadi G. Spinal muscular atrophy: From animal model to clinical trial. Functional Neurology. 2010;25(2):73-79.
82. Lee H, Shamy G, Elkabetz Y, et al. Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. Stem Cells. 2007;25(8): 1931-1939.
83. Bai L, Hecker J, Kerstetter A, et al. Myelin repair and functional recovery mediated by neural cell transplantation in a mouse model of multiple sclerosis. Neuroscience Bulletin. 2013;29(2):239-250.
84. Ascherio A, Munger K. Environmental risk factors for multiple sclerosis - part I: The role of infection. Annals of Neurology. 2007;61(4):288-299.
85. Ascherio A, Munger K. Environmental risk factors for multiple sclerosis - part II: noninfectious factors. Annals of Neurology. 2007;61(6):504-513.
86. Nguyen H, Byers B, Cord B, et al. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell. 2011;8(3): 267-280.
87. Araki A, Uda M, Hoki Y, et al. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature. 2013;494 (7435):100-104.
88. Zhao T, Zhang Z, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011;474(7435):212-215.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=865&id=32&aid=8399